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Editorial Team

CONTENTS

ORIGINAL ARTICLES

REVIEW ARTICLES

CASE STUDIES

ORIGINAL ARTICLE

CONTENTS

Evaluation of E-cadherin expression in invasive ductal breast cancer

Ilona V. Chelpanova[1], Liliya I. Volos[1], Andrii P. Dudash[2], Yevgen V. Paltov[1], Artur V. Poliiants[3], Olha V. Dudok[1], Yulia V. Hnidyk[1]

[1]STATE NON-PROFIT ENTERPRISE «DANYLO HALYTSKY LVIV NATIONAL MEDICAL UNIVERSITY», LVIV, UKRAINE
[2]WESTERN UKRAINIAN HISTOLOGICAL LABORATORY, LVIV, UKRAINE
[3] INTERNATIONAL MEDICAL CENTER LLC, KYIV, UKRAINE

ABSTRACT

Aim: To evaluate E-cadherin expression in various clinical and pathological prognostic scenarios to determine its significance in the development of molecular subtypes of invasive ductal breast cancer.

Materials and Methods: A comprehensive morphological and immunohistochemical study of 80 cases of invasive ductal carcinoma (IDC) was conducted to determine the molecular phenotype. The expression of E-cadherin, ER, PR receptors, c-erbB2, and Ki-67 was evaluated according to the manufacturer's standardized protocols using appropriate positive and negative controls. The degree of tumor malignancy was determined using the modified Scarff-Bloom-Richardson system. Semi-quantitative assessment of E-cadherin expression was performed using the Qureshi scale. Pearson's criterion was used for statistical analysis. Differences were considered statistically significant at $p < 0.05$.

Results: Low E-cadherin expression was associated with stage 3, pT3, and G2/G3 grades of IDBC malignancy, confirming its unfavorable prognostic significance and correlation with the molecular profile. High E-cadherin expression was characteristic of ER-positive luminal A tumors, regardless of menopause, indicating a regulatory role for ER expression. The low proliferative activity of luminal IDBC cells was explained by high E-cadherin expression, which increased adhesive properties. Low E-cadherin expression is also a prognostic marker for TNBC.

Conclusions: E-cadherin is a potent tumor suppressor in breast cancer. Its role in disease progression is confirmed by the correlation between partial or complete loss of E-cadherin expression and poor prognosis for patients.

KEY WORDS: E-cadherin expression, morphological and immunohistochemical study, invasive ductal breast cancer, molecular phenotype

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INTRODUCTION

Breast cancer (BC) is a significant global health problem, being the most commonly identified cancer among women and ranking second in terms of mortality from cancer. According to the World Health Organization strategy, the key objective in the fight against BC is to achieve an annual reduction in global mortality, potentially preventing millions of deaths worldwide by 2040.

The strategy, which aims to reduce global breast cancer mortality by 2.5% annually, envisages preventing 25% of deaths by 2030 and 40% by 2040 among women under the age of 70. Experts emphasize that achieving these goals requires strengthening health measures for early detection, rapid diagnosis, and a comprehensive approach to breast cancer treatment [1]. And also taking into account the current trends in modern reconstructive and cosmetic surgery, such as augmentation mammoplasty, which is not usually associated with an increased risk of cancer, although it has an aesthetic purpose, may contribute to the early detection and timely diagnosis of breast cancer [2]. The current understanding of (BC) defines it as a spectrum of diseases with diverse morphology, unique molecular profiles, clinical courses, and responses to treatment [3]. In the context of the development of personalized medicine based on individual patient characteristics, despite the expansion of therapeutic options, traditional clinical-pathological and already known molecular prognostic markers are insufficient to reflect this degree of heterogeneity. For a successful, so-called "targeted" approach to therapy and improved cancer treatment efficacy, both additional molecular markers and prognostic biomarkers are critically important.

Accurate prognostic stratification is critically important because it allows patients with a good prognosis, who can avoid potentially toxic systemic therapy, to be distinguished from patients with a poor prognosis who require more intensive forms of treatment. It is for this second group that the use of prognostic markers allows the most appropriate therapy to be determined, which will increase the chances of success and reduce the risk of side effects. Although HER2/neu and hormone receptors are the main prognostic factors in the routine diagnosis of breast cancer for targeting targeted therapy and provide some prognostic information, they do not solve all problems. Luminal tumors (ER-positive, HER2/neu-negative) account for a significant proportion of BC cases—almost 60% in the early stages with negative lymph nodes and over 50% of all molecular subtypes [4]. HER2/neu-positive (12-20%) and triple-negative (15-20%) BC are recognized as aggressive tumors associated with poor outcomes; unfavorable prognostic parameters of the disease are also found in younger patients [5]. In addition to breast cancer subtypes, histological assessment provides significant prognostic information, according to the 2013 St Gallen consensus [6].

Given the rising costs of healthcare and the emergence of new targeted therapies, the use of biomarkers has become an integral part of breast cancer diagnosis, predicting treatment response, and monitoring disease progression during and after treatment.

Over the past decade, numerous research centers have been actively investigating new prognostic factors based on molecular characteristics of tumors in patients with BC. In modern oncology, the priority area of molecular genetic research is the identification of genomic abnormalities that affect tumor development, malignancy, metastatic potential, and progression rate.

As a result, molecular morphopathology becomes crucial for tumor prediction. It analyzes the presence or absence of oncogenes and tumor growth suppressors (molecular biological markers) in cells, since differences in the expression of these markers can explain the varying aggressiveness of tumors that are comparable in prevalence and histological structure [7]. Analysis of molecular biological markers in tumor tissue provides a deeper understanding of its biological characteristics, such as growth rate, invasive and metastatic potential, and chemoresistance.

E-cadherin (epithelial cadherin) is one of the key markers that functions as a potent suppressor of tumor invasion and metastasis, providing cell adhesion, and its selective loss in human carcinomas can lead to dedifferentiation and increased invasiveness, confirming its role as a tumor suppressor [8]. The main cell adhesion molecule in epithelial tissues, E-cadherin, is encoded by the CDH1 gene located on chromosome 16q22.1 [9, 10]. This polypeptide, consisting of 728 amino acid residues, is expressed on the surface of epithelial cells. Among the most important features of the multistage process of metastasis is the deformation of adhesive contacts of neoplastic cells, which is caused by a violation of cadherin expression [11]. In addition, epithelial-mesenchymal transformation also plays a critical role in this process. Although epithelial-mesenchymal transformation (EMT) is similar to the processes of embryonic development, its decisive difference lies in its uncontrolled nature [12]. It is this transformation in epithelial tumors that provides their ability to invade and metastasize [13]. The significant role of E-cadherin as a tumor suppressor encoded by the CDH1 gene [14] is emphasized by the fact that its loss or abnormal expression promotes the invasion of neoplastic cells. In cases of breast cancer, a decrease in E-cadherin levels is observed in approximately 50% of invasive ductal carcinomas and reaches 90% in invasive lobular carcinoma, predominantly among tumors with a triple-negative phenotype [12]. A number of authors also point to a correlation between aberrant E-cadherin expression and disease stage, metastatic potential, and frequent absence of ER expression [15].

Morphological studies of breast cancer, especially with the introduction of immunohistochemical (IHC) methods, have become crucial, as their results allow not only to predict the course of the disease, but also to determine the direction of antitumor therapy [16]. Thus, preliminary data indicate the important role of E-cadherin protein expression.

It has been established that reduced expression of E-cadherin during tumor development triggers a cascade of signaling mechanisms, providing tumor cells with a more invasive phenotype, increasing their ability to migrate and survive, and promoting the development of distant metastases.

Research into the correlation between E-cadherin expression, lymph node metastasis status, clinical and pathological parameters, and the molecular phenotype of invasive ductal breast cancer is becoming increasingly important. The assessment of E-cadherin expression is recognized as a key prognostic marker for the course of breast cancer. Therefore, any deviations associated with abnormal expression or dysfunction of these cell adhesion molecules can have profound destructive consequences.

AIM

This study aim was to evaluate E-cadherin expression in various clinical and pathological prognostic scenarios

Table 1. Antibody panel for IHC testing

Antibody	Clone	Immunized animal	Manufacturer	Localization in the cell
E-cadherin	Clone HECD-1	monoclonal mouse antibodies	Master diagnostica	Membrane
ER	Clone SP1	monoclonal rabbit antibodies	Dako	Nucleus
PR	PgR 636	monoclonal mouse antibodies	Dako Flex	Nucleus
c-erbB2	Clone SP3	monoclonal rabbit antibodies, to Her2/neu	Thermo scientific	Membrane
Ki-67	Clone MIB-1	monoclonal mouse antibodies	Dako	Nucleus

Source: compiled by the authors of this study

to determine its significance in the development of molecular subtypes of invasive ductal breast cancer.

MATERIALS AND METHODS

The study covered 80 cases of invasive ductal breast cancer using immunohistochemical examination and analysis of E-cadherin expression. Immunohistochemical analysis of E-cadherin revealed immunoreactivity of varying degrees of intensity depending on the age of the patients, disease stage, tumor size, G malignancy grade, lymph node involvement, and different molecular subtypes of breast cancer.

In all cases, the diagnosis of the breast invasive ductal carcinoma was verified histologically. The histological type of cancer was determined in accordance with WHO recommendations [17]. Tumor grading according to the degree of malignancy was performed based on modified criteria by P. Scarff, H. Bloom, and W. Richardson [18].

After studying the clinical and pathomorphological information and dividing the sample into molecular subtypes according to the 2015 St. Gallen consensus [19], the following observation groups were formed: luminal A subtype (21 cases); luminal B subtype (19); Her2/neu (20); triple-negative (20).

The Bioethics Committee of Danylo Halytsky Lviv National Medical University (protocol No. 3 dated March 11, 2020) has established that all animals were housed in a vivarium and procedures for cleaning, inspection, marking and all other manipulations were carried out in accordance with the provisions of the «European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes» (Strasbourg, 1986), the «General Ethical Principles of Experiments on Animals» adopted by the First National Congress on Bioethics (Kyiv, 2001), the Law of Ukraine No. 3447-IV «On the Protection of Animals from Cruel Treatment» in accordance with the Directive of the Council of the European Union 2010/63/EU on compliance with the regulations, laws, and administrative provisions of the EU Member States on the protection of animals used for scientific purposes [20,21].

Histological studies of surgical material were performed using a Leica DM750 universal light microscope (Leica Microsystems GmbH) by a standard method [22]. Immunohistochemical examination was performed on serial paraffin sections of tumor tissue (invasive ductal breast cancer) according to the manufacturer's protocols. After deparaffinization, rehydration, temperature unmasking of antigens, and suppression of endogenous peroxidase activity, the sections were incubated with monoclonal antibodies in humid chambers at 23-25°C for 30 minutesThe DAKO EnVision+System visualization system was used. To identify the reaction, a solution of chromogen 3-diaminobenzidine tetrachloride ("DAKO", USA) was applied under microscopic control for 20 seconds to 3 minutes, with a brown color appearing. Then, we additionally stained with Mayer's hematoxylin for 1-3 minutes, followed by dehydration and fixation with balsam according to the standard procedure [23,24].

The immunohistochemical status of E-cadherin, estrogen receptor (ER), progesterone receptor (PR), c-erbB2 oncoprotein, and Ki-67 proliferation index expression was studied using the manufacturer's protocols with the necessary controls. For samples with Her2/neu 2+ status, fluorescence in situ hybridization (FISH) was performed based on the results of immunohistochemical examination. The antibody data panel is presented in Table 1.

ASSESSMENT OF IMMUNOHISTOCHEMICAL STAINING

Positive ER and PR expression was established when ≥1% of neoplastic cells showed positive nuclear expression of any intensity [25]. ER and PR status were assessed according to the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines for ER and PR IHC testing. The threshold between low and high Ki-67 nuclear expression was set at ≥20% positive cells according to the 2015 St. Gallen Consensus. For Her2/neu IHC, only

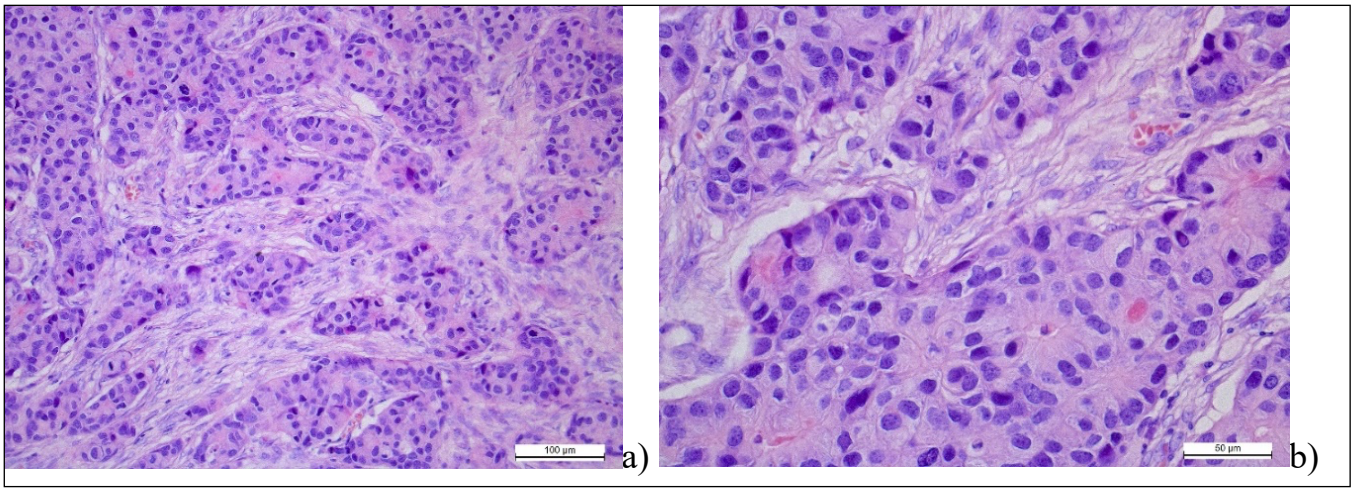

Fig. 1. Invasive ductal breast carcinoma: solid structures in the invasive component. Hematoxylin and eosin staining. a) ×200; b) ×400
Picture taken by the authors

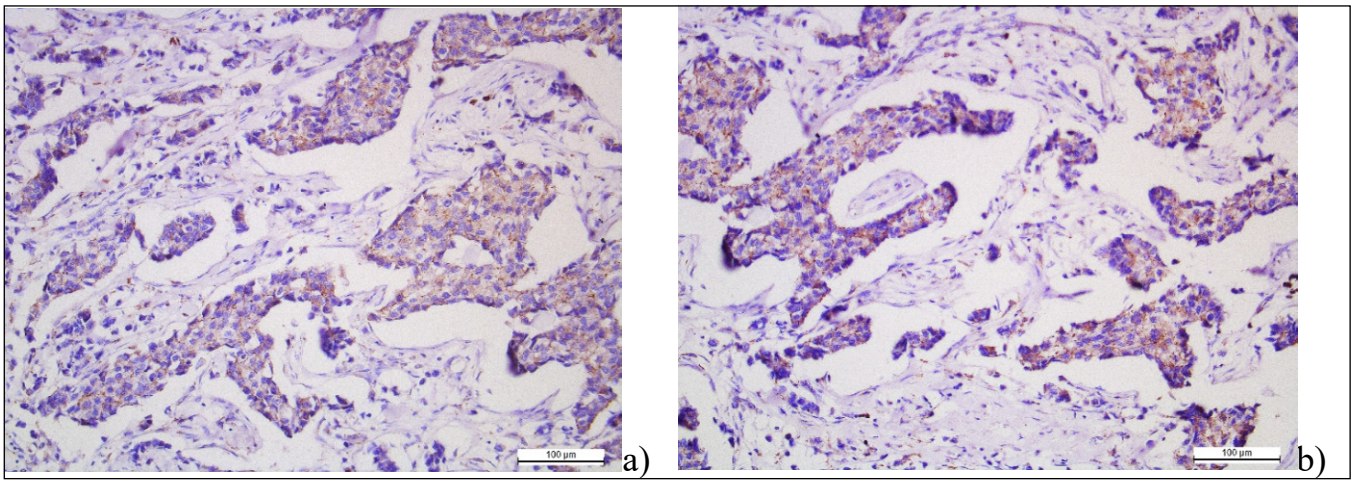

Fig. 2. Invasive ductal breast carcinoma : a) no immunoreactivity with antibodies to E-cadherin (×200); b) membrane positivity in <10% of tumor cells (0 points). ×200
Picture taken by the authors

membrane staining was considered, and more than 10% strong membrane positivity was accepted as positive (3+) Her2/neu according to CAP recommendations.

E-cadherin reaction was evaluated using a scoring system developed by Qureshi et al. [26], in which E-cadherin expression was assessed according to the percentage of positive cells and staining intensity in five fields of view at ×400 magnification. Scoring: 0 – no staining or membrane positivity in <10% of tumor cells; 1 – incomplete and weak membrane staining in >10% of tumor cells; 2 – complete membrane staining with weak or moderate intensity in >10% of tumor cells; 3 – strong membrane staining in >10% of tumor cells. According to this assessment, the reaction was considered negative for scores 0 and 1, weakly positive for scores 2, and strongly positive for scores 3. Cytoplasmic staining was considered nonspecific and was not included in the assessment. The presence of E-cadherin staining in epithelial cells of normal ducts and acini served as an internal positive control. All specimens were evaluated by two pathologists to ensure consistency.

STATISTICAL PROCESSING OF RESEARCH RESULTS

Statistical analysis of the results was performed using the R Commander program. The data are presented as percentages with 95% confidence intervals (% [95% CI]), calculated using Fisher's angular transformation criterion-φ. The comparison of the degree of E-cadherin expression at different clinical and pathological parameters and different molecular phenotypes was evaluated using Pearson's criterion. For all types of analysis, differences were considered significant at $p<0.05$.

RESULTS

Epithelial cadherin (E-cadherin) is an important member of the cadherin family, playing a major role

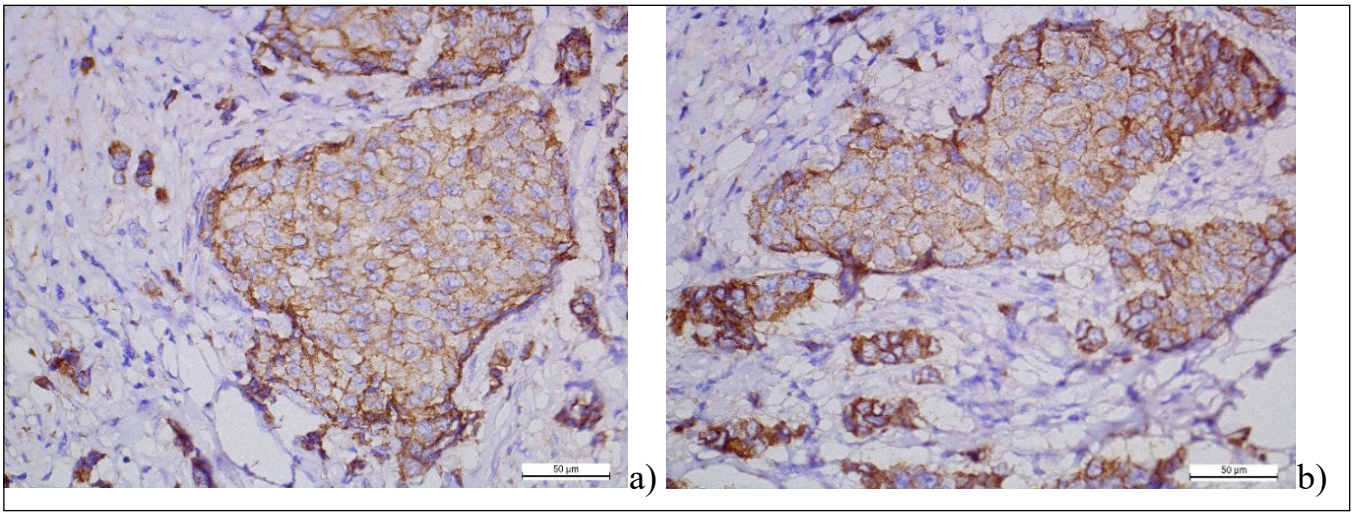

Fig. 3. Invasive ductal breast carcinoma , E-cadherin expression: incomplete (a) and weak (b) membrane staining >10% of tumor cells (1 point). ×400
Picture taken by the authors

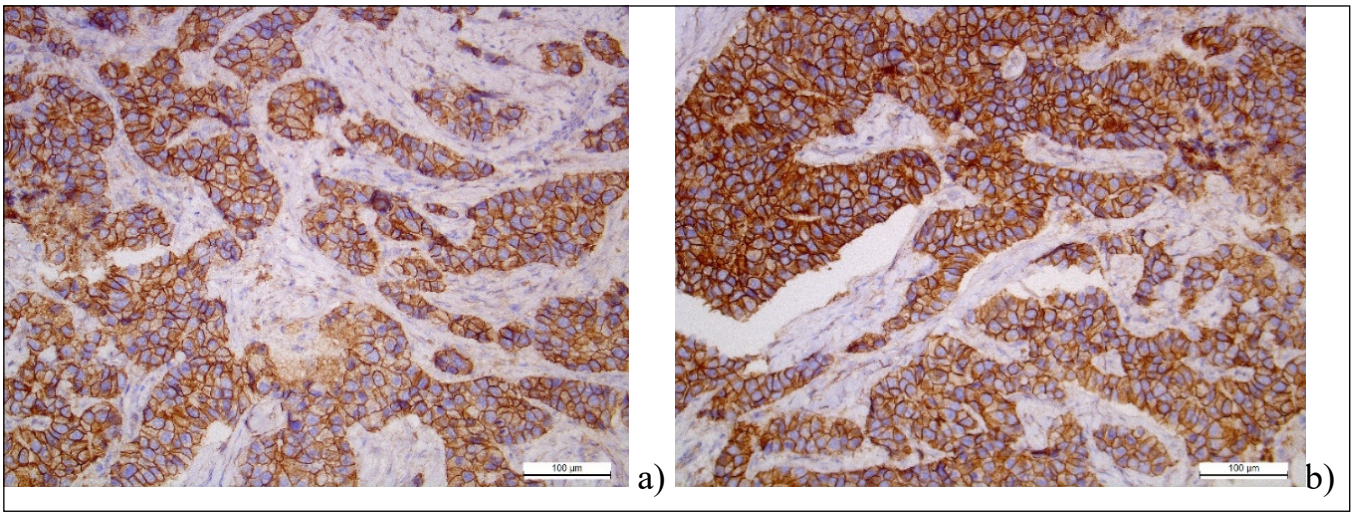

Fig. 4. Invasive ductal breast carcinoma, E-cadherin expression: complete membrane staining with weak (a) or moderate (b) intensity in >10% of tumor cells (2 points). ×200.
Picture taken by the authors

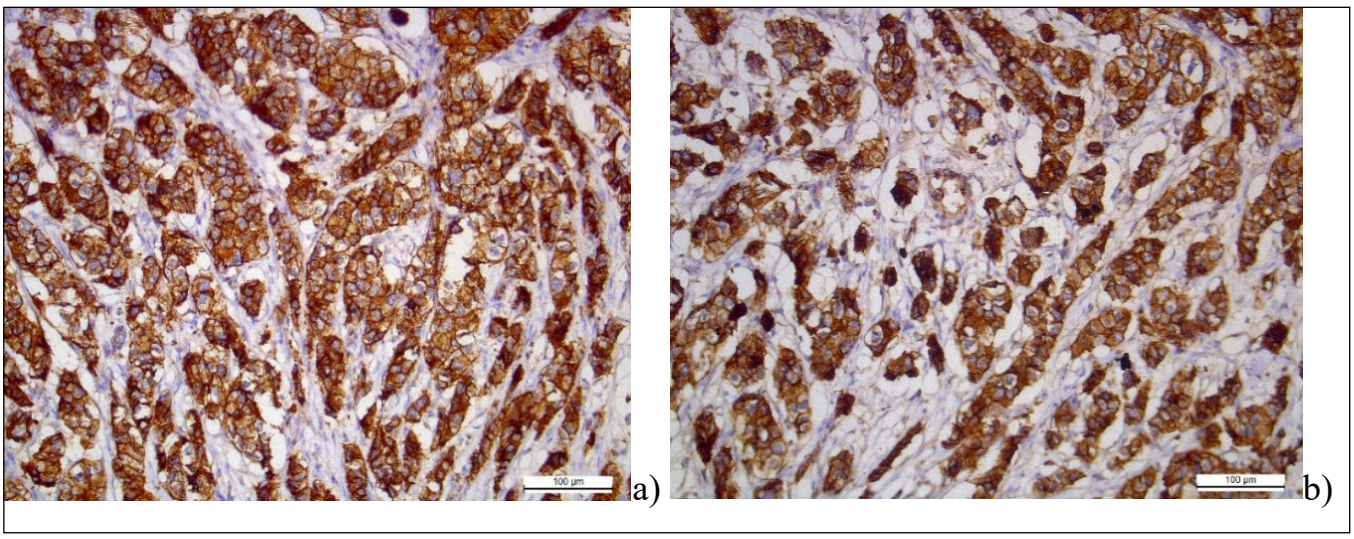

Fig. 5. Invasive ductal breast carcinoma, E-cadherin expression: strong membrane staining in >10% of tumor cells (3 points). ×200
Picture taken by the authors

in cell adhesion and acting as a powerful suppressor of invasion and metastasis. One of the most important features of the metastasis process is the deformation of adhesive contacts of neoplastic cells, mediated by impaired expression of cadherins. Another important feature of the metastasis process is the implementation of epithelial-mesenchymal transformation, which ensures the tumor's ability to invade and metastasize. Selective loss of E-cadherin can cause dedifferentiation and invasiveness in human carcinomas, so this marker is considered a tumor suppressor.

Prior to IHC analysis, the specimens were examined using routine hematoxylin and eosin staining (Fig. 1). E-cadherin was expressed as membrane staining. E-cadherin expression was graded as "0" – absence (Fig. 2a), membrane positivity in <10% of tumor cells (Fig. 2b), "1" – incomplete and weak membrane staining (Fig. 3a,b), "2" – complete membrane staining with weak or moderate intensity (Fig. 4a,b) and "3" – strong membrane staining in >10% of tumor cells (Fig. 5) based on the intensity of epithelial cell membrane staining.

Comparison of E-cadherin expression and lymph node metastasis showed that positive E-cadherin expression varied in intensity and included both low and high expression.

High expression levels were found in cases of non-metastatic tumors and tumors with micrometastases in the lymph nodes ($p<0.05$). It was found that E-cadherin expression in ductal breast cancer tissue was significantly lower in the presence of lymph node metastases than in the absence of metastatic lymph node involvement ($p<0.05$), i.e., low E-cadherin expression was associated with metastases in lymph nodes.

E-cadherin expression showed insignificant differences in terms of patient age, i.e., whether patients were in menopause or premenopause. Thus, in patients under 50 years of age, negative and low E-cadherin expression was found in 54% of cases, while high expression of 3 points was diagnosed in 46% of patients, $p>0.05$. Patients over 50 years of age were characterized by both high positive expression of E-cadherin and low and negative expression, but there was no significant difference in the age of patients, $p>0.05$.

E-cadherin expression showed significant differences in disease stages according to TNM classification. In stage T1 and tumor size less than 2 cm, high E-cadherin expression was diagnosed in 68% of cases ($p<0.005$). In stage T3 breast cancer and with a tumor size greater than 5 cm, negative and low expression was diagnosed in 78% of cases ($p<0.05$), i.e., patients with stage T1 were significantly associated with positive E-cadherin expression, while patients with stage T3 were significantly associated with low and negative E-cadherin expression.

A similar pattern was observed in the study of E-cadherin expression in patients with clinical stage 1 with a tumor size of up to 2 cm in the largest diameter without metastatic lymph node involvement and clinical stage 3 with a tumor size of more than 5 cm in the largest diameter, with metastases in regional and distant lymph nodes, in moderately differentiated and poorly differentiated tumors, respectively, $p<0.05$ and $p<0.001$.

Significant results were observed between E-cadherin expression and G differentiation grade, $p<0.05$. In patients with moderately differentiated G2 and poorly differentiated G3 tumors, negative and low E-cadherin expression significantly prevailed over cases with high expression levels. Thus, low cell adhesion indices were determined in 65% of patients with G2 tumors, and high positive E-cadherin expression was found in 35% of patients, i.e., G2 tumors were significantly characterized by a predominance of negative and low E-cadherin expression, $p<0.01$. G3 tumors similarly manifested negative and low expression in 77%, which was more than 3 times higher than low-differentiated tumors with a high degree of E-cadherin expression (23%), $p<0.01$.

The association of E-cadherin expression with tumor receptor status and molecular-genetic phenotype of carcinomas was studied. Distribution of patients by receptor status: luminal A phenotype (21), luminal B phenotype (19), triple-negative breast cancer (20), Her2/neu-positive phenotype (20).

High expression of E-cadherin was common in ER-positive tumors, particularly in 64% of patients with luminal A phenotype carcinoma. It should be noted that high E-cadherin expression was determined in both premenopausal and postmenopausal patients, but patients over 50 years of age were significantly associated with positive E-cadherin expression.

In patients with luminal B breast cancer, E-cadherin testing showed low and negative expression in 55% and high levels in 45%. In age groups up to 50 years and over 50, low and negative expression slightly prevailed ($p>0.05$). A similar pattern was observed in the group of patients with Her2/neu-positive phenotype. Thus, 58% of patients showed negative and low expression, while 42% showed positive high expression. Low and negative expression also prevailed regardless of whether patients were in menopause or premenopause, and there were no significant differences with the subgroup in which positive high expression was observed ($p>0.05$).

With regard to triple-negative breast cancer, it should be noted that a significant predominance of patients with low and negative E-cadherin expression was found ($p<0.01$). In 70% of cases, low cell adhesion rates were observed, although most patients were over 50 years of age ($p<0.05$). In 30% of patients, high E-cadherin

expression was observed, mainly in the age group also over 50 years ($p<0.05$).

DISCUSSION

The incidence of breast cancer continues to rise, and it is currently the most common form of malignant tumor among women worldwide [27, 28]. The spread of breast cancer metastases affects the prognosis of the disease and is the main cause of mortality [29]. Breast cancer recurrence and metastasis are serious clinical problems.

Changes in cell adhesion are the main mechanism of malignant tumor invasion and metastasis. Changes in adhesion molecules can reduce tumor cell adhesion, promoting tumor infiltration and metastasis. Thus, decreased cell adhesion is an important factor leading to tumor metastasis [30]. Epithelial cadherin (E-cadherin) is a member of the cadherin family and plays an important role in the process of cell adhesion. Selective loss of E-cadherin can cause dedifferentiation and invasiveness in human carcinomas, leading to this marker being classified as a tumor suppressor. Consistent with this role in breast cancer progression, partial or complete loss of E-cadherin expression has been found to correlate with poor prognosis in patients [30- 33].

To determine the nature of E-cadherin protein expression and its possible clinical significance, 80 cases of invasive ductal carcinoma of the breast were studied. E-cadherin expression was detected using immunohistochemistry, and clinical and pathological features and molecular subtypes of invasive ductal carcinoma of the breast were compared according to E-cadherin expression levels. Research have shown that E-cadherin is closely associated with the infiltration and metastasis of ductal carcinoma of the breast and can be used as a marker for predicting metastasis to lymph nodes. High expression of E-cadherin has been detected in invasive tumor cells without metastasis. The negative expression rate was significantly higher in patients with breast cancer with local metastases to regional lymph nodes than in patients without lymph node metastases, and the differences were statistically significant ($p<0.05$).

Paredes J, Figueiredo et al. showed in their studies that, when epithelial-mesenchymal transformation occurs in cancer cells, E-cadherin expression decreases or demonstrates functional loss, causing decreased cell adhesion, loss of polarity, and infiltration of surrounding tissue [34]. E-cadherin has become one of the focal points of research among all cadherins, and it has been shown that E-cadherin is involved in the early onset, infiltration, and metastasis of various tumors [35, 36].

Loss of E-cadherin expression has an unfavorable prognostic significance. Its absence is often associated with metastatic lymph node status, tumor recurrence, low differentiation, and advanced tumor stage [37]. The results of this study showed significant differences in E-cadherin expression in stages T1 and T3 according to the TNM classification, i.e., when the tumor size was less than 2 cm and when it was greater than 5 cm. Positive E-cadherin expression was diagnosed in patients with stage T1 ($p<0.005$), while low and negative E-cadherin expression was observed in patients with stage T3 disease ($p<0.05$).

A similar pattern was observed in the study of E-cadherin expression in patients with clinical stage 1 with a tumor size of up to 2 cm in the largest diameter without metastatic lymph node involvement and clinical stage 3 with a tumor size of more than 5 cm in the largest diameter, with metastases in regional and distant lymph nodes, in moderately differentiated and poorly differentiated tumors, respectively, $p<0.05$ and $p<0.001$.

Significant results were observed between E-cadherin expression and G differentiation grade, $p<0.05$. In patients with moderately differentiated G2 and poorly differentiated G3 tumors, negative and low E-cadherin expression prevailed several times over cases with high expression. Positive immune response decreased with tumor dedifferentiation. In poorly differentiated tumors, E-cadherin expression was weak, with membrane positivity in less than 10% of tumor cells, equal to 0 points or absent (Fig. 2). Not all cells were stained, and positive cells showed abnormal staining patterns, with only focal and punctate membrane positivity, which was equal to 1 point (Fig. 3). In moderately differentiated tumors, E-cadherin expression was heterogeneous: complete membrane staining with weak or moderate intensity in more than 10% of tumor cells and equal to 2 points (Fig. 4). In contrast, high E-cadherin expression prevailed in patients with highly differentiated carcinomas, with strong membrane staining in more than 10% of tumor cells, equal to 3 points (Fig. 5).

The results of this study demonstrated that E-cadherin expression was associated with the molecular type of invasive ductal carcinoma of the breast. In the luminal A phenotype, E-cadherin expression was high in 64% of patients, predominantly in those over 50 years of age, indicating that ER-positive expression may be involved in the regulation of E-cadherin expression. The literature also shows that low tumor activity of invasive ductal breast cancer cells of the luminal subtype is accompanied by an increase in the adhesive properties of these cells due to high levels of E-cadherin expression [38].

Today, according to researchers, E-cadherin is considered an independent marker of triple-negative breast cancer, a molecular subtype characterized by

poor prognosis and short life expectancy [39]. This study found a significant predominance of patients with low and negative E-cadherin expression ($p<0.01$). Low cell adhesion rates were observed in 70% of cases, although most patients were over 50 years of age ($p<0.05$). In the triple-negative phenotype, E-cadherin showed low expression, which was closely associated with invasion and metastasis.

E-cadherin expression in HER-2-positive and luminal B phenotypes was low in most observations and even negative in both premenopausal and postmenopausal patients, In contrast, high expression was determined in 45% of observations of the luminal B phenotype and in 42% of Her2/neu-positive cases. In addition, low and negative expression prevailed regardless of whether patients were menopausal or premenopausal, and there were no significant differences with the subgroup in which positive high expression was observed ($p>0.05$). The different oncogenicity of HER-2-positive and luminal B phenotypes is associated with changes in adhesive contacts, which is due to disturbances in E-cadherin expression.

CONCLUSIONS

The results of the study and data from the literature allowed us to analyze the immunoreactivity of E-cadherin depending on the age of patients, stage of disease, tumor size, degree of malignancy G, involvement of lymph nodes in the tumor process, as well as in different molecular subtypes of breast cancer.

It has been established that low expression of E-cadherin or its absence was associated with moderately differentiated and poorly differentiated tumors of stage T3, clinical stage 3, and the presence of metastases in the lymph nodes. Loss of E-cadherin expression has an unfavorable prognostic significance.

E-cadherin expression was associated with the molecular type of invasive ductal carcinoma of the breast. High E-cadherin expression was common in ER-positive luminal A phenotype tumors and was detected in both premenopausal and postmenopausal patients, suggesting that ER-positive expression may be involved in the regulation of E-cadherin expression. Low tumor activity of invasive ductal breast cancer cells of the luminal subtype is accompanied by an increase in the adhesive properties of these cells due to high levels of E-cadherin expression.

E-cadherin is considered an independent marker of triple-negative breast cancer and is characterized by an unfavorable prognosis and short life expectancy for patients. Triple-negative cancer was associated with a significant predominance of patients with low and negative E-cadherin expression ($p<0.01$).

Thus, E-cadherin is a potent tumor suppressor in breast cancer. Consistent with this role in breast cancer progression, partial or complete loss of E-cadherin expression has been found to correlate with poor prognosis in patients.

Prospects for further research are related to determining whether there are differences in E-cadherin expression in primary breast cancer cells and their metastases. Assessment of the E-cadherin tumor marker, which is involved in cell adhesion, may be a useful method for assessing the risk of metastasis in patients with breast cancer.

REFERENCES

1. World Health Organization. Breast cancer. https://www.who.int/news-room/fact-sheets/detail/breast-canceraw areness-month-2025 [Accessed 16 April 2025]
2. Dadayan VA, Adamovych OO, Simonov VF et al. Stromal-cellular remodeling of breast tissue after silicone implant damage. Reports of Morphology. 2024;30(2):44-52. doi:10.31393/morphology-journal-2024-30(2)-06. DOI
3. Volos LI, Dudash AP. Invasive ductal breast carcinoma: morphological features of molecular subtypes. Medical University of Lublin, 2021. The modern stage of the development of medical education in Ukraine and EU countries: Collective monograph. Riga, Latvia: «Baltija Publishing»; 2021, p. 50-66. doi:10.30525/978-9934-26-090-2-3. DOI
4. Fernández-Tortolero Á, Reigosa-Yániz A. Subtypes of luminal breast carcinoma according to the Saint Gallen Consensus in a group of Venezuelan patients. Biomedica. 2021;41(3):531-540. doi: 10.7705/biomedica.5496. DOI
5. Youn I, Ko EY, Lee JE et al. Prognosis of Breast Cancer in Women in Their 20s: Clinical and Radiological Insights. Diagnostics (Basel). 2025;15(16):2072. doi: 10.3390/diagnostics15162072. DOI
6. Ehinger A, Malmström P, Bendahl PO et al. South and South-East Swedish Breast Cancer Groups. Histological grade provides significant prognostic information in addition to breast cancer subtypes defined according to St Gallen 2013. Acta Oncol. 2017;56(1):68-74. doi: 10.1080/0284186X.2016.1237778. DOI
7. Volos LI, Dudash AP. Osoblyvosti ekspresiyi SOKh-2 v riznykh molekulyarnykh pidtypakh invazyvnoho protokovoho raku hrudnoi zalozy [Features of the COX-2 Expression in Different Molecular Subtypes of Invasive Ductal Breast Cancer]. Ukr ž med bìol sportu. 2022;1(35):68-78. doi: 10.26693/jmbs07.01.068. (Ukrainian) DOI

8. Frixen UH, Behrens J, Sachs M et al. E-cadherin-mediated cell-cell adhesion prevents invasiveness of human carcinoma cells. J Cell Biol. 1991;113(1):173-185. doi:10.1083/jcb.113.1.173. DOI
9. Paredes J, Figueiredo J, Albergaria A et al. Epithelial E- and P-cadherins: role and clinical significance in cancer. Biochim Biophys Acta. 2012;1826:297-11. doi: 10.1016/j.bbcan.2012.05.002. DOI
10. Sethi S, Sarkar FH, Ahmed Q et al. Molecular markers of epithelial-to-mesenchymal transition are associated with tumor aggressiveness in breast carcinoma. Transl Oncol. 2011;4:212-16. doi: 10.1593/tlo.10244. DOI
11. Esquível C, Ribeiro R, Ribeiro AS et al. AdhesionScore: A Prognostic Predictor of Breast Cancer Patients Based on a Cell Adhesion-Associated Gene Signature. Cancers. 2025; 17(23):3731. doi: 10.3390/cancers17233731. DOI
12. Zeisberg M, Neilson EG. Biomarkers of epithelial-mesenchymal transitions. J Clin Investig. 2009;119(6):1429-37. doi: 10.1172/JCI36183. DOI
13. Pietilä M, Ivaska J, Mani SA. Whom to blame for metastasis, the epithelial-mesenchymal transition or the tumor microenvironment?. Cancer Lett. 2016;380(1):359-368. doi: 10.1016/j.canlet.2015.12.033. DOI
14. Pecina-Slaus N. Tumor suppressor gene E-cadherin and its role in normal and malignant cells. Cancer Cell Int. 2003;3(1):17. doi: 10.1186/1475-2867-3-17. DOI
15. Kowalski PJ, Rubin MA, Kleer CG. E-cadherin expression in primary carcinomas of the breast and its distant metastases. Breast Cancer Res. 2003; 5:217-22. doi: 10.1186/bcr651. DOI
16. Chand P, Garg A, Singla V, Rani N. Evaluation of Immunohistochemical Profile of Breast Cancer for Prognostics and Therapeutic Use. Niger J Surg. 2018;24(2):100-106. doi: 10.4103/njs.njs_2_18. DOI
17. Tan PH, Ellis I, Allison K et al. The 2019 World Health Organization classification of tumours of the breast. Histopathology. 2020;77(2):181-185. doi: 10.1111/his.14091. DOI
18. Bailleux C, Chardin D, Gal J et al. Metabolomic Signatures of Scarff-Bloom-Richardson (SBR) Grade in Non-Metastatic Breast Cancer. Cancers (Basel). 2023;15(7):1941. doi: 10.3390/cancers15071941. DOI
19. Coates AS, Winer EP, Goldhirsch A et al. Panel Members. Tailoring therapies--improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015. Ann Oncol. 2015;26(8):1533-46 doi: 10.1093/annonc/mdv221. DOI
20. European Convention for the protection of vertebrate animals used for experimental and other scientific purposes. Strasburg: Council of Europe. 1986;123:52.
21. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals Used for Scientific Purposes. Off J Eur Union. 2010;53(L276):33–79.
22. Aescht E, Büchl-Zimmermann S, Burmester A et al. Romeis mikroskopische technik. Heidelberg: Spektrum Akademischer Verlag. 2010. doi: doi: 10.1007/978-3-8274-2254-5. DOI
23. Magaki S, Hojat SA, Wei B et al. An Introduction to the Performance of Immunohistochemistry. Methods Mol Biol. 2019;1897:289-98. doi: 10.1007/978-1-4939-8935-5_25. DOI
24. Nguyen T. Immunohistochemistry: A Technical Guide to Current Practices. Cambridge: Cambridge University Press. 2022, p.272.
25. Allison KH, Hammond MEH, Dowsett M et al. Estrogen and Progesterone Receptor Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Guideline Update. Arch Pathol Lab Med. 2020;144(5):545-63. doi: 10.5858/arpa.2019-0904-SA. DOI
26. Qureshi HS, Linden MD, Divine G, Raju UB. E-Cadherin status in breast cancer correlates with histologic type but does not correlate with established prognostic parameters. Am J Clin Pathol. 2006;125(3): 377-85. doi: 10.1309/WMX7DRWTFVQP2LQT. DOI
27. Miller KD, Siegel RL, Lin CC et al. Cancer treatment and survivorship statistics. CA Cancer J Clin. 2016;66(4):271-89. doi: 10.3322/caac.21349. DOI
28. Volos LI, Dudash AP. Tumor-infiltrating lymphocytes in nonluminal invasive ductal breast carcinoma. Azerbaijan Med J. 2022;1:131-37. doi: 10.34921/amj.2022.1.021. DOI
29. Chen W, Zheng R, Baade PD et al. Cancer statistics in China 2015. CA Cancer J Clin. 2016;66:115-32. doi: 10.3322/caac.21338. DOI
30. Li DM, Feng YM. Signaling mechanism of cell adhesion molecules in breast cancer metastasis: Potential therapeutic targets. Breast Cancer Res Treat. 2011;128:7-21. doi: 10.1007/s10549-011-1499-x DOI

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CONFLICT OF INTEREST

The Author declares no conflict of interest

CORRESPONDING AUTHOR
Ilona V. Chelpanova
Danylo Halytsky Lviv National Medical University
52. Pekarska St, 79010 Lviv, Ukraine
e-mail: ilona.med75@gmail.com

ORCID AND CONTRIBUTIONSHIP
Ilona V. Chelpanova: 0000-0001-5215-814X B C D
Liliya I. Volos: 0000-0002-1733-589X A E F
Andrii P. Dudash: 0000-0002-7934-8995 B C D
Yevgen V. Paltov: 0000-0003-2611-0294 B E
Artur V. Poliiants: 0000-0002-2622-4753 B C F
Olha V. Dudok: 0000-0001-9513-3460 C E
Yulia V. Hnidyk: 0000-0002-6830-7143 C E

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ORIGINAL ARTICLE

CONTENTS

Renal protective effects of Eprosartan in sepsis: Targeting NF-κB and apoptotic pathways

Abeer J. Abdulredha[1], Murooj L. Majeed[2]

[1]DEPARTMENT OF PHARMACOLOGY, FACULTY OF PHARMACY, UNIVERSITY OF KUFA, NAJAF, IRAQ

[2]DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS, FACULTY OF MEDICINE, UNIVERSITY OF KUFA, NAJAF, IRAQ

ABSTRACT

Aim: To reveal possible protective impact of Eprosartan on sepsis-induced acute kidney injury within the sepsis model.

Materials and Methods: Albino male Swiss mice (n=40) were allocated into four distinct groups: (I) Normal group, (II) Cecal ligation & puncture group, (III) Vehicle group, and (IV) CLP + Eprosartan group (60 mg/kg one hour before CLP intraperitoneally). Blood and tissue biochemical/routine indicators, renal function, SA-AKI-related pathophysiological processes, and nuclear factor kappa B p65 gene expression in septic mice were assessed by histological hematoxylin and eosin staining, quantitative real-time polymerase chain reaction, and Enzyme-Linked Immunosorbent Assay.

Results: Our findings highlight that Eprosartan reversed CLP-provoked increased serum blood urea nitrogen, creatinine (as well as kidney injury molecule levels. It also significantly inhibited the elevated concentrations of tumor necrosis factor alpha and caspase-3 within the tissue. Additionally, NF-κB gene expression level was notably lessened in the group of CLP+ Eprosartan than that of CLP ($p<0.05$). Eprosartan treatment attenuated considerable tubular injuries in the sepsis murine group $p<0.05$.

Conclusions: our findings unveil that Eprosartan could serve as a promising therapeutic agent in the context of sepsis-induced AKI.

KEY WORDS: cecal ligation & puncture, Eprosartan, sepsis, NF-κB p65 gene expression, sepsis-associated acute kidney injury

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INTRODUCTION

Sepsis is a life-threatening condition caused by and leading to infection-induced organ dysfunction syndrome [1]. Sepsis is a life-threatening medical condition that occurs when infection leads to systemic impaired function of the tissues and organs in response to this infection, leading to immunosuppression [2]. Particularly, sepsis is a leading reason for intensive care unit (ICU) admission nationwide; it was present in 291 of every 1000 ICU admissions [3]. This is often due to uncontrolled immune response, cytokine storm, and oxidative stress that cause multiple organ failure, eventually leading to death [4]. They frequently result from microorganisms such as bacteria, viruses, and fungi that can lead to organ dysfunction in most cases [5]. Sepsis is related to several morbidities, such as the heart, kidney, liver, and central nervous system [6]. A common outcome associated with the clinical scenario of sepsis is acute kidney injury (AKI) [7]. The pathogenesis of AKI in the context of sepsis is multifaceted. Among those contributing factors, oxidative stress and inflammation arise as pivotal etiological agents of septic AKI [8]. Sepsis-associated acute kidney injury (SA-AKI) originates from intricate and heterogeneous mechanisms that culminate in renal injury. These mechanisms may either arise directly from the infectious agent and corresponding host immune response or they may represent indirect ramifications of sepsis or its therapeutic intervention. Various pathophysiological mechanisms may interact and participate in AKI in patients suffering from sepsis, encompassing systemic and renal inflammation, macrocirculatory anomalies, microcirculatory dysfunction, metabolic reprogramming, macrocirculatory impairment, and dysregulation of the renin-angiotensin-aldosterone system (RAAS) [9]. Inflammation, recognized as a critical element of sepsis, appears to exert a significant influence on the pathogenesis of SA-AKI. Specifically, pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) have the potential to stimulate toll-like receptors (TLRs). TLR-2 and TLR-4 are expressed on the surfaces of tubular epithelial cells within the

renal system [10, 11]. The engagement of TLR-2 and TLR-4 initiates a cascade of inflammatory responses, which is marked by the secretion of pro-inflammatory cytokines, including interleukin (IL): IL-1α, IL-6, IL-8, and tumor necrosis factor alpha (TNF-α) [12, 13]. Eprosartan was specifically chosen because angiotensin II plays a crucial role in the pathogenesis of sepsis-induced renal injury through activation of the nuclear factor kappa B (NF-κB) pathway and promotion of inflammatory, oxidative, and apoptotic responses; therefore, blocking its action with Eprosartan was hypothesized to provide renoprotective effects.

AIM

The aim of this research is to reveal possible protective impact of Eprosartan on sepsis-induced acute kidney injury within the sepsis model.

MATERIALS AND METHODS

ANIMALS

The current investigation was conducted utilizing a cohort of forty albino Swiss mice weighing between 25 and 30 grams and aged 8 to 12 weeks, procured from the University of Kufa's science college. These mice were accommodated in the animal housing facility within designated enclosures, maintained under a controlled photoperiod of 12 hours of light and 12 hours of darkness, at a stable room temperature of 25°C, with humidity levels between 60% and 65%, and were provided with unrestricted access to food and water *ad libitum*.

CECAL LIGATION AND PUNCTURE

Within the current research, the cecal ligation and puncture **(**CLP) used in earlier studies, including those done by [14, 15], was employed to induce sepsis in the animals. In this case, CLP produces a persistently draining, multifocal infectious source located in the peritoneal cavity. It is introduced by making a midline incision measuring approximately 1.5 cm while the subject is under general anesthesia, wherein xylazine of (20mg/ml) and ketamine of (100mg/ml) are mixed (2:1) and administered [16]. The caecum is ligated, and the caecum is situated below the ileocecal junction and punctured twice with a cutting cannula to inflict kidney organ damage during the acute sepsis phase, first 24 hrs. After that process, the puncture hole is used to squeeze a tiny amount of fecal material from behind the site of perforation. Thereafter, to prevent leakage, the anterior abdominal wall is closed with a single button stitch and tissue adhesive after the cecum is secured back in the stomach. The sham mice were given the same procedures as the experimental groups, except that CLP was not done.

EXPERIMENTAL GROUPS

Mice were classified into the subsequent four distinct groups (n=10):

1. Sham group: Evidently, mice exhibited no apparent signs of disease.
2. CLP cohort: Mice belonging to this cohort experienced a CLP surgical procedure.
3. Vehicle group: Mice classified within this category were administered an equivalent volumetric measurement of the solvent dimethyl sulfoxide (DMSO) intraperitoneally; CLP was performed after 1 hour, and then the animals were sacrificed after 24 hours.
4. Eprosartan group: The mice in this group were given Eprosartan 60 mg/kg intraperitoneally [17]; CLP was performed after 1 hour, then the animals were sacrificed after 24 hours.

SAMPLE PREPARATION AND TISSUE ISOLATION

After 24 hours, mice were euthanized under anesthesia, and blood was collected using the direct heart puncture method. The blood was left in a gel tube rack for about twenty minutes to allow for clot formation, after which it was centrifuged at 10,000×g for about 10 minutes. Then the supernatant was retained at -20 °C for Enzyme-Linked Immunosorbent Assay (ELISA) and the analysis of renal function [18]. After blood sample collection, the abdominal cavity was opened along the midline of the abdomen. After removing the kidney capsule and perirenal fat, the kidneys were carefully dissected and washed, and the right parts of the kidney tissue were fixed in 10% formaldehyde for histological investigation: Hematoxylin and eosin (H&E) [19], while the left kidney of different groups was collected and divided into two sections. The initial segment was subjected to homogenization at 7.4 pH in a cold phosphate-buffered saline (PBS) solution, followed by centrifugation at 3000×g. The resultant supernatant was employed for measurement of TNF-α and caspase 3 by ELISA, the second section was stored at -80 °C for real-time PCR tests for gene expression measurement [20].

EVALUATION OF RENAL HISTOLOGY

After being fixed for twenty-four hours with 4% paraformaldehyde, the right kidney was embedded in

Table 1. Sequences of primers and the housekeeping gene

Target Gene	Primer Direction	Sequence (5′→3′)
NF-κB/p65	Forward	GGCCTCATCCACATGAACTT
	Reverse	CACTGTCACCTGGAAGCAGA
HKG	Forward	TCTTGGGCTACACTGAGGAC
	Reverse	TGTTGCTGTAGCCGTATTCA

paraffin. H&E and periodic acid-Schiff (PAS) reagents were used to stain the 4 μm thick sections cut from the wax blocks containing renal tissue. Using a standard light microscope, the pathological alterations in the kidney tissue of mice were seen. The percentage of injured tubules was used to score tissue damage, which was examined in a blinded manner: 0 is "no harm"; 1 is 0%–25%; 2 – 25%–50%; 3 – 50%–75%; 4 – >75% [21].

EVALUATION OF RENAL FUNCTION

Measurement of serum BUN and creatinine (Cr) concentrations was conducted utilizing Biolis colorimetric assay kits (Biolis, Japan).

ASSAY FOR ENZYME-LINKED IMMUNOSORBENT

Instructions of the ELISA kit (Sunlong, China; TNF-α, catalog no. SL0547Mo; caspase-3, catalog no. SL0679Mo; KIM-1, catalog no. SL0339Mo) were followed while processing tissue samples kept in a refrigerator at -80°C for evaluation of TNF-α and caspase-3, in addition to measurement of serum Kidney Injury Molecule (KIM-1) levels. A microplate reader detected the absorbance at 450 nm to create a standard curve and determine the concentration.

QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION ANALYSIS

By the manufacturer's instructions, total RNA was extracted using TRIzol, and the RNA recovery kit's instructions were closely followed to purify the recovered RNA. The objective of this process was to assess the target genes' mRNA expression levels. We used the ABI 7500 real-time PCR machine and the Power SYBR Green PCR master mix to run real-time PCR in triplicate on cDNA that was produced by a reverse transcription reaction. Every process was carried out in compliance with the manufacturer's instructions. Table 1 displays the primer sequences that were employed, with GAPDH acting as the internal control. The 2^-ΔΔCt technique determined the target genes' relative expression levels.

STATISTICAL ANALYSIS

The statistical analysis in this study was performed using GraphPad Prism version 9.3.1. To investigate differences between groups, a one-way ANOVA was used. Subsequently, the Bonferroni method for multiple comparisons was utilized to conduct post hoc tests. Additionally, the Kruskal-Wallis test was used to analyze non-parametric variables of histopathological outcomes. All tests were deemed statistically significant when p was less than 0.05. All data are presented as mean ± SEM.

RESULTS

EFFECT OF EPROSARTAN ON RENAL FUNCTION

The results indicated that the CLP cohorts had remarkably heightened BUN and Cr levels ($p<0.001$), contrasting with the sham cohort. Additionally, BUN (Fig.1) and Cr (Fig.2) levels of Eprosartan groups had significantly decreased ($p<0.001$), contrary to the CLP cohort.

EFFECT OF EPROSARTAN ON INFLAMMATORY CYTOKINES

According to the findings, the CLP cohort exhibited a notably heightened level of TNF-α ($p< 0.001$), which was in stark contrast to the sham cohort. Furthermore, the TNF-α (Fig.3) concentration of the Eprosartan cohort was observed to be significantly diminished ($p< 0.001$) in comparison to the CLP cohort.

EFFECT OF EPROSARTAN ON APOPTOTIC FACTOR (CASPASE-3)

The results indicated that caspase-3 tissue levels within the CLP cohort were remarkably raised ($p<0.001$), contrary to the sham cohort. Additionally, the Eprosartan group had significantly lower levels ($p<0.001$) of caspase-3, contrasting with the CLP group (Fig.4).

EFFECT OF EPROSARTAN SERUM KIM-1

The results indicated that the CLP cohort had remarkably heightened serum KIM-1 levels compared to the sham cohort. Additionally, KIM-1 levels of the Eprosartan group had significantly decreased, contrary to the CLP cohort (Fig.5).

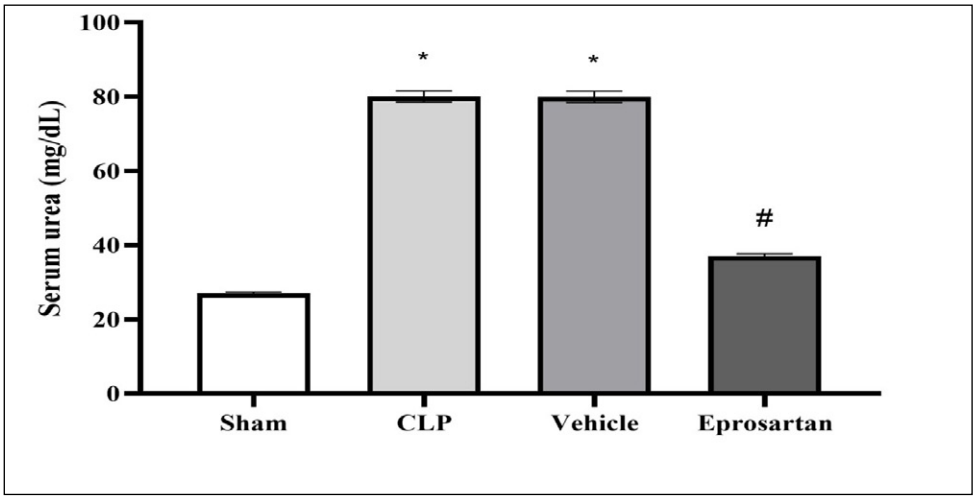

Fig. 1. Mean ± SEM concentrations of the urea in the different experimental cohorts; *: $p < 0.001$, *vs.* Sham group; #: $p < 0.001$, *vs.* CLP or vehicle group, CLP: cecal ligation & puncture
Source: Own materials

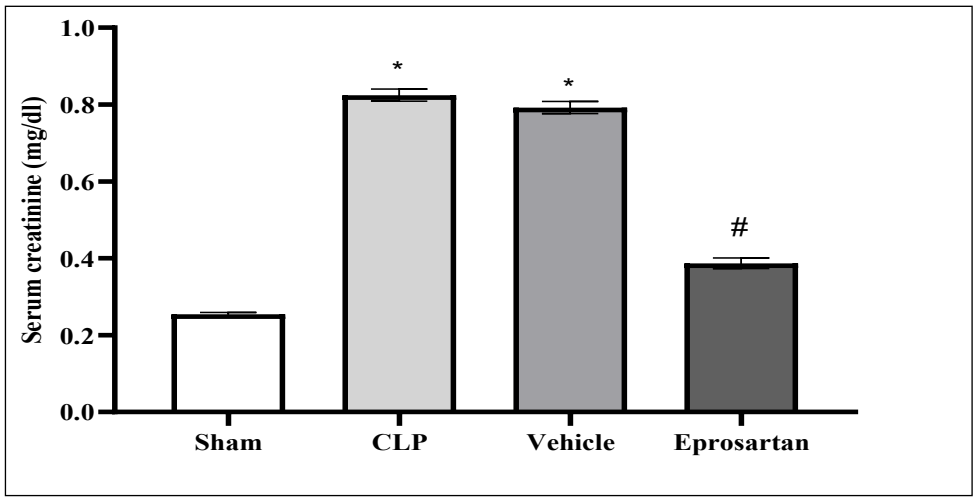

Fig. 2. Mean ± SEM concentrations of serum creatinine in the different experimental cohorts; *: $p < 0.001$, *vs.* Sham group; #: $p < 0.001$, *vs.* CLP or vehicle group, CLP: cecal ligation & puncture
Source: Own materials

IMPACT OF EPROSARTAN ON RENAL HISTOPATHOLOGICAL DAMAGE

As it illustrated by figures 6&7, significant pathological alterations were observed in both the CLP and vehicle cohorts, encompassing interstitial edema, loss of the brush border, vacuolar degeneration, cast formation, inflammation, vascular congestion/hemorrhage, and tubular necrosis. Nevertheless, the renal injury induced by CLP were markedly ameliorated through pretreatment with Eprosartan (Fig. 6-7).

EFFECT OF EPROSARTAN ON mRNA EXPRESSION OF NF-KB P65 GENE

As shown in figure 8, the CLP group had a lower ΔCT than the sham group, indicating a significant increase in NF-κB p65 gene mRNA expression ($p<0.001$). Additionally, there is a substantial ΔCT surge ($p<0.001$) in the Eprosartan group compared to the CLP group, representing a decrease in NF-κB p65 gene mRNA expression.

DISCUSSION

Polymicrobial sepsis is a life-threatening situation characterized by the dysfunction of multiple organs resulting from the aberrant response of the body towards microbial invasion [22]. Sepsis in the United States is regarded as the third most common cause of death and contributes significantly to mortality rates [23]. A plethora of experimental and clinical investigations show that the immunosuppressive state induced by sepsis is typified by decreased antimicrobial effector functionalities, thereby heightening vulnerability to infections [24]. The immunosuppression associated with sepsis is multifaceted and is believed to arise from compromised cytokine production and a reduction in the phagocytic capabilities of myeloid cells. The present investigation elucidated that concentration of the pro-inflammatory cytokine TNF-α was markedly elevated in the CLP cohort in comparison to the ostensibly healthy cohort. This investigation corroborates prior findings [25], which demonstrated that in models of sepsis, the concentrations of

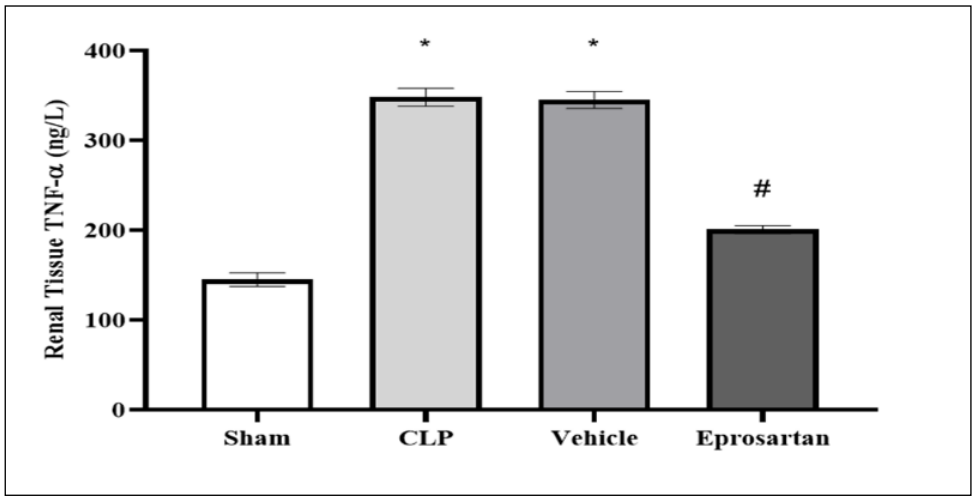

Fig. 3. Mean ± SEM concentrations of renal tissue TNF-α (ng/L) among the various experimental cohorts; *: $p < 0.001$, *vs.* Sham group; #: $p < 0.001$, *vs.* CLP or vehicle group, CLP: cecal ligation & puncture; TNF-α: tumor necrosis factor alpha
Source: Own materials

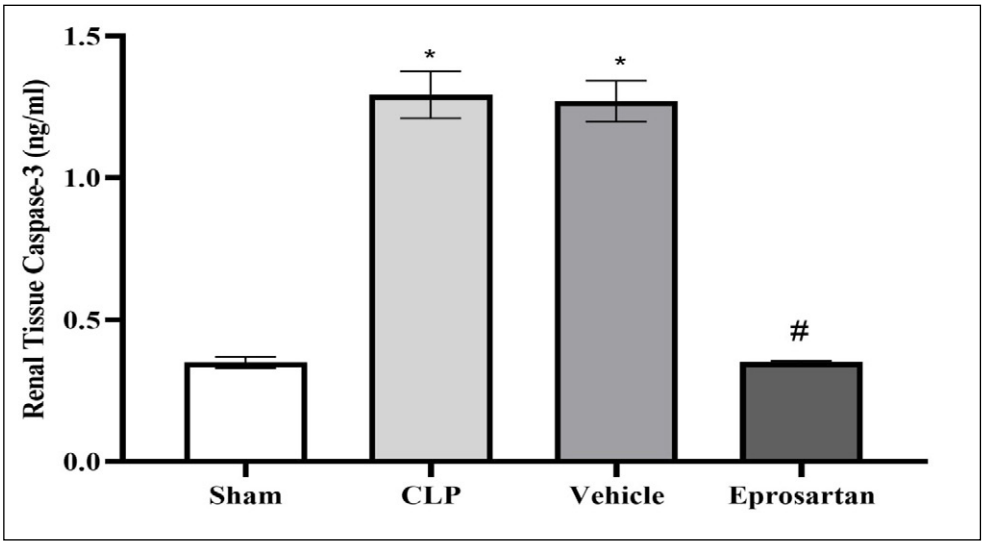

Fig. 4. Mean ± SEM caspase-3 (ng/mL) levels of the experimental groups; *: $p < 0.001$, vs. Sham group; #: $p < 0.001$, *vs.* CLP or vehicle group, CLP: cecal ligation & puncture

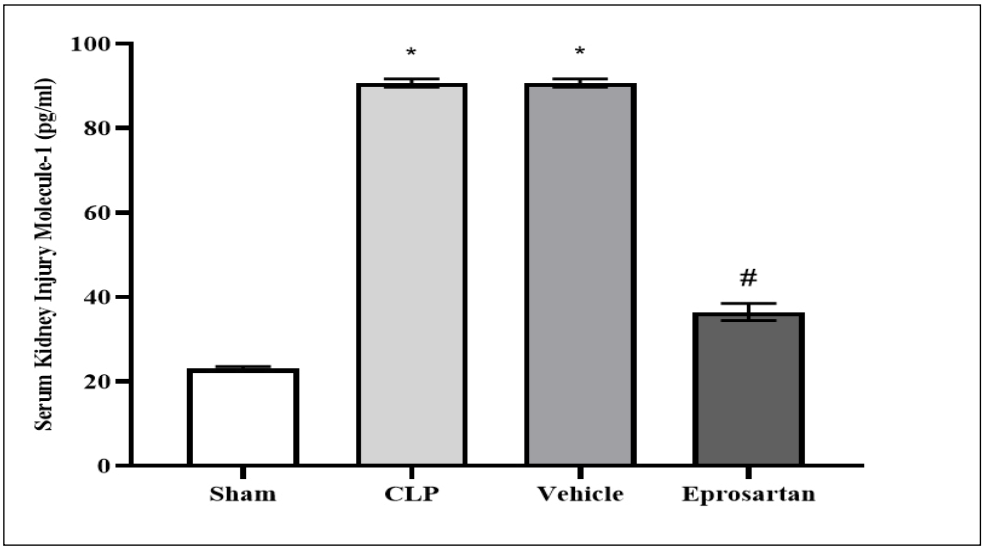

Fig. 5. Mean ± SEM concentrations of serum KIM-1 (pg/mL) in the different experimental cohorts; *: $p < 0.001$, *vs.* Sham group; #: $p < 0.001$, *vs.* CLP or vehicle group, CLP: cecal ligation & puncture
Source: Own materials

proinflammatory cytokines, particularly TNF-α, were elevated in CLP murine. Also, this result, allied with a study [26], showed that renal ischemia-reperfusion injury (IRI) is a major cause of AKI, characterized by significant inflammation that exacerbates tissue damage. Another study investigated the effect of resveratrol in IRI rats. They found that the level of TNF-α became altered (increased significantly) in ischemic rats [27]. Within the confines of the present study, concerning the impact of Eprosartan on TNF-α

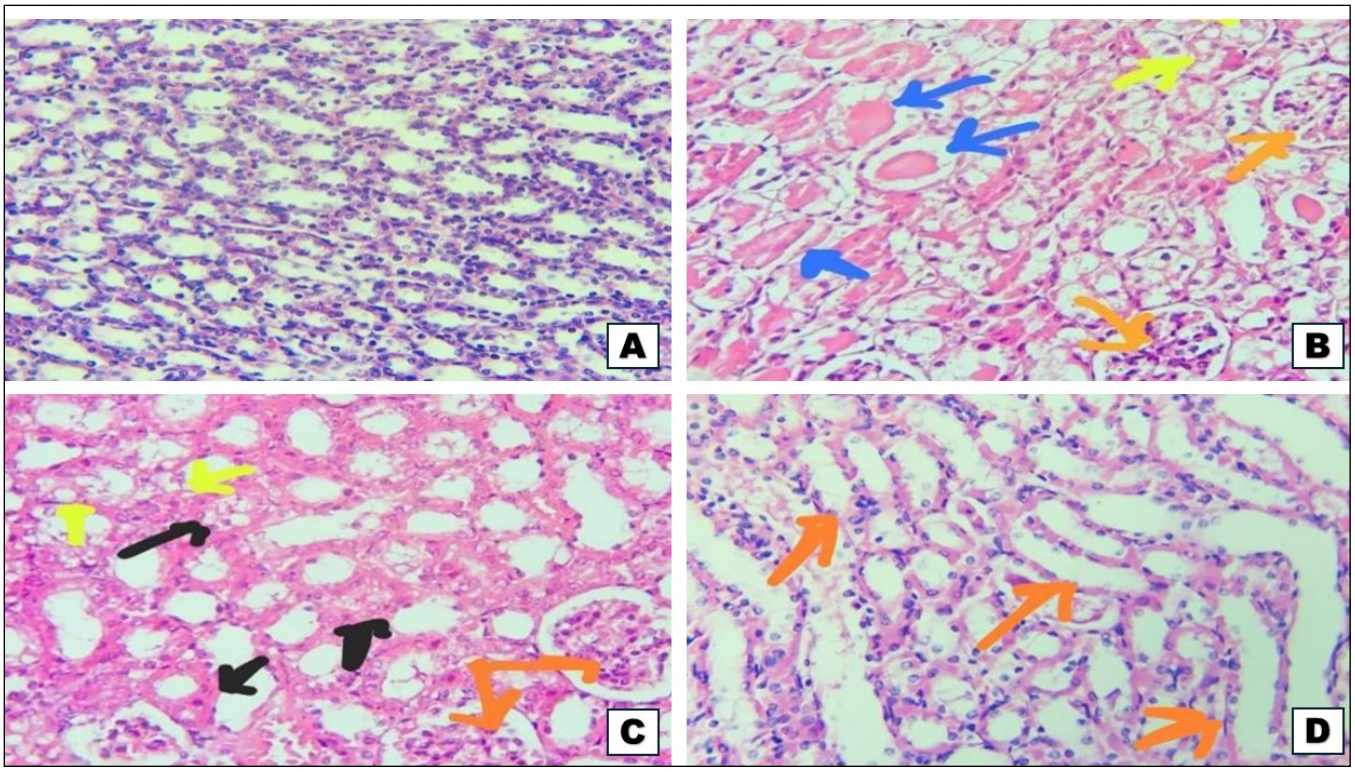

Fig. 6. Eprosartan mitigates the pathological impairment of renal tissues in septic rodent models. H&E staining (400x): **A)** Sham group, mouse kidney with normal renal tubules; **B)** CLP group, mouse kidney with 95% renal tubule damage. Cytoplasmic vacuoles (yellow arrows), eosinophilic casts (blue arrows), and a normal glomerulus (orange arrows); **C)** vehicle groups, mice kidneys with 90% renal tubule damage. Cytoplasmic swelling and increased cytoplasmic eosinophilia (black arrows), cytoplasmic vacuoles (yellow arrows), and a normal glomerulus (orange arrows); **D)** Eprosartan group, mice kidney with 40 % renal tubule damage. Normal tubules (orange arrows)
Source: Own materials

levels, it was observed to significantly diminish TNF-α levels in the renal tissue in comparison to the CLP cohort. To the utmost extent of our knowledge, this was the first study to show how this agent affected the renal TNF-α in mice with sepsis in the CLP model. This observation may be ascribed to NF-κB's noticeable downregulation alongside its related cytokines that promote inflammation [17]. The current work found that CLP and vehicle groups had significantly greater tissue levels of caspase-3 than the sham group. Similar results found that the sepsis group had higher caspase-3 levels than the physiological normal state [28]. Moreover, this work concurs with a prior study that examined renal damage induced by renal IRI and employed a new effective therapeutic approach. They found that renal IRI caused a significant surge in kidney markers of apoptosis, caspase-3, compared to the sham group [29, 30]. Also, in the present study, concerning the effect of Eprosartan on the level of caspase-3, the concentration of caspase-3 was remarkably lower within the Eprosartan cohort opposing to the CLP cohort. This study, as far as we know, was the first to validate the agent's effect on caspase-3 in the murine CLP pattern of sepsis. The pro-apoptotic mechanisms are efficiently thwarted by the role of Eprosartan in the downregulation of the NF-κB pathway, the upregulation of Bcl2, and the downregulation of BAX, in addition to stabilizing the permeability of the mitochondrial membrane and prohibiting cell death via the stimulation of the Sirtuin 1/PGC1 α /Sirtuin 3 pathway [17]. The present study proved that KIM-1 level was notably increased within the CLP cohort in comparison to the sham cohort. This work agrees with a previous study that confirmed levels of KIM-1 in *Lyn* mice who underwent sepsis were raised remarkably when compared with Lyn murine [31]. Additionally, the current study aligns with another research effort that demonstrated the upregulation of the inflammatory marker KIM-1 in both the renal IRI and vehicle groups, when compared to the sham groups [29, 30]. Moreover, concerning the influence of Eprosartan on the KIM-1 concentration, it is observed that this agent significantly attenuates the KIM-1 level when juxtaposed with the CLP cohort. To the extent of our knowledge, this investigation represents the inaugural work elucidating the impact of this pharmacological agent on renal KIM-1 levels within the CLP sepsis model in mice. The rationale underlying such an observation may be attributed

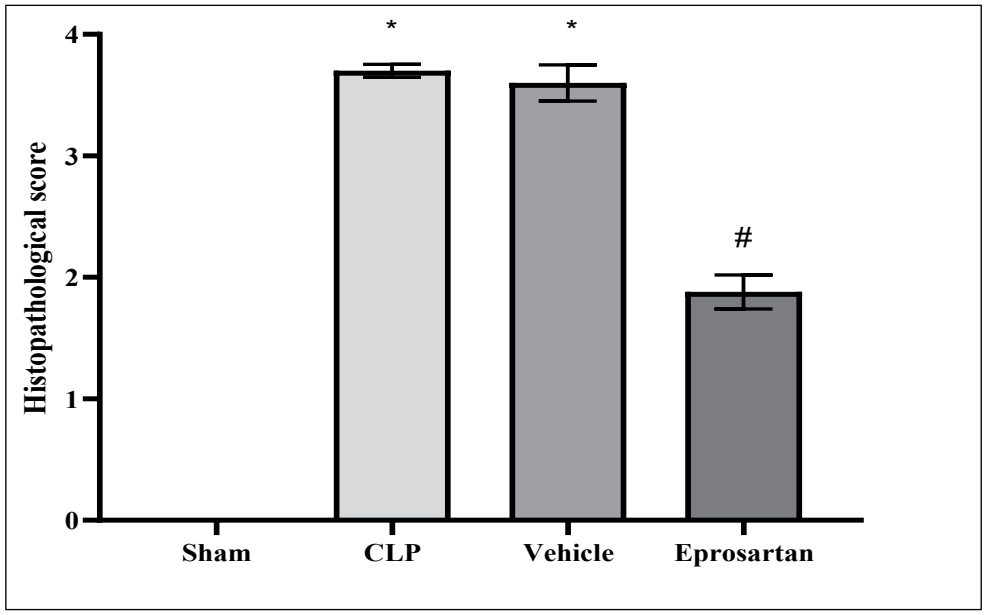

Fig. 7. Quantification of renal tissue damage scores of mice across all groups. *: $p < 0.001$, compared to Sham group; #: $p < 0.001$, compared to CLP or vehicle group. CLP: cecal ligation & puncture
Source: Own materials

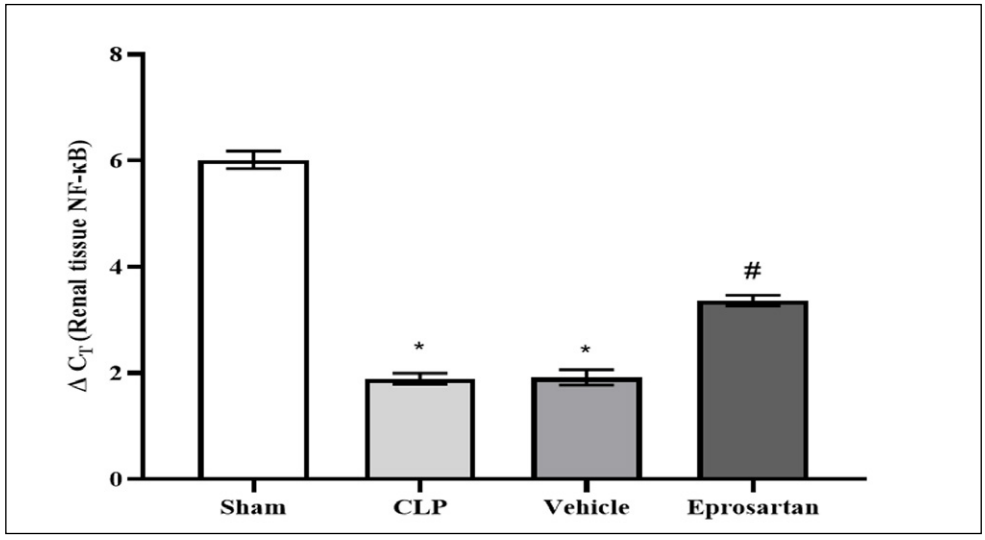

Fig. 8. Mean ± SEM NF-κB p65 mRNA expression levels in the experimental groups, *: $p<0.001$, *vs.* Sham group; #: $p <0.001$, *vs.* CLP or vehicle group. CLP: cecal ligation & puncture; ΔCT: delta cycle threshold; NF-κB: nuclear factor kappa B
Source: Own materials

to its inhibitory effect on ERK phosphorylation [32]. Collier and Schnellmann have elucidated that the proposed mechanism underlying acute renal injury encompasses the phosphorylation of STAT3 and (ERK1/2) [33]. Furthermore, the present investigation elucidated that the mRNA expression levels of NF-κB p65 exhibited a notable elevation in the CLP cohort in comparison to the sham cohort. This work corroborates findings from a preceding study, which indicated that the phosphorylation level of p65 within the renal tissue of the sham cohort was reduced, whereas the CLP cohort demonstrated a significant increase that was statistically significant when juxtaposed with the sham cohort [34]. Moreover, concerning the influence of Eprosartan on the expression levels of NF-κB, it markedly diminished p65 expression in renal tissues when compared to the CLP group. To our utmost knowledge, this investigation represents the inaugural demonstration of the impact of this pharmacological agent on renal NF-κB p65 expression within the CLP model of sepsis in murine subjects. The justification for such an observation may be ascribed to its anti-inflammatory properties and the reduction of IL-6 via the regulation of the upstream NF-κB signaling pathway. Eprosartan significantly reduces NF-κB expression, accompanied by downstream inflammatory cytokines in renal tissue samples [17]. Also, the CLP, along with vehicle groups, exhibited significant histopathological changes in comparison to the sham group. The renal tissue of the sham group mice had normal architecture, while the kidneys obtained from the mice in the CLP cohort exhibited signs of hemorrhage, severe inflammation, increased cytoplasmic eosinophilia, eosinophilic casts, and cytoplasmic vacuoles, as well as loss of brush border. These observations are consistent with those obtained by

[35] during their study on groups of CLP mice. They reported that CLP causes inflammation, necrosis, hemorrhage, and degeneration in kidney tissue. Notably, within the current investigation, the Eprosartan group reduced renal tissue injury when compared to the CLP. In a mouse model of sepsis for the Eprosartan group were arranged from no change to moderate changes, such as a marked decrease in inflammation, vascular congestion, cytoplasmic eosinophilia, and hemorrhage. As far as we know, this research was the first to show the protective effect of Eprosartan on the renal tissues in a mouse model of sepsis. This result may be attributed to eprosartan, which significantly plummeted the levels of inflammatory and apoptotic factors [17].

CONCLUSIONS

Findings revealed that Eprosartan preserves and improves renal function following sepsis by reducing NF-κB–driven inflammation and modulating apoptotic factors, particularly caspase-3, suggesting a potential therapeutic strategy for sepsis-related AKI.

REFERENCES

1. Zhang X, Su C, Zhao S, Li J, Yu F. Combination therapy of Ulinastatin with Thrombomodulin alleviates endotoxin (LPS)-induced liver and kidney injury via inhibiting apoptosis, oxidative stress and HMGB1/TLR4/NF-κB pathway. Bioengineered. 2022;13(2):2951-2970. doi: 10.1080/21655979.2021.2024686. DOI
2. Cao C, Yu M, Chai Y. Pathological alteration and therapeutic implications of sepsis-induced immune cell apoptosis. Cell Death Dis. 2019;10(10):782. Published 2019 Oct 14. doi:10.1038/s41419-019-2015-1. DOI
3. Fleischmann-Struzek C, Mellhammar L, Rose N, et al. Incidence and mortality of hospital- and ICU-treated sepsis: results from an updated and expanded systematic review and meta-analysis. Intensive Care Med. 2020;46(8):1552-1562. doi:10.1007/s00134-020-06151-x. DOI
4. Zhang H, Feng YW, Yao YM. Potential therapy strategy: targeting mitochondrial dysfunction in sepsis. Mil Med Res. 2018;5(1). doi: 10.1186/s40779-018-0187-0. DOI
5. Dai W, Zheng P, Luo D, et al. LPIN1 Is a Regulatory Factor Associated with Immune Response and Inflammation in Sepsis. Front Immunol. 2022;13:820164. doi:10.3389/fimmu.2022.820164. DOI
6. Algahtani MM, Alshehri S, Alqarni SS, et al. Inhibition of ITK Signaling Causes Amelioration in Sepsis-Associated Neuroinflammation and Depression-like State in Mice. Int J Mol Sci. 2023;24(9):8101. doi: 10.3390/ijms24098101. DOI
7. Heinzl MW, Resl M, Klammer C, et al. Subclinical kidney injury is caused by a moderate single inflammatory event. Shock. 2022;58(1):14-19. doi:10.1097/SHK.0000000000001942. DOI
8. Wang B, Xu J, Fu P, Ma L. MicroRNAs in septic acute kidney injury. Burns Trauma. 2023;11:tkad008. doi:10.1093/burnst/tkad008. DOI
9. Zarbock A, Nadim MK, Pickkers P, et al. Sepsis-associated acute kidney injury: consensus report of the 28th Acute Disease Quality Initiative workgroup. Nat Rev Nephrol. 2023;19(6):401-417. doi: 10.1038/s41581-023-00683-3. DOI
10. Peerapornratana S, Manrique-Caballero CL, Gómez H, Kellum JA. Acute kidney injury from sepsis: current concepts, epidemiology, pathophysiology, prevention and treatment. Kidney Int. 2019;96(5):1083-1099. doi: 10.1016/j.kint.2019.05.026. DOI
11. Chang YM, Chou YT, Kan WC, Shiao CC. Sepsis and Acute Kidney Injury: A Review Focusing on the Bidirectional Interplay. Int J Mol Sci. 2022;23(16):9159. doi: 10.3390/ijms23169159. DOI
12. El-Zayat SR, Sibaii H, Mannaa FA. Toll-like receptors activation, signaling, and targeting: an overview. Bull Natl Res Cent. 2019;43(1):1-12. doi: 10.1186/s42269-019-0119-0. DOI
13. Kumar V. Toll-like receptors in sepsis-associated cytokine storm and their endogenous negative regulators as future immunomodulatory targets. Int Immunopharmacol. 2020;89(Pt B):107087. doi: 10.1016/j.intimp.2020.107087. DOI
14. Drechsler S, Osuchowski M. Cecal ligation and puncture. In: Ward PA (ed.). Sepsis: Methods and Protocols. New York, NY: Springer US;2021:1-8. doi: 10.1007/978-1-0716-1656-7_1. DOI
15. Zigam QA, Al-Zubaidy AA, Abbas WJ, Al-Mudhafar RH. Cardioprotective Effects of Octreotide against Sepsis-Induced Cardiotoxicity in Mice. Arch Razi Inst. 2023;78(1):53-61. doi:10.22092/ARI.2022.358339.2201. DOI
16. Navarro KL, Huss M, Smith JC, Sharp P, Marx JO, Pacharinsak C. Mouse Anesthesia: The Art and Science. ILAR J. 2021; 62(1-2): 238-273. doi:10.1093/ilar/ilab016. DOI
17. Lotfi B, Bagheri Y, Abdollahpour A, et al. Protective effect of Eprosartan against ischemic acute renal injury: Acting on NF-κB, caspase 3, and Sirtuin 1. Int Immunopharmacol. 2023;115:109690. doi: 10.1016/j.intimp.2023.109690. DOI
18. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals [published correction appears in J Pharmacol Pharmacother. 2017;8(3):153. doi: 10.4103/0976-500X.215702. DOI
19. Chen D, Ma S, Ye W, et al. Kaempferol reverses acute kidney injury in septic model by inhibiting NF-κB/AKT signaling pathway. J Food Biochem. 2023;47(1):e1353449. doi:10.1111/jfbc.1353449. DOI

20. Taha IA, Al-Drobie BF, Alghanim KM. The role of fixation on antibodies expression in immunohistochemical staining. Maaen J Med Sci. 2024;3(1):1. doi:10.55810/2789-9136.1035. DOI
21. Shi L, Zha H, Pan Z, et al. DUSP1 protects against ischemic acute kidney injury through stabilizing mtDNA via interaction with JNK. Cell Death Dis. 2023;14(11):724. Published 2023 Nov 7. doi: 10.1038/s41419-023-06247-4. DOI
22. Hollenberg SM, Singer M. Pathophysiology of sepsis-induced cardiomyopathy. Nat Rev Cardiol. 2021;18(6):424-434. doi: 10.1038/s41569-020-00492-2. DOI
23. Prest J, Nguyen T, Rajah T, Prest AB, Sathananthan M, Jeganathan N. Sepsis-Related Mortality Rates and Trends Based on Site of Infection. Crit Care Explor. 2022;4(10):e0775. doi: 10.1097/CCE.0000000000000775. DOI
24. Nascimento DC, Viacava PR, Ferreira RG, et al. Sepsis expands a CD39+ plasmablast population that promotes immunosuppression via adenosine-mediated inhibition of macrophage antimicrobial activity. Immunity. 2021;54(9):2024-2041.e8. doi: 10.1016/j.immuni.2021.08.005. DOI
25. Ibadi MH, Majeed S, Ghafil FA, Hadi NR. Effects of CDDO-EA in sepsis-induced acute lung injury: mouse model of endotoxaemia. Wiad Lek. 2024;77(3):497-505. doi: 10.36740/WLek202403119. DOI
26. Alkhafaji GA, Janabi AM. GIP/GLP-1 dual agonist tirzepatide ameliorates renal ischemia/reperfusion damage in rats. Int J Appl Pharm. 2025;7(2):165-173 doi:10.22159/ijap.2025v17i2.53156. DOI
27. Alaasam ER, Janabi AM, Al-Buthabhak KM, et al. Nephroprotective role of resveratrol in renal ischemia-reperfusion injury: a preclinical study in Sprague-Dawley rats. BMC Pharmacol Toxicol. 2024;25(1):82. doi:10.1186/s40360-024-00809-8. DOI
28. Miliaraki M, Briassoulis P, Ilia S, et al. Survivin and caspases serum protein levels and survivin variants mRNA expression in sepsis. Sci Rep. 2021;11(1):1049. doi: 10.1038/s41598-020-78208-2. DOI
29. Jallawee HQ, Janabi AM. Potential nephroprotective effect of dapagliflozin against renal ischemia reperfusion injury in rats via activation of autophagy pathway and inhibition of inflammation, oxidative stress and apoptosis. South East Eur J Public Health. 2024:488-500. doi: 10.70135/seejph.vi.1009. DOI
30. Jallawee H, Janabi AM. Trandolapril improves renal ischemia-reperfusion injury in adult male rats via activation of the autophagy pathway and inhibition of inflammation, oxidative stress, and apoptosis. J Biosci Appl Res. 2024;10(6):114-127. doi: 10.21608/jbaar.2024.315239.1077. DOI
31. Li N, Lin G, Zhang H, et al. Lyn attenuates sepsis-associated acute kidney injury by inhibition of phospho-STAT3 and apoptosis. Biochem Pharmacol. 2023;211:115523. doi: 10.1016/j.bcp.2023.115523. DOI
32. Mukaddam-Daher S, Menaouar A, Paquette PA, et al. Hemodynamic and cardiac effects of chronic Eprosartan and moxonidine therapy in stroke-prone spontaneously hypertensive rats. Hypertension. 2009; 53(5): 775-781. doi:10.1161/HYPERTENSIONAHA.108.126524. DOI
33. Collier JB, Schnellmann RG. Extracellular Signal-Regulated Kinase 1/2 Regulates Mouse Kidney Injury Molecule-1 Expression Physiologically and Following Ischemic and Septic Renal Injury. J Pharmacol Exp Ther. 2017;363(3):419-427. doi:10.1124/jpet.117.244152. DOI
34. Sun S, Wang J, Wang J, Wang F, Yao S, Xia H. Maresin 1 Mitigates Sepsis-Associated Acute Kidney Injury in Mice via Inhibition of the NF-κB/STAT3/MAPK Pathways. Front Pharmacol. 2019;7(10):1323. doi:10.3389/fphar.2019.01323. DOI
35. Arifin A, Purwanto B, Indarto D, et al. Improvement of renal functions in mice with septic acute kidney injury using secretome of mesenchymal stem cells. Saudi J Biol Sci. 2024; 31(3): 103931. doi: 10.1016/j.sjbs.2024.103931. DOI

Ethics approval

In the present investigation, laboratory mice were employed as the experimental subjects. All sacrificial procedures were conducted under a combination of ketamine and xylazine-based anesthesia, with diligent efforts made to mitigate individual distress. The experimental methodologies and protocols employed in this study received approval on August 29, 2024, under reference number (20553) from the committee responsible for the ethics of laboratory animal care and use.

CONFLICT OF INTEREST

The Authors declare no conflict of interest

AUTHOR CONTRIBUTIONS

Abeer J Abdulredha and Murooj L Majeed contributed equally as co-first authors

CORRESPONDING AUTHOR

Abeer J. Abdulredha
Department of Pharmacology,
Faculty of Pharmacy, University of Kufa, Najaf, Iraq
e-mail: abeer.alamri@student.uokufa.edu.iq

ORCID AND CONTRIBUTIONSHIP
Abeer J. Abdulredha: 0009-0007-8640-432X A B C D E F
Murooj L. Majeed: 0009-0006-6697-7529 A B C D E F

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

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ORIGINAL ARTICLE

CONTENTS

Incidence and outcomes of postoperative complications after liver cirrhosis – related surgery

Oleksii Petiunin[1], Vasyl Syplyviy[1], Kamel Jbarah Hammad Al Mashni[2], Dmytro Myroshnychenko[1], Vitaly Makarov[1], Anna Novikova[1], Bohdan Porovai-Nevoit[1]

[1]KHARKIV NATIONAL MEDICAL UNIVERSITY, KHARKIV, UKRAINE

[2]HEBRON GOVERNMENTAL HOSPITAL, HEBRON, PALESTINE

ABSTRACT

Aim: To investigate incidence and outcomes of postoperative complications after cirrhosis-related surgery.

Materials and Methods: Retrospective review of medical records of 344 liver cirrhosis (LC) patients who underwent cirrhosis-related surgical treatment was done. Types and incidence of postoperative complications, causes of death in operated patients, incidence of postoperative complications and mortality based on type of surgery were determined.

Results: Postoperative complications developed in 108 (31.4%) patients. Acute-on-chronic liver failure (ACLF) developed in 60 (17.4%) patients was the most common complication. ACLF was the main cause of death - 28 (49.1%) patients with developed complications died. The lowest number of postoperative complications and the lowest mortality were observed after extraperitonization of right hepatic lobe with intraoperative laser irradiation: complications developed in 3 (9.1%) patients, 1 (3.0%) patient died. Complications after endoscopic interventions developed in 10 (15.4%) patients, 6 (9.2%) died, after roentgen-endovascular interventions complications developed in 10 (15.4%) patients, all of them died. Devascularization and transection interventions complications developed in 31 (31.0%) patients, 21 (21%) died. In patients, who underwent distal splenorenal shunt - complications developed in 54 (66.7%) patients, 19 (23.4%) patients died.

Conclusions: The most common complication of the early postoperative period after LC-related surgery is ACLF, which develops in 17.4% of patients and is the cause of death in 49.1% of cases. The incidence and outcomes of postoperative complications of cirrhosis-related surgery, depends on the liver function, timing and type of surgery.

KEY WORDS: liver cirrhosis, postoperative complications, mortality, adverse outcomes, cirrhosis-related surgery

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INTRODUCTION

Liver cirrhosis (LC), a complex and progressive disease, imposes a significant global health burden, characterized by irreversible liver tissue scarring and various life-threatening complications, is one of the leading causes of morbidity and mortality [1,2].

Patients with LC have an increased risk of in-hospital mortality or postoperative complication after surgery [3-13]. This increased risk is attributed to adverse effects of liver disease, encompassing coagulation dysfunction, altered metabolism of anesthesia and sedatives, immunologic dysfunction, hemorrhage related to varices, malnutrition and frailty, impaired wound healing, as well as diminished portal blood flow, overall hepatic circulation, and hepatic oxygen supply during surgical procedures [14].

Patients with cirrhosis had significantly higher 30-day mortality than noncirrhotic patients with chronic hepatitis B (4.4% vs 1.3%), or with no chronic liver disease (0.8%), postoperative mortality was almost 6 times higher after emergent rather than elective surgery (17.2% vs. 2.1%) [15].

Patients with cirrhosis have significantly higher mortality rates after emergency surgery, more surgical complications and reoperations, and reduced days-alive-out-of-hospital at 90-days [16, 17]. Among gastrointestinal surgeries, the highest postoperative mortality is after colorectal resection (13%–37%), esophagectomy (11%–25%), and pancreaticoduodenectomy (11.9%–17%). The lowest postoperative mortality is after laparoscopic cholecystectomy and elective uncomplicated hernia repair (0% in most studies). High mortality was reported after coronary artery bypass graft and valvular heart surgery, whereas low mortality was reported after elective hip and knee

replacements. Patients with Child-Turcotte-Pugh class C disease or high MELD score had very high mortality across all surgical procedures [18,19].

AIM

The aim of our study was to investigate incidence and outcomes of postoperative complications after cirrhosis-related surgery.

MATERIALS AND METHODS

We retrospectively reviewed the medical records of 344 liver LC patients who underwent curative cirrhosis-related surgical treatment in the clinical bases of the Department of Surgery No. 4 of Kharkiv National Medical University and Zaitsev Institute of General and Emergency surgery of the National Academy of Medical Sciences of Ukraine. The age of the patients ranged from 9 to 66 years. The average age of the patients was 43.5 years. By gender, the patients were categorized as follows: 206 (59.9%) mails and 138 (40.1%) femails. In 81 (23.5%) patients distal splenorenal shunt (DSRS) was done, in 33 (9.6%) patients - extraperitonization of right hepatic lobe with intraoperative laser irradiation (ERHLILI), devascularization and transection interventions (DTI) – in 100 (29,1%) patients ((gastrotomy with gastroesophageal varices (GOV) suturing- in 36 (10.4%) patients, skeletonisation of the abdominal part of the esophagus and cardia with extra-organs GOV suturing and esophagophrenofundoplication - in 14 (4.1%) patients, devascularisation of the Lesser curvature of the stomach by highly selective vagotomy type, pyloroplasty - in 7 (2.1%) patients, proximal gastric resection with removal of GOV conglomerate - in 2 (0.6%) patients, ligation of left gastric artery and vein, splenic artery - in 41 (11.9%) patients)), endoscopic interventions (EI) - in 65 (18.9%) patients ((endoscopic sclerotherapy (ES) – in 56 (16.3%) patients, endoscopic variceal ligation (EVL) - in 9 (2.6%) patients)), roentgen-endovascular interventions (REI) – in 65 (18.9%) patients - ((splenic artery embolization (SAE) in 43 (12.5%) patients, SAE and left gastric artery embolization - in 17 (4.9%) patients, SAE and common hepatic artery embolization - in 5 (1.5%) patients)).

Indications for DSRS were various manifestations of portal hypertension (PH): bleeding from GOV, ascites, hypersplenism syndrome. Indications for ERHLILI was clinically significant PH and ascites. DTI were done in patients with persistent GOV bleeding and ineffective conservative means and sclerotherapy. EI were done in high degree of surgical risk patients and ineffective conservative means to stop acute GOV bleeding or to prevent the recurrence of bleeding. REI were indicated in cases of acute GOV bleeding and clinical manifestations caused by increased activity of the reticuloendothelial system (hypersplenism).

Types and incidence of postoperative complications, causes of death in operated patients, incidence of postoperative complications and mortality based on type of surgery were determined. IBM SPSS Statistics 29.0.2.0 (IBM Corp, NY) was used for statistical analysis. Categorical data were summarized and presented as numbers and percentages of a group.

ETHICS

The authors adhered to the ethical principles of the Helsinki Declaration of the World Medical Association and international standards for publications in medical journals, including the recommendations of the ICMJE (International Committee of Medical Journal Editors).

RESULTS

Postoperative complications developed in 108 (31.4%) patients. The most common complication was acute – on – chronic liver failure (ACLF), which developed in 60 (17.4%) patients, from which 8 (2.3%) patients developed hepato-renal failure (Table 1).

In 11 (3.1%) patients, ACLF manifested itself without other complications, and in 30 (8.7%) patients with other complications. The combination of ACLF with DSRS thrombosis was observed in 3 (0.9%) patients, with thrombosis of the veins of the portal system in 2 (0.4%), with intra-abdominal bleeding in 3 (0.9%), with spontaneous bacterial peritonitis (SBP) in 3 (0.9%), with acute gastric ulcers in 4 (1.2%), with abdominal eventeration in 3 (0.9%), with pneumonia in 15 (4.3%) patients. DSRS thrombosis in 2 (0.6%) cases was diagnosed postmortem at autopsy - hepatic coma dominated in the clinical course. In the remaining 3 (0.9%) patients, variceal bleeding on the background of hepatic failure was a sign of DSRS thrombosis and veins of the portal system. At the same time, acute gastric ulcers were the cause of bleeding in 4 (1.2%) patients due to ACLF.

Recurrent variceal bleeding developed in 35 (10.1%) patients. Ascites developed in 10 (2.9%) patients, indicating liver failure and impaired liver function in the postoperative period. 3 (0.9%) patients postoperatively developed SBP.

Hematoma in the site of DSRS was diagnosed in 4 (1.2%) patients on the basis of a decrease in the level of hemoglobin and erythrocytes of the blood, the

Table 1. Postoperative complications in LC patients

Complication	Number of patients	%
ACLF	60	17.4
Recurrent variceal bleeding	35	10.1
Pneumonia	24	6.9
Ascites	10	2.9
Hematoma of laparotomy wound	6	1.8
Thrombosis of DSRS, veins of the portal system	5	1.4
Acute gastric ulcers	4	1.2
Hematoma in the site of DSRS	4	1.2
Intra-abdominal bleeding	3	0.9
Spontaneous bacterial peritonitis	3	0.9
Abdominal eventeration	3	0.9
Postoperative pancreatitis	3	0.9
Acute fibrinolysis	3	0.9
Ischemic stroke	1	0.3

Source: compiled by the authors of this study

presence of a palpable infiltrate in the left hypogastrium (anastomosis), and blood discharge through the drainage tube leading to the anastomosis zone.

Hematoma of the postoperative wound developed in 6 (1.8%) patients. In 3 (0.9%) patients, the combination of ascites and hematoma of the laparotomy wound caused eventeration.

Acute fibrinolysis complicated the course of the postoperative period in 3 (0.9%) patients. In 1 (0.3%) case fibrinolytic bleeding developed during surgery and, despite treatment, resulted in patient death. In 2 (0.6%) patients, intra-abdominal bleeding was stopped during relaparotomy performed first 24 hours following surgery.

Pneumonia in the postoperative period was diagnosed in 24 (6.9%) patients. At the same time, in 7 (2.0%) patients it was an independent complication, and in the remaining 17 (4.9%) it was a manifestation of pulmonary injury as a result of multiple organ failure. Postoperative pancreatitis as a consequence of pancreatic traumatization during splenic vein isolation was diagnosed in 3 (0.9%) DSRS patients.

In 1 (0.3%) patient in the postoperative period the development of acute appendicitis was observed, in 1 (0.3%) - acute cholecystitis, which required surgical treatment. In both cases, the diagnosis was confirmed histologically.

Of the 108 patients who developed postoperative complications, 57 (52.8%) died. The most common cause of death was ACLF, which caused death of 28 (49.1%) patients (Table 2).

At the same time, at autopsy in 3 (0.9%) patients SBP was diagnosed, and in 2 (0.6%) – DSRS thrombosis.

In 24 (42.1%) patients, the cause of death was recurrence of bleeding from GOV. The development of bleeding causes decompensation of a liver function and rapid patient's death.

In 3 (5.2%) patients, bleeding from acute gastric ulcers on the background of liver failure caused their death. At the same time, bleeding from acute ulcers developed on the 2nd-4th day postoperatively on background of severe liver failure.

In 1 (1.8%) patient, the cause of death was acute fibrinolysis, in 1 (1.8%) - ischemic stroke.

The incidence of postoperative complications and mortality were different depending on the type of surgical intervention performed (Table 3).

The lowest number of postoperative complications and the lowest mortality were observed after ERHLILI surgery: complications developed in 3 (9.1%) patients, 1 (3.0%) patient died. EI complications developed in 10 (15.4%) patients, 6 (9.2%) died. REI complications developed in 10 (15.4%) patients, all of them died. DTI complications developed in 31 (31.0%) patients, 21 (21%) died. The largest number of postoperative complications and the highest mortality were observed in patients, who underwent DSRS surgery - complications developed in 54 (66.7%) patients, of which 19 (23.4%) died.

DISCUSSION

Patients with cirrhosis are at increased risk of perioperative morbidity and mortality. Severity of liver

Table 2. Causes of deaths in LC patients in the postoperative period

Cause of death	Number of patients	%
ACLF	28	49.1
Recurrent variceal bleeding	24	42.1
Bleeding from acute gastric ulcers secondary to liver failure	3	5.2
Acute fibrinolysis	1	1.8
Ischemic stroke	1	1.8
Total	57	100

Source: compiled by the authors of this study

Table 3. Incidence of postoperative complications and mortality in LC patients based on type of surgery

Type of surgery	Number of patients	Postoperative complications		Mortality	
		n	%	n	%
DSRS	81	54	66.7	19	23.4
ERHLILI	33	3	9.1	1	3.0
DTI	100	31	31.0	21	21.0
EI	65	10	15.4	6	9.2
REI	65	10	15.4	10	15.4
Total	344	108	31.4	57	16.6

Source: compiled by the authors of this study

dysfunction, medical comorbidities and the type and complexity of surgery, including whether it is elective versus emergent, are all determinants of perioperative mortality and morbidity in patients with cirrhosis. There are major limitations to the existing clinical research on risk assessment and perioperative management that warrant further investigation [20-23].

In our study, postoperative complications developed in 108 (31.4%) patients. ACLF, the most common complication, developed in 60 (17.4%) patients, while recurrent variceal bleeding developed in 35 (10.1%) patients, pneumonia - in 24 (6.9%) patients, ascites - in 10 (2.9%) patients, hematoma of the postoperative wound - in 6 (1.8%) patients, thrombosis of DSRS, veins of the portal system - in 5 (1.4%) patients, acute gastric ulcers, as well as, hematoma in the site of DSRS - in 4 (1.2%) patients, intra-abdominal bleeding, SBP, abdominal eventeration, postoperative pancreatitis, acute fibrinolysis - in 3 (0.9%) patients each, ischemic stroke 1 (0.3%) patient. In our opinion, ACLF was related to extra-hepatic precipitating event (major surgery) in the context of a pre-existing liver condition (LC).

ACLF also was a leading cause of death in postoperative period - 28 (49.1%) of patients, who developed complications died. 24 (42.1%) patients died because of recurrent variceal bleeding, which causes decompensation of a liver function. Bleeding from acute gastric ulcers on the background of liver failure caused death in 3 (5.2%) patients, acute fibrinolysis, as well as, ischemic stroke - in 1 (1.8%) patient.

Morbidity and mortality vary between types of surgery with further studies required in patients with more advanced liver disease. Patient-specific considerations and practicing precision medicine may allow for improved postoperative outcomes [22].

The severity of liver decompensation, timing of surgery, and type of surgery are the most important determinants of the surgical outcome in patients with cirrhosis [24].

The results of published studies indicated that the postoperative mortality rate for patients with cirrhosis is 8.3–25% [23, 25, 26]. In our study, the in-hospital mortality of the LC patients was 16.6%.

The incidence of postoperative complications and mortality were different depending on the type of surgical intervention performed. The lowest number of postoperative complications and the lowest mortality were observed after ERHLILI surgery: complications developed in 3 (9.1%) patients, 1 (3.0%) patient died. This result was due to the fact that only compensated LC patients underwent this type of surgery, the ERHLILI itself is not highly traumatic and prolonged.

EI complications developed in 10 (15.4%) patients, 6 (9.2%) died. REI complications developed in 10 (15.4%) patients, all of them died. Although these surgical interventions do not belong to the category of major surgery and are short in duration, we can explain such outcomes by the fact that they were performed in patients with decompensated LC to stop acute variceal bleeding as an emergency.

DTI complications developed in 31 (31.0%) patients,

21 (21%) died. This type of surgery, belongs to major surgery, was done in emergency only for decompensated LC patients with acute variceal bleeding.

The largest number of postoperative complications and the highest mortality were observed in patients, who underwent DSRS - complications developed in 54 (66.7%) patients, of which 19 (23.4%) died. Such adverse outcomes also can be explained as DSRS is a major surgery, taking long time, which was done for mainly decompensated patients.

CONCLUSIONS

Thus, when analyzing the immediate results of surgical treatment of liver cirrhotic patients, it was found that the most common complication of the early postoperative period is ACLF, which develops in 17.4% of patients and is the cause of death in 49.1% of cases. The incidence and outcomes of postoperative complications of cirrhosis-related surgery, depends on the liver function, timing and type of surgery.

REFERENCES

1. Jadaun SS, Saigal S. Surgical Risk Assessment in Patients with Chronic Liver Diseases. J Clin Exp Hepatol. 2022;12(4):1175-1183. doi: 10.1016/j.jceh.2022.03.004. DOI
2. Seo Kwak E, Alharbi A, Bosaily A et al. Inpatient complications and mortality in cirrhotic patients undergoing bariatric surgery: a comprehensive analysis. Hosp Pract (1995). 2025;53(1):2455921. doi: 10.1080/21548331.2025.2455921. DOI
3. Garatti A, Daprati A, Cottini M Russo CF et al. Cardiac Surgery in Patients With Liver Cirrhosis (CASTER) Study: Early and Long-Term Outcomes. Ann Thorac Surg. 2021;111(4):1242-1251. doi: 10.1016/j.athoracsur.2020.06.110. DOI
4. Jo HH, Min C, Kyoung DS et al. Adverse outcomes after surgeries in patients with liver cirrhosis among Korean population: A population-based study. PLoS One. 2021;16(6):e0253165. doi: 10.1371/journal.pone.0253165. DOI
5. Kaltenbach MG, Mahmud N. Assessing the risk of surgery in patients with cirrhosis. Hepatol Commun. 2023;7(4):e0086. doi: 10.1097/HC9.0000000000000086. DOI
6. Klein J, Spigel Z, Kalil J et al. Postoperative Mortality in Patients With Cirrhosis: Reconsidering Expectations. Am Surg. 2022;88(2):181-186. doi: 10.1177/0003134820988825. DOI
7. Liu F, Li ZW, Liu XR et al. The Effect of Liver Cirrhosis on Patients Undergoing Cardiac Surgery. Glob Heart. 2023;18(1):54. doi: 10.5334/gh.1270. DOI
8. Mahmud N, Fricker Z, Hubbard RA et al. Risk Prediction Models for Post-Operative Mortality in Patients With Cirrhosis. Hepatology. 2021;73(1):204-218. doi: 10.1002/hep.31558. DOI
9. Mahmud N, Fricker Z, Lewis JD et al. Risk Prediction Models for Postoperative Decompensation and Infection in Patients With Cirrhosis: A Veterans Affairs Cohort Study. Clin Gastroenterol Hepatol. 2022;20(5):e1121-e1134. doi: 10.1016/j.cgh.2021.06.050. DOI
10. Martin Mateos R, Garcia de la Filia Molina I, Albillos A. Pre-surgical risk assessment in patients with cirrhosis. Acta Gastroenterol Belg. 2020;83(3):449-453.
11. Morris SM, Abbas N, Osei-Bordom DC et al. Cirrhosis and non-hepatic surgery in 2023 - a precision medicine approach. Expert Rev Gastroenterol Hepatol. 2023;17(2):155-173. doi: 10.1080/17474124.2023.2163627. DOI
12. Pantea R, Meister P, Neuhaus JP et al. Chirurgie bei Patienten mit Leberzirrhose [Surgery in patients with liver cirrhosis]. Chirurg. 2021;92(9):838-845. doi: 10.1007/s00104-020-01319-z. (German) DOI
13. Saleh ZM, Solano QP, Louissaint J et al. The incidence and outcome of postoperative hepatic encephalopathy in patients with cirrhosis. United European Gastroenterol J. 2021;9(6):672-680. doi: 10.1002/ueg2.12104. DOI
14. Ostojic A, Mahmud N, Reddy KR. Surgical risk stratification in patients with cirrhosis. Hepatol Int. 2024;18(3):876-891. doi: 10.1007/s12072-024-10644-y. DOI
15. Johnson KM, Newman KL, Green PK et al. Incidence and Risk Factors of Postoperative Mortality and Morbidity After Elective Versus Emergent Abdominal Surgery in a National Sample of 8193 Patients With Cirrhosis. Ann Surg. 2021;274(4):e345-e354. doi: 10.1097/SLA.0000000000003674. DOI
16. Skovsen AP, Jensen TK, Gögenur I, Tolstrup MB. A high rate of mortality in liver cirrhosis patients after emergency abdominal surgery. Eur J Trauma Emerg Surg. 2025;51(1):117. doi: 10.1007/s00068-025-02787-w. DOI
17. Snitkjær C, Jensen KK, Henriksen NA et al. Umbilical hernia repair in patients with cirrhosis: systematic review of mortality and complications. Hernia. 2022;26(6):1435-1445. doi: 10.1007/s10029-022-02598-7. DOI
18. Newman KL, Johnson KM, Cornia PB et al. Perioperative Evaluation and Management of Patients With Cirrhosis: Risk Assessment, Surgical Outcomes, and Future Directions. Clin Gastroenterol Hepatol. 2020;18(11):2398-2414.e3. doi: 10.1016/j.cgh.2019.07.051. DOI
19. Ng ZQ, Tan P, Theophilus M. Colorectal Surgery in Patients with Liver Cirrhosis: A Systematic Review. World J Surg. 2023;47(10):2519-2531. doi: 10.1007/s00268-023-07069-3. DOI

20. Goldberg D, Wilder J, Terrault N. Health disparities in cirrhosis care and liver transplantation. Nat Rev Gastroenterol Hepatol. 2025;22(2):98-111. doi: 10.1038/s41575-024-01003-1. DOI
21. Newman KL, Johnson KM, Cornia PB et l. Perioperative Evaluation and Management of Patients With Cirrhosis: Risk Assessment, Surgical Outcomes, and Future Directions. Clin Gastroenterol Hepatol. 2020;18(11):2398-2414.e3. doi: 10.1016/j.cgh.2019.07.051. DOI
22. Morris SM, Abbas N, Osei-Bordom DC et al. Cirrhosis and non-hepatic surgery in 2023 - a precision medicine approach. Expert Rev Gastroenterol Hepatol. 2023;17(2):155-173. doi: 10.1080/17474124.2023.2163627. DOI
23. Jo HH, Min C, Kyoung DS et al. Adverse outcomes after surgeries in patients with liver cirrhosis among Korean population: A population-based study. PLoS One. 2021;16(6):e0253165. doi: 10.1371/journal.pone.0253165. DOI
24. Abbas N, Makker J, Abbas H, Balar B. Perioperative Care of Patients With Liver Cirrhosis: A Review. Health Serv Insights. 2017;10:1178632917691270. doi: 10.1177/1178632917691270. DOI
25. Hanje AJ, Patel T. Preoperative evaluation of patients with liver disease. Nat Clin Pract Gastroenterol Hepatol. 2007;4(5):266-76. doi: 10.1038/ncpgasthep0794. DOI
26. Ziser A, Plevak DJ, Wiesner RH et al. Morbidity and mortality in cirrhotic patients undergoing anesthesia and surgery. Anesthesiology. 1999;90(1):42-53. doi: 10.1097/00000542-199901000-00008. DOI

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CORRESPONDING AUTHOR

Oleksii Petiunin
Kharkiv National Medical University
4 Nauky avenue, 61022 Kharkiv, Ukraine
e-mail: oh.petiunin@knmu.edu.ua

ORCID AND CONTRIBUTIONSHIP

Oleksii Petiunin: 0000-0001-9411-994X A B C D E F
Vasyl Syplyviy: 0000-0002-6052-1444 B C E
Kamel Jbarah Hammad Al Mashni: 0009-0006-5735-2340 A B C D E
Dmytro Myroshnychenko: 0000-0001-5581-6494 A B C D E
Vitaly Makarov: 0000-0002-4224-0294 B C D E
Anna Novikova: 0009-0006-9048-6498 B C D
Bohdan Porovai-Nevoit: 0009-0006-3956-7372 B C D

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ORIGINAL ARTICLE

CONTENTS

Study of changes in the length of the anterior talo-fibular ligament in patients with symptoms of the ankle-foot joint instability

Yurii Hrubar[1], Iryna Ya. Hrubar[2], Markiian Yu. Hrubar[3], Yuliana Yu. Hrubar[3], Nadiia M. Hrabyk[2], Olha V. Hulka[2]

[1] HORBACHEVSKY TERNOPIL NATIONAL MEDICAL UNIVERSITY, TERNOPIL, UKRAINE

[2]TERNOPIL VOLODYMYR HNATIUK NATIONAL PEDAGOGICAL UNIVERSITY, TERNOPIL, UKRAINE

[3]TERNOPIL REGIONAL CLINICAL HOSPITAL, TERNOPIL, UKRAINE

ABSTRACT

Aim: To study changes in the length of the anterior talo-fibular ligament (ATFL) by measuring it before and during manual inversion loading in the presence of symptoms of chronic ankle-foot joint instability.

Materials and Methods: The study was conducted in patients of two age groups: 20-29 and 30-39 years. The length of the ligament was determined sonographically in the neutral position and in the position of maximum inversion.

Results: According to the results of the study, the average length of the uninjured ATFL in men in the neutral position was 19.08±0.3 mm. With inversion loading, the length of the ligament increased to 19.65±0.3 mm. In women, the average length of the uninjured ATFL in the neutral position was 16.92±0.2 mm, with inversion loading - 17.37±0.2 mm. With joint instability, the average length of the ATFL on the side of the injury in the neutral position was 19.13±0.36 mm, with inversion loading - 20.35±0.44 mm. The statistical difference in the length of the ATFL between the neutral position and inversion loading on the side of the injury was 1.25±0.04 mm ($p<0.001$).

Conclusions: The results of our study show that sonography with inversion loading is a highly sensitive, specific, and accurate imaging method that can be used to diagnose chronic ankle-foot joint instability.

KEY WORDS: sonography, ankle-foot joint instability, anterior talo-fibular ligament, inversion load test

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INTRODUCTION

Acute ankle-foot joint sprains are one of the most common musculoskeletal injuries. This type of injury is particularly common among physically active young people, both in everyday life and in sports [1]. 85% of ankle-foot joint sprains involve the lateral collateral ligament complex. More than 65% of cases involve isolated anterior talo-fibular ligament injuries. Sudden inversion and internal rotation, combined with plantar flexion, place significant stress on the anterior talo-fibular ligament, leading to its rupture [2]. In 20% of cases, this type of injury requires emergency medical attention, but up to 64% of injured individuals do not seek medical attention at all [3]. Recent studies have shown that up to 70% of individuals who sustain an acute lateral ankle-foot joint sprain may develop symptoms of chronic ankle instability (CAI) within a short period of time after the initial injury [4].

To prevent these complications, high-quality diagnostics and subsequent selection of treatment tactics, which should be started quickly and in full, remain important. To date, limited evidence has been shown for many widely used special orthopedic tests for ankle-foot joint injuries [5]. Among non-invasive instrumental diagnostic methods, sonography, stress radiography, and magnetic resonance imaging are most often used. The results of stress radiographs are questioned because they are seriously affected by the radiography technique, the magnitude of the force applied during the examination, and the patient's reaction to the examination [6]. Magnetic resonance imaging is the most accurate diagnostic method for assessing ankle-foot joint injuries, given its high resolution and accuracy. However, MRI does not show the dynamic function of the ligaments, and its use in acute trauma is considered inappropriate [7].

Fig. 1. Ankle-foot joint ultrasound device
Picture taken by the authors

Today, sonography is becoming increasingly important in assessing the condition of the ligamentous apparatus of the joints, and the sensitivity of ultrasound in detecting injuries to the anterior calcaneal-fibular ligament is up to 93% [8]. Dynamic examination in the form of stress tests makes it possible to sonographically assess the degree of joint instability, simultaneously determine the condition of the ligaments of the contralateral joint and conduct a comparative analysis [9]. Thus, in case of ankle-foot joint damage, ATFL ultrasound assessment is a highly reliable procedure due to the practicality of both dynamic and static assessment of the ligament condition [10].

AIM

Study of changes in the length of the anterior talo-fibular ligament by measuring it before and during manual inversion loading in age groups most frequently exposed to its damage in the presence of symptoms of chronic ankle-foot joint instability.

MATERIALS AND METHODS

SELECTION OF PARTICIPANTS

The control group (CG) consisted of students of Ternopil National Medical University and Ternopil National Pedagogical University, who voluntarily participated in the study. The average length of the anterior talo-fibular ligament on both limbs in a neutral position and with inversion loading was studied. The average value of the length of the anterior talo-fibular ligament of the right and left ankle-foot joint in each position was recorded in the protocol. The average age was 22.63±0.11 years in men and 22.54±0.12 years in women. The experimental group (EG) initially included 59 patients with signs of chronic ankle-foot joint instability, who underwent examination in the clinic from 2019 to 2024. Of these, 37 (62.72%) were men, 22 (39.28%) were women; the average age was 32.3±2.8 years. At the final stage of the study, 49 patients remained in the experimental group for one reason or another. In the process of studying the stability of the ankle-foot joint in the study groups, we used standard inclusion and exclusion criteria approved by the International Ankle Consortium.

RESEARCH METHODOLOGY

Sonography of the ligament was performed using a Siemens Acuson S2000 ultrasound console with a high-frequency broadband linear transducer with an operating frequency of 7–12 MHz, using standard acoustic gel.

Patient positioning. Measurements of the anterior talo-fibular ligament length in both groups were

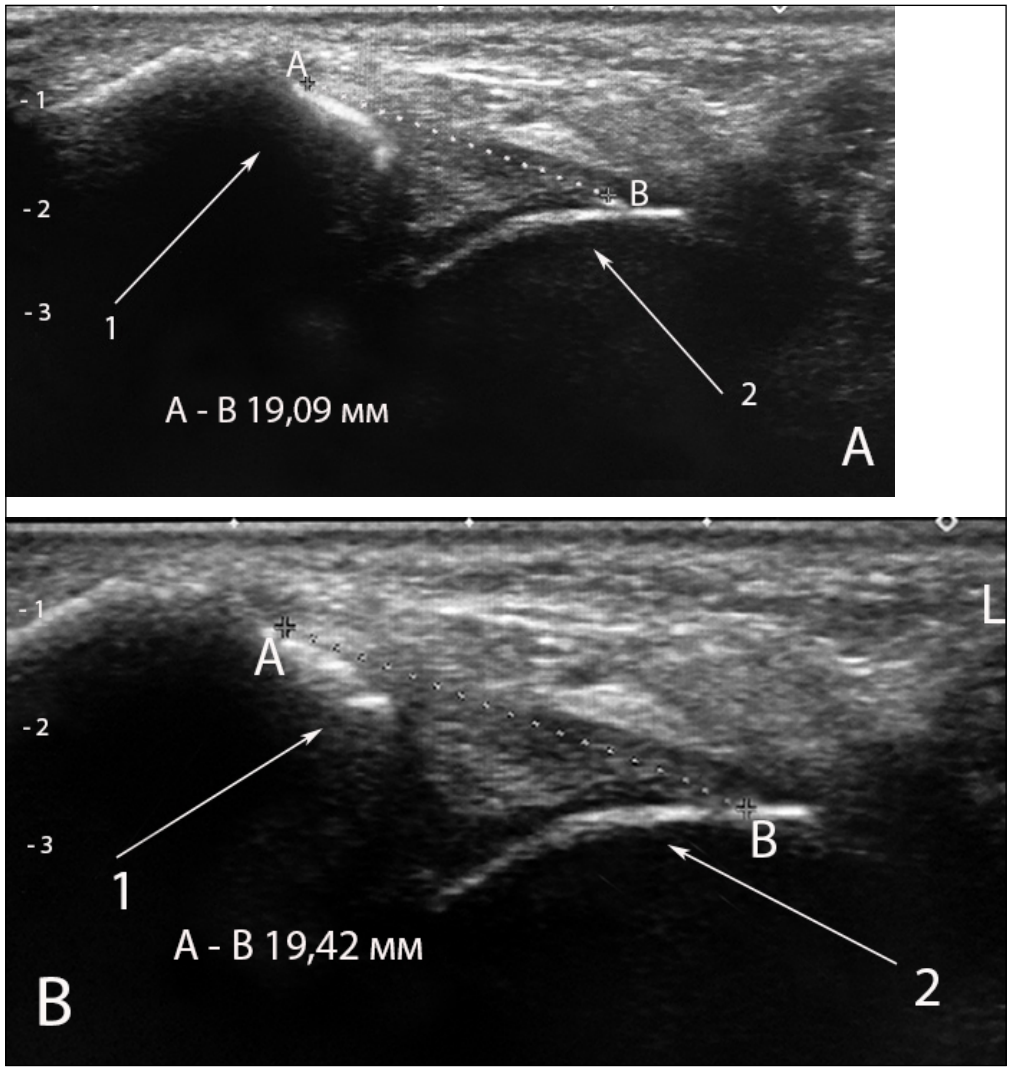

Fig. 2. Length measurement of the anterior talo-fibular ligament (ATFL) in a neutral position during sonography in a control patient. A. Dimensions of the anterior talo-fibular ligament in a neutral position in a patient of the control group (A-B measurement points of the anterior talo-fibular ligament. 1 - fibula; 2 - talus). B. Dimensions of the anterior talo-fibular ligament during inversion loading in a patient of the control group (A-B measurement points of the anterior talo-fibular ligament. 1 - fibula; 2 - talus)
Picture taken by the authors

performed in two positions (neutral and maximum inversion), three times per position in each patient, to obtain the average ligament length.

In the first position, the subjects held the examined limb on the ankle-foot joint ultrasound device (Fig. 1) in a position of flexion in the knee joint at an angle of 45°, and the ankle-foot joint area hung freely in a position of plantar flexion to an angle of 20°. This position was defined as the neutral resting position and the baseline value for each subsequent measurement of the anterior talo-fibular ligament length [11].

During the sonographic examination, the distance from the anterolateral edge of the lateral bone, corresponding to the origin of the anterior talo-fibular ligament, to the anterolateral angle of the lateral surface of the talus, corresponding to the anatomical site of its fixation, was measured. The length of the ligament was measured on a longitudinal ultrasound image using a Preset of the sonographic apparatus, which allowed measurements to be made with an accuracy of two decimal places. (Fig. 2. A, B).

In the second position (maximum inversion loading position): the limb was in a neutral position, the researcher performed maximum inversion at the ankle-foot joint while holding the foot in a position of plantar flexion. The length of the anterior talo-fibular ligament in the inversion loading position was determined on the longitudinal ultrasound image as a straight line between the above-described bony landmarks (Fig. 3. A, B.).

STATISTICAL ANALYSIS

Statistical analysis was performed using Statistica 8.0 software. Results were presented as average values with 95% confidence interval. The Shapiro-Wilk test was used to confirm whether the data were normally distributed. When the data showed a normal distribution, the Student's t-test was performed to compare continuous data. Otherwise, the Mann-Whitney U-test was performed. The chi-square test or Fisher's exact test was used to compare categorical data. The Wilcoxon signed-rank test was performed to compare the ultrasound results for the right and left ankles.

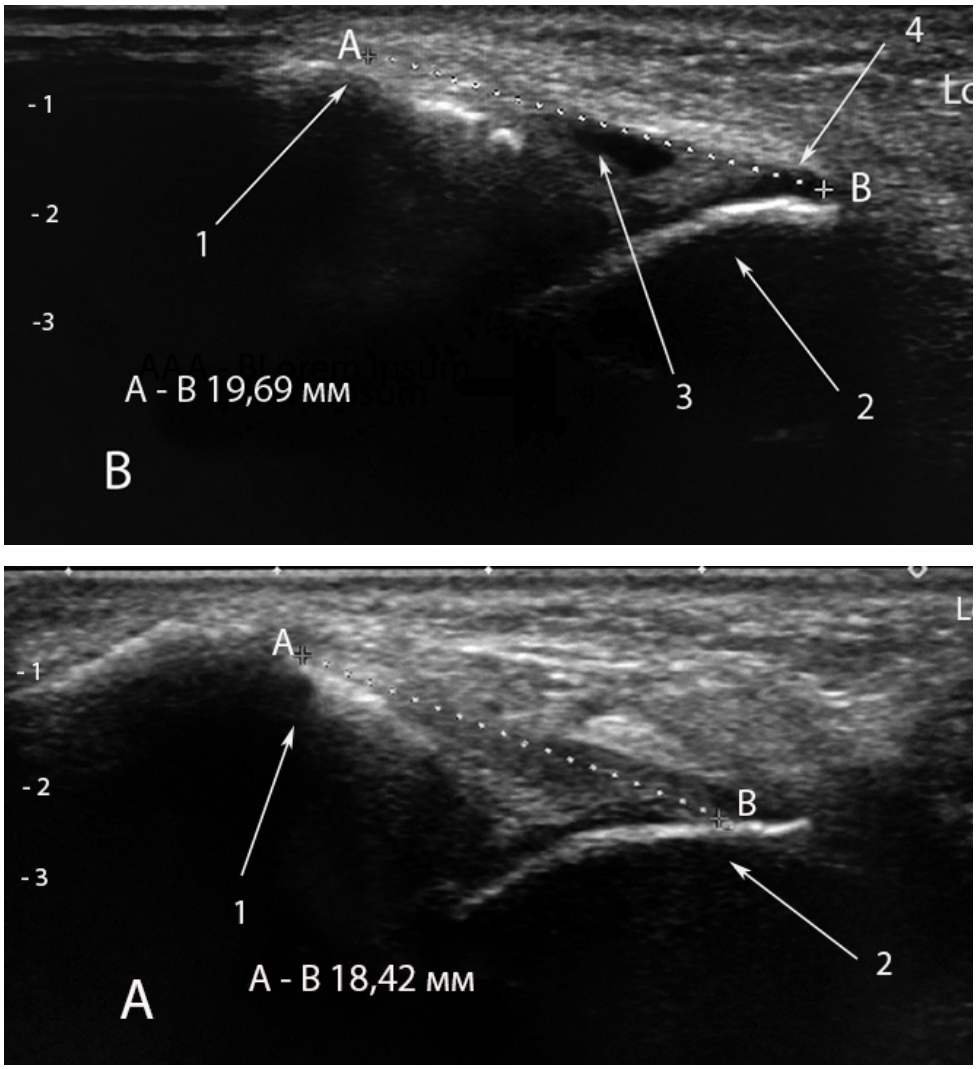

Fig. 3. Anterior talo-fibular ligament of the experimental group patient with chronic ankle-foot joint instability during sonography. A. Dimensions of the anterior talo-fibular ligament in a neutral position in a patient of the experimental group with chronic ankle-foot joint instability (A-B measurement points of the anterior talo-fibular ligament. 1 - fibula; 2 - talus). B. Dimensions of the anterior talo-fibular ligament at maximum inversion loading in a patient of the experimental group with chronic instability of the ankle-foot joint (A-B measurement points of the anterior talo-fibular ligament. 1 - fibula; 2 - talus; 3 - free fluid in the joint cavity; 4 - place of detachment of the anterior talo-fibular ligament from the talus)
Picture taken by the authors

ETHICS

This work complies with the principles of the Declaration of Helsinki.

RESULTS

In the first stage of the research, sonographic measurements of the length of the intact anterior talo-fibular ligament of the control group (age 20-29 years) were compared with the length of the ligament on the contralateral side of the experimental group (age 30-39 years) in a neutral position and with inversion loading in order to determine the effect of the patients' age on changes in its length.

In men of the control group, the average ATFL length in the neutral position was 19.11±0.32 mm, and with inversion loading - 19.68±0.33 mm ($p<0.001$). The elongation of the ligament was 0.57±0.01 mm. In men of the experimental group, the average ATFL length in the neutral position was 19.00±0.40 mm, and with inversion loading - 19.61±0.33 mm ($p<0.001$). The elongation of the ligament was 0.56±0.01 mm. The data obtained by us indicate that there is no statistically significant difference between male patients of the control and experimental groups in terms of ATFL length in different states ($p>0.05$) (Table 1).

In women of the control group, the average length of the ATFL in the neutral position was 16.97±0.19 mm, and with inversion loading - 17.42±0.20 mm ($p<0.001$). The elongation of the ligament was 0.45±0.01 mm. In women of the experimental group, the average length of the ATFL in the neutral position was 16.99±0.22 mm, with inversion loading - 17.45±0.24 mm ($p<0.001$). The elongation of the ligament was 0.46±0.01 mm.

These data indicate that there is no statistically significant difference between female patients in the control and experimental groups in terms of ATFL length in different states ($p>0.05$) (Table 1).

In men of the CG and EG, the length of the ATFL in the neutral position and with inversion loading was greater than in women of the same groups at the level of statistical probability ($p<0.001$). The intraclass correlation coefficients for neutral and tense ligament lengths were 0.85-0.9, indicating good reliability of the study results.

Table 1. Length indicators of the intact ATFL in the neutral and inversion positions in patients of the study groups

Gender	Men			Women			
Group	ATFL length neutral position, mm	ATFL length inversion loading, mm	ATFL elongation, mm	ATFL length neutral position, mm	ATFL length inversion loading, mm	ATFL elongation, mm	P (m/w)
CG	19.11±0.32	19.68±0.33	0.57±0.01	16.97±0.19	17.42±0.20	0.45±0.01	<0.001
EG	19.00±0.40	19.61±0.33	0.56±0.01	16.99±0.22	17.45±0.24	0.46±0.01	<0.001
Statistical difference CG/EC	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	
CG statistical difference (neutral position/inversions)		<0.001			<0.001		
EG statistical difference (neutral position/inversions)		<0.001			<0.001		

Source: compiled by the authors of this study

Table 2. Average length of intact ATFL in neutral position and with inversion loading in patients of the study groups

Position	Average length of the anterior talo-fibular ligament		
	Control group	Experimental group	Statistical difference CG/EG
Neutral position, mm	18.04±0.12	17.99±0.18	p>0.05
Inversion loading, mm	18.55±0.13	18.53±0.13	p>0.05
Length change, mm	0.51±0.007	0.51±0.006	p>0.05

Source: compiled by the authors of this study

Table 3. Average ATFL length indicators in neutral position and at maximum inversion loading in the experimental group

Side	Contralateral side	Damaged side	Statistical difference contralateral/injured
Position	Average length of the anterior talo-fibular ligament		
Neutral position, mm	17.99±1.06	19.13±0.36	p<0.001
Inversion loading, mm	18.53±1.12	20.35±0.44	p<0.001
Length change, mm	0.51±0.05	1.25±0.04	p<0.001
Statistical difference between the positions	p<0.05	p<0.001	

Source: compiled by the authors of this study

The generalized average length of the ATFL in the control group in the neutral position was 18.04±0.12 mm, and at maximum inversion - 18.55±0.13 mm, the change in length was 0.51±0.007 mm. In the experimental group, these indicators were as follows: in the neutral position, the length of the ligament was 17.99±0.18 mm, with inversion loading - 18.53±0.13 mm, and the elongation was 0.51±0.006 mm. There is no statistical difference between the indicators of the control and experimental groups (p>0.05). This gives us reason to believe that the length of the intact ATFL in the neutral position and with inversion loading in both study groups does not change with age (Table 2).

In the second stage of the study, the length of the anterior talo-fibular ligament on the contralateral side and the side of the injury in the neutral position and at maximum inversion loading was compared in the experimental group (Table 3).

In the experimental group, the average values of the length of the anterior talo-fibular ligament in the neutral position on the contralateral side were 17.99±1.06 mm, and with maximum inversion - 18.53±1.12 mm (p<0.05). The change in the length of the ligament was 0.51±0.05 mm. The average values of the ATFL length in the neutral position on the side of injury were 19.13±0.36 mm, with maximum inversion - 20.35±0.44 mm. The change in its length was 1.25±0.04 mm on average (p<0.001).

DISCUSSION

This article is a continuation of the study of the condition of the lateral ligament complex of the ankle-foot joint when it is damaged in young people, which we have been conducting since 2019.

During physical examination, the standard tests used to detect lateral instability are the front box, talus,

and inversion stress test [12]. According to Beynon A., physical examination is limited by subjectivity and difficulties in recognizing the degree of ligament stability, as well as identifying the specific injured ligament [13]. Dynamic sonography has a high potential for detecting ankle-foot joint ligament instability, but the value parameters of the change in length of the anterior talo-fibular ligament during inversion loading in patients with signs of chronic lateral instability in different age groups have not been fully determined.

Our study methodology meets the current requirements for performing sonographic inversion loading. The internal rotation moment at plantar flexion to an angle of 30° is considered optimal for assessing increased translation of the ankle-foot joint with ATFL insufficiency, when using stress ultrasound examination [14].

The results of our study show that ATFL lengths in both neutral and inversion loading can be effectively measured using manual stress sonography. Thus, the average ATFL length in the neutral position in men of the control and experimental groups is 19.11±0.32 and 19.00±0.40 mm, in women, respectively, 16.97±0.19 - 16.99±0.22 mm. They are in the range of the stated values from 19.2 to 20.31±3.12 mm which were measured in bone-ligamentous preparations of cadaveric ankle-foot joints [15]. By measuring ATFL length on 50 cadaveric joints, Khawaji B. et al. found that ATFL length ranged from 18.81 mm in dorsiflexion to 21.06 mm in plantar flexion [16].

In addition, the average length of the ATFL, both for men and women in our study, is consistent with the figures of Dong Y. et al. who reported that changes in the length of the ATFL in vivo in healthy ankle-foot joints range from 16 mm in the neutral position to 20.8 mm during active plantar flexion of the ankle-foot joint and supination [17].

In MRI examinations, Szaro P. et al. indicate that the length of the ATFL can vary from 13-14 mm to 21-22 mm and the values obtained depend on the methodology of the study and variations in the structure of the ligament bundles [18].

In sonography, the average length of the ATFL of the non-tensioned ligament is 19.50 ± 1.81, and of the tensioned ligament is 21.08 ± 1.98 [19].

We showed a statistically significant difference comparing the changes in ATFL length in asymptomatic subjects 0.51±0.07 mm and patients with anterolateral instability 1.25±0.04 mm ($p<0.001$). The average change in ATFL length between neutral state and inversion loading in the symptomatic group was almost 2.5 times greater compared to asymptomatic patients. In an ultrasound study, Cho J.H. et al found that the ATFL ratio (tension/rest) was 1.3±0.1 mm [20]. Similar results were obtained in the studies of Kamada K et al. [21]. Our results are consistent with the data of Kikumoto T. et al. who concluded that the change in ATFL length during inversion loading with plantar flexion of the foot was statistically significantly greater than in healthy individuals [22].

CONCLUSIONS

During the study, it was found that the length of the intact ATFL in a neutral position and under inversion loading in patients of the studied groups does not change with age.

The average values of the ATFL length in a neutral position on the side of the injury were 19.13±0.36 mm, with maximum inversion - 20.35±0.44 mm. The change in its length was on average 1.25±0.04 mm ($p<0.001$), which may indicate chronic instability of the ankle-foot joint.

Thus, inversion loading sonography is a highly sensitive, specific, and accurate imaging method that can be used to diagnose chronic ankle-foot joint instability.

A potential limitation of this study was that there was some subjective assessment of the ATFL during the sonographic examination. We also did not determine the force applied during inversion loading. Future studies should aim to standardize testing methods and ligament length measurements to provide an objective definition of chronic ankle-foot joint instability.

REFERENCES

1. Miklovic TM, Donovan L, Protzuk OA et al. Acute lateral ankle sprain to chronic ankle instability: a pathway of dysfunction. Phys Sportsmed. 2018;46(1):116-122. doi: 10.1080/00913847.2018.1409604. DOI
2. Chen RP, Wang QH, Li MY et al. Progress in diagnosis and treatment of acute injury to the anterior talofibular ligament. World J Clin Cases. 2023;11(15):3395-3407. doi: 10.12998/wjcc.v11.i15.3395. DOI
3. Cavazos GJJ, Harkless LB. The epidemiology, evaluation, and assessment of lateral ankle sprains in athletes. J Sports Med Therapy 2021;6(2):008-017. doi: 10.29328/journal.jsmt.1001052. DOI
4. Gribble PA, Bleakley CM, Caulfield BM et al. Evidence review for the 2016 International Ankle Consortium consensus statement on the prevalence, impact and long-term consequences of lateral ankle sprains. Br J Sports Med. 2016;50(24):1496-1505. doi: 10.12998/wjcc.v11.i15.3395. DOI

5. Larkins LW, Baker RT, Baker JG. Physical Examination of the Ankle: A Review of the Original Orthopedic Special Test Description and Scientific Validity of Common Tests for Ankle xamination. Arch Rehabil Res Clin Transl. 2020;2(3):100072. doi: 10.1016/j.arrct.2020.100072. DOI
6. Cao M, Liu S, Zhang X et al. Imaging diagnosis for anterior talofibular ligament injury: a systemic review with meta-analysis. Acta Radiol. 2023;64(2):612-624. doi: 10.1177/02841851221080556. DOI
7. Park EH, de Cesar Netto C, Fritz J. MRI in Acute Ankle Sprains: Should We Be More Aggressive with Indications? Foot Ankle Clin. 2023;28(2):231-264. doi: 10.1016/j.fcl.2023.01.011. DOI
8. Hosseinian SHS, Aminzadeh B, Rezaeian A et al. Diagnostic Value of Ultrasound in Ankle Sprain. J Foot Ankle Surg. 2022;61(2):305-309. doi: 10.1053/j.jfas.2021.08.008. DOI
9. Park JW, Lee SJ, Choo HJ et al. Ultrasonography of the ankle joint. Ultrasonography. 2017;36(4):321-335. doi: 10.14366/usg.17008. DOI
10. Hattori S, Saggar R, Mullen J et al. Role of Ultrasound in Evaluating Ligament Injuries Around the Ankle: A Narrative Review. Diagnostics (Basel). 2025;15(3):392. doi: 10.3390/diagnostics15030392. DOI
11. Hrubar Y, Hrubar I, Hrabyk N et al. Change of the anterior talus-fibular ligament length of asymptomatic patients during inversion stress test. Wiad Lek. 2024;77(12):2435-24413. doi: 10.36740/WLek/195548. DOI
12. Aiyer A, Murali S, Kadakia AR. Advances in Diagnosis and Management of Lateral Ankle Instability: A Review of Current Literature. J Am Acad Orthop Surg Glob Res Rev. 2023;7(12):e23.00251. doi: 10.5435/jaaosglobal-d-23-00251. DOI
13. Beynon A, Le May S, Theroux J. Reliability and validity of physical examination tests for the assessment of ankle instability. Chiropr Man Therap. 2022;30(1):58. doi: 10.1186/s12998-022-00470-0. DOI
14. Hattori S, Onishi K, Chan CK et al. Optimal assessment for anterior talofibular ligament injury utilizing stress ultrasound entails internal rotation during plantarflexion. J ISAKOS. 2024;9(3):302-308. doi: 10.1016/j.jisako.2024.02.001. DOI
15. Schoonover C, Wright B. Variation in the anterior talofibular ligament and the impact of sex, height, weight, and age. Clin Anat. 2023;36(5):830-835. doi: 10.1002/ca.24056. DOI
16. Khawaji B, Soames R. The anterior talofibular ligament: A detailed morphological study. Foot (Edinb). 2015;25(3):141-147. doi: 10.1016/j.foot.2015.05.004. DOI
17. Dong Y, Qian Y, Liu L et al. Anatomical Study on the Reconstruction of the Anterior Talofibular Ligament. J Foot Ankle Surg. 2021;60(5):908-911. doi: 10.1053/j.jfas.2021.03.004. DOI
18. Szaro P, Ghali Gataa K, Solidakis N, Pękala P. Morphometric relationships between dimensions the anterior talofibular ligament and calcaneofibular ligament in routine magnetic resonance imaging. J Exp Orthop. 2021;8(1):90. doi: 10.1186/s40634-021-00406-2. DOI
19. Yokoe T, Tajima T, Kawagoe S et al. The Ratio of Stress to Nonstress Anterior Talofibular Ligament Length on Ultrasonography: Normative Values. Orthop J Sports Med. 2021;9(11):23259671211056305. doi: 10.1177/23259671211056305. DOI
20. Cho JH, Lee DH, Song HK et al. Value of stress ultrasound for the diagnosis of chronic ankle instability compared to manual anterior drawer test, stress radiography, magnetic resonance imaging, and arthroscopy. Knee Surg Sports Traumatol Arthrosc. 2016;24(4):1022-1028. doi: 10.1007/s00167-015-3828-9. DOI
21. Kamada K, Hoshino Y, Yamamoto T et al. Diagnostic strategies for chronic lateral ankle instability: a narrative review. Ann Jt. 2024;9:41. doi: 10.21037/aoj-24-31. DOI
22. Kikumoto T, Akatsuka K, Nakamura E et al. Quantitative evaluation method for clarifying ankle plantar flexion angles using anterior drawer and inversion stress tests: a cross-sectional study. J Foot Ankle Res. 2019;12:27. doi: 10.1186/s13047-019-0337-y. DOI

CONFLICT OF INTEREST

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CORRESPONDING AUTHOR

Yurii Hrubar
Horbachevsky Ternopil National Medical University
1 Maidan Voli, 46001 Ternopil, Ukraine
e-mail: hrubar@ukr.net

ORCID AND CONTRIBUTIONSHIP

Yurii Hrubar: 0000-0002-4221-2250 B C D
Iryna Ya. Hrubar: 0000-0002-0809-1299 A D E
Markiian Yu. Hrubar: 0000-0002-4696-0213 B D E
Yuliana Yu. Hrubar: 0000-0003-0951-9485 A D F

Nadiia M. Hrabyk: 0000-0002-8882-9782 C D F
Olha V. Hulka: 0000-0002-8364-5941 A D F

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ORIGINAL ARTICLE

CONTENTS

Exploring the anti-tumor potential of saxagliptin in A549 lung adenocarcinoma cells

Sama J. Shubbar[1], Ahsan F. Bairam[1]
DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY, FACULTY OF PHARMACY, UNIVERSITY OF KUFA, NAJAF, IRAQ

ABSTRACT

Aim: To evaluate the anti-tumor potential of SAXA on A549 cells and assess its combinatory effects with CP on cell viability and apoptosis markers.

Materials and Methods: Four primary groups were utilized from A549 lung cancer cell lines: unprocessed Cells (control), cells subjected to CP treatment, cells subjected to SAXA treatment, and cells treated with CP plus SAXA, thereby obtained a combination of varying concentrations of CP and SAXA. Five used concentrations (62.5, 125, 250, 500 and 1000) µg/mL for SAXA and 0.9, 1.87, 3.75, 7.5, and 15 µg/mL for CP with four duplicates employed for each treated group. Incubated for 72hr., cells gathered, centrifuged, and supernatants were eliminated, while particles were gathered to determine BCL2 and BAX levels using ELISA test kits

Results: SAXA dramatically reduced A549 cell viability in a dose-dependent manner. The combination of SAXA and CP also displayed cytotoxicity; however, no synergistic effect was found above CP alone. Notably, the combined treatment dramatically lowered BCL2 levels ($p < 0.001$), but BAX levels remained stable ($p > 0.05$).

Conclusions: SAXA showed promising anti-cancer action against A549 cells. Although the combination with CP did not boost cytotoxicity, the observed pro-apoptotic reduction in BCL2 implies potential therapeutic efficacy.

KEY WORDS: SAXA, A549 cell-line, Cisplat, MTT assay, Anticancer.

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INTRODUCTION

Cancer is a group of diseases which identified by several defining characteristics, that include persistent cell proliferation, resistance to programmed cell death, promotion of blood vessel development, and invasion of surrounding healthy cells [1]. lung cancer is a type of cancer that begins when aberrant cells proliferate uncontrollably in the lungs [2]. Lung cells normally replicate under controlled condition in order to help in tissue repair and maintain general health. An increase in the frequency of cell division and a lack of control over cellular development, however, may lead to the development of a tumor [3]. Pulmonary carcinoma represents primary reason of Cancer related fatality and incidence globally, with approximately 2 million diagnoses and 1.8 million deaths reported. After prostate and breast cancer, respectively, lung neoplasms are the second most prevalent cancer diagnoses in both men and women [4]. Men are slightly more likely than women to be diagnosed with invasive cancer throughout their lifetime (41.6%) compared to (39.6%). also, there is thought that males are more likely to develop most types of cancer because they are exposed to more carcinogenic environmental and lifestyle variables, such smoking, yet a new study indicates that other unchangeable characteristics also have a significant effect [5]. In accordance with the most recent estimate from the global cancer observatory "GLOBOCAN", there were 2,206,771 recent cases of pulmonary carcinoma detected globally in 2020 [4]. An estimated 33,873 new cases of cancer were reported in Iraq in 2020, with 14,070 cases involving men and 19,803 involving women. 19,786 patients lost their lives by lung cancer [6]. Surgery, radiation, immunotherapy, and chemotherapy, including CP, are among the available therapeutic options [7]. Platinum based chemotherapy (CP) is typically used as the initial treatment for patients with non-small cell lung cancer [8-9]. In biological systems, anticancer medications cause oxidative stress, which results in lipid peroxidation and the production of several electrophilic aldehydes. Since oxidative stress prevents cancer cells from proliferating, its effects can increase the effectiveness of anticancer treatments [10]. CP's use and efficacy are restricted by two intrinsic problems: adverse effects and

drug resistance [11]. CP treatment adverse consequences include vestibulopathy, peripheral neuropathies (which are less common in younger patients), neurotoxicity that manifests as ototoxicity, and severe nephrotoxicity that results in end-stage dialysis [12]. It has been noted that 50% of CP-treated individuals either quickly develop multidrug resistance or develop intrinsic resistance [13]. Dipeptidyl peptidase-4 (DPP-4) inhibitors have recently been shown to have profound anticancer effects on cancer cells. Specifically, the US Federal Drug Agency (FDA) authorized sitagliptin, an anti-diabetic drug, as a DPP-4 inhibitor in 2006 [14]. DPP-4 inhibitors are a group of oral diabetic medications that work by blocking the DPP-4 enzyme. Numerous bioactive peptides, such as Glucagon like Peptide-1" GLP-1" and glucose dependent Insulinotropic Polypeptide "GIP", are disabled by the ubiquitous enzyme DPP-4. In addition to slowing stomach emptying time, inhibiting incorrect post-meal glucagon release, and reducing food intake, GLP-1 primarily works via inducing glucose-dependent insulin release from the pancreatic islets. Consequently, its blockage may have a variety of impacts on glucose regulation [15]. Recent study in 2024 showed that linagliptin (a member of DPP4 inhibitors) has anti-tumor effect on lung cancer by decreasing the cancer cell viability when treated with linagliptin IC50. Additionally, BCL2 level reduced following treatment of A549 cells with IC50 of linagliptin [7]. Other study in 2023 revealed that sitagliptin has an anti-apoptotic effect against Hepatocellular carcinoma based on BCL2 measurement on HepG2 cell line [14].

AIM

The purpose of this article is to evaluate the anti-tumor potential of SAXA on A549 cells and assess its combinatory effects with CP on cell viability and apoptosis markers.

MATERIALS AND METHODS

CHEMICALS AND CELL LINE

The Iraq Biotech Cell Bank Unit in Basrah provided the A549 lung cancer cells, passage number 20. A459 cells were generally obtained in January 1972 from a human alveolar-cell cancer in a 58-year-old white man. They were continuously propagated in vitro for more than three years, producing more than 1,000 cell generations that are frequently used as models for lung cancer [16]. HBL100 cells which is provided from Iraq Biotech Cell Bank Unit in Basrah. The milk of an apparently healthy lady was used to create the epithelial HBL-100 cell line in vitro. It has transformational properties from the start and progresses throughout vitro maintenance until it becomes tumorigenic in nude mice. This immortal cell line is a helpful model for researching the transition of human epithelial cells to cancer [17]. MTT (3-(4,5 Dimethylthiazole-2-yl)-2,5-Diphenyl-2H-tetrazolium Bromide) pigment powder, Dimethyl sulfoxide (DMSO) and RIPA buffer for lysis was ordered from Sigma, USA. Gibco, USA provided the phosphate buffer saline (PBS), 10% fetal bovine serum (FBS), and Roswell Park Memorial Institute-1640 (RPMI-1640). The UK-based Flow Laboratories provided the trypan-blue stain. The suppliers of streptomycin and benzoylpenicillin were Troge, Germany. Capricorn, USA, was the source of trypsin-EDTA. CP was ordered from Pfizer, USA. SAXA had been purchased from Targetmol pharmaceuticals Co, USA. From Bioassay Technology Laboratory of China.

CELL CULTURE AND MTT ASSAY

Trypsin-EDTA as a proteolytic protein, phosphate buffer saline (PBS), and fetal bovine serum (FBS) were used to extract the A549 and HBL100 cell lines. The cells were then incubated in 96 well plate using Roswell Park Memorial Institute-1640 liquid medium containing 100units/mL Penicillin, and 100µg/mL Streptomycin. The sample was incubated for 24 hours at 37 °C, with 5 % Co2, and 95 % humidity, to facilitate formation of the monolayer of cells (80 % growth phase). After that, 200 µL of medium—which contains the test medications and control group—was used to substitute the prior medium [18]. Four main groups were used: cells that were left untreated (control), cells that were treated with CP, cells that were treated with SAXA, and cells that were treated with CP + SAXA, which obtained a combination of CP and SAXA at varying concentrations. Five different concentrations (62.5, 125, 250, 500 and 1000 µg/mL) for SAXA and 0.9, 1.87, 3.75, 7.5, and 15 µg/mL for CP with four duplicates employed for each treated group. The non-specific conversion between formazan and the studied medications was assessed using a blank (which contained only medium). Incubation for 72 hr., then the (4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2Htetrazolium bromide (MTT) test [19] used for determining cell viability, and Non-linear regression utilizing four-parameter logistic Hill equation was utilized to compute dose-effect curves. For every group, the IC50 (concentration required for 50% suppression of cell viability) was determined using GraphPad Prism 10. Subsequent equation was employed to get the percentile of cell viability:

Cell Viability % = (AS-AB)/(AC-AB) × 100 %

Sample absorbance represented by AS, control absorbance represented by AC, and Ab is the absorbing

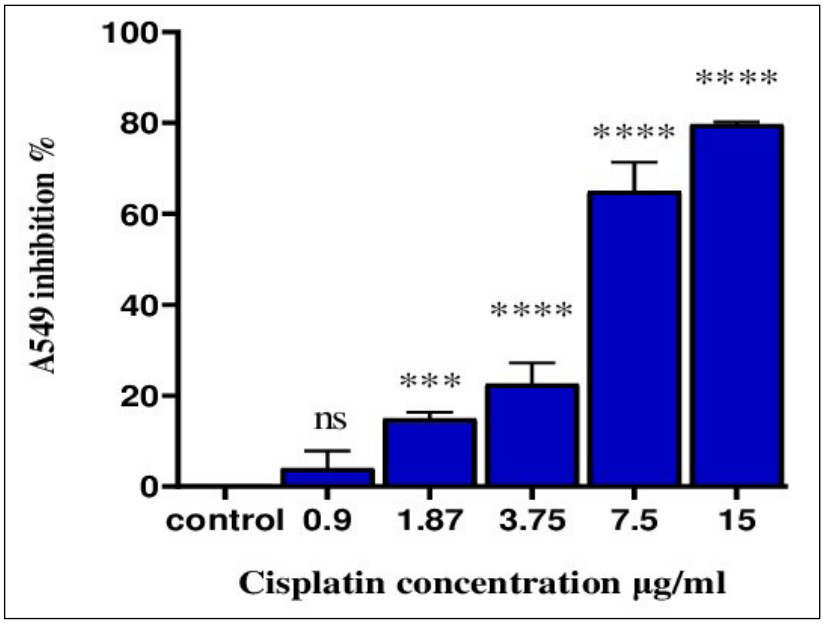

Fig. 1. Cytotoxic effect of different cisplatin concentrations on"A549" cell line. For the examination, (one way) Anova was used. Data are displayed as mean ± SD, ns (P>0.05), ***P<0.001, ****P<0.0001, n=4, 72 hours incubation
Source: Own materials

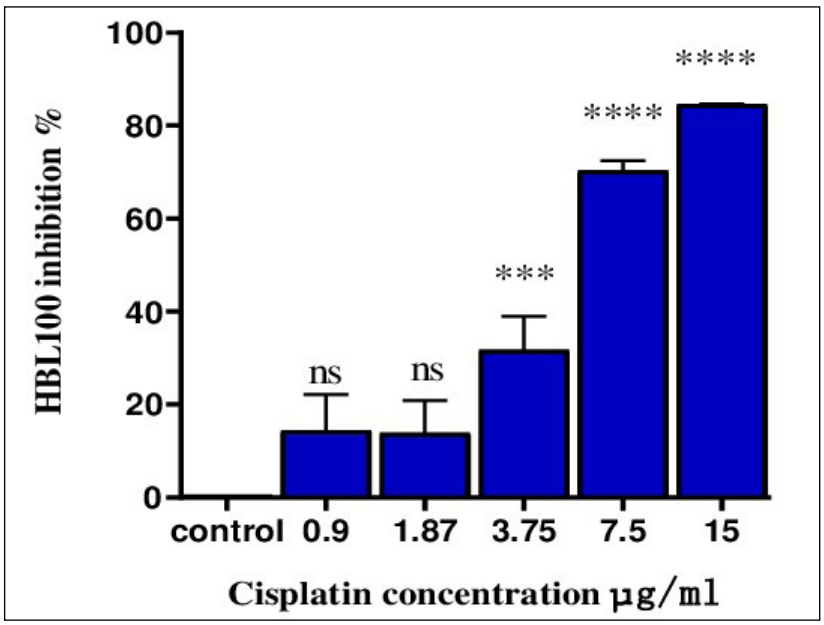

Fig. 2. Cytotoxic effect of different cisplatin concentrations against HBL100 cell line. For the examination, (one way) Anova was used. Data displayed as mean ± SD, ns =P>0.05, ***P<0.001, ****P<0.0001, n=4, 72 hours incubation
Source: Own materials

capacity of the blank. Four copies of each determination were made. The following formula was used to estimate the inhibition rate, or the percentage of cytotoxicity [20].

Inhibition%=100-Viability x 100%

EVALUATION OF BCL2 CONCENTRATION

A549 and HBL100 cells were cultivated in four flasks and subjected to the IC50 of Cp, SAXA, and IC50 of CP plus SAXA for 36 hours. Following treatment, the supernatant was extracted from the cells after they were harvested and centrifuged. After extracting the proteins from the cell pellets using a lysis buffer, they were inserted into an Eppendorf sterile tube (1.5 mL) and kept at –20 °C until they could be examined with a Bcl2 ELISA test kit. ELISA kit for human Bcl2 ordered from SUNLOG Biotech CO "Hang Zhou, China" was utilized for examination. The testing procedure was conducted in accordance with the SUNLOG Biotech CO protocol. The absorbance value for each well was measured with a microplate reader calculated to 450 nm.

EVALUATION OF BAX CONCENTRATION

The extracted protein that mentioned in the previous paragraph was examined with a BAX ELISA assay kit, which is obtained from SUNLOG Biotech CO (Hang Zhou, China). The SUNLOG Biotech CO methodology was followed for conducting the test. To determine each well's absorbance value, a microplate reader set to 450 nm was used.

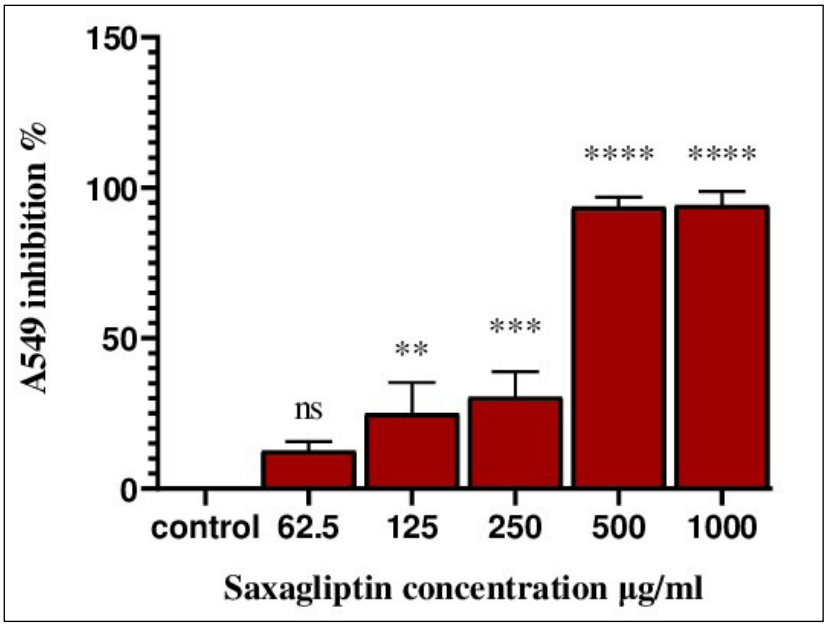

Fig. 3. Cytotoxic effect of different concentrations of saxagliptin against "A549" cell line . For the examination, (one way) Anova was used. Data displayed as mean ± SD, ns=P 0.05, **p<0.01, ***P<0.001, ****p<0.0001, n=4, 72 hours incubation
Source: Own materials

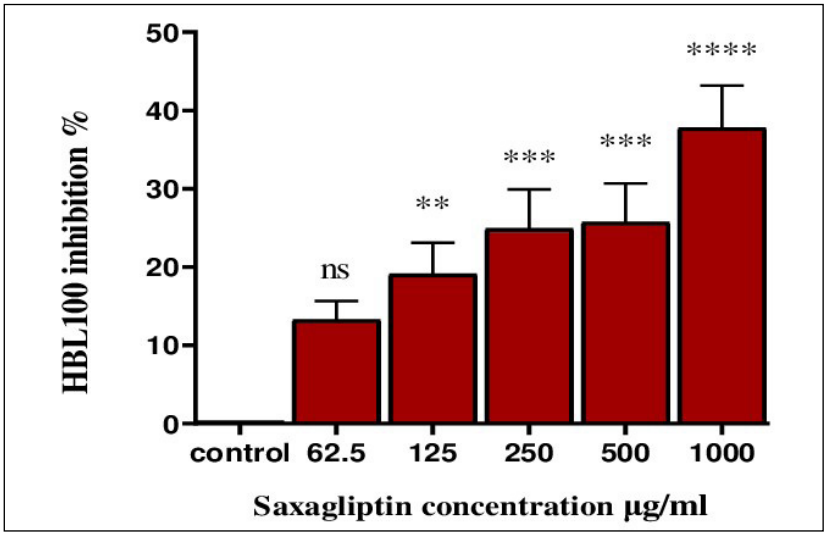

Fig. 4. Cytotoxic effect of different concentrations of saxagliptin against HBL100 cell line. For the examination, (one way) Anova was used. Data displayed as mean ± SD, ns=P>0.05, **P<0.01, ***p 0.001, ****P<0.0001, n= 4, 72 hours incubation
Source: Own materials

STATISTICAL ANALYSIS

The results were gathered and analyzed utilizing GraphPad Prism Edition10 and Microsoft Office Excel2019. Significant variations between the data means were evaluated using a post hoc (Tukey) analysis and one-way ANOVA test. A p-value of 0.05 or lower signifies a statistically significant variance.

RESULTS

CISPLATIN CYTOTOXICITY AGAINST A549 AND HBL100 CELL LINE

Cisplatin showed no significant cytotoxicity in concentration 0.9 µg/mL for both A549 and HBL100 cell lines. At 1.87 µg/mL, CP revealed significant cytotoxic activity in A549 cell line (p= 0.0003) while in HBL100 there is no significant cytotoxicity. Other concentrations (3.75, 7.5, 15 µg/mL) displayed a significant inhibition in A549 and HBL100 cell lines viability (P < 0.0001) in comparison to control group, Figures (1-2).

SAXAGLIPTIN CYTOTOXIC EFFECT AGAINST A549 AND HBL100 CELL LINES

SAXA demonstrated dose-dependent cytotoxicity against A549 lung cancer cells and HBL100 normal cell lines. SAXA demonstrated no notable cytotoxicity at 62.5 µg/mL in both A549 (lung cancer) and HBL100 (non-tumorigenic breast) cell lines; nevertheless, significant decrease of cell viability commenced at 125 µg/mL (p<0.01). SAXA had notable cytotoxic effects on A549 and HBL100 cell lines at a dose of 250 µg/mL (P>0.001). In addition, SAXA at a dose of 500 µg/mL significantly inhibited cell viability in the A549 and HBL100 cell lines, with p-values of < 0.0001 and <0.001,

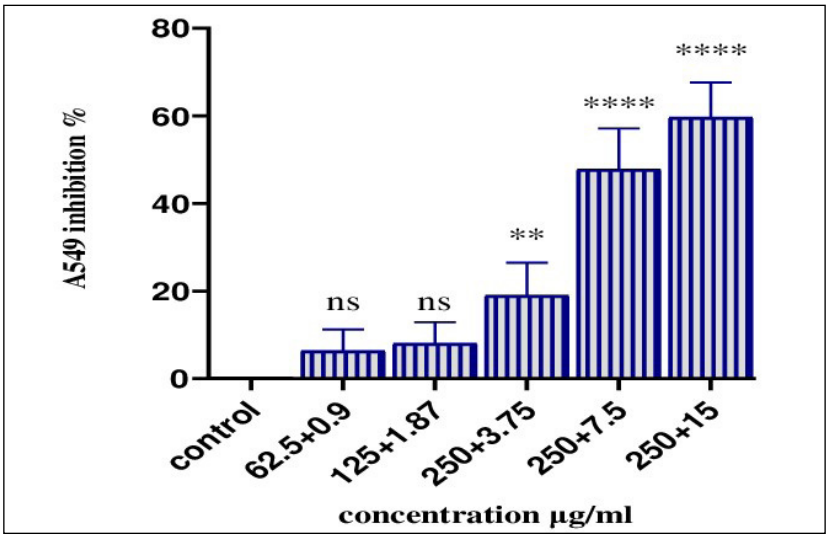

Fig. 5. Cytotoxicity Of Different Concentrations Of saxagliptin plus cisplatin against "A549" Cell Line. For the examination, (one-way) Anova was used. Data displayed as mean ± SD, ns=P>0.05, **p<0.01, *** P<0.0001, n= 4, 72-hours incubation
Source: Own materials

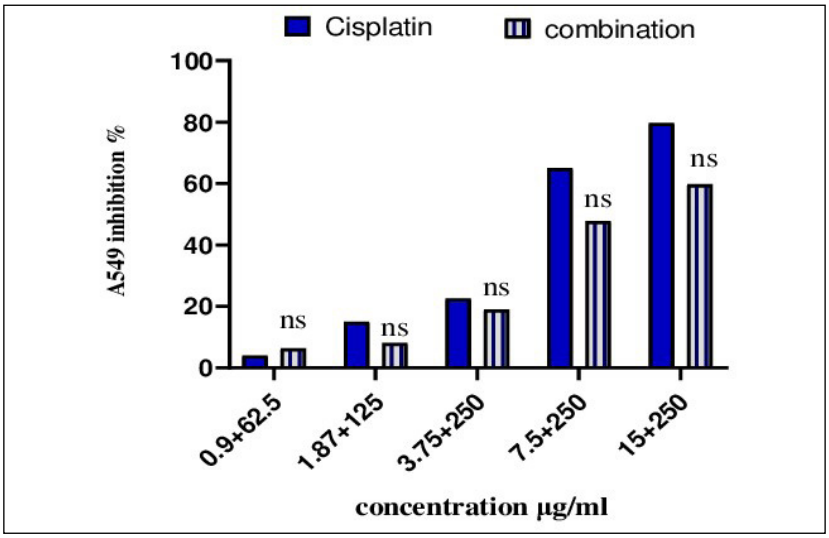

Fig. 6. Comparison between cytotoxicity of cisplatin alone against cisplatin plus saxagliptin Combination on "A549" Cell line. For the examination, (one way) Anova was used. Data displayed as mean ± SD, ns=P>0.05, n=4, 72 hours incubation
Source: Own materials

respectively. Ultimately, both cell lines exhibited comparable responses at the maximum dose (1000 µg/mL, p<0.0001), Figures (3-4).

CYTOTOXIC ACTIVITY OF A COMBINATION OF CISPLATIN PLUS SAXAGLIPTIN ON A549 CELL LINE

The combination of CP and SAXA demonstrated a dose-proportional cytotoxic effect on A549 lung adenocarcinoma cells, with significant anti-cancer activity observed at higher concentrations. At the lowest tested doses (0.9 µg/mL CP + 62.5 µg/mL SAXA and 1.87 µg/mL CP + 125 µg/mL SAXA), no significant cytotoxicity was detected. However, at 3.75 µg/mL CP + 250 µg/mL SAXA, a statistically significant cytotoxic effect was observed (P < 0.01). Most notably, at the highest tested concentrations (7.5 µg/mL CP + 250 µg/mL SAXA and 15 µg/mL CP + 250 µg/mL SAXA), the combination induced highly significant cytotoxicity (P < 0.0001) compared to the control group, Figure (5).

COMPARISON BETWEEN THE CYTOTOXICITY OF CISPLATIN ALONE AGAINST CISPLATIN PLUS SITAGLIPTIN COMBINATION ON THE A549 CELL LINE

Figure (6) demonstrates a comparison between the cytotoxicity of CP alone versus SAXA plus CP combination against A549 cells at various concentrations. Combination between SAXA and CP did not significantly increase the cytotoxic effect against A549 (P>0.05), in comparison with CP alone.

MEASUREMENT OF HUMAN BCL2 LEVELS

CISPLATIN AND SAXAGLIPTIN EFFECT ON BCL2 LEVEL

The results of the investigation showed that after treating A549 cells with IC50 of CP and SAXA, the level of BCL2 decreased with P value (<0.001) for CP and (<0.01) for SAXA compared with control group, Figure (7).

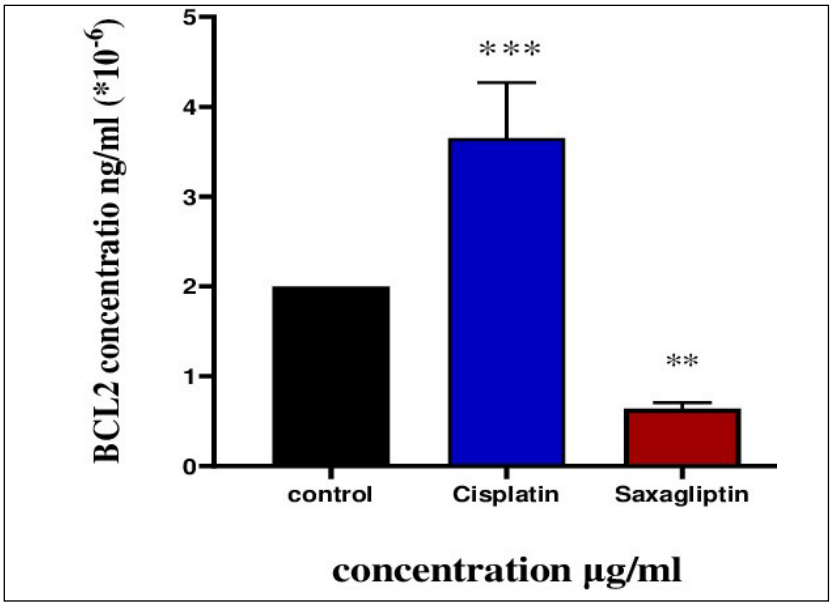

Fig. 7. Cisplatin and saxagliptin activity on BCL2 concentration in "A549" cell line for the examination, one way Anova was used. Data displayed as mean ± SD, **p<0.01, ***P<0.001 Compared with Control
Source: Own materials

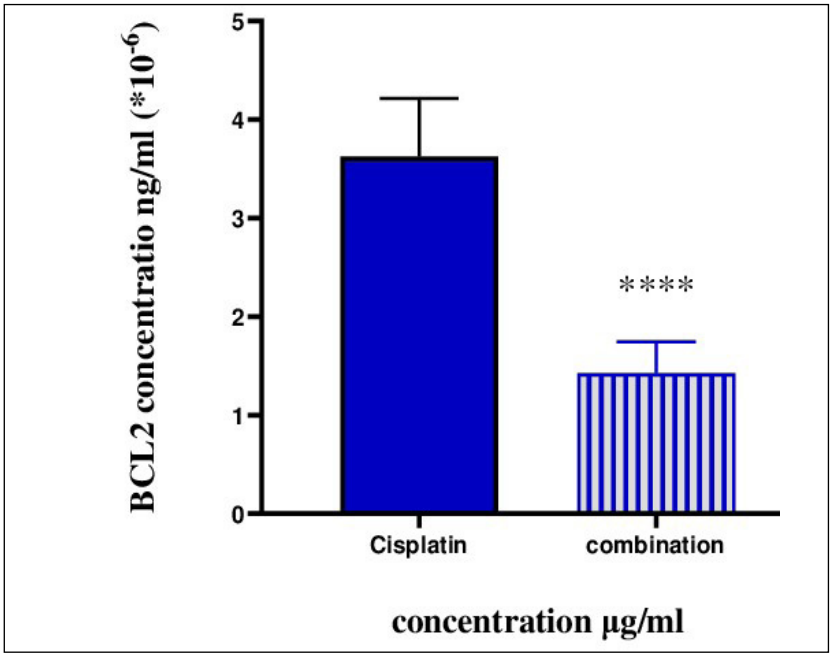

Fig. 8. Comparison between the activity of cisplatin alone and cisplatin plus saxagliptin on BCL2 concentration in "A549" Cell Line. For the examination, (one way) Anova was used. Data displayed as mean ± SD, ****P<0.0001 Compared to Cisplatin
Source: Own materials

COMPARISON BETWEEN THE EFFECTS OF CISPLATIN ALONE AGAINST CISPLATIN PLUS SAXAGLIPTIN COMBINATIONS ON THE BCL2 LEVEL

The results of the study showed that after treating A549 cells with IC50 of the combination CP plus SAXA, the level of BCL2 decreased p<0.0001 in comparison to the CP alone, Figure (8).

CISPLATIN AND SAXAGLIPTIN EFFECT ON BCL2 LEVEL

The results of the investigation showed that after treating A549 cells with IC50 of CP, there is no significant increase in BAX level (P > 0.05) in comparison to the control group. At the same time, the findings showed there is a Significant increase In BAX level P<0.0001 after exposing A 549 to the Ic50 Of SAXA, Compared with Control group, Figure (9).

COMPARISON BETWEEN THE EFFECTS OF CISPLATIN ALONE AGAINST CISPLATIN PLUS SAXAGLIPTIN COMBINATIONS ON THE BAX LEVEL

The results of the study revealed that after treating A549 cells with IC50 of the combination CP plus SAXA, there is no significant increase in BAX level P>0.05 in comparison to the CP alone, Figure (10).

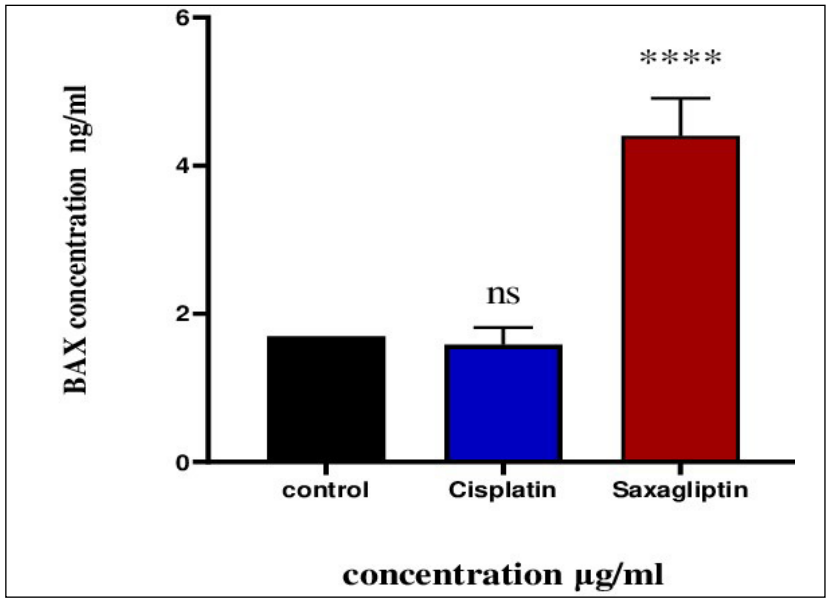

Fig. 9. Cisplatin and saxagliptin "activity" On BAX concentration in "A549" Cell Line. For the examination, (one way) Anova was used. Data displayed as mean ± SD, ****P<0.0001, ns (P>0.05) Compared with control
Source: Own materials

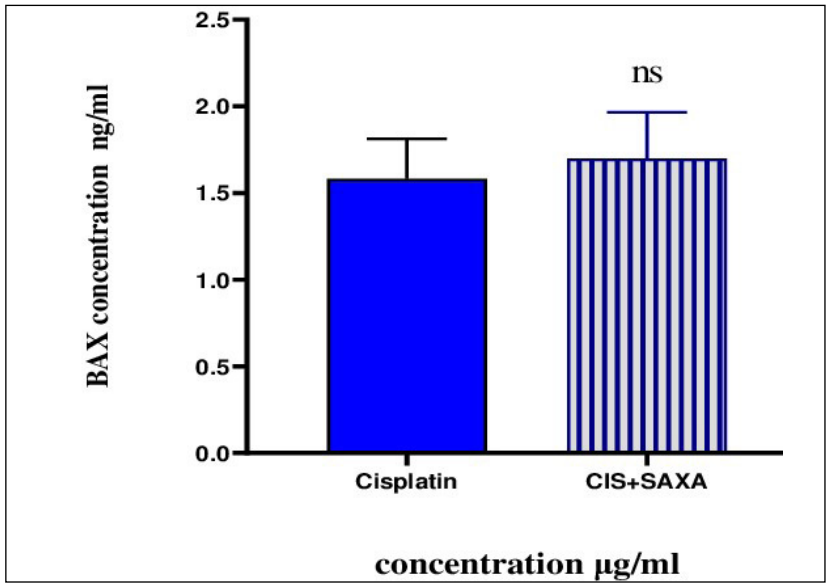

Fig. 10. Comparison between the activity of cisplatin alone and cisplatin plus saxagliptin on BAX Concentration In "A549" Cell Line for the examination, (one way). Anova was used. Data Displayed as mean ± SD, ns (P>0.05) Compared with Cisplatin
Source: Own materials

DISCUSSION

The primary obstacles in the treatment of cancer are drug side effects and treatment resistance, which cause Over 90% of deaths occur in patients receiving chemotherapy [21]. The current research sought to evaluate the cancer suppression activity of SAXA on Lung Cancer cells (A549), alone and combined with CP and comparison with HBL100 cells. This was achieved by employing the MTT assay to evaluate the cancer cells' toxicity and vitality. The anticancer properties of CP have already received approval, and this Chemotherapy Is commonly utilized in the treatment of pulmonary carcinoma. CP's main biological target is DNA [22]. As part of its cytotoxic effect on tumor cells, CP disrupts DNA synthesis and repair processes in cancer cells. It creates 1–2 and 1–3 intra-strand adducts and inter-strand cross-links between purine bases by forming covalent bonds at the N7 position of adenine and guanine. DNA adduct halts the cell cycle at the G2 phase and trigger apoptosis by inhibiting DNA replication [23]. Our study found that SAXA, a drug typically used to treat diabetes, shows promising anti-cancer effects against A549 lung cancer cells in the lab. This discovery adds to a growing body of research suggesting that DPP-4 inhibitors—commonly prescribed for type 2 diabetes - might also play a role in fighting cancer. A prior study by [24] indicates that the Expression of DPP 4 enzyme was significantly elevated in pulmonary carcinoma when compared with normal lung tissue. Therefore, it seems That DPP 4 Inhibitors may be able to impede the growth of lung cancer by blocking the related DPP4 enzyme. The current study findings indicate that SAXA exhibit significant

anti-cancer activity against A549 cells in comparison to the control group, as assessed by MTT assay. These findings agree with that stated by [25], the MTT assay was utilized to assess the anti-neoplastic properties Of DPP 4 Inhibitors SAXA and sitagliptin (SITA) on human ovary (A2780), human breast (MCF-7), and human prostate (PC-3 and LNCaP) cancer cell lines. The results indicated that both drugs exhibited significant beneficial effects on cancer in comparison with Control, which functioned as a Cytotoxic chemical to cancerous cells. Furthermore, retrospective studies indicated that sitagliptin may decrease the risk of breast cancer in type 2 diabetic patients after one year of use, and it may also reduce the risk of prostate and oral cancer. The effects of sitagliptin may be influenced by the dosage and duration of your treatment [26]. Additionally, a study published in 2024 showed that DPP-4 suppression has the potential to augment anti-cancer immune responses through the enhance functioning of cDC1s (type 1 conventional dendritic cells). For instance, sitagliptin improved cDC1 antigen presentation, which Aided T cell activation and the consequent suppression of tumors [27]. Given the safety profile of DPP-4 inhibitors in diabetic patients, their repurposing for cancer therapy could offer a low-toxicity adjunct treatment option.

EFFECT OF COMBINATION THERAPY ON MTT ASSAY

Using combination of CP plus SAXA demonstrated a concentration-dependent cytotoxic effect on A549 lung cancer cell line, with significant anti-cancer activity seen at higher concentrations. At the lowest tested concentrations (0.9 µg/mL CP + 62.5 µg/mL SAXA and 1.87 µg/mL CP + 125 µg/mL SAXA), no significant cytotoxicity was observed, suggesting that a threshold concentration must be reached for the drugs to exert a measurable anti-cancer effect. However, at 3.75 µg/mL CP+ 250 µg/mL SAXA, a statistically significant cytotoxic effect was observed ($P<0.05$). Most notably, at the highest tested concentrations (7.5 µg/mL CP + 250 µg/mL SAXA and 15 µg/mL CP + 250 µg/mL SAXA), the combination induced highly significant cytotoxicity ($P < 0.0001$) compared to the control group.

EFFECT OF COMBINATION THERAPY VERSUS CISPLATIN ALONE ON MTT ASSAY

The observed lack of a significant increase in the cytotoxic effect of CP when combined with SAXA against A549 cells suggests a potential no synergism between the two drugs. This finding aligns with prior research indicating that dipeptidyl peptidase-4 (DPP-4) inhibitors, such as sitagliptin, may interfere with the anticancer activity of chemotherapeutic agents [28] demonstrated that sitagliptin, when used alone, modulated proteins associated with metastasis and apoptosis in SKOV-3 ovarian cancer cells. However, in combination with paclitaxel, sitagliptin reduced the cytotoxic efficacy of the chemotherapy drug at certain concentrations. No synergism could be attributed to the complex biological roles of DPP-4, which is involved not only in glucose metabolism but also in immune regulation, cell adhesion, and apoptosis [29-30]. DPP-4 inhibitors may interfere with these pathways, potentially counteracting the pro-apoptotic or cytotoxic effects of chemotherapy agents like CP or paclitaxel. One possible explanation for the no synergism effect is that DPP-4 inhibition may alter intracellular signaling pathways that are critical for CP-induced apoptosis. For instance, DPP-4 has been implicated in the regulation of NF-κB, PI3K/Akt, and MAPK pathways, which are also targeted by CP [31], If SAXA modulates these pathways in a way that promotes cell survival or reduces drug uptake, it could diminish CP's efficacy. Additionally, SAXA might influence the tumor microenvironment by affecting immune cell activity or cytokine production, indirectly reducing CP's cytotoxic impact. Further mechanistic studies are needed to elucidate the exact molecular interactions between SAXA and CP. Evaluating changes in key apoptotic markers (e.g., caspase activation, Bcl-2 family proteins) or drug transport mechanisms (e.g., copper transporter CTR1, which is involved in CP uptake) could provide insights into the observed no synergism. Additionally, exploring different dosing regimens or sequences of administration might help determine whether the interaction is schedule dependent. Based on that, the combination of SAXA and CP does not enhance cytotoxicity in A549 cells and may exhibit antagonism, consistent with previous findings on DPP-4 inhibitors and chemotherapeutic agents. These results highlight the need for careful evaluation when combining antidiabetic drugs like SAXA with anticancer therapies, as their interaction may inadvertently reduce treatment efficacy. Future studies should investigate whether this effect is cell line-specific or a broader phenomenon affecting other cancer types.

EFFECT ON BCL2

This study revealed that A549 cells were somewhat resistant to CP treatment. This was explained by the fact that A459 cells treated with CP had higher levels of BCL2 than the control group. These results are similar to study that displayed the development of CP resistance in a variety of malignancies was facilitated by Bcl-2. Such as in non-small cell lung cancer (NSCLC), THE

cytoplasmic repressor/activator protein-1-mediated CP resistance was linked to an increase in Bcl-2 level [32]. These results are supported by a study displayed CP resistance by A549 cells which are outlined by the increasing BCL2 level in comparison to control group in A549 cells exposed to CP's IC50 [7]. Concerning the effect of SAXA on BCL2 level, our findings indicate that SAXA exerts varying effects on BCL2 expression based upon the cell type. SAXA treatment in A549 cells results in a notable reduction in the anti-apoptotic protein BCL2. The reduction in BCL2 level indicates the pro apoptotic effect of SAXA that had selectivity toward cancer cells. In parallel with previous findings, LINA inhibits colorectal cancer cell growth by facilitating cell apoptosis via cell cycle arrest and the inhibition of BCL-2 expression [33]. However, when A549 cells were treated with CP plus SAXA combination, the BCL2 level decreased dramatically compared with those treated with CP's IC50 alone. This result potentiating CP cytotoxicity on cancer cells and exhibited synergistic effect on A549 cells. These results are supported by a study that demonstrated reduced BCL2 level in HepG2 liver cancer cell line when exposed to sitagliptin plus CP in comparison to control and CP alone [14].

EFFECT ON BAX

This study showed that A549 cells were somewhat resistant to CP treatment. This was explained by the fact that A459 cells treated with CP had no change than the control group. This aligns with previous research by [34], who observed that CP alone did not induce a noticeable increase in BAX (a pro-apoptotic protein) levels, suggesting a mechanism behind CP resistance in tumor cells. Resistance to CP is a well-documented challenge in cancer therapy, often attributed to defective apoptotic signaling, enhanced DNA repair mechanisms, or increased drug efflux [35]. Concerning the impact of SAXA on A549 cells, our results show that SAXA has a pro-apoptotic effect on A549 cells, as demonstrated by the substantial rise in BAX levels after treatment with its IC50. This indicates that SAXA may facilitate programmed cell death in this lung adenocarcinoma cell line. Remarkably, other cancer types have also been shown to have the pro-apoptotic effect of DPP4 inhibition, which is SAXA's target. [36] a study on Papillary thyroid carcinoma (PTC) cells, revealing that DPP4 silencing resulted in decreased levels of the anti-apoptotic marker BCl-2 and diminished phosphorylation of ERK1/2, JNK1, and P38 MAPK pathways. Concurrently, there was an increase in the expression of E-cadherin and the pro-apoptotic protein BAX. Additionally, our study showed that there was no discernible change in BAX levels as compared to the control when A549 cell line were treated with IC50 of the combination of SAXA and CP. This observation is consistent with the results of the MTT assay, which demonstrated that the two medications did not exhibit any synergistic cytotoxic effects. Notably, no recent studies addressing this specific interaction on BAX levels in this cell type were found, making this study an original contribution to our understanding of the combined impact of SAXA and CP on BAX expression in A549 cell lines. To conclude, SAXA, a DPP-4 inhibitor, demonstrated anti-cancer effects against A549 cells in the MTT assay, indicating its potential as a therapeutic agent. However, when combined with CP, no synergism effect was observed, reducing the expected cytotoxic impact. This suggests that SAXA may interfere with CP's mechanism of action, possibly by modulating survival pathways or altering drug uptake. Despite the no synergism, SAXA has a pro-apoptotic effect on A549 cells, as demonstrated by the substantial rise in BAX levels and reduction in BCL2 level, SAXA plus CP combination exhibited pro-apoptotic activity, as indicated by reduced BCL2 levels (an anti-apoptotic protein).

FUTURE DIRECTIONS

Further research is needed to:

- Elucidate the exact molecular mechanisms (e.g., immune modulation, apoptosis induction, or metabolic effects).
- Validate these findings in vivo using animal models.
- Assess the efficacy of SAXA and other DPP-4 inhibitors in lung cancer patients, particularly in combination with standard therapies.
- Assess the alternative combinations (If sequential, rather than concurrent, administration improve efficacy?

REFERENCES

1. Debela DT, Muzazu SG, Heraro KD, Ndalama MT, Mesele BW, Haile DC, et al. New approaches and procedures for cancer treatment: current perspectives. SAGE Open Med. 2021 Aug 12;9:20503121211034366. doi: 10.1177/20503121211034366. DOI
2. World Health Organization. Lung cancer – fact sheet. WHO; 26 June 2023 https://www.who.int/news-room/fact-sheets/detail/lung-cancer (Access: January 2025).
3. Popper HH. Progression and metastasis of lung cancer. Cancer Metastasis Rev. 2016;35(1):75–91. doi:10.1007/s10555-016-9618-0. DOI

4. Thandra CK, Barsouk A, Saginala K, Aluru SJ, Barsouk A. Epidemiology of lung cancer. Contemp Oncol (Pozn). 2021;25(1):45-52. doi:10.5114/wo.2021.103829 DOI
5. Tamura T, Kurishima K, Nakazawa K, Kagohashi K, Ishikawa H, Satoh H, et al. Specific organ metastases and survival in metastatic non-small-cell lung cancer. Mol Clin Oncol. 2015 Jan;3(1):217-221. doi:10.3892/mco.2014.410. DOI
6. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021 May;71(3):209-249. doi:10.3322/caac.21660. DOI
7. Al Khafaji AM, Bairam AF. Anticancer and antioxidant effects of sitagliptin and linagliptin against lung cancer cell lines: an in vitro study. Trop J Nat Prod Res. 2024;8(5): 7042-7047. doi:10.26538/tjnpr/v8i5.14. DOI
8. Du L, Morgensztern D. Chemotherapy for advanced-stage non–small cell lung cancer. Cancer J. 2015 Sep;21(5):366-370. doi:10.1097/PPO.0000000000000141. DOI
9. Fennell DA, Summers Y, Cadranel J, Benepal T, Christoph DC, Lal R, et al. Cisplatin in the modern era: the backbone of first-line chemotherapy for non-small cell lung cancer. Cancer Treat Rev. 2016 Mar;44: 42-50. doi:10.1016/j.ctrv.2016.01.003. DOI
10. Aldossary SA. Review on pharmacology of cisplatin: clinical use, toxicity and mechanism of resistance of cisplatin. Biomed Pharmacol J. 2019;12(1):7-15. doi:10.13005/bpj/1608. DOI
11. Yu Z, Cao W, Ren Y, Zhang Q, Liu J. ATPase copper transporter A, negatively regulated by miR-148a-3p, contributes to cisplatin resistance in breast cancer cells. Clin Transl Med. 2020 Mar;10(1):57-73. doi:10.1002/ctm2.19. DOI
12. Tchounwou PB, Dasari S, Noubissi FK, Ray P, Kumar S. Advances in our understanding of the molecular mechanisms of action of cisplatin in cancer therapy. J Exp Pharmacol. 2021;13:303-328. doi:10.2147/JEP.S267383. DOI
13. Lugones Y, Loren P, Salazar LA. Cisplatin resistance: genetic and epigenetic factors involved. Biomolecules. 2022 Sep 24;12(10):1365. doi:10.3390/biom12101365. DOI
14. Alameen R, Bairam A, Al-Haddad M. Antioxidant and apoptotic activities of sitagliptin against hepatocellular carcinoma: an in vitro study. F1000Res. 2023;12:962. doi:10.12688/f1000research.139277.1. DOI
15. Richter B, Bandeira-Echtler E, Bergerhoff K, Lerch CL. Do dipeptidyl peptidase-4 (DPP-4) inhibitors improve outcomes in people with type 2 diabetes mellitus? Cochrane Clin Answers. 2013 Jan 2. doi: 10.1002/cca.55. DOI
16. Giard DJ, Aaronson SA, Todaro GJ, Arnstein P, et al. In vitro cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. J Natl Cancer Inst. 1973 Nov;51(5):1417-1423. doi:10.1093/jnci/51.5.1417. DOI
17. de Fromentel CC, Nardeux PC, Soussi T, Lavialle C, et al. Epithelial HBL-100 cell line derived from milk of an apparently healthy woman harbours SV40 genetic information. Exp Cell Res. 1985 Sep; 160(1): 83–94. doi:10.1016/0014-4827(85)90238-1. DOI
18. van Meerloo J, Kaspers GJL, Cloos J. Cell sensitivity assays: the MTT assay. In: Cree I, editor. Cancer Cell Culture. 1st edn. Totowa: Humana Press; 2011:237-245. doi: 10.1007/978-1-61779-080-5_20. DOI
19. Antiproliferative potential of ethanol leaf extract of Motandra guineensis (Thonn.) A.DC. (Apocynaceae) against human melanoma and ovarian cancer cells. Trop J Nat Prod Res. 2024 Mar 30;8(3). doi:10.26538/tjnpr/v8i3.33. DOI
20. He Y, Zhu Q, Chen M, Huang Q, et al. The changing 50% inhibitory concentration (IC50) of cisplatin: a pilot study on the artifacts of the MTT assay and the precise measurement of density-dependent chemoresistance in ovarian cancer. Oncotarget. 2016 Sep 23;7(43):70803-70821. doi:10.18632/oncotarget.12223. DOI
21. Bukowski K, Kciuk M, Kontek R. Mechanisms of multidrug resistance in cancer chemotherapy. Int J Mol Sci. 2020 May 2;21(9):3233. doi: 10.3390/ijms21093233. DOI
22. Jung Y, Lippard SJ. Direct cellular responses to platinum-induced DNA damage. Chem Rev. 2007 Apr 25;107(5):1387-1407. doi: 10.1021/cr068207j. DOI
23. Ranasinghe R, Mathai ML, Zulli A. Cisplatin for cancer therapy and overcoming chemoresistance. Heliyon. 2022 Sep;8(9):e10608. doi: 10.1016/j.heliyon.2022.e10608. DOI
24. Wu CY, Ghule SS, Liaw CC, Achudhan D, Fang SY, Liu PI, et al. Ugonin P inhibits lung cancer motility by suppressing DPP-4 expression via promoting the synthesis of miR-130b-5p. Biomed Pharmacother. 2023 Nov;167:115483. doi: 10.1016/j.biopha.2023.115483. DOI
25. Yeğin D, Ulukaya E. Investigation of cytotoxic and apoptotic effects of verapamil in combination with docetaxel, gemcitabine, and carboplatin in human breast cancer cell lines. Uludag Univ Med J. 2025 Jan 12;50(3):509-518. doi:10.32708/uutfd.1549517. DOI
26. Dhas Y, Biswas N, Dhanaraj MR, Jones LD, Ashili S. Repurposing metabolic regulators: antidiabetic drugs as anticancer agents. Mol Biomed. 2024 Sep 28;5(1). doi: 10.1186/s43556-024-00204-z. DOI
27. Ng II, Zhang J, Tian T, Peng Q, Huang Z, Xiao K, et al. Network-based screening identifies sitagliptin as an antitumor drug targeting dendritic cells. J Immunother Cancer. 2024 Mar;12(3):e008254. doi: 10.1136/jitc-2023-008254. DOI
28. Kosowska A, Garczorz W, Kłych-Ratuszny A, Aghdam MRF, et al. Sitagliptin modulates the response of ovarian cancer cells to chemotherapeutic agents. Int J Mol Sci. 2020 Nov 26;21(23):8976. doi: 10.3390/ijms21238976. DOI
29. Zhang J, Chen Q, Zhong J, Liu C, Zheng B, Gong Q. DPP-4 inhibitors as potential candidates for antihypertensive therapy: improving vascular inflammation and assisting the action of traditional antihypertensive drugs. Front Immunol. 2019 May 9;10:1050. doi: 10.3389/fimmu.2019.01050. DOI

30. Zhang T, Tong X, Zhang S, Wang D, Wang L, Wang Q, et al. The roles of dipeptidyl peptidase 4 (DPP4) and DPP4 inhibitors in different lung diseases: new evidence. Front Pharmacol. 2021 Dec 9;12:731453. doi: 10.3389/fphar.2021.731453. DOI
31. Wang Y, Jin M, Cheng CK, Li Q. Tubular injury in diabetic kidney disease: molecular mechanisms and potential therapeutic perspectives. Front Endocrinol (Lausanne). 2023 Aug 2; 14: 1238927. doi: 10.3389/fendo.2023.1238927. DOI
32. Zheng Z, You H, Feng Y, Zhang Z. LncRNA KCNQ1OT1 is a key factor in the reversal effect of curcumin on cisplatin resistance in the colorectal cancer cells. Mol Cell Biochem. 2020 Aug 5;476(7):2575-2585. doi: 10.1007/s11010-020-03856-x. DOI
33. Mani RJ, Anand M, Agarwal K, Tiwari A, Hashmi QAR, Singh TV, et al. A systematic review of molecular pathway analysis of drugs for potential use in liver cancer treatment. Drugs Drug Candidates. 2023 Apr 3;2(2):210-231. doi: 10.3390/ddc2020013. DOI
34. Zhao Z, Wu Q, Xu Y, Qin Y, Pan R, Meng Q, et al. Groenlandicine enhances cisplatin sensitivity in cisplatin-resistant osteosarcoma cells through the BAX/Bcl-2/Caspase-9/Caspase-3 pathway. J Bone Oncol. 2024 Oct;48:100631. doi: 10.1016/j.jbo.2024.100631. DOI
35. Dasari S, Tchounwou PB. Cisplatin in cancer therapy: molecular mechanisms of action. Eur J Pharmacol. 2014 Oct;740:364-378. doi: 10.1016/j.ejphar.2014.07.025. DOI
36. Hu X, Chen S, Xie C, Li Z, Wu Z, You Z. DPP4 gene silencing inhibits proliferation and epithelial-mesenchymal transition of papillary thyroid carcinoma cells through suppression of the MAPK pathway. J Endocrinol Invest. 2021 Jan 2;44(8):1609-1623. doi: 10.1007/s40618-020-01455-7. DOI

CONFLICT OF INTEREST

The Authors declares no conflict of interest

CORRESPONDING AUTHOR

Sama J. Shubbar
Department of Pharmacology and Toxicology,
Faculty of Pharmacy, University of Kufa, Najaf, Iraq
e-mail: samashubbar@gmail.com

ORCID AND CONTRIBUTIONSHIP

Sama J. Shubbar: 0009-0008-6364-191X B C D E
Ahsan F. Bairam: 0000-0002-0832-6502 A F

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

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ORIGINAL ARTICLE

CONTENTS

Possible somatosensory modulation of tinnitus in a patient with temporomandibular joint disorder: An fMRI/TMJ-MRI case report (3-Month Follow-Up)

Vasil Pehnyo[1], Nataliia Savychuk[1], Oleksandr Bida[2], Stanislav Riebienkov[3], Ivan Riabko[4], Roman Sulik[1]
[1]SHUPYK NATIONAL HEALTHCARE UNIVERSITY OF UKRAINE, KYIV, UKRAINE
[2]BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE
[3] NATIONAL CHILDREN'S SPECIALIZED HOSPITAL "OKHMATDYT", KYIV, UKRAINE
[4]TARAS SHEVCHENKO NATIONAL UNIVERSITY OF KYIV, KYIV, UKRAINE

ABSTRACT

Aim: To explore whether changes in mandibular/condylar position are associated with alterations in brain activation in a patient with chronic subjective tinnitus and temporomandibular disorder (TMD) during a 3-month follow-up.

Materials and Methods: Single-patient case report (39-year-old; 2-year tinnitus; diagnosed TMD). TMJ magnetic resonance imaging (TMJ MRI) and functional magnetic resonance imaging(fMRI) of the brain were acquired in centric occlusion and after fabrication of an anterior repositioning appliance. fMRI was processed in FMRIB Software Library (FSL) (FEAT 6.0); Z-images were cluster-corrected with $Z>3.1$ and cluster-wise $p<0.05$ (Gaussian random field theory). Primary metrics: Activation Index (AI; Z-max), cluster size (voxels). Discriminative ability between states was explored with ROC analysis.

Results: Mean (AI) decreased from 5.51 ± 0.67 (occlusion) to 5.05 ± 0.70 (splint), $p<0.05$. A large cluster in the left superior temporal gyrus present in occlusion ($AI=6.54$; 16 799 voxels) disappeared with the splint. Regional reductions were also observed in anterior cingulate and prefrontal/insular cortices, while some right temporal/frontal clusters persisted with lower AI. Receiver Operating Characteristic (ROC) analysis suggested the AI (Z-max) had the highest discriminative ability between states (Area Under the Curve (AUC)≈0.69), whereas voxel-wise uncorrected/false discovery rate(FDR)-corrected p values were weak classifiers.

Conclusions: In this clinical case, mandibular repositioning with an anterior repositioning appliance was associated with decreased cortical hyperactivation in auditory/affective regions and disappearance of a major left temporal cluster. Findings support a plausible somatosensory contribution to tinnitus modulation in TMJ disorder and motivate hypothesis-driven prospective studies. Causality cannot be inferred from a single-case design.

KEY WORDS: tinnitus, temporomandibular joint disorders, fMRI, TMJ MRI, anterior repositioning appliance, somatosensory tinnitus

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INTRODUCTION

Subjective tinnitus is a disorder often associated with increased neural activity in the central auditory system and related areas of the brain [1-8]. In recent decades, functional MRI (fMRI) studies have made it possible to better understand the neurophysiological basis of tinnitus, in particular the mechanisms underlying its reduction or intensification in patients [9], and a strong association between TMD and tinnitus; one meta-analysis reported ~4.45-fold higher odds of tinnitus in individuals with TMD than in those without [10]

In the general population, the prevalence of tinnitus ranges from 15-20% to 44.5%.In patients with Temporomandibular Disorders (TMD), this figure can increase from 25 to 59%, which indicates a possible association between these conditions [10, 11, 14]. Such a high level of prevalence emphasizes the need for a deeper study of both the pathophysiological mechanisms of tinnitus and the associated risk factors, including TMD. At the present stage, numerous studies confirm that the TMD can significantly influence the occurrence and course of tinnitus through somatosensory and neuronal integration [10-12, 15, 16].

Despite the number of scientific studies, the role of the TMD in the development of tinnitus has not yet been sufficiently studied. Consideration of such clinical cases allows not only to better understand the pathophysiology of tinnitus, but also emphasizes the importance of a multidisciplinary approach to the diagnosis and treatment of this disorder. This is especially true in the context of the latest findings, which demonstrate the complex interaction of somatic and neuronal mechanisms in the formation of symptoms [8, 10, 16, 17].

Scientists distinguish the following mechanisms of tinnitus development in patients with TMD: somatosensory, reflex, muscular, anatomical, psychophysiological, central sensitization. Particular attention is paid to the role of central sensitization, which, according to recent studies, can increase the activity of the auditory tracts even in the absence of external stimuli [13, 14, 17]. For example, studies using fMRI have found increased activity in the auditory and sensorimotor areas of the cerebral cortex in patients with tinnitus, suggesting a complex neuronal mechanism of this disorder [15, 18-23].

In our opinion, among the theories presented, the competence of a dentist may include somatosensory, reflex, anatomical, muscular hypotheses.

AIM

To explore whether changes in mandibular/condylar position are associated with alterations in brain activation in a patient with chronic subjective tinnitus and temporomandibular disorder (TMD) during a 3-month follow-up.

MATERIALS AND METHODS

Patient O., born in 1985, applied to the Department of Orthopedic Dentistry, Digital Technologies and Implantology, Faculty of Dentistry of the Shupyk National Health University of Ukraine on October 22, 2024 with complaints of a constant feeling of tension in the jaw and bruxism, as well as a subjective feeling of constant tinnitus, which has been observed over the past 2 years, after collecting complaints and anamnesis.

After filling out the normative consent and familiarizing himself with the examination plan (approved *by the Commission on Ethics and Academic Integrity of the* Shupyk National Health University of Ukraine (protocol No. 13/10), an examination was carried out according to the generally accepted methodology. The treatment tactics were as follows, taking into account the neurological component, the use of an interdisciplinary approach involved specialists in related specialties, including a neurologist and an orthopedist. This strategy allows us to take into account the relationship between somatosensory symptoms and neurophysiological mechanisms of tinnitus [20-22].

A anterior repositioning appliance on the upper jaw was fabricated to achieve the therapeutic reference position(TRP) according to Gelb/Lotzmann principles. TRP was determined by symptom-guided deprogramming with Kois deprogrammer[28], bilateral palpation, and verification of reduced joint loading and muscle tenderness. The appliance was made from hard acrylic dental material; with uniform lingual occlusal contacts and anterior guidance preventing posterior interferences. The patient was instructed to wear the device nighttime only for at least 12 weeks, with check-ups at 1,3,10 and 12 weeks for occlusal equilibration. Adherence and adverse events were recorded.

Additional diagnostic of the state of the TMJ were carried out using MRI of the TMJ and determination of the activity of the cerebral cortex using fMRI of the brain, after the manufacture of a repositioning appliance on the upper jaw.

The results of the audiogram (Fig. 1) indicate normal hearing in the patient. The right ear has hearing thresholds for air conduction (AC) in the range of up to 20 dB at all frequencies, and bone conduction (BC) is also in the range of up to 20 dB, indicating normal air and bone conduction. Similarly, the left ear shows hearing thresholds for AC and BC up to 20 dB at all frequencies. on palpation at the level of C6-C7, percussion of the spinous processes, sharp pain, palpation of paravertebral T5-T6 on the left sharply painful, painful rotation. Clinical diagnosis: facet syndrome T5-T6 on the left, C6-C7 on the left. Tinnitus.

The examination according to the generally accepted method revealed the presence of integral dentition, the CPV index was 23%, the bite was orthognathic, hypermobility of the articular heads on both sides was noted, without signs of scaling and (or) crepitus.

Functional diagnosis of TMJ included magnetic resonance imaging (MRI), which was performed in the position of centric occlusion and open mouth, for a detailed analysis of the biomechanics of the joint[16]. The MRI of the TMJ and fMRI of the cerebral cortex was performed on a Siemens MAGNETOM Aera (1.5 Tesla) device with a 12-channel head coil.

The results of MRI of the TMJ in the position of centric occlusion were found in the right TMJ: Degeneration and partial rupture of the intermediate part of the articular disc, compression of the articular disc. Minimal anterior displacement of the disc, violation of the biomechanics of the joint. Left TMJ: Degeneration and erosion of the intermediate part of the articular disc, compression of the articular disc. Minimum anterior displacement (Fig. 2).

After analyzing the complaints and laboratory research methods carried out, it was decided to determine the curative position for TMJ, according to the theory of Gelb (1960)[18]. Next, a anterior repositioning appliance was made for the upper jaw. The treatment tactics corresponded to modern approaches to the treatment of TMJ dysfunction, based on the stabilization of occlusion and the restoration of joint function.

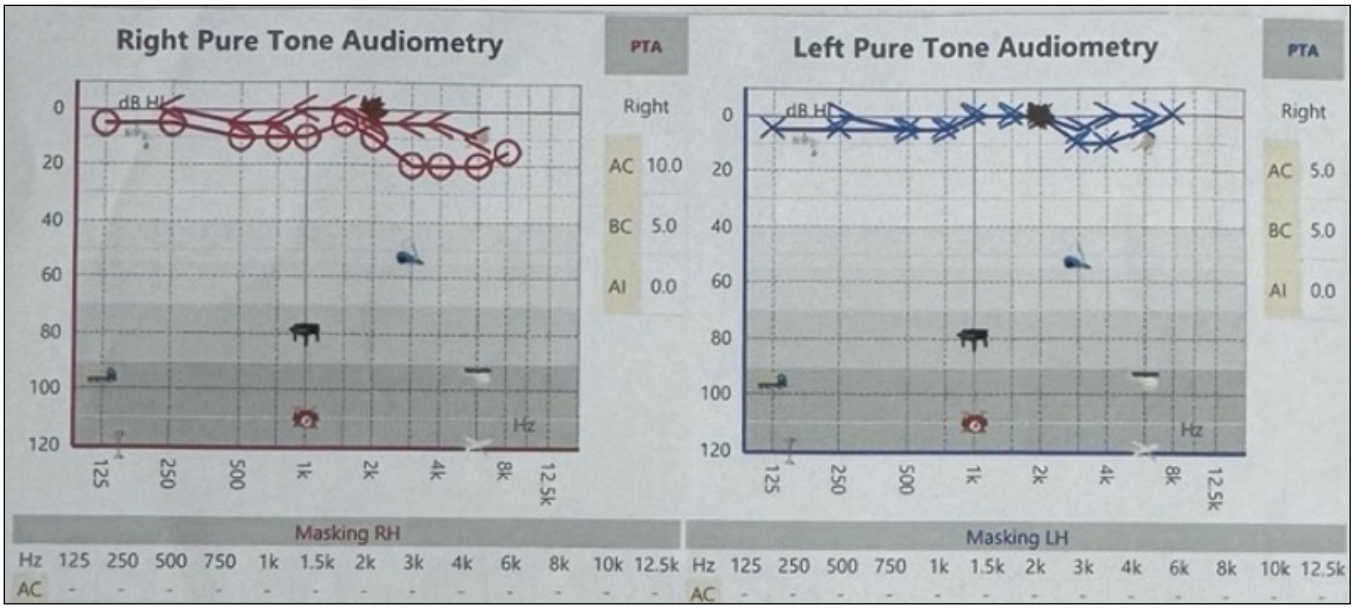

Fig. 1. Results of the audiogram of the examined patient
Picture taken by the authors

In particular, the theories of Gelb (1960) and Lotzmann (1980-1990) were used, which predict reducing the load on the affected joint by optimizing the position of the lower jaw [19].

The results of MRI of the TMJ in the position with the repositioning splint were found in the right TMJ: degeneration and partial rupture of the intermediate part of the articular disc, lack of compression of the articular disc. Reduction of anterior displacement of the disc. Left TMJ: Degeneration and erosion of the intermediate part of the articular disc, lack of compression of the articular disc. Reduction of the minimum anterior displacement (Fig. 3).

RESULTS

Analysis of statistical indicators for the "Occlusion" and "Splint" states revealed the following features: in "Occlusion", the mean Activation index (AI) z-max is 5.51(mean=5.51) with a standard deviation of 0.67(σ=0.67), and the Number of voxels in cluster has an average of 3086 (mean=3086) with a large standard deviation of 3842(σ=3842), which indicates a lot of variability. For the "Splint" position, the mean activation index is lower at 5.05(mean=5.05) with a standard deviation of 0.70(σ=0.70), with the number of voxels in the cluster also varying significantly, with a mean of 3166(mean=3166) and a standard deviation of 7859(σ=7859). These data indicate within-subject differences between the two jaw-position states in this patient's brain activity. The results of the fMRI examination are presented in Table 1 and Table 2.

Following the obtained fMRI statistics, fMRI data processing was carried out using the FEAT (FMRI Expert Analysis Tool) software package version 6.00, which is part of the FSL (FMRIB's Software Library) Statistical images Z (T/F Gaussianized) were threshold using clusters defined by Z>3.1 and the (adjusted) cluster significance threshold P=0.05[26]. This was followed by a statistical analysis for logistic regression (the position of the lower jaw was determined by an independent change, the fMRI indicators were used as a metric both for the usual closure of the teeth and for the position after treatment), and an ROC curve was constructed for the sample of diagnostic-effective indicators of the fMRI study (Fig. 4).

The resulting ROC curves indicated the diagnostic efficacy of five fMRI scores for classification between Splint (control) and Occlusion (pathology) conditions, where each significant cluster was considered as a separate observation. The highest discriminatory ability was shown by the Activation index (AI) Z-MAX) with an AUC ≈ 0.69, which indicates its high information content. Number of voxels in cluster (AUC ≈ 0.60) and Corrected p value, whole cluster -ln(P) (AUC ≈ 0.64) had lower but moderate classification ability. False Discovery Rate-corrected p value of voxel (AUC ≈ 0.37) and Uncorrected p value of voxel (AUC ≈ 0.36) turned out to be statistically weak and practically do not allow to distinguish between groups.

Thus, among the proposed results of fMRI, Activation index, Corrected p value whole cluster -ln(P) and Number of voxels in cluster can be considered as key markers[26, 27].

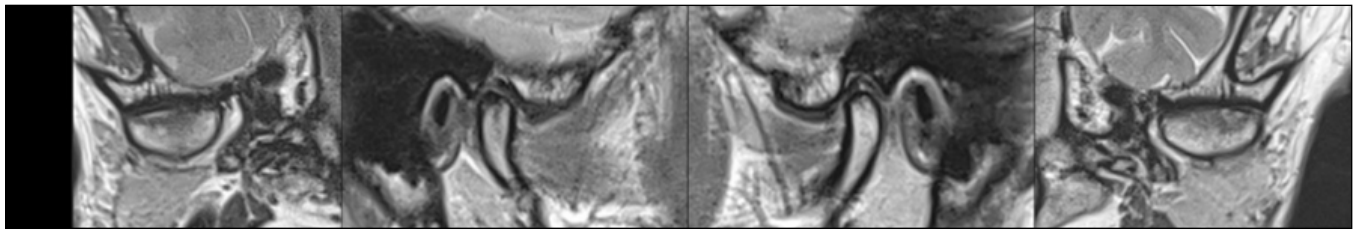

Fig. 2. MRI-TMJ _right and left in the paracoronary and parasagittal planes
Picture taken by the authors

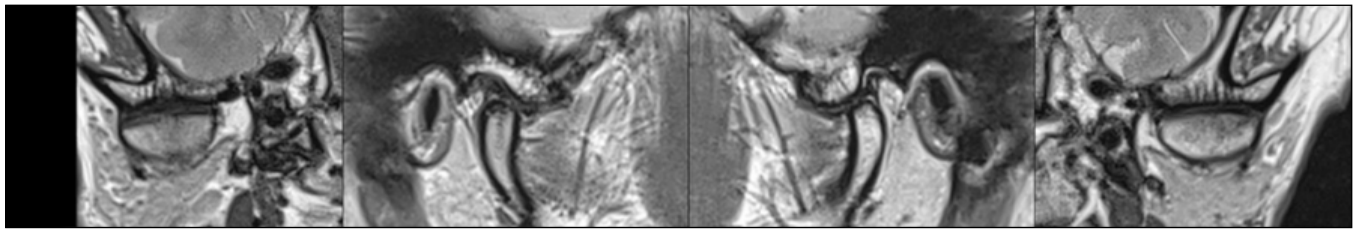

Fig. 3. MRI-TMJ _right and left in the paracoronary and parasagittal planes
Picture taken by the authors

It should be noted that the data obtained were obtained with the cluster-forming threshold Z>3.1; observed Z-max up to 7.5; cluster-wise FWE p<0.05 (GRF).

The assessment of changes in functional activation between the Occlusion and Splint states by *the Activation index (AI) Z-MAX* and *Number of voxels in cluster revealed* significant topographic and quantitative differences in the following anatomical areas:

1. **The Right Superior Temporal Gyrus** is activated in both states with a higher AI Z-max in Occlusion at 1.08 (6.76 vs 5.68), with a slight decrease in the number of voxels by 289 (5344 vs 5633).
2. **The Right Superior Frontal Gyrus** shows an increase in AI Z-max of 1.92 (6.41 vs 4.49) and an increase in the number of voxels by 4433 (5334 vs 901) in the Occlusion state
3. **Left Superior Temporal Gyrus** is only activated in Occlusion with AI Z-max 6.54 and a voxel count of 16799, completely absent in Splint
4. **The Left Anterior Cingulate Cortex** is only seen in Occlusion with AI Z-max 5.62 and 10269 voxels, while Splint lacks this activation.
5. **Right Temporal Pole** only appears in Splint with AI Z-max 4.87 and 1594 voxels, missing from Occlusion
6. **Left Middle Temporal Gyrus** is only activated in Splint with AI Z-max 5.16 and 1315 voxels, not detected in Occlusion
7. **Right Precentral Gyrus** is only present in Splint with AI Z- max 5.11 and 1203 voxels
8. **Left Insula** is observed in Splint with AI Z-max 4.63 and 1132 voxels, inactive in Occlusion
9. **Right Middle Frontal Gyrus** is only active in Splint, has AI Z-max 5.08 and 1062 voxels
10. **Right Paracentral Lobule** only appears in Splint with AI Z-max 4.79 and 843 voxels
11. **Left Superior Frontal Gyrus** is only activated in Splint with AI Z-max 4.52 and 728 voxels.

DISCUSSION

In this single-patient fMRI/TMJ-MRI case with a within-subject comparison of Occlusion versus Splint states, mandibular repositioning was associated with a measurable reduction in cortical activation metrics, including a decrease of the mean Activation Index (AI; Z-max) and the disappearance of a very large Left Superior Temporal Gyrus cluster that was present in occlusion.

These findings are consistent with the broader neuroimaging literature suggesting that tinnitus is not limited to a single "auditory locus" but involves distributed changes across auditory and non-auditory networks, including cortical regions implicated in auditory perception, attention, salience, and affective processing [3–7,20,21]. Contemporary frameworks derived from human neuroimaging emphasize network-level contributions to tinnitus perception and persistence, including maladaptive patterns of local activity and altered connectivity across cortical systems [3,6,20,21]. Our observation that the Left Superior Temporal Gyrus cluster (auditory association territory) was prominent in occlusion and then absent in the splint state aligns with reports that tinnitus can be accompanied by abnormal cortical activity patterns in auditory areas and that these patterns may vary with state-dependent modulation [2–4,7,20,21]. Although causality cannot be inferred, the directionality of change (reduced activity markers

Table 1. Results of fMRI of the brain before treatment (highlighted in color according to ROC analysis)

Anatomical region	Activation index (AI) Z-MAX	Corrected p value. whole cluster -ln(P)	Number of voxels in cluster	False Discovery rate-corrected p-value of voxel	Uncorrected p-value voxel	Talairach Daemon Atlas Coordinates mni		
						x	y	z
Left Cerebrum. Temporal Lobe. Superior Temporal Gyrus. White Matter.	6.54	23.4	16799	0.000	4.15e-24	-60	-48	155
Left Cerebrum. Limbic Lobe. Anterior Cingulate. White Matter.	5.62	16.4	10269	0.000	4.06e-17	-13	35	19
Right Cerebrum. Temporal Lobe. Superior Temporal Gyrus. White Matter.	6.76	10	5344	0.000	9.16e-11	66	-20	4
Right Cerebrum. Frontal Lobe. Superior Frontal Gyrus. White Matter.	6.41	10	5334	0.000	9.47e-11	67	179	91
Left Cerebrum. Temporal Lobe. Fusiform Gyrus. White Matter.	6.35	9.43	4929	0.000	3.74e-10	-53	-8	-31
Right Cerebrum. Temporal Lobe. Inferior Temporal Gyrus. White Matter.	5.54	7.65	3789	0.000	2.23e-08	54	-9	-33
Left Cerebrum. Occipital Lobe Middle. Temporal Gyrus. White Matter.	5.34	7.59	3750	0.000	2.58e-08	-52	-73	19
Right Cerebrum. Frontal Lobe. Medial Frontal Gyrus. White Matter.	5.14	3.8	1689	0.000	0.00016	14	59	-2
Left Cerebrum. Occipital Lobe. Cuneus. Gray Matter. Brodmann area 18.	5.68	3.67	1631	0.001	0.000213	-11	-70	17
Left Brainstem. Midbrain.	5.61	3.39	1501	0.001	0.00041	-11	-20	9
Left Cerebrum. Parietal Lobe. Sub-Gyral. White Matter.	5.92	3.29	1456	0.001	0.000516	-25	-34	59
Right Cerebrum. Frontal Lobe. Sub-Gyral. White Matter.	5.65	3.06	1355	0.002	0.000874	20	11	55
Right Cerebrum. Temporal Lobe. Superior Temporal Gyrus. Gray Matter. Brodmann area 39.	4.35	3.01	1336	0.002	0.000967	57	-61	23
Left Cerebrum. Limbic Lobe. Uncus. White Matter.	5.9	2.78	1237	0.003	0.00165	-31	-1	-25
Left Cerebrum. Frontal Lobe. Medial Frontal Gyrus. Gray Matter. Brodmann area 6.	5.98	2.61	1166	0.004	0.00243	-3	1	57
Left Cerebrum. Frontal Lobe. Sub-Gyral. White Matter.	5.12	2.5	1120	0.004	0.00314	-31	-13	43
Left Cerebrum. Frontal Lobe. Superior Frontal Gyrus. Gray Matter. Brodmann area 6.	5.23	2.49	1114	0.004	0.00325	-16	22	55
Right Cerebrum. Limbic Lobe. Posterior Cingulate. Gray Matter. Brodmann area 30.	4.43	1.91	886	0.015	0.0123	5	-47	16
Right Cerebellum. Posterior Lobe. Pyramis. Gray Matter.	4.94	1.8	845	0.018	0.0158	48	-83	55
Left Brainstem. Pons.	4.6	1.78	835	0.018	0.0168	-10	-31	-34
Left Cerebrum. Parietal Lobe. Precuneus.	5.1	1.7	808	0.021	0.0198	90	56	115
Left Cerebellum. Posterior Lobe. Declive. Gray Matter.	4.91	1.42	707	0.038	0.0376	115	57	52

Note:

Activation index (AI) Z-MAX - a numerical variable that displays the maximum activation index value in a given region of the brain.

Number of voxels in cluster - the number of voxels in the cluster where statistically significant activation is observed. A higher number of voxels may indicate a more significant change in brain activity.

Corrected p value, whole cluster -ln(P) is the logarithm of the negative value of the corrected p-value for the whole cluster. A lower value of p indicates a greater statistical significance of changes in brain activity.

False Discovery rate-corrected p value of voxel - thecorrect value of p for the voxel, adjusted according to the False Discovery Rate (FDR) control method, which reduces the likelihood of false positives.

Uncor-rected P value voxel - p-value calculated for a specific voxel without correction for multiple comparisons.

Talairach Daemon Atlas Coordinates mni – coordinates, according to the parameters of mni and the Tailarach atlas

Source: compiled by the authors of this study

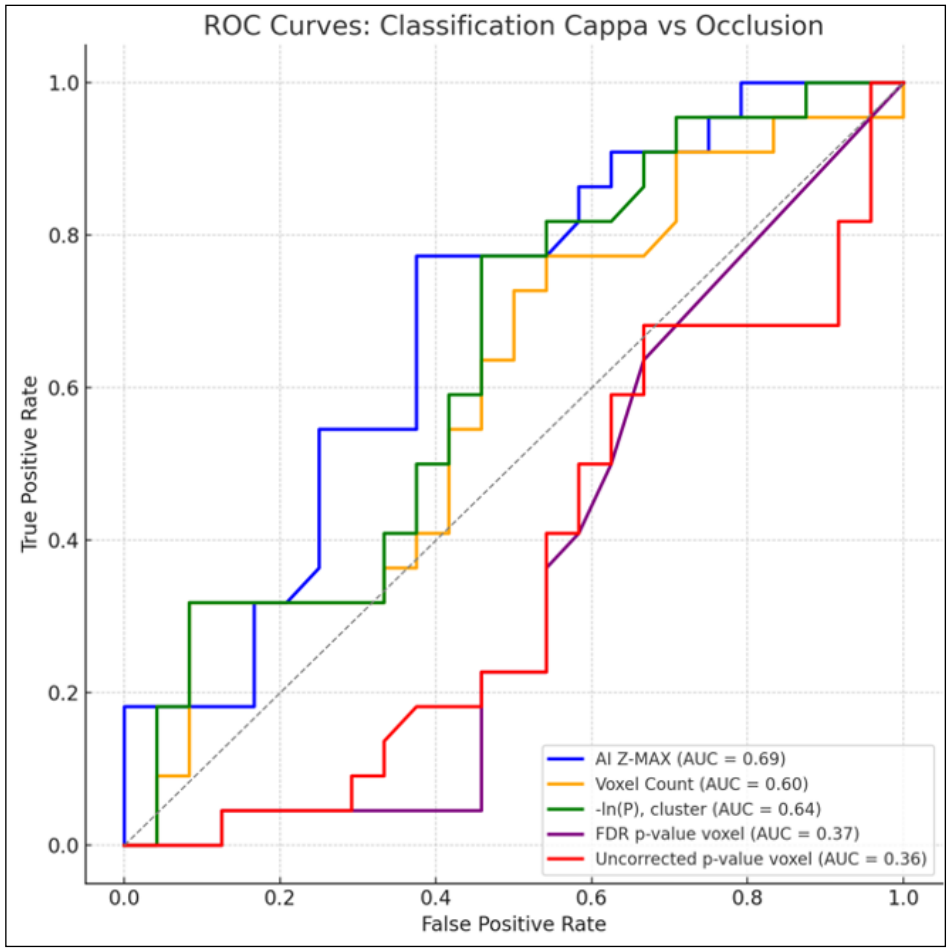

Fig. 4. ROC-Curves fMRI results
Picture taken by the authors

with mandibular repositioning) is compatible with the concept that at least some tinnitus phenotypes may express a modifiable cortical signature under specific physiological or somatosensory conditions [3,6,20,21].

Beyond auditory cortex, the affective and salience-related components of tinnitus are increasingly recognized as key determinants of distress and symptom burden [3,6,22,24]. In our case, Left Anterior Cingulate Cortex activation was present in occlusion and absent in the splint state, and regional reductions were also described in prefrontal/insular territories.

These findings are broadly concordant with the literature linking tinnitus-related distress and chronicity to engagement of limbic–cortical circuits and "aversive memory" or salience networks rather than purely peripheral auditory damage [22,24]. The ACC and frontal systems are often discussed as part of a distributed network supporting attention to internally generated percepts and the affective appraisal of tinnitus [3,20–22,24]. Therefore, attenuation of activity markers in these regions after mandibular repositioning may reflect a shift in the balance between auditory representation and top-down modulatory control, a pattern that is conceptually compatible with network models proposed in neuroimaging reviews and meta-analyses [3,20,21].

The clinical context—temporomandibular disorder with tinnitus—also supports interpreting the observed state-dependence through a somatosensory lens. Systematic reviews and meta-analyses consistently report an association between TMD and tinnitus and emphasize the clinical relevance of multidisciplinary evaluation for patients with orofacial complaints and ear symptoms [10–12,14,18]. In particular, contemporary evidence syntheses describe higher tinnitus prevalence/odds in individuals with TMD and underscore that somatosensory factors can influence tinnitus severity and treatment response [10–12,14,18]. Interventional literature similarly supports the plausibility of somatosensory tinnitus modulation: an RCT evaluating orofacial treatment within a multidisciplinary program and subsequent mechanistic work suggest that improving TMD status can mediate reductions in somatic tinnitus severity [8,9]. A broader systematic review of physical therapy interventions in subjective tinnitus further supports that targeting somatic contributors may be beneficial in selected patients [16], and recent controlled trials of manual therapy report effects on somatosensory tinnitus/dizziness outcomes [15]. Against this background, our within-subject fMRI

Table 2. Results of fMRI of the brain after treatment (highlighted in color according to ROC analysis)

Anatomical region	Activation index (AI) Z-MAX	Correced p-value, whole cluster -ln(P)	Number of voxels in cluster	False Discovery rate-corected p-value of voxel	Uncorreced p-value voxel	Talairach Daemon Atlas Coordinates mni		
						x	y	z
Left Cerebrum. Temporal Lobe. Superior Temporal Gyrus. Gray Matter Brodmann area 42.	5.86	40.5	39682	0.000	3.09e-41	-64	-26	17
Right Cerebrum. Temporal Lobe. Superior Temporal Gyrus. White Matter.	5.68	9.88	5633	0.000	1.33e-10	65	-36	11
Right Cerebrum. Frontal Lobe. Medial Frontal Gyrus. Gray Matter Brodmann area 6.	6.23	6.22	3171	0.000	5.96e-07	84	128	132
Right Cerebrum. Frontal Lobe. Superior Frontal Gyrus. Gray Matter Brodmann area 10.	4.81	6.02	3037	0.000	9.54e-07	28	58	18
Left Cerebrum. Frontal Lobe. Sub-Gyral. White Matter.	5.05	4.93	2411	0.000	1.18e-05	-28	40	-3
Left Cerebrum. Sub-lobar. Extra-Nuclear. White Matter.	5.48	4.55	2204	0.000	2.82e-05	-17	3	22
Left Cerebrum. Limbic Lobe. Parahippocampal Gyrus. White Matter.	4.98	4.22	2029	0.000	6.01e-05	-34	-21	-24
Left Cerebrum. Sub-lobar. Extra-Nuclear. White Matter. Corpus Callosum.	5.39	4.01	1921	0.000	9.7e-05	-14	31	6
Right Cerebrum. Parietal Lobe. Precuneus.	6	3.47	1650	0.001	0.000336	48	-76	43
Right Cerebrum. Frontal Lobe. Middle Frontal Gyrus. Gray Matter Brodmann area 6.	6.16	3.17	1502	0.002	0.00068	54	5	38
Right Cerebrum. Frontal Lobe. Middle Frontal Gyrus. Gray Matter Brodmann area 10.	3.93	2.82	1340	0.003	0.00151	46	46	13
Left Cerebrum. Occipital Lobe. Middle Occipital Gyrus. White Matter.	4.36	2.48	1189	0.007	0.00328	-44	-73	7
Right Cerebrum. Temporal Lobe. Inferior Temporal Gyrus. White Matter.	6.13	2.32	1119	0.009	0.00475	62	-53	-15
Inter-Hemispheric.	4.2	1.87	929	0.023	0.0135	1	-20	13
Right Cerebellum. Anterior Lobe. Gray Matter. Dentate	4.21	1.85	920	0.023	0.0142	16	-57	-23
Right Cerebrum. Frontal Lobe. Superior Frontal Gyrus. White Matter.	4.49	1.8	901	0.024	0.0158	24	52	-8
Right Cerebrum. Frontal Lobe. Precentral Gyrus White Matter.	5.45	1.76	886	0.024	0.0172	43	-6	55
Right Cerebrum. Parietal Lobe. Sub-Gyral. White Matter	4.98	1.6	822	0.032	0.025	32	-56	42
Left Cerebrum Temporal Lobe. Middle Temporal Gyrus. Gray Matter Brodmann area 39.	5.06	1.58	813	0.032	0.0263	-35	-62	31
Left Cerebrum. Frontal Lobe. Middle Frontal Gyrus.	4.31	1.57	810	0.032	0.0268	-43	45	17
Right Cerebrum. Sub-lobar. Claustrum. Gray Matter.	4.57	1.56	804	0.032	0.0277	32	17	5
Left Cerebrum. Temporal Lobe. Middle Temporal Gyrus. White Matter.	4.94	1.4	742	0.043	0.0401	-56	-56	2
Right Cerebrum. Frontal Lobe. Precentral Gyrus White Matter.	4.34	1.39	738	0.043	0.0411	33	-15	54
Right Cerebrum. Limbic Lobe. Cingulate Gyrus White Matter.	4.48	1.36	728	0.044	0.0436	11	16	24

Note:

Activation index (AI) Z-MAX - a numerical variable that displays the maximum activation index value in a given region of the brain.

Number of voxels in cluster - the number of voxels in the cluster where statistically significant activation is observed. A higher number of voxels may indicate a more significant change in brain activity.

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Talairach Daemon Atlas Coordinates mni – coordinates, according to the parameters of mni and the Tailarach atlas.

Source: compiled by the authors of this study

differences between jaw-position states provide a neuroimaging correlate that is directionally compatible with the clinical concept of somatosensory tinnitus in the setting of TMD [8–12,15,16,18].

At the same time, our results also highlight the complexity of brain responses to mandibular repositioning. Several regions were reported as present only in the splint state (e.g., Right Temporal Pole, Left Middle Temporal Gyrus, Right Precentral Gyrus, Left Insula, Right Middle Frontal Gyrus, Right Paracentral Lobule, Left Superior Frontal Gyrus), while some right temporal/frontal clusters persisted across both states with reduced AI.

Such patterns do not necessarily contradict "reduction of hyperactivity"; rather, they may indicate network reconfiguration in which decreases in dominant auditory/affective clusters co-occur with recruitment of regions involved in sensorimotor integration, attentional control, or contextual processing, which are frequently implicated in contemporary tinnitus network accounts [3,5,6,20,21,24]. Importantly, the neuroimaging literature emphasizes heterogeneity across tinnitus phenotypes and the need to interpret regional activations within broader network dynamics rather than as isolated markers [3,6,20,21]. Therefore, state-dependent emergence of additional clusters in the splint condition may represent compensatory or adaptive redistribution of processing demands, especially in a single-case design.

Methodologically, the use of cluster-based inference (cluster-forming threshold $Z>3.1$; cluster-wise significance control) is aligned with standard approaches to fMRI statistical mapping [25].

The exploratory ROC analysis indicated that AI (Z-max) showed the highest discriminative ability between states (AUC≈0.69), whereas voxel-wise p-value metrics were weak classifiers.

While AUC≈0.69 is only moderate by conventional diagnostic-accuracy interpretation [26], it is noteworthy in a within-subject, cluster-as-observation exploratory setting and supports prioritizing AI-like summary metrics in future hypothesis-driven work, as also discussed in methodological literature on ROC interpretation and performance quantification [26,27]. Overall, these analytic results support the notion that a small set of fMRI-derived summary indicators (AI, cluster-level measures) may be more stable for state discrimination than voxel-wise p-value features in this context [25–27].

This report has clear limitations. Most importantly, a single-case design precludes causal inference and generalization; spontaneous fluctuations, regression to the mean, and non-specific effects (including placebo-related mechanisms) may contribute to observed clinical or neural changes [17].

Additionally, tinnitus is heterogeneous, and neuroimaging findings vary across studies depending on inclusion criteria, hearing status, analytic pipelines, and the specific symptom dimension studied (perception vs distress vs comorbidities) [3,6,20,21]. For these reasons, the present observations should be viewed as hypothesis-generating. Future work should test preregistered hypotheses in prospective cohorts enriched for TMD-related/somatosensory tinnitus, incorporate standardized tinnitus outcomes alongside repeated neuroimaging time points, and examine whether changes in TMJ biomechanics on MRI covary with reproducible changes in network-level brain metrics [8–12,15,16,20,21]. Such studies would help clarify whether mandibular repositioning reliably modulates auditory–affective circuitry in a subset of patients and whether fMRI-derived markers such as AI can support monitoring of treatment-related neurofunctional change.

CONCLUSIONS

The mandibular position with the anterior repositioning appliance was associated with a decrease in the mean Activation Index (AI) in the brain - from 5.51 ± 0.67 to 5.05 ± 0.70 ($\Delta = -8.3\%$; $p<0.05$) - and the disappearance of a large cluster of the left superior temporal gyrus (16799 voxels), indicating a significant decrease in pathological neuronal hyperactivity associated with tinnitus. The exploratory discriminative signal of AI as a neuroimaging biomarker is confirmed by an AUC ≈ 0.69 (the highest among the five indicators), which makes it promising for monitoring the therapeutic effect in future studies. The most pronounced regional changes are observed in key auditory-sensory and affective areas, such as Right Superior Temporal Gyrus: $\Delta AI = 1.08$ with a slight decrease in cluster volume of 289 voxels, which may indicate a mitigation of pathological activation of the auditory pathways; Left Superior Temporal Gyrus: complete disappearance of ΔAI activation =6.54, which correlates with a probable decrease in subjective noise perception; Prefrontal Cortex: $\Delta AI = 1.92$ and contraction of the voxel cluster ($\Delta = 4433$), possibly increasing top-down inhibition and decreased emotional reactivity to tinnitus; Insular Cortex: $\Delta AI = 1.67$ with the formation of a control cluster at 1132 voxels, which may reflect an overbalancing of somatosensory integration; Anterior Cingulate Cortex: complete disappearance of ΔAI activation = 5.62, which indicates a decrease in affective discomfort.

Our data are supported, in part, by current research (e. g, Fan 2022 [1] and Vanneste et al. (2016)[22], indicating a decreased in neural hyperactivity and improved sensory integration. Further research should be aimed at clarifying the mechanisms of action and assessing the long-term effect of this method. This approach has the potential to be integrated into comprehensive tinnitus therapy, especially in patients who experience significant emotional or cognitive discomfort.

REFERENCES

1. Chen J, Fan L, Cai G et al. Altered amplitude of low-frequency fluctuations and degree centrality in patients with acute subjective tinnitus: a resting-state functional magnetic resonance imaging study. J Integr Neurosci. 2022;21(4):116. doi:10.31083/j.jin2104116. DOI
2. Boyen K, De Kleine E, Van Dijk P, Langers DRM. Tinnitus-related dissociation between cortical and subcortical neural activity in humans with mild to moderate sensorineural hearing loss. Hear Res. 2014;312:48–59. doi:10.1016/j.heares.2014.03.001. DOI
3. Elgoyhen AB, Langguth B, De Ridder D. Tinnitus: perspectives from human neuroimaging. Nat Rev Neurosci. 2015;16(10):632–642. doi:10.1038/nrn4003. DOI
4. Eggermont JJ. The auditory cortex and tinnitus – a review of animal and human studies. European J Neuroscie. 2015;41(5):665–676. doi:10.1111/ejn.12759. DOI
5. Ghodratitoostani I, Zana Y, Delbem ACB. Theoretical tinnitus framework: a neurofunctional model. Front Neurosci. 2016;10:370. doi:10.3389/fnins.2016.00370. DOI
6. Haider HF, Bojić T, Ribeiro SF, Paço J. Pathophysiology of subjective tinnitus: triggers and maintenance. Front Neurosci. 2018;12:866. doi:10.3389/fnins.2018.00866. DOI
7. Han Q, Zhang Y, Liu D et al. Disrupted local neural activity and functional connectivity in subjective tinnitus patients: evidence from resting-state fMRI study. Neuroradiology. 2018;60(3):291–299. doi:10.1007/s00234-018-2087-0. DOI
8. van der Wal A, Michiels S, Van de Heyning P et al. Treatment of Somatosensory Tinnitus: A Randomized Controlled Trial Studying the Effect of Orofacial Treatment as Part of a Multidisciplinary Program. J Clin Med. 2020;9(3):705. doi:10.3390/jcm9030705. DOI
9. van der Wal A, Michiels S, Van de Heyning P et al. Reduction of Somatic Tinnitus Severity is Mediated by Improvement of Temporomandibular Disorders. Otol Neurotol. 2022;43(3):e309–e315. doi:10.1097/MAO.0000000000003446. DOI
10. De La Torre Canales G, Christidis N, Grigoriadis A et al. Associations between temporomandibular disorders and tinnitus - a systematic review. Cranio. 2025;43(6):969-985. doi:10.1080/08869634.2024.2404270. DOI
11. Dipalma G, Inchingolo AD, Pezzolla C et al. The association between temporomandibular disorders and tinnitus: evidence and therapeutic perspectives from a systematic review. J Clin Med. 2025;14(3):881. doi:10.3390/jcm14030881. DOI
12. Saczuk K, Kal W, Kaczała A et al. The Coexistence of Tinnitus and Temporomandibular Disorder: A Narrative Review on the Importance of an Interdisciplinary Approach. J Clin Med. 2024;13(23):7346. doi: 10.3390/jcm13237346. DOI
13. Myrhaug H. Parafunktionen im Kauapparat als Ursache eines otodentalen Syndroms. [Parafunctions in the masticatory system as a cause of otodental syndrome]. Quintessenz. 1969;20:117–121. (German)
14. Bury M, Nijakowski K, Majewska A et al. The Co-Occurrence of Temporomandibular Disorders in Patients Diagnosed with Tinnitus: A Systematic Review with Meta-Analysis. J Clin Med. 2025;14(6):1836. doi: 10.3390/jcm14061836. DOI
15. Bökel A, Fobbe A, Lesinski-Schiedat A, Sturm C. Exploring the Effects of Manual Therapy on Somatosensory Tinnitus and Dizziness: A Randomized Controlled Trial. J Clin Med. 2025;14(13):4579. doi: 10.3390/jcm14134579. DOI
16. Michiels S, Naessens S, Van de Heyning P et al. The Effect of Physical Therapy Treatment in Patients with Subjective Tinnitus: A Systematic Review. Front Neurosci. 2016:10:545. doi: 10.3389/fnins.2016.00545. DOI
17. Turner JA, Deyo RA, Loeser JD et al. The importance of placebo effects in pain treatment and research. JAMA. 1994;271(20):1609–1614.
18. Peter N, Serventi J, Neff P et al. (2025). Tinnitus in patients with orofacial complaints. Head Face Med. 2025;21(1):31. doi: 10.1186/s13005-025-00505-w. DOI
19. Bumann A, Lotzmann U. Funktionsdiagnostik und Therapieprinzipien. [Functional diagnostics and therapy principles]. 8th ed. Stuttgart: Thieme. 2015, p.372. doi:10.1055/b-002-54091. (German) DOI
20. Hu J, Cui J, Xu JJ et al. The neural mechanisms of tinnitus: a perspective from functional magnetic resonance imaging. Front Neuroscie. 2021;15:621145. doi:10.3389/fnins.2021.621145. DOI
21. Chang Y, Lee H, Kim K. Neural networks of tinnitus: a systematic review and meta-analysis of functional neuroimaging studies. NeuroImage: Clinical. 2020;27:102302. doi:10.1016/j.nicl.2020.102302. DOI
22. Vanneste S, Plazier M, van der Loo E et al. The neural correlates of tinnitus-related distress. NeuroImage. 2010;52(2):470–480. doi:10.1016/j.neuroimage.2010.04.029. DOI
23. Schecklmann M, Landgrebe M, Langguth B. Phenotypic characteristics of hyperacusis in tinnitus. PLoS One. 2014;9(1):e86944. doi:10.1371/journal.pone.0086944. DOI
24. De Ridder D, Elgoyhen AB, Romo R, Langguth B. Phantom percepts: tinnitus and pain as persisting aversive memory networks. Proc Natl Acad Sci U S A. 2011;108(20):8075–8080. doi:10.1073/pnas.1018466108. DOI
25. Worsley KJ. Statistical analysis of activation images. Functional MRI: An Introduction to Methods. New York: Oxford University Press. 2001, p.251–270. http://ndl.ethernet.edu.et/bitstream/123456789/45780/1/315.pdf [Accessed 13 April 2025]
26. Çorbacıoğlu ŞK, Aksel G. Receiver operating characteristic curve analysis in diagnostic accuracy studies: a guide to interpreting the area under the curve value. Turk J Emerg Med. 2023;23(4):195–198. doi:10.4103/tjem.tjem_182_23. DOI

27. Peres ASC, Lemos TW, Barros AKD et al. Performance quantification of clustering algorithms for false positive removal in fMRI by ROC curves. Sci Flo Brazil. 2017;33(1):31–41. doi:10.1590/2446-4740.03215. DOI
28. Revilla-León M, Zeitler JM, Kois DE, Kois JC. Utilizing an additively manufactured Kois deprogrammer to record centric relation: A simplified workflow and delivery technique. J Prosthet Dent. 2024;132(1):20-25. doi: 10.1016/j.prosdent.2022.04.034. DOI

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Vasil Pekhno
Shupyk National Healthcare University of Ukraine
9 Dorohozhytska St, 04112 Kyiv, Ukraine
e-mail: Pekhnyo@ukr.net

ORCID AND CONTRIBUTIONSHIP

Vasil Pehnyo: 0000-0002-0075-6225 A B C D E F
Nataliia Savychuk: 0000-0001-9532-665X E F
Oleksandr Bida: 0000-0002-6038-6545 B
Stanislav Riebienkov: 0000-0001-8116-5277 B
Ivan Riabko: 0009-0000-6748-8686 C
Roman Sulik: 0000-0002-0487-3357 E

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

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ORIGINAL ARTICLE

CONTENTS

Physical and sports rehabilitation of patients with amputations using adaptive badminton

Valentyn V. Bondarenko[1], Kostiantyn V. Prontenko[2], Hanna V. Bykova[3], Anton M. Chernikov[1], Ihor O. Sidelnikov[1], Larysa V. Kozibroda[4], Oleksandr S. Herasymenko[5]

[1]NATIONAL ACADEMY OF INTERNAL AFFAIRS, KYIV, UKRAINE
[2]S.P. KOROLIOV ZHYTOMYR MILITARY INSTITUTE, ZHYTOMYR, UKRAINE
[3]STATE UNIVERSITY OF TRADE AND ECONOMICS, KYIV, UKRAINE
[4]IVAN BOBERSKYI LVIV STATE UNIVERSITY OF PHYSICAL CULTURE, LVIV, UKRAINE
[5]DROHOBYCH IVAN FRANKO STATE PEDAGOGICAL UNIVERSITY, DROHOBYCH, UKRAINE

ABSTRACT

Aim: To assess the impact of adaptive badminton training sessions on the functional status, quality of life, and health of patients with amputations.

Materials and Methods: The research conducted in 2025 involved 26 combatants who were in the final stages of their rehabilitation after limb amputations due to blast and shrapnel wounds. The dynamics of functional status, quality of life, and health indicators were determined using the "SF-36 Health Survey" socio-psychological questionnaire and the "WAM" functional status self-assessment method.

Results: A significant improvement in overall physical and psychological health components was observed in patients with amputations as a result of participating in adaptive badminton training sessions. These indicators improved by 5.92 and 6.18 points, reaching 65.06 ± 0.97 and 76.60 ± 0.65 points, respectively. The most pronounced positive changes were recorded in the indicators of physical functioning, vitality, and social functioning. Positive dynamics were also found in the well-being, activity, and mood of the participants in the rehabilitation activities; the overall "WAM" indicator improved by 0.8 points and reached 4.77 ± 0.12 points.

Conclusions: It was established that adaptive badminton have a positive effect on the health and functional status of patients with amputations. The results of the research indicate a significant improvement in the physical and psychological components of health in the study participants. Positive changes in well-being, activity, and mood were also observed among participants in rehabilitation activities, indicating the advisability of wider implementation of adaptive badminton training sessions in physical and sports rehabilitation for combatants.

KEY WORDS: adaptive badminton, health, patients with amputations, physical and sports rehabilitation, functional status, quality of life

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INTRODUCTION

Military aggression and large-scale combat operations have led to a significant increase in the number of military personnel, law enforcement officers, and civilians with limb amputations caused by gunshot and shrapnel wounds [1, 2]. The return of such individuals to a whole life exacerbates the need for effective physical, psychological, and social rehabilitation [3]. In view of the above, physical and sports rehabilitation is essential and effective. It is a system of measures based on physical exercises aimed at restoring and adapting the body's functional capabilities, improving physical and mental health, and overall health [4]. One type of physical and sports rehabilitation is adaptive physical culture and sports. Performing physical exercises during adaptive physical culture training sessions contributes to: the restoration of motor activity; correction of impaired functions; development of new motor skills considering amputations; improvement of psycho-emotional and functional status, and quality of life. Training sessions in adaptive sports are an effective tool for combating stress, post-traumatic stress disorder, anxiety, and social isolation [5, 6].

Analysis of current scientific literature supports the conclusion that adaptive badminton is among the most effective and efficient methods for physical and sports rehabilitation in individuals who have lost limbs or sustained musculoskeletal injuries [7]. This sport is a modified form of classic badminton, aimed at involving people with musculoskeletal disorders, in particular those with limb amputations due to blast and shrapnel injuries. It combines elements of sports training,

physical therapy, and psychological rehabilitation and promotes the restoration of motor skills, balance, cognitive functions, and social integration of participants [8].

Scientists [9] claim that adaptive badminton training sessions activate the vestibular apparatus, sensorimotor connections, and proprioceptive sensitivity; develop concentration, spatial perception, and quick thinking; promote motivation for regular physical activity and reduce pain and anxiety. At the same time, such training sessions require developing the ability to control movement while accounting for changes in biomechanics, since people with amputations may have a shifted center of mass and a different pattern of compensatory movements [10]. The type of amputation determines how badminton is adapted. In particular, for individuals with lower-limb amputations, the emphasis is on stabilizing the body, controlling the center of gravity, using the prosthesis, and developing balance. In the absence of an upper limb, the focus is on learning the correct racket grip (using special cuffs-fasteners), compensating for actions through torso movement, and developing the back muscles. In the case of bilateral amputation, the work is performed while sitting, with emphasis on maintaining a stable pelvic position, making short movements, and developing stroke technique. In cases of combined injuries, a single recovery trajectory is used. [11].

Experts in adaptive sports note that the methodology for teaching adaptive badminton involves mastering the basics of the game; safety rules during training sessions; biomechanical principles of movement and strokes; principles of adapting movements to the type of amputation; methods of self-control and recovery after exercise [12]. Scientists [13] argue that it is essential to develop skills in maintaining proper body position, coordinating movements with the racket and shuttlecock, maintaining balance during movement, executing serves, returns, and basic strokes, and applying breathing and recovery techniques. The training methodology for athletes with amputations in adaptive badminton is based on the comprehensive use of interactive, corrective-rehabilitative, psychosocial, and other methods. The choice of a particular technique depends on the type of amputation, the level of functional preparedness, the phase of the rehabilitation process, and the participant's psycho-emotional state [14].

Scientists [15] are convinced that the introduction of adaptive badminton into rehabilitation programs for people with amputations and musculoskeletal injuries helps expand participants' functional capabilities, promotes faster recovery of physical and mental health, and supports more effective coping with stress. The expected results of implementing such programs include: improvement in static and dynamic balance; restoration of motor control during prosthesis use; development of strength, coordination, and precision of motor actions; formation of basic playing skills and motivation for training; and creation of conditions for social integration through joint motor activity. The need to study the impact of adaptive badminton as a means of physical and sports rehabilitation on the functional state, quality of life, and health of people with amputations made the topic of our research relevant.

AIM

The aim is to assess the impact of adaptive badminton training sessions on the functional status, quality of life, and health of patients with amputations.

MATERIALS AND METHODS

PARTICIPANTS

The research, conducted in 2025, involved 26 combatants (military personnel and law enforcement officers) who were in the final stage of their rehabilitation after lower/upper limb amputations (categories SL and SU) due to blast and shrapnel wounds. Based on the decisions of a multidisciplinary rehabilitation commission, these patients were recommended to undergo physical and sports rehabilitation through adaptive sports, particularly adaptive badminton.

Rehabilitation measures were implemented in rehabilitation centers in Kyiv and the Kyiv Oblast. Adaptive badminton training sessions were conducted at the sports facilities of the National Academy of Internal Affairs (NAIA) three times a week. The results were processed at the Department of Special Physical Training of the NAIA and the Department of Physical Education and Sports Rehabilitation of S. P. Koroliov Zhytomyr Military Institute. Training sessions were conducted under medical supervision and accompanied by rehabilitation specialists.

RESEARCH METHODS

The research employed a range of theoretical, empirical, and statistical methods. The dynamics of functional status, quality of life, and health indicators were determined using the "SF-36 Health Survey" socio-psychological questionnaire [16] and the "WAM" functional status self-assessment method [17]. The participants were tested upon arrival at the training bases of the educational institutions and after 10 weeks of systematic training. According to the methodology for assessing

quality of life and health indicators, participants in rehabilitation activities must answer 36 questions from the "SF-36 Health Survey" questionnaire. The assessment was conducted by comparing pre- and post-course results from adaptive badminton training sessions. The methodology does not provide for normative or critical values. For each item, several answer options are offered, which are scored from 0 to 100 points. A score of 0 indicates the worst possible quality of life, and 100 indicates the highest. The results are presented across eight scales, grouped into two areas that determine the components of physical and psychological health. The Physical Health (PH) component comprises the following indicators: Physical Functioning (PF), Role-Physical (RP) Functioning, Bodily Pain (BP), and General Health (GH). The Mental Health (MH) component includes the following indicators: Vitality (VT); Social Functioning (SF); Role-Emotional (RE) Functioning; Mental Health (MH) [16].

The "WAM" functional status self-assessment method is designed to assess well-being, activity, and mood quickly. According to the instructions, you need to compare your condition with a series of signs on a multi-step scale. The scale includes indices (3 2 1 0 1 2 3). It is located between thirty pairs of statements that are opposite in meaning and reflect mobility, speed, and pace of functions (activity), strength, health, fatigue (well-being), as well as characteristics of emotional state (mood). The test participant should choose the number that most accurately reflects their current state. The processing and interpretation of the results obtained involves decoding the answers. In particular, index 3, which corresponds to poor well-being, low activity, and bad mood, is taken as 1 point; index 2 is taken as 2 points; index 1 is taken as 3 points, and so on up to index 3 on the opposite side of the scale, which is equal to 7 points. Positive functional status is assessed with high scores, whereas negative functional status is assessed with low scores. Based on the points obtained, the arithmetic mean is calculated for the overall score and separately for activity, well-being, and mood [17].

STATISTICAL METHODS

The methods of mathematical statistics were used to process the data obtained. The reliability of the difference between the indicators was determined using the Student's t-test. The results were presented as $X \pm m$, where X is the arithmetic mean, m is the standard error. The reliability of the difference was set at $p<0.05$. All statistical analyses were performed using STATISTICA 6.1 software package (number AGAR909E415822FA), 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 NAIA. 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

The results of the study of the dynamics of the indicators of the physical and mental components of health and the functional status of the participants before and after 10 weeks of adaptive badminton training sessions are presented in Table 1. Based on the data analysis, a significant improvement in the overall indicator of the physical component of health was observed ($p \leq 0.01$). At the initial stage, this indicator was 59.14 ± 1.49 points, and at the final stage, it was 65.06 ± 0.97 points. The effectiveness of physical functioning in participants in rehabilitation activities also improved significantly ($p \leq 0.01$). The survey showed that, on the eve of the adaptive badminton training course, this indicator was 49.42 ± 1.60 points, and after 10 weeks it was 56.35 ± 1.56. The role-physical functioning also improved significantly ($p \leq 0.05$), and at the end of the course, the participants rated it at 58.88 ± 1.38 points. The indicator characterizing bodily pain did not change significantly ($p \geq 0.05$), but improved by 3.92 points. The results of processing the self-assessment of general health responses indicated significant changes ($p \leq 0.05$). In particular, at the initial stage, this indicator was rated 64.81 ± 1.10 points, and at the final stage, 68.85 ± 1.18 points.

Positive dynamics were observed in the indicators of the mental component of health. In particular, the overall indicator improved by 6.18 points during the training sessions, reaching 76.60 ± 0.65 ($p \leq 0.001$). Among the elements of this component, the highest level of dynamism was observed in the data on the state of well-being and the assessment of a person's vitality (7.07 points). Slightly lower, but reliable, results were observed for indicators of social functioning ($p \leq 0.01$). At the initial stage, 74.12 ± 1.81 points were recorded,

Table 1. Dynamics of indicators of functional status, quality of life, and health of persons with amputations (n = 26), points

Indicators	Stages of research	X ± m	t	p
The Physical Health Component				
Physical Functioning – PF	initial	49.42±1.60	3.10	p≤0.01
	final	56.35±1.56		
Role-Physical Functioning – RP	initial	53.11±1.60	2.73	p≤0.05
	final	58.88±1.38		
Bodily Pain – BP	initial	69.23±2.97	1.06	p≥0.05
	final	73.15±2.20		
General Health – GH	initial	64.81±1.10	2.50	p≤0.05
	final	68.85±1.18		
Overall indicator of the physical health component	initial	59.14±1.49	3.33	p≤0.01
	final	65.06±0.97		
The Mental Health Component				
Vitality – VT	initial	65.28±1.29	4.40	**p≤0.001**
	final	72.35±0.96		
Social Functioning – SF	initial	74.12±1.81	3.46	**p≤0.01**
	final	81.73±1.25		
Role-Emotional Functioning – RE	initial	71.69±1.61	2.47	**p≤0.05**
	final	76.80±1.27		
Mental Health – MH	initial	70.58±1.30	3.24	**p≤0.01**
	final	75.38±0.71		
Overall indicator of the mental health component	initial	70.42±0.72	6.32	**p≤0.001**
	final	76.60±0.65		
"WAM" Functional Status				
Well-being	initial	3.88±0.16	3.48	**p≤0.01**
	final	4.62±0.14		
Activity	initial	4.08±0.16	2.16	**p≤0.05**
	final	4.54±0.14		
Mood	initial	3.96±0.17	4.29	**p≤0.001**
	final	5.12±0.21		
"WAM" overall indicator	initial	3.97±0.08	5.55	**p≤0.001**
	final	4.77±0.12		

Note: X – arithmetic mean; m – standard error; t – Student's t-test value; p – p-value
Source: compiled by the authors of this study

and at the final stage, 81.73 ± 1.25. Positive changes were also found in self-assessment of mental health ($p \leq 0.01$). Before the adaptive badminton course, participants rated their mental health at 70.58 ± 1.30 points; after completion, it was 75.38 ± 0.71. Reliable results were obtained for indicators that assess the level of role-emotional functioning ($p \leq 0.05$). At the initial stage, this indicator was 71.69 ± 1.61 points, and at the final stage, it was 76.80 ± 1.27 points.

The study of the results of self-assessment of the functional status of participants using the "WAM" method also gives grounds to note positive dynamics. There is a clear pattern of changes in well-being, activity, and mood during rehabilitation activities. Significant changes were observed in well-being and mood. According to the survey, participants' well-being before the start of training sessions was rated at 3.88 ± 0.16 points, and after completion at 4.62 ± 0.14 points ($p \leq 0.01$). The smallest, but still significant, changes were recorded in activity indicators – 4.08 ± 0.16 and 4.54 ± 0.14 points, respectively ($p \leq 0.05$). A positive trend in mood self-assessment was observed – 3.96 ± 0.17 points at the initial stage and 5.12 ± 0.21 points at the final stage ($p \leq 0.001$). The overall "WAM" indicator improved by 0.8 points and reached 4.77 ± 0.12 points ($p \leq 0.001$). This can be explained by the presence of internal

attitudes toward the rehabilitation process and by recognition of the participant's personal role in it. A significant factor influencing the psycho-emotional state of individuals is the process of adaptation and attitude to changed living conditions as a result of acquired injuries, in particular amputations.

DISCUSSION

The relevance of the research is confirmed by publications by other scientists [18], who argue that the development of adaptive sports is aimed at improving services in the field of physical culture and sports and building barrier-free environments. The use of adaptive sports in rehabilitation systems for people with limb amputations increases functional mobility, psychological stability, and daily activities [19, 20]. Other scientists are convinced that the psychosocial role of adaptive badminton is to develop a sense of bodily control, increase self-esteem, restore social skills, foster teamwork, and motivate individuals to lead an active lifestyle [7, 12].

Experts [9, 13] recommend using special exercises during adaptive badminton training sessions, including: balancing on a prosthesis with a racket; passing the shuttlecock while maintaining position; strengthening core stability; moving in short distances; balancing with eyes closed; moving with changing support; holding the ball on the racket, etc. Other researchers emphasize the need to strictly adhere to the sequence of stages in implementing the rehabilitation program using adaptive badminton, namely: introductory-adaptive, initial, developmental, and game-reinforcement. In view of the above, scientists emphasize the need to adhere to the methodological principles of the adaptive badminton training process, including: safety, consideration of the type of amputation, prosthetics, psychological state, comprehensiveness, and repeatability. The main task is to ensure the formation of functional motor skills and adaptive mechanisms necessary for performing technical elements of the game, as well as to promote social integration and psychological recovery of participants.

Overall, our results confirm improvements in functional status, quality of life, and health among individuals during adaptive badminton training sessions using both methods. Such results may be due to the effects of physical exertion experienced by participants during specific motor actions in this sport. Moderate physical exercise helps reduce cortisol levels and stimulates endorphin production. Team spirit, emotional support, and a positive psychological atmosphere on the court have beneficial effects on participants' psycho-emotional state, improving mood, reducing tension, anxiety, and depression, and helping participants overcome stress more effectively, owing to their complex effects on the hormonal profile and nervous system.

The results obtained confirm the findings of other scientists [21, 22] regarding the positive effects of moderate physical exercise on functional status, quality of life, and health in veterans, combatants, and persons with disabilities. Involving such persons in adaptive badminton training sessions is one of the most essential tools for their rehabilitation and social integration. The results of our research do not contradict the conclusions of other scientists, but rather expand and complement them.

CONCLUSIONS

It has been established that adaptive physical culture and adaptive sports are essential means of physical and sports rehabilitation for people with amputations. A significant improvement in the overall physical health indicator ($p \leq 0.01$) was found. At the initial stage, this indicator was 59.14 ± 1.49 points; at the final stage, it was 65.06 ± 0.97 points. Significant changes were found in the effectiveness of physical functioning ($p \leq 0.01$), role-physical functioning ($p \leq 0.05$), and overall health in participants in rehabilitation activities ($p \leq 0.05$). Positive but not significant changes were found in the bodily pain indicator ($p \geq 0.05$). Positive dynamics were observed in the indicators of the mental component of health. The overall indicator of the mental element improved by 6.18 points during the training sessions, reaching 76.60 ± 0.65 points ($p \leq 0.001$). Among the aspects of this component, the highest dynamics were recorded in the data on the level of life activity. The increase was 7.07 points and reached 72.35 ± 0.96 points at the final stage ($p \leq 0.001$). Slightly lower, but also significant improvements were observed in indicators of social functioning ($p \leq 0.01$), mental health ($p \leq 0.01$), and role-emotional functioning ($p \leq 0.05$).

Positive changes in participants' functional status during rehabilitation were observed ($p \leq 0.001$). An increase in the overall "WAM" indicator to 4.77 ± 0.12 points ($p \leq 0.001$) was noted, as well as improvements in well-being ($p \leq 0.01$), activity ($p \leq 0.05$), and mood ($p \leq 0.001$). The results of the research justify the broader adoption of adaptive badminton training sessions in physical and sports rehabilitation for people with amputations.

PROSPECTS FOR FURTHER RESEARCH

We see prospects for further research into the impact of adaptive swimming training sessions on the functional status, quality of life, and health of combatants with musculoskeletal injuries.

REFERENCES

1. Biloshytska OK, Bespalova OY, Seminska NV, Galkin OY. Concept of a multifunctional prosthetic and rehabilitation center with an innovative educational component for patients with limb amputation in the context of war in Ukraine. Wiad Lek. 2025;78(6):1160-1167. doi:10.36740/WLek/207373. DOI
2. Epstein CS, Prokhorenko G, Los D, Pavchak R. Combat orthopedic trauma care: Challenges and innovations in Ukraine's wartime response. Surgery. 2025;182:109313. doi:10.1016/j.surg.2025.109313. DOI
3. Griban G, Zablotska OS, Asauliuk IO et al. Peculiarities of rehabilitation of patients with musculoskeletal disorders: what has changed during the war in Ukraine. Wiad Lek. 2025;78(9):1813-1820. doi:10.36740/WLek/206076. DOI
4. Dupuis F, Perreault K, Hébert LJ et al. Group Physical Therapy Programs for Military Members With Musculoskeletal Disorders: A Pragmatic Randomized Controlled Trial. J Orthop Sports Phys Ther. 2024;54(6):417-426. doi:10.2519/jospt.2024.12342. DOI
5. Tinney MJ, Caldwell ME, Lamberg EM. Adaptive Sports and Recreation in Persons with Limb Loss/Limb Deficiency. Phys Med Rehabil Clin N Am. 2024;35(4):769-793. doi:10.1016/j.pmr.2024.06.004. DOI
6. Lee KK, Uihlein MJ. Adaptive Sports in the Rehabilitation of the Disabled Veterans. Phys Med Rehabil Clin N Am. 2019;30(1):289-299. doi:10.1016/j.pmr.2018.08.001. DOI
7. Soares LFL, Mollo Tormin L, Carvalho KS, Alves ACJ. Assistive technology for Para-badminton athletes: the application of the matching person and technology theoretical model in occupational therapy. Disabil Rehabil Assist Technol. 2024;19(4):1170-1177. doi:10.1080/17483107.2022.2154398. DOI
8. Bravo-Sánchez A, Abián-Vicén J, Jiménez F, Abián P. Influence of badminton practice on calcaneal bone stiffness and plantar pressure. Phys Sportsmed. 2020;48(1):98-104. doi:10.1080/00913847.2019.1635050. DOI
9. Alberca I, Chénier F, Astier M et al. Impact of Holding a Badminton Racket on Spatio-Temporal and Kinetic Parameters During Manual Wheelchair Propulsion. Front Sports Act Living. 2022;4:862760. doi:10.3389/fspor.2022.862760. DOI
10. Wang Y, Mei Q, Liew BXW et al. Influence of gender, limb dominance, training experience, and loading conditions on arch characteristics in badminton players. Sci Rep. 2025;15(1):21060. doi:10.1038/s41598-025-06910-0. DOI
11. Hawkins C, Coffee P, Soundy A. Considering how athletic identity assists adjustment to spinal cord injury: a qualitative study. Physiotherapy. 2014;100(3):268-274. doi:10.1016/j.physio.2013.09.006. DOI
12. Alberca I, Watier B, Chénier F et al. Trying to use temporal and kinematic parameters for the classification in wheelchair badminton. PLoS One. 2025;20(3):e0315939. doi:10.1371/journal.pone.0315939. DOI
13. Alberca I, Chénier F, Watier B et al. Impact of holding a badminton racket on spatiotemporal parameters during manual wheelchair propulsion based on forward and backward propulsion. Disabil Rehabil Assist Technol. 2025;20(5):1530-1538. doi:10.1080/17483107.2025.2459885. DOI
14. Ma S, Soh KG, Japar SB et al. Effect of core strength training on the badminton player's performance: A systematic review & meta-analysis. PLoS One. 2024;19(6):e0305116. doi:10.1371/journal.pone.0305116. DOI
15. Phomsoupha M, Laffaye G. The science of badminton: game characteristics, anthropometry, physiology, visual fitness and biomechanics. Sports Med. 2015;45(4):473-495. doi:10.1007/s40279-014-0287-2. DOI
16. Yarlas A, Bayliss M, Cappelleri JC et al. Psychometric validation of the SF-36® Health Survey in ulcerative colitis: results from a systematic literature review. Qual Life Res. 2018;27(2):273-290. doi:10.1007/s11136-017-1690-6. DOI
17. Rodriguez-Ayllon M, Cadenas-Sánchez C, Estévez-López F et al. Role of Physical Activity and Sedentary Behavior in the Mental Health of Preschoolers, Children and Adolescents: A Systematic Review and Meta-Analysis. Sports Med. 2019;49(9):1383-1410. doi:10.1007/s40279-019-01099-5. DOI
18. Rayes R, Ball C, Lee K, White C. Adaptive Sports in Spinal Cord Injury: a Systematic Review. Curr Phys Med Rehabil Rep. 2022;10(3):145-153. doi:10.1007/s40141-022-00358-3. DOI
19. Farrokhi S, Mazzone B, Moore JL et al. Physical Therapy Practice Patterns for Military Service Members with Lower Limb Loss. Mil Med. 2019;184(11-12):e907-e913. doi:10.1093/milmed/usz107. DOI
20. Ladlow P, Phillip R, Etherington J et al. Functional and Mental Health Status of United Kingdom Military Amputees Postrehabilitation. Arch Phys Med Rehabil. 2015;96(11):2048-2054. doi:10.1016/j.apmr.2015.07.016. DOI
21. Tow S, Gober J, Nelson MR. Adaptive Sports, Arts, Recreation, and Community Engagement. Phys Med Rehabil Clin N Am. 2020;31(1):143-158. doi:10.1016/j.pmr.2019.09.003. DOI
22. Talbot LA, Brede E, Metter EJ. Psychological and Physical Health in Military Amputees During Rehabilitation: Secondary Analysis of a Randomized Controlled Trial. Mil Med. 2017;182(5):e1619-e1624. doi:10.7205/MILMED-D-16-00328. DOI

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Kostiantyn V. Prontenko
S. P. Koroliov Zhytomyr Military Institute
22 Myr Avenue, 10023 Zhytomyr, Ukraine
e-mail: prontenko-kostya@ukr.net

ORCID AND CONTRIBUTIONSHIP
Valentyn V. Bondarenko: 0000-0002-0170-2616 A B
Kostiantyn V. Prontenko: 0000-0002-0588-8753 D
Hanna V. Bykova: 0000-0003-1006-0296 C
Anton M. Chernikov: 0009-0001-1495-3414 B D
Ihor O. Sidelnikov: 0009-0002-5993-5073 C D
Larysa V. Kozibroda: 0000-0001-8232-425X E
Oleksandr S. Herasymenko: 0000-0001-7642-2160 F

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

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ORIGINAL ARTICLE

CONTENTS

DNA repair RAD 18 rs373572 and OGG1 rs1052133 genes polymorphisms association with histological characterization of renal cell carcinoma

Iftikhar Khdhair Abbas Altemimi[1], Binan Adil Mohammed Ameen[1], Mona N. Al-Terehi[2], Liwaa Mahdi Hussein[1]
[1]DEPARTMENT OF PATHOLOGY AND FORENSIC MEDICINE, FACULTY OF MEDICINE, UNIVERSITY OF KUFA, NAJAF, IRAQ
[2]BIOLOGY DEPARTMENT, COLLEGE OF SCIENCE, UNIVERSITY OF BABYLON, HILLA, IRAQ

ABSTRACT

Aim: Validate the association RAD18 Arg302Gln (rs373572) and OGG1 Ser326Cys (rs1052133) - with Renal Cell Carcinoma (RCC) susceptibility and histopathological characterization.

Materials and Methods: present study compromised of 37 patients with RCC and control group consisted of 28 healthy apparently individuals. A case control study was conducted using Hand E staining and allele-specific PCR for genes genotyping.

Results: The cohort comprised cases with subtypes clear cell, chromophobe, and variant RCC. A significant link was detected between OGG1 rs1052133 and RCC risk ($p = 0.006$), with the GG genotype being strongly linked to disease $p=0.005$. Haplotype demonstrated a significant relations of the GC with RCC ($p=0.013$). LD test revealed a significant association between RAD18 and OGG1 loci ($\chi^2 = 9.214$, $p = 0.027$), with r- value of 0 in cases and 0.14 in controls. Histopathological correlations revealed that OGG1 rs1052133 genotypes demonstrated a highly significant association with RCC $p<0.001$. RAD18 rs373572 genotypes were significantly closed link with tumor stage $p=0.001$. TNM classification revealed a significant relationship with RAD18 genotypes $p=0.017$.

Conclusions: A significant relationship between OGG1 rs1052133 and both RCC susceptibility and cancer histological subtype, proposed a potential role in the pathogenesis of disease. RAD18 rs373572 showed relevance to tumor stage and TNM classification, indicating a possible impact in disease progression rather than initiation.

KEY WORDS: DNA repair system, genes polymorphisms, RAD 18 rs373572, OGG1 rs1052133, renal cell carcinoma, histopathological characterization

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ABBREVIATIONS

RCC: Renal Cell Carcinoma
LD: Linkage Disequilibrium
PRR: Post-Replication Repair System
FFPE: Formalin-Fixed Paraffin-Embedded
HWE: Hardy-Weinberg Equilibrium

INTRODUCTION

Renal cell carcinoma (RCC) is the most commonly reported malignancy of the urinary system, accounting for over 90% of kidney cancers and approximately 2% of all cancer-related deaths worldwide [1-2]. The impact of DNA repair genes in tumorigenesis and cancer development has been extensively investigated [3]. The DNA repair p comprises some critical pathways, including base excision repair, mismatch repair and homologous recombination repair, all of which are vital for maintaining genomic integrity. Deficiencies or mutations in these systems can lead to genomic instability and the accumulation of mutations, thereby contributing to carcinogenesis [4]. Furthermore, DNA repair pathways have become key targets in cancer therapy. As well as, poly (ADP-ribose) polymerase inhibitors have enhanced efficacy in cancers with BRCA1/2 mutations, highlighting the therapeutic potential of targeting DNA repair deficiencies [5]. Renal cell carcinoma is well observed in both male and female, about 81610 patients of kidney and renal pelvis tumor were reported in the United States in 2024 with 14390 deaths. These accounts for about 4.1% of malignancies in adult [6]. The ratio between both sexes is 1.9:1. [7] RCC is different from kidney tumors that consist of renal pelvis or renal medulla, and it just applies to cancer that generate in the kidney bed lining. This summary does not classify non-RCCs of the kidney, as well as renal pelvis or renal

medulla cancer. The Genetic pathogenic variations have detected as the inherited tumor risk cause in several RCC-prone families; these variations are identified for only 5–8% of RCC cases [8-9], other undiscovered genes might have relations in the progression of familial RCC in addition to non-genetic risk factors. Numerous Studies have estimated a new genes variation which didn't associate with RCC hereditary, the results showed that the rate of germline changes in classic RCC genes aligns with prior detect and reported of other pathogenic variants, several of these variations observed in DNA repair encoding genes. The other pathogenic alteration rate was about (12.8-17.0) [10-14]. The other pathogenic incidence changes are more than would be founded in the different population. However, these studies are not population-based, and they are volubility enriched for cases of cancer who have been suggested for germline measurement. Many factors like Endogenous and environment like exposure to ionizing radiation, ultraviolet and some chemicals can lead to DNA damage that can be repaired by repair processing [15]. Most persistent DNA injury are efficiently took out by base and nucleotide excision repair pathways [16]. Meanwhile, several DNA injuries can stay at replication because DNA repair systems have limited capacity, which stimulate gaps in the newly synthesized strand. These gaps are repaired by post-replication repair system (PRR) [17]. Human *Rad18* gene is located on chromosome 3p24-25, the Rad18 protein link with the human Rad6 protein (HHR6A and HHR6B) that used by PRR [18]. The mutation in Rad18 or Rad6 lead to more sensitivity to different mutagens [19]. Oxoguanine glycosylase (OGG1) is the primary molecules used in the excision of modified nucleotide 8-oxoguanine (8-oxoG), a DNA lesion caused by exposure to reactive oxygen species, this enzyme linked with sequences rich in 8-O guanine in the promoter region, that lead to changing in DNA conformation, the downstream gene transcription activation, the recruitment of transcription factors [20]. The OGG1 involvement mechanism in the renal inflammatory through enhance binding of NF-κB/RelA with cis-elements resulted rapid production of inflammatory cell accumulation and chemokines/cytokines in the airways [21]. OGG1 also interacts directly with other proteins and impacts downstream biological mechanisms. It also enhance transformation of TGF-β1-induced cell via interacting with Smad7 [22]. OGG1 has been well investigated in cancer, but in renal tumors is less found. It is a common substitution mutation locus in renal cancer like RCC [23]. Researches have referred that the OGG1 gene is related to DNA injury with chronic kidney disease patients, and in its pathological process [24].

AIM

The present study aims to detect The RAD 18 Arg302Gln (rs373572) and OGG1 Ser326Cys (rs1052133) gene variations in renal cell carcinoma and association with histological characterization

MATERIALS AND METHODS

SAMPLE COLLECTION

This study included 37 cases with renal cell carcinoma (RCC), all of whom attended a Al-Sadir teaching hospital, Najaf city, Iraq, prior to receiving any treatment (chemotherapy or radiotherapy), control group consisted of 28 healthy apparently individuals. Formalin-fixed paraffin-embedded (FFPE) tumor tissue samples and relevant clinical data were collected from each case and blood samples were collected from control group for DNA extraction after obtaining written informed consent. DNA concentration and purity were detected via NanoDrop. The single nucleotide polymorphisms (SNPs) analyzed in this study were RAD18 Arg302Gln (rs373572), as previously reported by [25], and OGG1 Ser326Cys (rs1052133), as reported by [26]. PCR Conditions and Electrophoresis: For amplification of the RAD18 gene variant, PCR was implemented using allele-specific primers under the following conditions: annealing at 58 °C for 40 seconds and extension at 72 °C for 40 sec, for 35 cycles. The allele-specific PCR produced a 146 bp band for the Gln allele, a 106 bp band for the Arg allele, and a 206 bp control band. For the OGG1 Ser-326Cys polymorphism, PCR performed with annealing at 64°C for 1 minute and extension at 72 °C for 1 minute, over 30 cycles. The resulting products included bands of 194 bp, 252 bp, and a 406 bp internal control. PCR products were visualized by agarose gel electrophoresis under UV light following ethidium bromide staining.

ETHICAL APPROVAL

The study was approved by the Ethical Committee of the College of Science, University of Babylon (Approval No. B24006 IN 11/5/2024). DNA isolation and Oligonucleotides: whole DNA was isolated from FFPE tissue via the Geneaid™ DNA Isolation Kit Tissue (GET150), with a protocol modification involving overnight incubation with proteinase K to enhance lysis efficiency.

DATA ANALYSIS

Descriptive data were represented as mean ± standard deviation (SD) for age and as percentages for sex, histological classifications, and genotypes. Statistical asso-

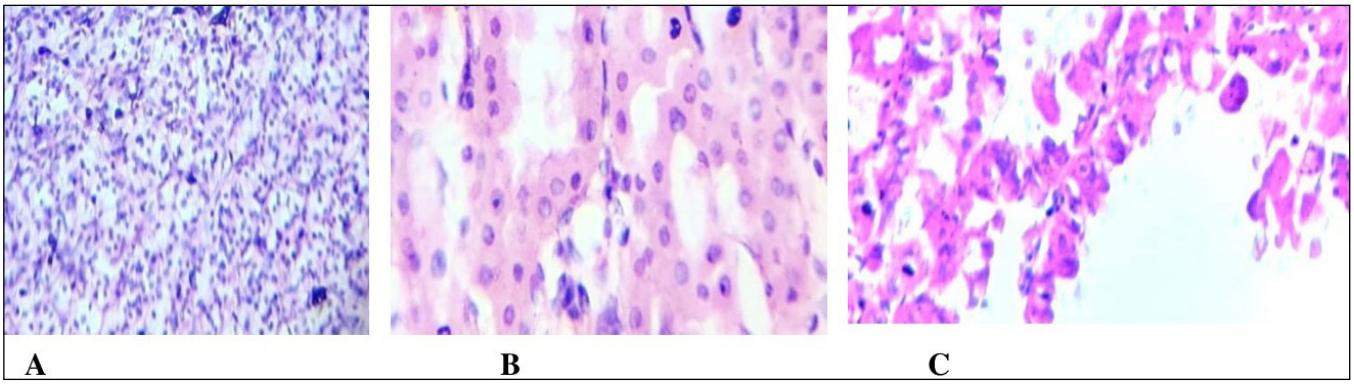

Fig. 1. Histopathological characterization of RCC types (A) 10X Clear cell variant of renal cell carcinoma, (B) 25X chromophobe variant of renal cell carcinoma and (C) 10X papillary variant of renal cell carcinoma

ciations were calculated using odds ratios, Chi-square tests, Fisher's exact tests, with a significance threshold of $p < 0.05$. Linkage disequilibrium and haplotype analysis were conducted using SHEsis software.

RESULTS

The present study was carried out to investigate the association between histopathological changes and critical SNPs in the OGG1 and RAD18 DNA repair genes in renal carcinoma (RCC) cases. The results revealed a significant association between cases age and the incidence of RCC. In addition, the male: female ratio was 2.6, indicating a higher prevalence of RCC among males compared to females, table (1).

Histopathological characterizations of renal carcinoma (RCC) were analyzed in the current study. Tumor grading showed that Grade I was the most prevalent, found in 81.08% of cases, while Grade III was the least frequent, accounting for only 2.70%. Three histological subtypes were observed: clear cell, chromophobe, and papillary carcinoma. Among these, clear cell carcinoma was the most common subtype, representing 75.67% of cases, the least common was papillary carcinoma at 10.81%. Tumor classification based on size and extent (T stage) demonstrated that T1 tumors (including subtypes T1a and T1b) were found in about 83.78% of cases, while T2 and T3 were less frequent. All cases were categorized under Nx and Mx. Overall, belong to the TNM staging system, 83.78% of the cases were categorized as T1NxMx, table (2).

The histopathological changes in the current study are illustrated in figure (1). Figure (1A) depicts renal cell carcinoma (RCC) of the chromophobe subtype, characterized by eosinophilic cells with prominent borders and reticular cytoplasm. Figure (1B) clarifies the papillary subtype, showing distinct papillary architecture with fibrovascular cores lined by neoplastic cells. Figure (1C)

The histopathological changes of this study are clarified in figure (1), figure A shows 10X Clear cell variant of RCC of the kidney show proliferating clear cells in diffuse growth patterns infiltrate the renal parenchyma tissue elements. Figure B explores 25X chromophobe variant of renal cell carcinoma of kidney show atypical malignant cells With dense eosinophilic cytoplasm and pleomorphic nuclei arranged in sheets and solid growth pattern, and figure C elucidates 10X papillary variant of renal cell carcinoma of kidney show atypical malignant papillary architecture composed of fibrovascular core with atypical malignant cells arranged in single layer pattern of growth. In this study, two SNPs RAD18 rs373572 and OGG1 rs1052133 were identified for their association with renal carcinoma. The rs373572 variant did not find a statistically significant association with renal carcinoma (χ^2=1.82, p=0.204), has odds ratio (OR) of 1.645 (95% CI: 0.796~3.399), suggesting no substantial difference in allele distribution between cases and controls. In contrast, the rs1052133 variant in the OGG1 gene revealed a significant protective association (χ^2=8.464, p=0.006), with an OR of 0.3 (95% CI: 0.13–0.689). The C allele was less frequent in cases 15.7% compared to controls 38.4%, refer to a potential protective role of the C allele against RCC table (3).

The genotypes distribution for RAD18 rs373572 GA did not statistically significant relation to RCC (χ^2=4.807, p=0.092). In spite of the heterozygous GA genotype observed more frequently in cases 64.8% than in controls 73%, the AA genotype was exclusively found in the case group 16.2% and didn't find in controls, suggesting a possible but not statistically confirmed trend. The GG genotype was found in 18.9% of cases and 26.9% of controls. Conversely, OGG1 rs1052133 GC exhibit a statistically significant association with RCC (χ^2=10.194, p=0.005). The GG genotype was markedly more prevalent among cases 81.5% than controls 46.1%, while the heterozygous GC genotype and the CC genotype were

Table 1. Distribution of study samples by sex and mean of age

Categories	Case	Control	p
Age	55.75±11.24	31.50±1.99	0.000
Sex Male Female	 27(72.22)% 10(27.77%)	 25 (89.30)% 3(10.70)%	0.093

Table 2. Classification renal carcinoma cases according to the histopathological changes

Cases	Histolopathogical changes		
Grade	I	II	III
	30 (81.08%)	6(16.21%)	1(2.70)
Cell type	Clear	Chromophobe	Papillary
	28(75.67%)	5(13.51%)	4(10.81%)
T	T1	T2	T3
	(a)12 (32.43%) (b)19 (51.35%)	(a)5 (13.51%) (b) 0	(a) 1 (2.70) (b) 0
N	Nx	N0	N1
	37 (100%)	0	0
M	Mx	M0	M1
	37 (100%)	0	0
TNM	T1NxMx	T2NxMx	T3NxMx
	31(83.78%)	5(13.51%)	1(2.70%)

Table 3. RAD18 (rs373572) and OGG1 (rs1052133) Single Locus Association Test in renal carcinoma and control group (odd ratio / p <0.05)

SNP	Chi²	Fisher's p	OR [95% CI]	Detail		
					G	A
rs373572	1.82	0.204	1.645 [0.796~3.399]	Case	38(0.513)	36(0.486)
				Control	33(0.634)	19(0.365)
					G	C
rs1052133	8.464	0.006	0.3 [0.13~0.689]	Case	64(0.842)	12(0.157)
				Control	32(0.615)	20(0.384)

Table 4. RAD18 (rs373572) and OGG1 (rs1052133) genotypes Test in renal carcinoma and control group (odd ratio/p<0.05)

SNP	Chi²	Fisher's p	Detail			
				GA	AA	GG
rs373572	4.8073	0.092	Case	24(0.648)	6(0.162)	7(0.189)
			Control	19(0.73)	0(0)	7(0.269)
				GG	CC	GC
rs1052133	10.194	0.005	Case	30(0.815)	5(0.135)	2(0.054)
			Control	12(0.461)	6(0.23)	8(0.307)

more common in the control group 30.7% and 23%, respectively than in cases 5.2% and 13.1%, respectively. These results suggest that the presence of the C allele may exert a protective effect against renal carcinoma table (4).

According to Hardy-Weinberg Equilibrium (HWE) analysis for RAD18 rs373572 presented no significant deviation in the case group ($\chi^2 = 3.29$, $p = 0.523$) or the control group ($\chi^2 = 8.618$, $p = 0.081$), although the control group approached borderline significance. In the same manner, when both groups were analyzed together, the finding ($\chi^2 = 9.45$, $p = 0.07$) showed statistically non-significant, suggesting that the allele frequencies at this locus are largely stable and consistent with HWE assumptions in the studied population (table 5). On the other hand, the OGG1 rs1052133 polymorphism demonstrated a significant differences from Hardy-Weinberg equilibrium in the case group

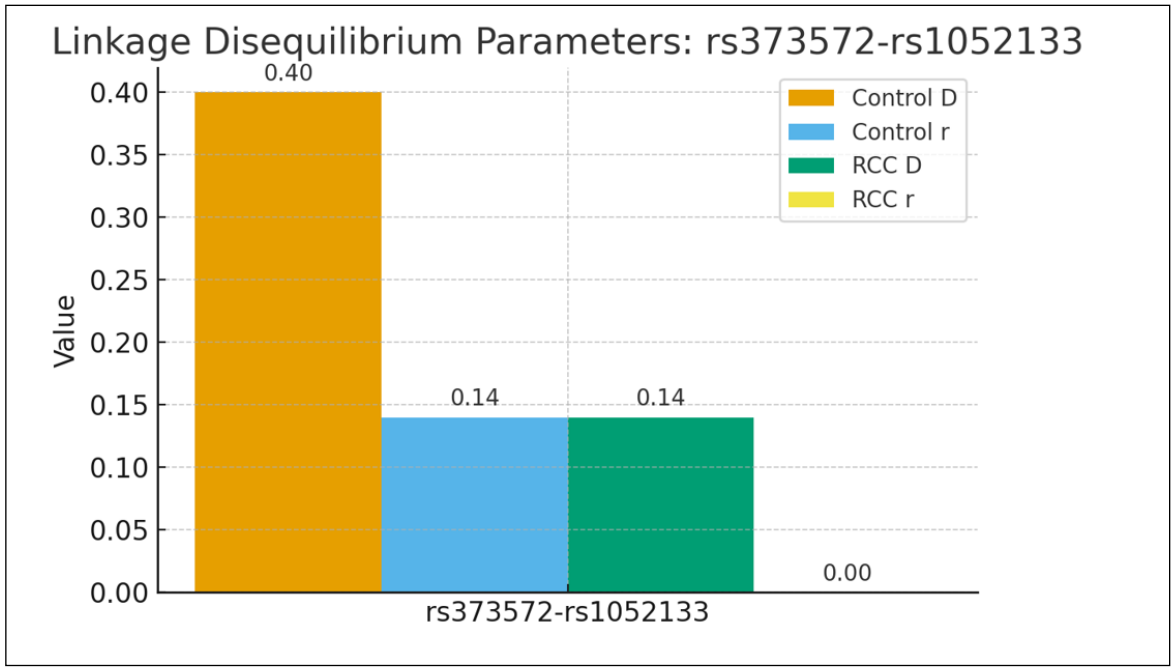

Fig. 2. Linkage Disequilibrium Analysis between RAD 18 (rs373572) and OGG1 (rs1052133) in case and control

Table 5. RAD18 (rs373572) and OGG1 (rs1052133) Hardy-Weinberg Equilibrium Test in renal carcinoma and control group (odd ratio/p<0.05)

SNP	Chi² in case	Fisher's p in case	Chi² in ctrl	Fisher's p in ctrl	Chi² in both	Fisher's p in both
rs373572	3.291	0.523	8.618	0.081	9.457	0.07
rs1052133	24.446	0.003	3.185	0.344	21.777	0.008

Table 6. Gene Interaction Analysis between RAD18 (rs373572) and OGG1 (rs1052133) in renal carcinoma and control group (odd ratio/p<0.05)

SNPs	Case Interaction	Control Interaction	diff	p
rs373572,rs1052133	-0.089	-0.095	0.005	0.456

Table 7. Haplotype Analysis of RAD18 (rs373572) and OGG1 (rs1052133) in renal carcinoma and control group (odd ratio / p <0.05)

Haplotype	Case(freq.)	Control(freq.)	Chi²	Fisher's p	OR [95% CI]
GG	31(0.418)	19(0.365)	0.365	0.583	1.252 [0.603~2.596]
AG	31(0.418)	13(0.25)	3.834	0.059	2.162 [0.992~4.714]
GC	7(0.094)	14(0.269)	6.706	0.014	0.283 [0.105~0.763]
AC	5(0.067)	6(0.115)	0.876	0.359	0.555 [0.16~1.927]

(χ^2 = 24.446, p = 0.003), refer to potential selection pressure or population stratification relation to renal carcinoma. However, the control group exhibited no significant deviation (χ^2 = 3.185, p = 0.344). The analysis of combined group also showed a significant deviation from HWE (χ^2 = 21.777, p = 0.008), reinforcing the link of this SNP with disease status and supporting its potential involvement in RCC susceptibility.

The interaction between SNP–SNP analysis of RAD18 rs373572 and OGG1 rs1052133 showed minimal interaction differences between cases and controls. The interaction coefficient was –0.089 in cases and –0.095 in controls, with a difference of 0.005 (p = 0.456). This statistically non-significant result mean that no synergistic or antagonistic interaction between these two variants in contributing to RCC susceptibility in the current study (table 6).

The haplotype analysis of both SNPs demonstrated variable distribution between cases and controls. The GC haplotype elucidated a significantly lower frequency in cases 0.094 compared to controls 0.269, with a statistically significant association p=0.014 and a protective odds ratio of 0.283 [95% CI: 0.105–0.736], suggesting a protective impact against renal carcinoma. Other haplotypes (GG, AG, and AC) did not report statistically significant differences between groups p>0.05, although the AG haplotype showed a trend toward increased risk (OR=2.162).

Table 8. Association of renal carcinoma cell type (clear, chromophobe and papillary) with RAD 18 (rs373572) and OGG1 (rs1052133) genotyping (chi square, p <0.05)

Genotyping's	Clear	Chromophobe	Papillary	P
RAD18				
GG	5 (20%)	2(40%)	0	0.398
GA	17(68%)	2(40%)	5(71.4%)	
AA	3(12%)	1(20%)	2(28.6%)	
A	0.54	0.6	0.35	0.9364
G	0.46	0.4	0.64	
OGG1				
CC	2(7.7%)	2(50%)	1(14.3%)	0.000056
CG	0	2(50%)	0	
GG	24(92.3%)	0	6(85.7%)	
C	0.07	0.75	0.14	0.52913
G	0.92	0.25	0.85	

Table 9. Association of renal carcinoma grades (I, II and III) with RAD 18 (rs373572) and OGG1 (rs1052133) genotyping (chi square, p <0.05)

Genotyping's	Grade I	Grade II	Grade III	P value
RAD18				
GG	7(22.58%)	0	0	0.00134
GA	22(70.96%)	1(20%)	1(100%)	
AA	2(6.45%)	4(80%)	0	
A	0.58	0.1	0.5	0.7579
G	0.42	0.9	0.5	
OGG1				
CC	3(10%)	2(3.33%)	0	0.58552
CG	2(6.66%)	0	0	
GG	25(83.33%)	4(6.66%)	1(100%)	
C	0.13	0.33	0	0.8067
G	0.86	0.66	1	

Linkage disequilibrium between RAD18 (rs373572) and OGG1 (rs1052133) showed different patterns in case and control groups figure (2). In the case group (Figure 2), LD was weak, with a D' value of 0.14 and r^2 of 0, this mean almost complete recombination and absence of linkage between the two loci. On the other hand, the control group (Figure 2) clarified a moderate level of LD, with D' = 0.4 and r^2 = 0.11, proposed a non-random association between study SNPs. This difference in LD structure between case and controls may reflect underlying genetic instability in RCC or selection pressure influencing haplotype integrity in the diseased state.

ASSOCIATION HISTOLOGICAL CHANGES OF RENAL CARCINOMA AND GENOTYPING OF RAD 18 (RS373572) AND OGG1 (RS1052133)

The genotypic distribution of both SNPs polymorphisms was estimated across RCC histological subtypes including clear cell, chromophobe, and papillary. For the RAD18 gene, the GA genotype was most frequent among clear cell RCC (68%) and papillary RCC (71.4%), whereas chromophobe RCC reported equal frequencies of GG and GA genotypes (40% each). However, the distribution of RAD18 genotypes among the different histological types was not statistically significant (p = 0.398), this mean no clear link between RAD18 variants and RCC subtype. On the other hand, OGG1 genotypic frequencies showed significant variation among RCC subtypes (p = 0.000056). The GG genotype was predominant in clear cell (92.3%) and papillary RCC (85.7%) but entirely absent in chromophobe RCC, which recorded a higher frequency of CC (50%) and CG (50%) genotypes. These results suggest a potential association between OGG1 polymorphism and the chromophobe subtype of RCC, possibly implicating a histotypes-specific role in DNA repair pathways table (8).

Table 10. Association of renal carcinoma classification TNM (T1NxMx T2NxMx and T3NxMx) with RAD 18 (rs373572) and OGG1 (rs1052133) genotyping (chi square, p <0.05)

Genotyping's	T1NxMx	T2NxMx	T3NxMx	P
RAD18				
GG	7(22.58%)	0	0	0.01774
GA	22(70.96%)	1(12.5%)	1(100%)	
AA	3(9.67%)	3(87.5%)	0	
A	0.56	0.13	0.5	0.7947
G	0.43	0.87	0.5	
OGG1				
CC	3(10%)	2(40%)	0	0.43326
CG	2(6.66%)	0	0	
GG	26(86.66)	3(60%)	1(100%)	
C	0.13	0.4	0	0.75109
G	0.87	0.6	1	

Table 11. Association of renal carcinoma classification according to sex (male and female) with RAD 18 (rs373572) and OGG1 (rs1052133) genotyping (chi square, p<0.05)

Genotyping's	Male	Female	P
RAD18			
GG	6(17.64%)	1(10%)	0.686297
GA	17(50%)	7(70%)	
AA	4(11.76)	2(20%)	
A	0.5	0.45	0.94355
G	0.5	0.55	
OGG1			
CC	4(11.76%)	1(10%)	0.71808
CG	1(2.94%)	1(10%)	
GG	22(64.70%)	8(80.0%)	
C	0.16	0.15	0.9819
G	0.83	0.85	

Both genes RAD18 and OGG1 genotypes distribution across RCC grades (I III) showed grade-specific variations in allelic and genotypic appearances. For RAD18, the GA genotype more frequent in grade I (70.96%) and was the only genotype found in grade III (100%), while the AA genotype was most frequent in grade II (80%). The GG genotype was not found in grade II and III. This distribution reported significant association with grade p=0.00134, imply a potential impact of RAD18 polymorphism in disease development. Whereas, OGG1 genotype frequencies did not exhibit significant variation regarding grades p=0.58552. The GG genotype remained predominant in all grades, especially in grade I and III (83.33%, 100%). The CC genotype found in grade I and II (10%, 33.3%), while CG was only found in grade I (6.66%). Allelic frequencies of both SNPs recorded no significant difference across tumor grades (RAD18 A allele p = 0.7579; OGG1 C allele p = 0.8067), this mean that while RAD18 genotypes may correlate with progression, allelic distribution alone may not be predictive table (9).

The RAD18 genotypes distribution across tumor grades demonstrated a statistically significant association (p = 0.01774). In the T1NxMx stage, the most frequent genotype was GA (70.96%), followed by GG (22.58%) and AA (9.67%). While, the T2NxMx stage clarified a predominance of the AA genotype 87.5%, with only one case carrying GA (12.5%) and none with GG. Notably, all cases in the T3NxMx stage carried the GA genotype 100%. The allele frequencies further supported this trend, with a higher A allele frequency in T1 and T3 stages, while the G allele was predominant in T2NxMx. Regarding the OGG1 gene, no statistically significant link was detected among tumor stages p=0.43326. In T1NxMx, GG was the dominant genotype 86.66%, followed by CC (10%) and CG (6.66%). In T2Nx-

Mx, 60% of individuals had the GG genotype and 40% had CC, with no heterozygous (CG) cases. In T3NxMx, only one case was present, carrying the GG genotype (100%). Allelic distribution showed a predominance of the G allele in all stages, especially in T3NxMx (100%) table (10).

The RAD18 and OGG1 genotypes distribution was detected according to sex. For the RAD18 gene, the most frequent genotype in both sexes was the heterozygous GA, found in 50.0% of males and 70.0% of females. The homozygous wild-type GG genotype appeared in 17.64% of males and 10.0% of females, whereas the mutant homozygous AA genotype was observed in 11.76% of males and 20.0% of females. Although of these differences in frequency, there was no significant link between RAD18 genotypes and gender p=0.686. Allelic distribution for RAD18 also reported comparable proportions between sexes: the A allele was found in 50.0% of males and 45.0% of females, while the G allele appeared in 50.0% of males and 55.0% of females. No significant gender-based difference was observed in allele frequency p=0.943. For the OGG1 gene, the GG genotype was the most common among both sexes, present in 64.70% of males and 80.0% of females. The CC genotype was observed in 11.76% of males and 10.0% of females, and the heterozygous CG genotype observed at lower frequencies (2.94% in males and 10.0% in females). There was no significant variation in genotype distribution between males and females p=0.718. Allele frequencies for OGG1 were also similar across sexes, with the G allele found in 83.0% of males and 85.0% of females, and the C allele in 16.0% and 15.0%, respectively. These differences were non-significant p=0.982 table (11).

DISCUSSION

The results revealed a significant association between cases age and the incidence of RCC. In addition, the male-to-female ratio was 2.6, indicating a higher prevalence of RCC among males compared to females, these finding are consistent with previous studies showing sex-based differences in the incidence of non-reproductive tumors. Especially, males are approximately twice as likely to develop kidney cancer and present higher mortality rates as female [27], potentially according to the protective role of sex hormones in females [28]. This findings further reported by an earlier study [29] found that females had slower tumor development and a 19% lower risk of RCC-specific mortality compared to males. furthermore, a correlation between age and sex in RCC incidence has also been illustrated in previous investigation [30]. Highlights neoplastic cells with clear cytoplasm arranged in a nested pattern interspersed with blood vessels [29]. RCC is not a uniform disorder but encompasses a group of histologically distinct tumors, each with unique clinical behavior, genetic variation, and therapeutic responses. In one report involving 843 RCC cases, the distribution included 488 clear cell, 274 papillary, and 81 chromophobe subtypes. The analyses of Genomic and phenotypic of these subtypes have presented distinct molecular profiles, demonstrate the progression of subtype-specific management and medication strategies [31]. Understanding the shared and unique features among RCC subtypes is essential for detection different processing and improving targeted therapies. Additionally, some reports propose that cancer cells reprogram their metabolism to improve cellular survival and proliferation, introduce novel diagnostic and medication targets. RCC subtypes metabolic network analyses have been suggested to uncover system-level alterations and detection potential metabolic markers for precise intervention strategies [32]. Despite of the RAD18 gene has not been extensively validated in the context of RCC, its role in DNA lesion tolerance and post-replication repair suggested a potential intervention to renal tumorigenesis. RAD18 encodes an E3 ubiquitin ligase critical for monoubiquitination of proliferating cell nuclear antigen, enhance translation synthesis and enabling replication past DNA injury [33]. Variations or Mutations in RAD18 gene may effect this function, leading to replication fork stalling, increased mutational burden, and instability genomic hallmarks of tumor development. In this study, certain RAD18 genotypes (especially GA and AA) demonstrate significant relationships with histological subtypes and stages of tumor, referring a potential impact in disease development. Moreover, RAD18 has been involved in oxidative DNA lesions response, which is especially relevant in RCC, a cancer highly influenced by hypoxia and oxidative stress [34]. The outputs focusing on the essential genetic contributions of DNA repair gene variations in RCC etiology and progression. The rs1052133 (Ser326Cys) polymorphism found to be affected in enzymatic function and genomic stability. The histological association to chromophobe RCC, where C alleles were more found, propose OGG1 variation may have a distinct role in tumors of different origins, likely due to differences in oxidative index susceptibility. On the other hand RAD18 is involved in post-replication repair by ubiquitination of PCNA, trigger translation DNA synthesis. In spite of no overall relation to RCC risk was observed, the AA genotype illustrated exclusive presence in stage II tumors, while GA was predominant in stages I and III, implying a potential link to tumor development rather than initiation. Other literatures

have linked RAD18 variation with other malignancies, such as esophageal and colorectal cancer, though data on RCC is limited [35-36]. The significant deviation from HWE in the case group of OGG1 imply selection pressure or disease linkage, supporting a functional impact of these alleles in RCC progression. The GC haplotype (RAD18 G + OGG1 C) was significantly less frequent in patients, indicating a protective synergistic effect. While, LD patterns differed between studies groups, stronger in controls possibly due to loss of LD in case associated genomic instability, a known phenomenon in tumorigenesis [37]. Regarding No sex-related differences in genotype or allele frequencies, aligning with prior RCC reports suggesting genetic risk factors act independently of sex, while hormonal or environmental factors may modulate expression [38]. Genetic variation like Ser326Cys (rs1052133) have been found to decrease OGG1 activity, defecting DNA repair and promoting mutagenesis. In this study, the GG genotype was predominant, but change genotype observation in specific histological and clinical subgroups propose a modulatory impact in RCC risk and development. Other studies prove the potential effect of OGG1 polymorphism in renal carcinogenesis like, Audebert et al. [39] observed OGG1 mutations in RCC tissues, highlighting a possible link between impaired DNA repair and renal cancer development. These outputs collectively underscore the relevance of genomic maintenance processing, especially DNA repair mechanisms, in RCC pathophysiology. The correlation of RAD18 and OGG1 variations with tumor stage and histological subtypes highlights their potential as markers for RCC progression and prognosis. Moreover, the interaction between DNA repair deficiency and oxidative index represents a promising area for medications targeting, particularly considering the kidney high susceptibility to oxidative damage.

CONCLUSIONS

The OGG1 rs1052133 variation is significantly related to a reduced risk of renal carcinoma and point to histological subtype specificity, while RAD18 rs373572 may influence tumor development rather than susceptibility. These findings prove a role for DNA repair gene variants especially OGG1 in modulating RCC risk and progression, providing a potential avenue for genetic screening and personalized risk assessment in RCC.

REFERENCES

1. Hsieh JJ, Purdue MP, Signoretti S, Swanton C, et al. Renal Cell Carcinoma. Nat Rev Dis Primers. 2017;9:3:17009. doi:10.1038/nrdp.2017.9. DOI
2. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2019. CA A. Cancer J Clin. 2019;69 (1):7-34. doi:10.3322/caac.21551 DOI
3. Gourley C, Balmaña J, Ledermann JA, Serra V, Dent R, Loibl S, et al. Moving from Poly (ADP-Ribose) Polymerase Inhibition to Targeting DNA Repair and DNA Damage Response in Cancer Therapy. J Clin Oncol. 2019; 37(25): 2257-2269. doi:10.1200/jco.18.02050. DOI
4. Gavande NS, Vander Vere-Carozza PS, Hinshaw HD, Jalal SI, et al. DNA Repair Targeted Therapy: The Past or Future of Cancer Treatment? Pharmacol. Ther. 2016;160:65-83. doi:10.1016/j.pharmthera.2016.02.003. DOI
5. Brown JS, O'Carrigan B, Jackson SP, Yap TA. Targeting DNA Repair in Cancer: Beyond PARP Inhibitors. Cancer Discov. 2017;7(1):20-37. doi:10.1158/2159-8290.cd-16-0860. DOI
6. American Cancer Society: Cancer Facts and Figures 2024. American Cancer Society, 2024. https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/2024-cancer-facts-figures.html (Access: June 21, 2024).
7. DeVita VT Jr, Lawrence TS, Rosenberg SA, et al. (eds). DeVita, Hellman, and Rosenberg's Cancer: Principles & Practice of Oncology. 11th edn. Wolters Kluwer, 2019.
8. Shuch B, Vourganti S, Ricketts CJ, et al. Defining early-onset kidney cancer: implications for germline and somatic mutation testing and clinical management. J Clin Oncol. 2014;32(5):431-7. doi: 10.1200/JCO.2013.50.8192. DOI
9. Gudbjartsson T, Jónasdóttir TJ, Thoroddsen A, et al. A population-based familial aggregation analysis indicates genetic contribution in a majority of renal cell carcinomas. Int J Cancer. 2002;100(4): 476-9. doi: 10.1002/ijc.10513. DOI
10. Carlo MI, Mukherjee S, Mandelker D, et al. Prevalence of Germline Mutations in Cancer Susceptibility Genes in Patients with Advanced Renal Cell Carcinoma. JAMA Oncol. 2018;4(9):1228-1235. doi: 10.1001/jamaoncol.2018.1986. DOI
11. Hartman TR, Demidova EV, Lesh RW, et al. Prevalence of pathogenic variants in DNA damage response and repair genes in patients undergoing cancer risk assessment and reporting a personal history of early-onset renal cancer. Sci Rep. 2020;10(1):13518. doi: 10.1038/s41598-020-70449-5. DOI
12. Abou Alaiwi S, Nassar AH, Adib E, et al. Trans-ethnic variation in germline variants of patients with renal cell carcinoma. Cell Rep. 2021;34(13):108926. doi: 10.1016/j.celrep.2021.108926. DOI
13. Nguyen KA, Syed JS, Espenschied CR, et al. Advances in the diagnosis of hereditary kidney cancer: Initial results of a multigene panel test. Cancer. 2017;123(22):4363-4371. doi: 10.1002/cncr.30893.

14. Smith PS, West H, Whitworth J, et al. Pathogenic germline variants in patients with features of hereditary renal cell carcinoma: Evidence for further locus heterogeneity. Genes Chromosomes Cancer. 2021;60(1):5-16. doi: 10.1002/gcc.22893. DOI
15. Heinen CD, Schmutte C, Fishel R. DNA repair and tumorigenesis: lessons from hereditary cancer syndromes. Cancer Biol Ther. 2002;5:477–85. doi: 10.4161/cbt.1.5.160. DOI
16. Lovett ST. Polymerase switching in DNA replication. Mol Cell. 2007;27:523-6. doi: 10.1016/j.molcel.2007.08.003. DOI
17. Callegari AJ, Kelly TJ. Shedding light on the DNA damage checkpoint. Cell Cycle. 2007; 6: 660–6. doi: 10.4161/cc.6.6.3984. DOI
18. Xin H, Lin W, Sumanasekera W, Zhang Y, Wu X, Wang Z. The human RAD18 gene product interacts with HHR6A and HHR6B. Nucleic Acids Res. 2000;28:2847-54. doi: 10.1093/nar/28.14.2847. DOI
19. Watanabe K, Tateishi S, Kawasuji M, Tsurimoto T, Inoue H, Yamaizumi M. Rad18 guides poleta to replication stalling sites through physical interaction and PCNA monoubiquitination. EMBO J. 2004;23:3886-96. doi: 10.1038/sj.emboj.7600383. DOI
20. Wang R, Hao W, Pan L, Boldogh I, Ba X. The roles of base excision repair enzyme OGG1 in gene expression. Cell Mol Life Sci. 2018;75(20):3741-3750. doi: 10.1007/s00018-018-2887-8. DOI
21. Ba X, Bacsi A, Luo J, et al. 8-oxoguanine DNA glycosylase-1 augments proinflammatory gene expression by facilitating the recruitment of site-specific transcription factors. J Immunol. 2014;192(5):2384-2394. doi: 10.4049/jimmunol.1302472. DOI
22. Wang Y, Chen T, Pan Z, et al. 8-Oxoguanine DNA glycosylase modulates the cell transformation process in pulmonary fibrosis by inhibiting Smad2/3 and interacting with Smad7. FASEB J. 2020; 34(10): 13461–13473. doi: 10.1096/fj.201901291RRRRR. DOI
23. Audebert M, Chevillard S, Levalois C, et al. Alterations of the DNA repair gene OGG1 in human clear cell carcinomas of the kidney. Cancer Res. 2000;60(17):4740-4744.
24. Corredor Z, da Silva Filho MI, Rodríguez-Ribera L, et al. Loci associated with genomic damage levels in chronic kidney disease patients and controls. Mutat Res Genet Toxicol Environ Mutagen. 2020; 852. doi: 10.1016/j.mrgentox.2020.503167. DOI
25. Das S, Purkayastha S, Roy H, Sinha A, Choudhury Y. Polymorphisms in DNA repair genes increase the risk for type 2 diabetes mellitus and hypertension. Biomol Concepts. 2018 Jun9;9(1):80-93. doi: 10.1515/bmc-2018-0008. PMID: 29886452. DOI
26. Niwa Y, Matsuo K, Ito H, Hirose K, Tajima K, et al. Association of XRCC1 Arg399Gln and OGG1 Ser326Cys polymorphisms with the risk of cervical cancer in Japanese subjects. Gynecol Oncol. 2005;99:43-9. doi: 10.1016/j.ygyno.2005.05.018. DOI
27. Peired AJ, Campi R, Angelotti ML, Antonelli G, et al. Sex and Gender Differences in Kidney Cancer: Clinical and Experimental Evidence. Cancers (Basel). 2021 Sep 13;13(18):4588. doi: 10.3390/cancers13184588. DOI
28. Hew MN, Zonneveld R, Kummerlin IP, Opondo D, de la Rosette JJ, Laguna MP. Age and gender related differences in renal cell carcinoma in a European cohort. J Urol. 2012;188:33-38. doi: 10.1016/j.juro.2012.02.2573. DOI
29. Rampersaud EN, Klatte T, Bass G, Patard JJ, et al. The effect of gender and age on kidney cancer survival: younger age is an independent prognostic factor in women with renal cell carcinoma. Urol Oncol. 2014 Jan;32(1):30.e9-13. doi: 10.1016/j.urolonc.2012.10.012. DOI
30. Qu Y, Chen H, Gu W, et al. Age-Dependent Association between Sex and Renal Cell Carcinoma Mortality: a Population-Based Analysis. Sci Rep. 2015;5:9160. doi: 10.1038/srep09160. DOI
31. Ricketts CJ, De Cubas AA, Fan H, Smith CC, et al. Cancer Genome Atlas Research Network; Spellman PT, Rathmell WK, Linehan WM. The Cancer Genome Atlas Comprehensive Molecular Characterization of Renal Cell Carcinoma. Cell Rep. 2018 Apr 3;23(1):313-326.e5. doi: 10.1016/j.celrep.2018.03.075. DOI
32. Pandey N, Lanke V, Vinod PK. Network-based metabolic characterization of renal cell carcinoma. Sci Rep. 2020;10:5955. doi: 10.1038/s41598-020-62853-8. DOI
33. Lou J, Yang Y, Gu Q, Price BA, Qiu Y, et al. Rad18 mediates specific mutational signatures and shapes the genomic landscape of carcinogen-induced tumors in vivo. NAR Cancer. 2021;3(1). doi: 10.1093/narcan/zcaa037. DOI
34. Yang Y, Durando M, Smith-Roe SL, Sproul C, Greenwalt AM, Kaufmann W, Oh S, Hendrickson EA, Vaziri C. Cell cycle stage-specific roles of Rad18 in tolerance and repair of oxidative DNA damage. Nucleic Acids Res. 2013 Feb 1;41(4):2296-312. doi: 10.1093/nar/gks1325. DOI
35. Kanzaki H, Ouchida M, Hanafusa H, Sakai A, Yamamoto H, Suzuki H, Yano M, Aoe M, Imai K, Date H, Nakachi K, Shimizu K. Single nucleotide polymorphism in the RAD18 gene and risk of colorectal cancer in the Japanese population. Oncol Rep. 2007 Nov;18(5):1171-5. PMID: 17914568.
36. Li P, He C, Gao A, Yan X, Xia X, Zhou J, Wu J. RAD18 promotes colorectal cancer metastasis by activating the epithelialmesenchymal transition pathway. Oncol Rep. 2020 Jul;44(1):213-223. doi: 10.3892/or.2020.7590. DOI
37. Slatkin M. Linkage disequilibrium - understanding the evolutionary past and mapping the medical future. Nat Rev Genet. 2008;9(6):477-85. doi: 10.1038/nrg2361. DOI
38. Lipworth L, Tarone RE, McLaughlin JK. The epidemiology of renal cell carcinoma. J Urol. 2006;176(6 Pt 1):2353-8. doi: 10.1016/j.juro.2006.07.130. DOI
39. Audebert M, Chevillard S, Levalois C, Gyapay G, et al. Alterations of the DNA Repair Gene OGG1 in Human Clear Cell Carcinomas of the Kidney1. Cancer Res. 2000;60(17):4740-4744.

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CORRESPONDING AUTHOR
Mona N. Al-Terehi
Biology Department, College of Science
University of Babylon,
Hilla, Iraq
e-mail: sci.muna.najah@uobabylon.edu.iq

ORCID AND CONTRIBUTIONSHIP
Iftikhar Khdhair Abbas Altemimi: 0009-0000-2018-1951 B C D
Binan Adil Mohammed Ameen: 0009-0000-0694-0771 C D E
Mona N. Al-Terehi: 0000-0002-9244-6709 A F
Liwaa Mahdi Hussein: 0000-0002-2310-2419 D E

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

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ORIGINAL ARTICLE

CONTENTS

Dynamics of cadets' morphofunctional development in the process of their academic training for law enforcement activities

Zoriana R. Kisil[1], Natalia E. Miloradova[2], Halyna V. Katolyk[1], Volodymyr P. Ostapovich[3], Sergii M. Myronets[4], Ihor Y. Hnyp[1], Ivan M. Okhrimenko[5]

[1]LVIV STATE UNIVERSITY OF INTERNAL AFFAIRS, LVIV, UKRAINE
[2]KHARKIV NATIONAL UNIVERSITY OF INTERNAL AFFAIRS, KHARKIV, UKRAINE
[3]VOLYN INSTITUTE NAMED AFTER VYACHESLAV LYPYNSKY, PRIVATE JOINT-STOCK COMPANY «HIGHER EDUCATIONAL INSTITUTION» «INTERNATIONAL ACADEMY OF PERSONNEL MANAGEMENT», LUTSK, UKRAINE
[4]STATE UNIVERSITY OF TRADE AND ECONOMICS, KYIV, UKRAINE
[5]NATIONAL ACADEMY OF INTERNAL AFFAIRS, KYIV, UKRAINE

ABSTRACT

Aim: To study the dynamics of morphofunctional development indicators in female cadets – future law enforcement officers – in the process of engaging in various types of motor activity during their academic training.

Materials and Methods: The research involved 56 female cadets in their first to third training years. Two groups of female cadets were formed: an experimental group (EG, n = 27), whose members attended the university's CrossFit sports club, and a control group (CG, n = 29), whose members did not engage in additional sports activities, but only participated in the traditional program during their sporting and mass-participation activities (SMPAs). Research methods: theoretical methods, biomedical methods, statistical methods.

Results: It was found that in female cadets who practiced CrossFit, all the parameters studied were significantly ($p \leq 0.05$-0.001) better at the end of the research than in female cadets who practiced using the traditional SMPAs method by the BMI – 1.07 kg/m^2, the SI – 4.36 %, the VI – 4.14 ml/kg, the RI – 2.99 c. u., HRRI – 7.4 s, HSTI – 6.8 c. u., PWC – 103.1 kgm/min, BF – by 0.4 %, and SHL – by 3.16 points. The analysis of the dynamics of the studied indicators during the academic training period showed that in the EG, unlike the CG, all studied indicators have a stable tendency to improve.

Conclusions: The data we obtained indicate that CrossFit training sessions effectively contribute to the improvement of the morphofunctional development of female cadets during their academic training for future law enforcement activities.

KEY WORDS: morphofunctional development, health, female cadets, CrossFit, law enforcement activity

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INTRODUCTION

Ukraine's European integration requires the establishment of appropriate standards for the functioning of state institutions and society, including ensuring equal rights and opportunities for women and men in Ukraine's law enforcement agencies and departments [1]. According to statistics, more than 20 % of law enforcement officers in the National Police of Ukraine are women [2]. This trend has been positive in recent years: as in many countries worldwide, Ukraine has seen a significant increase in the number of women in the police force. This is particularly evident in higher educational institutions with specific learning environment (HEIs with SLE), where future police officers are trained for law enforcement activities.

At the same time, the crime situation in the country and the legal regime of martial law require female law enforcement officers to continually enhance their mental and physical training for law enforcement activities under extreme conditions [3, 4]. According to many scientists [5-7], the issue of women's physical readiness for law enforcement activities is particularly acute, in particular during the forcible detention of offenders, the use of physical force, the pursuit of criminals, and other specific conditions of law enforcement activities. Taking into account the physiological and psychological characteristics of the female body, to develop the physical readiness of female cadets for their future professional activities, it is necessary to apply such means of physical training in HEIs with SLE that would simultaneously contribute to the comprehensive

development and improvement of the physical qualities of female cadets, improve their morphological and functional development, promote their health, and be modern, engaging, and accessible to women with different levels of physical fitness, which can be applied in various climatic conditions and training locations, taking into account the legal regime of martial law in Ukraine. According to several scientists [8-10], CrossFit is one of the modern training methods used to train police officers, both men and women, in many countries worldwide.

CrossFit for women is a high-intensity functional training program that combines strength training, cardio exercises, and gymnastics to develop strength, endurance, speed, agility, and flexibility, promoting rapid fat burning and the formation of a harmonious body. It is versatile, suitable for various training levels, and helps improve the figure and overall tone in women [11]. An interesting fact is that CrossFit was created by Greg Glassman and his wife Lauren Jenney in the United States in 2000, specifically for training police officers and firefighters, since the main idea of CrossFit training is to prepare the body for unpredictable situations, which is very important for representatives of law enforcement agencies and departments [12, 13]. According to experts [14], the advantages of CrossFit for female law enforcement officers are: high efficiency and versatility; development of all physical qualities; rapid weight loss due to intense training and a change in appearance; strengthening of the cardiovascular and respiratory systems; improvement of overall physical condition and well-being, increased stress resilience; CrossFit training sessions do not take much time and do not require special conditions for training. Considering that female law enforcement officers are physically inferior to men, which makes them more vulnerable when performing forceful actions in specific law enforcement conditions, many experts [15-18] believe that CrossFit training sessions during their academic training at HEIs with SLE will significantly improve the physical and psychological readiness of female cadets for their future professional activities.

AIM

The aim is to study the dynamics of morphofunctional development indicators in female cadets – future law enforcement officers – in the process of engaging in various types of motor activity during their academic training.

MATERIALS AND METHODS

PARTICIPANTS

The research, conducted in 2022-2025, involved 56 female cadets in their first to third training years at Lviv State University of Internal Affairs (LSUIA, Lviv, Ukraine), majoring in "Law" specialty. The female cadets were aged 17-22. Two groups of female cadets were formed: an experimental group (EG, n = 27), whose members attended the university's CrossFit sports club, and a control group (CG, n = 29), whose members did not engage in additional sports activities, but only participated in the traditional program during their sporting and mass-participation activities (SMPAs). The number of hours of motor activity per week for female cadets in both groups was the same: 4 hours of training sessions (2 sessions of 2 hours each) in physical training and 6 hours (3 sessions of 2 hours each) during their SMPAs (training sessions according to the traditional program or training sessions in the CrossFit sports club). The differences that formed the basis of the comparative analysis between the EG and the CG indicators were the content and focus of motor activity training sessions. The training sessions in the EG were conducted by a CrossFit coach using individual and group formats. In contrast, in the CG, they were conducted by a curator (training-year officer) using exclusively group formats based on established options (accelerated movement, exercises on gymnastics equipment, general development exercises). The inclusion criteria for participants were as follows: only female cadets who voluntarily selected a motor activity could participate; there were no health contraindications to participating in any motor activity. The exclusion criterion was female cadets' desire to withdraw from the research at any time. Female cadets from all groups were informed about the objectives of the research, after which they gave their written consent to participate in the research solely for scientific purposes. The research was conducted in three stages: the first at the end of the first training year, the second at the end of the second training year, and the third at the end of the third training year.

RESEARCH METHODS

Theoretical methods, biomedical methods, statistical methods. Theoretical methods were used to conduct the analysis, generalization and analytical review of literature sources on the outlined range of issues (28 sources (2005-2026) from MedLine, Scopus, Web of Science databases were analyzed).

Biomedical methods were used to investigate the effect of CrossFit training sessions compared to a traditional program during SMPAs. The following indicators were studied: body mass index (BMI), vital index (VI), strength index (SI), and Robinson index (RI), heart rate recovery time index (HRRTI), Harvard Step Test Index (HSTI), physical working capacity (PWC), subcutaneous fat level (body fat content (BF)), somatic health level (SHL).

Table 1. The dynamics of the morphofunctional development indicators of female cadets in the EG (n=27) and the CG (n=29) during the research period (X±m)

Research stages	EG (n=27)	CG (n=29)	Significance of the difference	
			t	p
BMI, kg/m^2				
1st	21.35±0.25	21.21±0.21	0.43	p>0.05
2nd	21.16±0.24	21.67±0.23	1.44	p>0.05
3rd	21.05±0.22	22.12±0.29	2.94	p≤0.01
t_{1-3} (p)	0.57 (p>0.05)	2.54 (p≤0.05)		
SI, %				
1st	48.51±1.13	48.34±1.24	0.10	p>0.05
2nd	52.12±1.07	50.19±1.18	1.21	p>0.05
3rd	55.82±0.98	51.46±1.11	2.94	p≤0.05
t_{1-3} (p)	4.89 (p≤0.001)	1.87 (p>0.05)		
VI, ml/kg				
1st	49.19±1.23	49.63±1.30	0.25	p>0.05
2nd	52.37±1.19	49.67±1.27	1.55	p>0.05
3rd	54.16±1.18	50.02±1.22	2.44	p≤0.05
t_{1-3} (p)	2.92 (p≤0.05)	0.34 (p>0.05)		
RI, c. u.				
1st	89.05±0.98	89.25±1.02	0.14	p>0.05
2nd	86.37±0.96	88.06±1.03	1.20	p>0.05
3rd	83.72±0.94	86.71±1.05	2.12	p≤0.05
t_{1-3} (p)	3.93 (p≤0.001)	1.74 (p>0.05)		
HRRTI, s				
1st	103.6±2.51	102.5±2.49	0.31	p>0.05
2nd	94.6±2.45	98.6±2.52	1.14	p>0.05
3rd	89.4±2.23	96.8±2.50	2.21	p≤0.05
t_{1-3} (p)	3.56 (p≤0.001)	1.62 (p>0.05)		
HSTI, c. u.				
1st	63.5±1.49	65.2±1.65	0.77	p>0.05
2nd	75.8±1.42	70.5±1.56	2.51	p≤0.05
3rd	83.2±1.41	76.4±1.50	3.30	p≤0.01
t_{1-3} (p)	9.91 (p≤0.001)	5.02 (p≤0.001)		
PWC_{170}, kgm/min				
1st	664.6±32.11	673.2±40.25	0.17	p>0.05
2nd	707.2±29.27	675.4±37.82	0.66	p>0.05
3rd	790.6±28.25	687.5±33.59	2.35	p≤0.05
t_{1-3} (p)	3.21 (p≤0.01)	0.48 (p>0.05)		
BF, %				
1st	18.3±0.13	18.5±0.17	0.93	p>0.05
2nd	18.1±0.12	18.3±0.16	0.96	p>0.05
3rd	18.0±0.08	18.4±0.14	2.48	p<0.05
t_{1-3} (p)	1.90 (p>0.05)	0.44 (p>0.05)		
SHL, points				
1st	4.08±0.71	4.19±0.87	0.11	p>0.05
2nd	6.18±0.55	5.45±0.71	0.81	p>0.05
3rd	9.29±0.48	6.13±0.65	3.91	p≤0.05
t_{1-3} (p)	6.08 (p≤0.001)	1.79 (p>0.05)		

Note: X – arithmetic mean; m – error of arithmetic mean; t – Student's test value; p – level of significance of the differences between studied indicators
Source: compiled by the authors of this study

The body mass index was calculated as body weight divided by height squared. The vital index was determined by the ratio of vital lung capacity to body weight. The ratio of the dynamometry of the stronger hand to body weight determined the strength index. The Robinson index was determined by the ratio of the product of the resting pulse and systolic blood pressure to 100. The heart rate recovery time index was calculated as the time required for the heart rate to return to baseline after 20 squats in 30 seconds. The somatic health level (SHL) of female cadets was determined by the sum of points for each of the five above-mentioned indices and was assessed as follows: 3 points or less – low SHL; 4-6 points – below average SHL; 7-11 points – average SHL; 12-15 points – below average SHL; 16-18 points – high SHL [19].

The Harvard Step Test Index allows assessing the functional state of the cardiorespiratory system and, in general, the level of physical fitness. In our research, we used a 3-minute HSTI with a 45 cm high step. The index was determined in c. u. according to the formula: $HSTI = (300 / (f2 + f3 + f4)) \times 100$, where f2, f3, and f4 are the HR indicators for 30 seconds at the 2nd, 3rd, and 4th minutes of rest. The assessment of the functional capacity of female cadets using a 3-minute step test was carried out as follows: 90 c. u. and above – excellent level of fitness; 80-89 – high level; 65-79 – average; 55-64 – below average; 55 and below – unsatisfactory level [20].

The physical working capacity of female cadets was determined using the PWC_{170} test with a bicycle ergometer. The female cadets performed two consecutive pedaling exercises (at a pace of 60 rpm) on a bicycle ergometer for 5 minutes each, with a 3-minute rest interval between them. In the last 30 seconds of the fifth minute of each exercise, the heart rate was counted. The power of the first and second exercises was determined using tables. The PWC_{170} value was calculated using the formula: $PWC_{170} = N1 + (N2 - N1) \cdot [(170 - f1) / (f2 - f1)]$, where N1 is the power of the first load (determined according to the table depending on the body weight of the study participant); N2 is the power of the second load (determined according to the table depending on N1); f1, f2 – HR at the end of the 1st and 2nd loads in 30 seconds [21].

Body fat content (in percent) was assessed using a caliper. Fat fold thickness was measured at four locations on the body: abdomen, front of the shoulder (biceps), back of the shoulder (triceps), and back under the shoulder blade. Based on the sum of these four measurements, a result was obtained, which was compared with the Jackson-Pollock table data [22], and the body fat content was assessed. The norm for women under 20 years of age is considered to be a fat content of 17.7 %, and for women under 25 years of age, 18.4 %.

STATISTICAL METHODS

Statistical methods 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 LSUIA. 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

The results of the study of the dynamics of the morphofunctional development indicators of female cadets in the EG and the CG during the research period are presented in Table 1.

The study of the BMI in female cadets of the EG and the CG shows that at the 1st stage, the indicators of both groups did not differ significantly ($p > 0.05$). At the 2nd stage, a difference was observed in the BMI indicators between female cadets in the EG and the CG, although it was not statistically significant ($p > 0.05$). Only at the 3rd stage of the research the BMI indicators in female EG cadets differ significantly from those in the CG, by 1.07 kg/m^2 ($p \leq 0.05$). During the research, the BMI in female cadets of the EG did not change ($p > 0.05$), while in the CG it significantly worsened by 0.91 kg/m^2 ($p \leq 0.01$), which indicates the advantage of CrossFit training sessions compared to the traditional SMPAs program in terms of improving the anthropometric data of female cadets.

The analysis of the SI, which reflects the development of strength qualities in female cadets in relation to their body weight, showed that at the 1st and 2nd stages, no

significant difference was found between the EG and the CG ($p > 0.05$). At the 3rd stage, the SI indicators in female cadets of the EG were significantly better than in the CG by 4.36 % ($p \leq 0.05$). The dynamics of the SI among female cadets in both groups are positive. Still, CrossFit has a more pronounced effect on the development of strength qualities in female cadets of the EG: in the EG, the SI indicators improved significantly by 7.31 % ($p \leq 0.001$), and in the CG, by 3.12 %, but the difference is not significant ($p > 0.05$). This underscores the effectiveness of CrossFit training sessions in enhancing the physical development of female cadets.

The study of the VI, which characterizes the functional capabilities of the respiratory system of female cadets, shows that the indicators of the EG and the CG at the 1st and 2nd stages are significantly the same ($p > 0.05$). At the 3rd stage, a significant difference was found between the VI indicators in female cadets of the EG and the CG – 4.14 ml/kg ($p \leq 0.05$). During the research period, the average VI value in female cadets in the EG improved by 4.97 ml/kg ($p \leq 0.05$), and in the CG by 0.61 ml/kg ($p > 0.05$). All of this underscores the advantage of CrossFit training sessions over the traditional SMPA program in improving the functional capabilities of the respiratory system of future law enforcement officers.

When studying the RI indicators characterizing the functioning of the cardiovascular system in female cadets, no significant difference between the EG and the CG indicators was observed at the 1st and 2nd stages of the research ($p > 0.05$). At the 3rd stage, the RI indicators in the EG were significantly ($p \leq 0.05$) better than in the CG, by 2.99 c. u. During the academic training period, the RI indicators in both groups improved, but in the EG the changes were significant ($p \leq 0.001$) and amounted to 5.33 c. u., while in the CG they were not significant ($p > 0.05$) and amounted to 2.54 c. u.

Analysis of the HRRI after 20 squats over 30 minutes showed that, at the 1st and 2nd stages, the HRRI indicators for female cadets in the EG and the CG did not differ significantly ($p > 0.05$). A significant improvement in the HRRI was observed in female cadets of the EG at the 3rd stage of the research. This value is 89.4 s and is significantly better than that of the CG (96.8 s), by 7.4 s ($p \leq 0.05$). During the research period, the HRRI improved by 14.2 s ($p \leq 0.001$) in the EG and by 5.7 s ($p > 0.05$) in the CG, which proves the effectiveness of CrossFit training sessions in strengthening the cardiovascular system of female cadets.

The functional capabilities of the cardiorespiratory systems of female cadets in the EG and the CG were also assessed using the 3-minute Harvard Step Test Index. At the 1st stage, there were no significant differences between them ($p > 0.05$). In the subsequent stages, the HSTI indicators in the EG were significantly better than those in the CG, by 5.3 c. u. in the 2nd stage ($p \leq 0.05$) and by 6.8 c. u. in the 3rd stage ($p \leq 0.01$), respectively. During the research period, the indicators improved significantly in both groups ($p \leq 0.001$), but in the EG the increase was 19.7 c. u., and in the CG it was 11.2 c. u.

To determine the physical working capacity of female cadets, we used the PWC_{170} test. Thus, the analysis of physical working capacity indicators at the 1st and 2nd stages of the research showed that the EG and the CG indicators did not differ significantly ($p > 0.05$). At the 3rd stage, the EG indicators were substantially better than those of the CG, by 103.1 kgm/min ($p \leq 0.05$). During the research period, the PWC_{170} indicators for female cadets in both groups improved; however, the changes in the EG were significant ($p \leq 0.01$), whereas in the CG they were not significant ($p > 0.05$). This indicates that CrossFit training sessions are more effective in improving the physical working capacity of female cadets during their academic training. The analysis of fat content in the cadets' bodies showed that, at the 1st and 2nd stages of the research, the EG and the CG indicators were not significantly different ($p > 0.05$). At the 3rd stage, the body fat content in the EG female cadets was considerably lower than in the CG by 0.4 % ($p \leq 0.05$). The fat tissue indicators in female cadets of both groups are within the age norm.

The comparative analysis of the SHL among female cadets showed that, in the 1st and 2nd stages of the research, the EG and the CG indicators did not differ significantly ($p > 0.05$). The somatic health level in female cadets in the EG at the 3rd stage was considerably better than in the CG by 3.16 points ($p \leq 0.001$). During the research, the SHL improved in both groups; however, in the EG, the difference between female cadets' indicators at the 1st and 3rd stages was 5.21 points ($p \leq 0.001$), whereas in the CG it was 1.94 points ($p > 0.05$). At the end of the research, the SHL among female cadets in the EG was assessed as average, whereas in the CG it was assessed as below average.

The research indicates that CrossFit training sessions have a more pronounced effect on the morphofunctional development and somatic health levels of female cadets during their academic training for future law enforcement activities, compared with the traditional SMPAs program.

DISCUSSION

Scientists [3] report that the number of female cadets is increasing annually, indicating their readiness to perform the demanding profession of law enforcement on par with men. Today, women in uniform perform the

same tasks as men and therefore must be trained on a par with them. At the same time, specific physiological and psychological characteristics of the female body, according to many scientists [15], should be considered when training women in HEIs with SLE. One of the main factors affecting the physiological characteristics of the female body is hormones. There are differences in hormone levels, such as testosterone and estrogen, between women and men. Women have significantly lower testosterone levels than men and higher estrogen levels. These hormones can affect women's physical abilities, including muscle development and endurance [16, 23]. The female body differs from the male body, particularly in physiological traits such as adipose tissue distribution and hormonal balance. Hence, it should be emphasized that this can affect their physical abilities, including muscle mass, stability, and movement speed. It is also essential to understand that some exercises may be more difficult for women due to their physiological characteristics, as well as a more delicate skeletal system, which makes them more vulnerable to injury.

Given that women have lower lung capacity, training should be aimed at improving their cardiorespiratory function. To do this, it is essential to include various types of aerobic training that increase lung capacity and improve blood circulation. When training with weights, it is necessary to stick to the permissible weight and perform the exercises correctly to avoid injury. It is also essential to pay attention to stretching and increasing flexibility to reduce the risk of injury and improve exercise effectiveness [14]. In addition, according to scientists [24], it is essential to account for the menstrual cycle in women, as it can affect their physical activity and endurance. During menstruation, women may experience pain and discomfort, which can worsen their training results. Therefore, women should be able to adapt their training schedules to their menstrual cycles, reduce their workloads, and rest during menstruation [25].

It is also important to consider the psychological characteristics of women. Women may be more prone to stress and anxiety, which can affect their physical activity and training performance. Therefore, it is necessary to create a supportive psychological environment, support women during training, and help them stay motivated [2, 5, 26]. Taking the above into account, as well as the experience of training women in the security forces of leading countries around the world, we proposed CrossFit training sessions for female cadets – future law enforcement officers – with the aim of developing and improving their physical qualities, improving their morphofunctional condition, promoting their health, and improving the effectiveness of their training for future professional activities. This is confirmed, in particular, by the data we have obtained, as positive dynamics are observed in most indicators.

Based on our research, we have established that CrossFit training sessions, with their numerous advantages, have had a positive impact on the physical development of female cadets during their academic training and on the functional capabilities of their primary physiological systems. At the same time, physical working capacity increased, and body fat decreased, as evidenced by a decrease in body mass index among female EG cadets. The results of our research extend and complement the conclusions of many scientists [3, 12-14, 18, 23, 27, 28] regarding the effectiveness of CrossFit in improving the professional performance of modern law enforcement officers.

CONCLUSIONS

We studied the dynamics of the morphofunctional development of female cadets in the process of various types of motor activity during their academic training. It was found that in female cadets who practiced CrossFit, all the parameters studied were significantly ($p \leq 0.05$-0.001) better at the end of the research than in female cadets who practiced using the traditional SMPAs method. Thus, at the 3rd stage of the research, the EG showed significantly better indicators than the CG: the BMI – 1.07 kg/m^2, the SI – 4.36 %, the VI – 4.14 ml/kg, the RI – 2.99 c. u., HRRI – 7.4 s, HSTI – 6.8 c. u., PWC – 103.1 kgm/min, BF – by 0.4 %, and SHL – by 3.16 points. The analysis of the dynamics of the studied indicators during the academic training period showed that in the EG, unlike the CG, all studied indicators have a stable tendency to improve. At the same time, the somatic health level in the EG female cadets at the end of the research corresponds to the average level (9.29 points), and in the CG – below average (6.13 points).

In general, as a result of CrossFit training sessions, the cardiovascular and respiratory systems of female cadets in the EG improved; the efficiency of these systems at rest and under load increased, and their reserve capacity increased. The recovery period after load decreased, working capacity improved, and body weight and fat content stabilized. The data we obtained indicate that CrossFit training sessions effectively contribute to the improvement of the morphofunctional development of female cadets during their academic training for future law enforcement activities.

PROSPECTS FOR FURTHER RESEARCH

It is planned to investigate gender differences in the manifestation of stress symptoms among future law enforcement officers during their academic training under crisis conditions.

REFERENCES

1.. Taylor BG, Maitra P, Mumford E, Liu W. Sexual harassment of law enforcement officers: Findings from a nationally representative survey. J Interpers Violence. 2022;37(11-12):NP8454-NP8478. doi:10.1177/0886260520978180. DOI
2.. Okhrimenko IM, Ozerskyi IV, Levytska LV et al. The impact of negative factors of professional activities on the health of law enforcement officers. Wiad Lek. 2024;77(6):1141-1146. doi:10.36740/WLek202406105. DOI
3.. Volianiuk OD, Klymenko IV, Rivchachenko OA et al. Peculiarities of psychophysical readiness formation in future law enforcement officers for their professional activities under martial law. Pol Merkur Lekarski. 2025;53(1):81-87. doi:10.36740/Merkur202501111. DOI
4.. Rentmeesters N, Hermans D. From incident to organizational response - a mixed-methods study of police officers' needs and expectations following exposure to potentially traumatic events. Police Practice and Research. 2025;1-21. doi:10.1080/15614263.2025.2608186. DOI
5.. Kisil ZR, Voluiko OM, Osodlo VI et al. Characterization of cadets' psychophysical health in war conditions. Wiad Lek. 2025;78(8):1447-1454. doi:10.36740/WLek/209496. DOI
6.. Okhrimenko IM, Fedyk AO, Zhygalkina NV et al. Changes in somatic and mental health indicators of instructor-officers under stress. Wiad Lek. 2024;77(2):293-298. doi:10.36740/WLek202402116. DOI
7.. Martenko YI, Malyshenko YL, Bushai IM et al. Impact of stressors of academic activities under martial law on the cadets' mental health. Pol Merkur Lekarski. 2025;53(2):250-255. doi:10.36740/Merkur202502114. DOI
8.. Lockie RG, Beitzel MM, Alvarez DA et al. Relationships between physical activity, general health and fitness, and job-specific fitness in law enforcement officers. J Strength Cond Res. 2026;40(1):e85-e94. doi:10.1519/JSC.0000000000005244. DOI
9.. Prontenko KV, Chornous VD, Aleksandrov YV et al. Mental cognitive processes and intellectual working capacity in cadets who were engaged in different types of physical activities. Wiad Lek. 2025;78(8):1462-1469. doi:10.36740/WLek/209498. DOI
10.. Wang Z, Huang WY, Liu Y, Ni G. Editorial: Accessible health programs promoting physical activity and fitness level. Front Public Health. 2023;11:1163686. doi:10.3389/fpubh.2023.1163686. DOI
11.. Oppewal A, Maes-Festen D, Hilgenkamp TIM. Small Steps in Fitness, Major Leaps in Health for Adults With Intellectual Disabilities. Exerc Sport Sci Rev. 2020;48(2):92-97. doi:10.1249/JES.0000000000000216. DOI
12.. Claudino JG, Gabbett TJ, Bourgeois F et al. CrossFit Overview: Systematic Review and Meta-analysis. Sports Med Open. 2018;4(1):11. doi:10.1186/s40798-018-0124-5. DOI
13.. Meyer J, Morrison J, Zuniga J. The Benefits and Risks of CrossFit: A Systematic Review. Workplace Health Saf. 2017;65(12):612-618. doi:10.1177/2165079916685568. DOI
14.. Prontenko K, Okhrimenko IM, Cherednichenko SV et al. Cadets' physical development and functional state during the different types of motor activity. Pol Merkur Lekarski. 2024;52(6):718-723. doi:10.36740/Merkur202406115. DOI
15.. Míguez-Burbano MJ, de Pool I, Hadrigan S et al. HIV knowledge and risk behaviors among women in law enforcement in Bogota, Colombia: potential role as community educators. J Urban Health. 2005;82(3):iv43-iv57. doi:10.1093/jurban/jti107. DOI
16.. Fekedulegn D, Innes K, Andrew ME et al. Sleep quality and the cortisol awakening response (CAR) among law enforcement officers: The moderating role of leisure time physical activity. Psychoneuroendocrinol. 2018;95:158-169. doi:10.1016/j.psyneuen.2018.05.034. DOI
17.. Okhrimenko IM, Martenko OL, Smirnova OM et al. Rukhova aktyvnist' yak zasib podolannya stresu u pravookhorontsiv pid chas yikhn'oyi profesiynoyi diyal'nosti v umovakh voyennoho stanu. [Motor activity as a means of overcoming stress in law enforcement officers during their professional activities under martial law]. Klinichna ta profilaktychna medytsyna. 2024;7:74-82. doi:10.31612/2616-4868.7.2024.09. (Ukrainian) DOI
18.. Klimek C, Ashbeck C, Brook AJ, Durall C. Are Injuries More Common With CrossFit Training Than Other Forms of Exercise?. J Sport Rehabil. 2018;27(3):295-299. doi:10.1123/jsr.2016-0040. DOI
19.. Stepanenko VV, Romanets LM, Petryshyn LY et al. Investigation of stress levels based on the type of behaviour in stressful situation among internally displaced persons after relocation from areas of active hostilities. Pol Merkur Lekarski. 2025;53(3):312-320. doi:10.36740/Merkur202503103. DOI
20.. Kim DH, Cho YH, Seo TB. Correlation between physical efficiency index using Harvard step test and heart rate variation in college students. J Exerc Rehabil. 2022;18(6):389-394. doi:10.12965/jer.2244400.200. DOI
21.. Prontenko KV, Griban GP, Aloshyna AI et al. The physical development and functional state as the important components of the students' health. Wiad Lek. 2019;72(12 cz 1):2348-2353.
22.. Kołodziejczak A, Jatczak A, Rotsztejn H. The correlation between the severity of symptoms and the thickness of the fat fold in cellulite-affected areas-A pilot study. J Cosmet Dermatol. 2022;21(11):5852-5858. doi:10.1111/jocd.15129. DOI
23.. Prontenko K, Griban G, Aloshyna A et al. Analysis of cadets' endurance development at higher military educational institutions during the kettlebell lifting training. Sport Mont. 2019;17(2):3–8. doi: 10.26773/smj.190601. DOI
24.. Wiecek M, Szymura J, Maciejczyk M et al. Effect of sex and menstrual cycle in women on starting speed, anaerobic endurance and muscle power. Physiol Int. 2016;103(1):127-132. doi:10.1556/036.103.2016.1.13. DOI

25.. Kanellakis S, Skoufas E, Simitsopoulou E et al. Changes in body weight and body composition during the menstrual cycle. Am J Hum Biol. 2023;35(11):e23951. doi:10.1002/ajhb.23951. DOI
26.. Quick KM, Paccione-Frometa JA, Wolff KT. Choosing the badge: motivational factors and their influence on job satisfaction among New York City police officers. Policing: An International Journal. 2025;1-20. doi:10.1108/PIJPSM-08-2025-0150. DOI
27.. Okhrimenko IM, Pop OY, Hresa NV et al. Motivational and value-based attitude of law enforcement officers to motor activity as a factor of a healthy lifestyle. Wiad Lek. 2024;77(6):1224-1229. doi:10.36740/WLek202406117. DOI
28.. Prontenko K, Bondarenko VV, Plisko VI, et al. Dynamics of indicators of cadets' daily motor activity in different training years. Pol Merkur Lekarski. 2024;52(4):433-438. doi:10.36740/Merkur202404108. DOI

CONFLICT OF INTEREST
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CORRESPONDING AUTHOR
Ivan M. Okhrimenko
National Academy of Internal Affairs
1 Solomyanska Square, 03035 Kyiv, Ukraine
e-mail: ivango-07@ukr.net

ORCID AND CONTRIBUTIONSHIP
Zoriana R. Kisil: 0000-0003-1405-4547 A
Natalia E. Miloradova: 0000-0002-0716-9736 C D
Halyna V. Katolyk: 0000-0002-2169-0018 B D
Volodymyr P. Ostapovich: 0000-0002-9186-0801 C D
Sergii M. Myronets: 0000-0002-9185-3206 E
Ihor Y. Hnyp: 0000-0002-6493-6399 B
Ivan M. Okhrimenko: 0000-0002-8813-5107 A F

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

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ORIGINAL ARTICLE

CONTENTS

Structural peculiarities of the vascular plexuses of the cerebral ventricles during postnatal ontogenesis

Larysa Ya. Fedoniuk[1], Yaroslav O. Bilyk[1], Oksana M. Matolinets[2], Nataliia V. Porokhovska[2], Oksana H. Popadynets[3], Nadiya S. Tokaruk[3], Olha M. Herman[1]

[1]I. HORBACHEVSKY TERNOPIL NATIONAL MEDICAL UNIVERSITY, TERNOPIL, UKRAINE

[2]DANYLO GALYTSKY LVIV NATIONAL MEDICAL UNIVERSITY, LVIV, UKRAINE

[3]IVANO-FRANKIVSK NATIONAL MEDICAL UNIVERSITY, IVANO-FRANKIVSK, UKRAINE

ABSTRACT

Aim: To study the structural features of vascular plexuses of the cerebral ventricles at different stages of ontogenesis.

Materials and Methods: The results of the study were obtained by examining the vascular plexuses of the ventricles of the human brain. Brain specimens from people aged up to 86 years were examined. Macro-, micro- and submicroscopic, light-optical, and statistical methods were used to establish the morphological features of the structure and innervation and to identify the nerve-receptor apparatus of the vessels and tissue substrate of the vascular plexuses of the human brain ventricles at different stages of ontogenesis.

Results: Research has shown that the vascular plexuses of the ventricles of the human brain consist of epithelium and connective tissue with a large number of blood vessels. The plexus has villous and non-villous parts. The epithelium is represented by light and dark cells of cubic and flattened shape. The stroma of the vascular p lexus consists of collagen fibrils, protofibrils, and fibers enclosed in the ground substance. Fibroblasts are located here singly and in groups. A large number of microvilli and cilia are found on the apical surface of epithelial cells. The nuclei of epithelial cells are located near the basement membrane of the epithelium and can take on various shapes. Contacts between epithelial cells are most often in the form of tight junctions. A small number of macrophages and mast cells are found at the border with epithelial cells. It should be noted that vascular plexuses, in terms of vascularization, are organs with dual blood supply (trophic and functional). The basis of the vascular plexus is formed by blood vessels, which, after entering the thickness of the plexus, branch off from the main vessels running along the plexus into a microcirculatory bed with a highly complex structure. We have established that the diameters of microvessels undergo changes during ontogenesis that correlate with changes in the plexus itself, occurring simultaneously with the development of the brain.

Conclussions: The basis of the vascular plexuses of the cerebral ventricles is formed by a collection of blood vessels – from muscular arteries to capillaries, which come into contact with the ependymal epithelium in the villi of the plexus. The microcirculatory bed is adapted to its connective tissue environment and is closely functionally related to the epithelium of the vascular organ. Capillaries with polar arrangement of fenestrated endothelial cells on the side of the vascular plexus epithelium predominate, indicating their active transport function and participation in the function of the blood-cerebrospinal fluid barrier as part of the blood-brain barrier. Microvessels undergo changes during ontogenesis that correlate with changes in the plexus itself, which occur simultaneously with the development of the brain and reflect the functional loads of the vascular plexus.

KEY WORDS: vascular plexuses, cerebral ventricles, ventricles of the brain, postnatal ontogenesis, microscopy, blood vessels, microcirculatory bed

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INTRODUCTION

The macroscopic anatomy of the vascular plexuses of the ventricles of the human brain is well described in the literature [1]. The vascular plexuses of the ventricles, as derivatives of the soft meninges, connect the circulatory and nervous systems and participate in cerebrospinal fluid dynamics [2, 3, 4]. The disruption of the functions of these structures is associated with the onset of some serious diseases of the central nervous system, in particular, hydrocephalus, intrauterine hydrocephalus, schizophrenia, epilepsy, and Alzheimer's disease, which is based on the atrophy of the epithelium of the vascular plexuses [5, 6].

The role of blood vessels in brain metabolism, cerebrospinal fluid formation and outflow, and in maintaining the stability of its pressure and physicochemical composition is universally recognized and indisputable.

Significant advances in modern neurosurgery, the growth of surgical interventions in the area of the cerebral ventricles and vascular plexuses, experimental

and clinical studies conducted on these structures, and attempts at their transplantation [7, 8, 9] increase curiosity in studying the morphology of neurovascular and tissue structures and their interactions, which is the main task in solving many unresolved issues in neurology and neurosurgery.

The relevance of the study is also determined by the scale of vascular pathology of the brain, which arises from sclerosis of the vascular walls and occupies a significant share in the list of diseases.

AIM

To study the structural features of the vascular plexuses of the cerebral ventricles at different stages of ontogenesis.

MATERIALS AND METHODS

The results of the study were obtained by studying the vascular plexuses of the ventricles of the human brain. Brain specimens from people aged up to 86 years were examined. The structure of the vascular plexuses of the ventricles of the human brain was studied using various methods at different stages of ontogenesis.

Anatomical dissection method. The brain, carefully removed from the skull of the cadaver, was placed in a desiccator with a formalin solution of increasing concentration (1% - 2 days, 3% - 2 days, 5-6% - 2 days, and 10% - 1 week). In addition, additional fixation was performed with a 3% formalin solution through the lower wall of the third ventricle (substantia perforata posterior) using a long needle, which was inserted to a depth of 1–1.5 cm and injected with 15–20 ml of the specified solution. Five brain specimens were fixed in this manner. The brain was in a "floating" state in the desiccator. Next, a series of horizontal cuts were made with a brain knife from the dorsal surface of the brain to the transverse fibers of the corpus callosum. Then the anterior horns of the lateral ventricles were opened and the brain knife was directed obliquely downward, opening the basal nuclei. Additionally, a scalpel and scissors were used to open the walls of the lower and posterior horns of the lateral ventricles, in which vascular plexuses were found. To demonstrate the central part of the lateral ventricles and the third ventricle, the corpus callosum was removed and the vault of the brain was dissected.

After isolating the epiphysis, the upper and posterior walls of the fourth ventricle were opened, revealing the vascular plexus of the fourth ventricle.

To study the structure of the microvascular bed of the ventricular vascular plexuses, the method proposed by V.V. Banin et al. was used.

For electron microscopic examination, material from the vascular plexuses extracted from the ventricular cavity immediately after opening was used. The material was placed in a Petri dish with a 2.5% solution of glutaraldehyde in 0.1M phosphate buffer. The vascular plexus was stretched on a transparent inert plastic plate with holes, after which the Petri dish was placed on the table of an MBS-9 binocular magnifier, where pieces measuring 0.3 x 0.5 cm were studied and cut out from various areas of the vascular plexus. These pieces were placed in fresh portions of 2.5% glutaraldehyde in 0.1 phosphate buffer (pH 7.2–7.4). This was followed by washing in phosphate buffer, followed by post-fixation with osmium fixative according to Milongi (1962) for 2 hours, washing and dehydration in alcohols of increasing concentration, impregnation, and casting in EPON-812 polymer resins. The sections were prepared on an LKB-III ultramicrotome. Contrasting was performed with uranyl acetate and lead citrate according to Reynolds (1963). The ultrathin sections were examined in a Hitaci HU-12A electron microscope.

The results obtained were subjected to computer processing using various variational, descriptive, and dispersion methods, Student's t-test, F-test, D-test, and ANOVA dispersion analysis.

The results obtained were processed using variational, descriptive, and dispersion methods. The results obtained were processed using variational, descriptive, and dispersion methods. The mounted preparations were photographed using a Zenit ET camera on low-sensitivity Micrat or RF-1 film, as well as an Olympus or Canon digital camera.

RESULTS

The vascular plexuses of the ventricles of the human brain are vascular organs consisting of a base and villi, which macroscopically in newborns and children appear as gray or dark red strands.

By the period of sexual maturity, the vascular plexuses are almost indistinguishable in appearance from those in adults. At this age, the vascular plexuses of all ventricles of the brain macroscopically appear as granular strands of red or yellowish-red color.

During this period, bubble-like, cystic indentations containing clear or yellowish fluid are observed in some vascular plexuses of the lateral and third ventricles of the brain. These formations can occur in various areas of the plexus in clusters or individually.

In the vascular plexuses of the ventricles of the brain in people over 60 years of age, cystic formations may appear as small bubbles or single large bubbles filled

with clear fluid. No cysts were observed in the vascular plexuses of the fourth ventricle.

In adults, the vascular plexus is a complex of blood vessels with accompanying connective tissue.

Microscopic analysis has revealed all the structural elements of vascular plexuses, including blood vessels of various diameters, nerve bundles, and fibers. It turns out that the initial structure of the organ is connective tissue stroma, which is a modified soft meningeal membrane located at the base of the plexus. Blood vessels and nerve fibers pass through it.

The entire stromal-vascular complex is covered on the outside by epithelium. Since the total area of the epithelial covering is larger than the area of the stromal-vascular complex, the surface becomes folded and villous.

Among the villi, one can distinguish between simple and more complex, large villi consisting of many lobes. Complex villi are mainly located at the edges of the plexus and consist of a large number of small villi forming lush branches.

The epithelium covering the vascular plexus is composed of cubic cells arranged in a single row. The epithelial lining and the surface of the villi are well visible when stained with Schiff's reagent. It is attached to the underlying basement membrane. The average height of the cells is 15 μm. In cross sections, the profile of the epithelial cells is polygonal, more often hexagonal. The nucleus is round, occupies a central position in the cell, has a nucleolus, and diffusely distributed chromatophilic granules. The cytoplasm contains the usual set of organelles.

So, the vascular plexuses of the ventricles of the brain consist of connective tissue, epithelium, and blood vessels. They are divided into a villous part, which contains a huge number of villi covered with a single layer of epithelium.

In preparations, you can see different types of villi containing links of the microcirculatory bed with twisted and loop-shaped passages. Villi can be of different sizes, from small to large, and are located singly or in various combinations. Large blood vessels are located in the center of large villi, surrounded by a wide-looped network of fibers, while capillaries pass through small villi, located in the center of the villi. Some capillaries have a wide lumen and can be located in close proximity to the epithelial lining, while others with a narrow lumen are located in the deeper layers of the vascular plexus. Many blood vessels are contained in the connective tissue stroma of the plexus. The vessels of the microcirculatory bed mainly flow towards the epithelium of the vascular plexus. Most of them have a straight course, compared to the microcirculatory bed of the villi, and some of them, which are thinner, meander, approaching or moving away from larger vessels.

The vascular plexuses of the ventricles of the human brain have a well-developed blood supply and a complex organization of the microcirculatory bed. This reflects the general principle of vascularization of the brain, which is in special hemodynamic conditions.

The third ventricle is supplied with blood by the lateral and medial choroidal arteries, which originate from the posterior cerebral artery and branches of the superior cerebellar artery, as well as branches of the anterior choroidal artery. They supply blood to the vascular covering – the vascular plexus of the third ventricle, the quadrigeminal plate, the optic tubercle, and the walls of the third ventricle. In the thickness of the plexuses, the arteries divide into arterioles, which spread throughout the vascular plexus.

The microvessels intertwine and anastomose with each other, ensuring adequate blood supply to all parts of the villous and non-villous parts of the plexus. At the edge of the plexus, they form microarcades, which in the cluster-like part deepen into the fringes of the villi. The microcirculatory bed makes up most of the vascular plexus: there are precapillaries, capillaries, and postcapillaries connecting the arteriolar and venular parts of the vascular plexus.

The vascular plexuses of the third and fourth ventricles, compared to the vascular plexuses of the lateral ventricles, are less rich in villi.

On semi-thin sections of the vascular plexus of the fourth ventricle, wide and narrow capillaries of the villi and tissue substrate with a thin endothelial lining are visible. Connective tissue elements accompany the bloodstream. The epithelial lining is represented by cubic cells arranged in a single row, with distinct, rounded or oval-shaped, centrally located nuclei. Prismatic-shaped cells are also found. Among the epithelial cells, there are cells with light and "dark" cytoplasm. They are in different phases of the secretory cycle.

The vessels that deliver blood to the microcirculatory bed of the vascular plexus are arterioles, which branch off from the villous arteries and their branches.

They usually occupy a more lateral, external position relative to the venules that accompany them.

Following the periphery of the vascular plexus, first-order arterioles (A1) branch and anastomose with each other: numerous second-order arterioles (A2) originate from them, whose diameter does not exceed 35-55 μm. which retain a single continuous layer of smooth muscle cells in their wall.

Arterioles and their branches are clearly directed towards the villi, where they break down into a huge number of capillary loops with a wide lumen, which

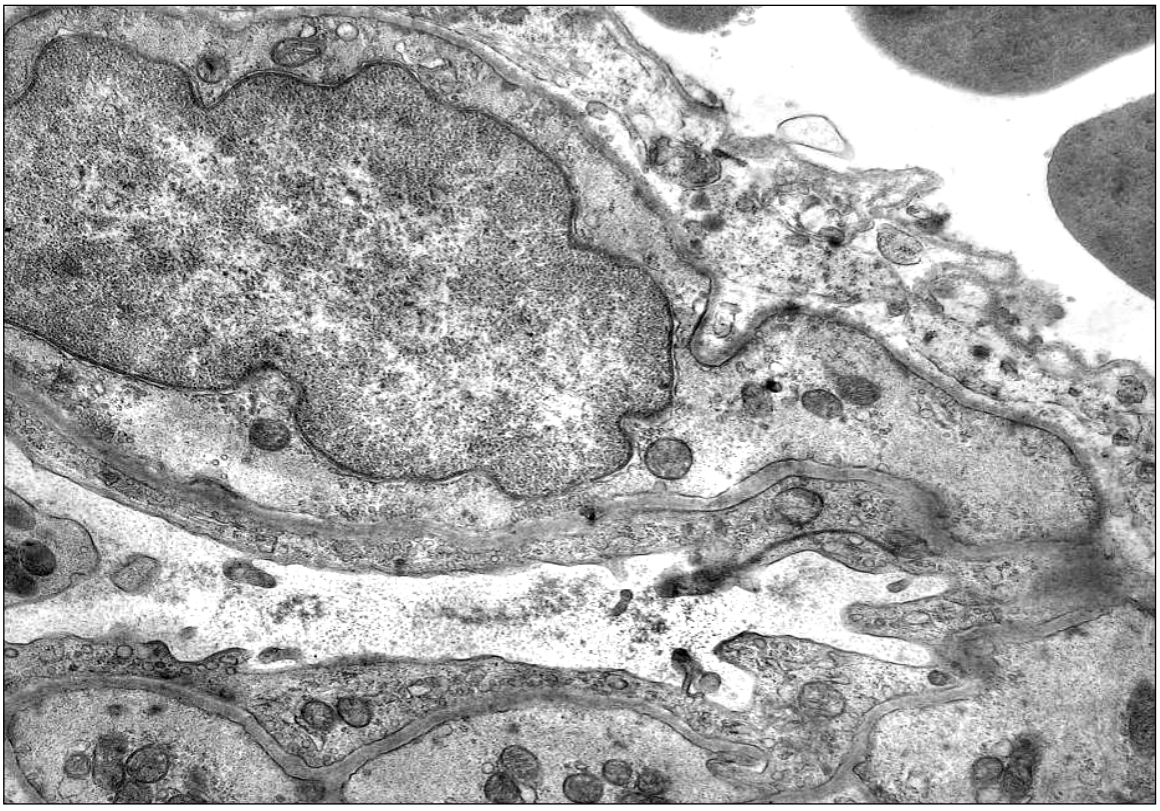

Fig. 1. Structure of the arteriolar wall of the vascular plexus of the cerebral ventricle. Electron micrograph. X 25000. Tunica media

enter the villi. Anastomoses between homogeneous arteriolar microvessels are quite common and have an arcuate appearance: the loops of vessels are distributed in the marginal zones of the vascular plexus as if in tiers.

The transition of arterioles into precapillaries (precapillary arterioles) occurs gradually. The latter directly form blood capillaries and ultimately form a cellular network.

Several precapillary arterioles branch off from each arteriolar arcade, which are mainly connected to the fragment of the capillary network located near this arcade.

Some of the precapillary arterioles supply blood to the capillaries that are part of adjacent fragments, which are topographically connected to neighboring arteriolar arches through their system of precapillaries. Blood from the capillaries collects in venules and veins.

These anatomical connections between the precapillaries and the exchange microvessels are important for changing the amount of blood flowing into the capillaries, which helps regulate cerebral blood flow, intracranial pressure, and the volume of cerebrospinal fluid that penetrates through the capillary wall into the ventricular lumen.

In the villus stalk, the arteriole branches off to secondary villi. In this case, each lobe of the complex villus has its own precapillary arteriole. Sometimes the afferent vessel continues without branching and reaches the top of the villus, passing into a marginal capillary loop. The other end of the loop becomes an efferent vessel – a postcapillary venule.

At the stage of blood delivery to the ependyma of the vascular plexus, there are muscle structures formed by smooth muscle cells at the points where the arterioles and precapillaries branch off from the main trunk.

This means that at the points where the arterial vessels of the plexus divide, there are areas with a more developed muscle layer.

As a result of their periodic contraction or relaxation, selective regulation of a small vascular basin, two or more capillaries, into which the corresponding precapillaries branch, is achieved.

Differentiation of vessels is complicated by the fact that precapillary arterioles do not have the wall typical of arterioles. There are almost no smooth muscle cells in it.

The inner surface of the arterioles of the vascular plexus of the cerebral ventricle is lined with a continuous layer of endothelial cells, which are thinned in the peripheral sections, except for the nucleus area, and are 40-50 μm long and up to 7 μm wide. Thick and thin microfilaments are present in their cytoplasm. The lamellar complex, cytoplasmic network, free ribosomes, and micropinocytic vesicles are located throughout the cell surface. Outside, the endothelial and subendothelial lining of the arterioles is surrounded by connective tis-

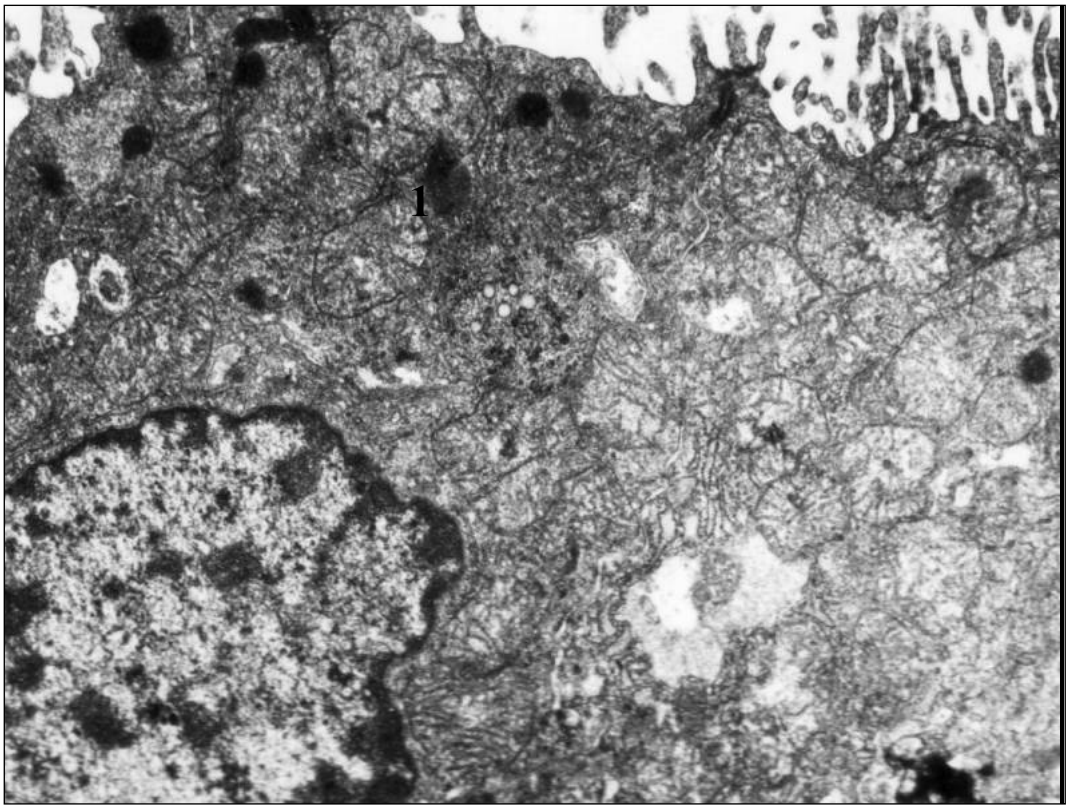

Fig. 2. Epithelial cell of the vascular plexus. Electron micrograph. X 20000

sue, with a layer of ground substance and a few fibrous elements, and there is an internal elastic membrane.

The microfilaments are well defined and directly adjacent to the basal surface of the endothelial cells. They are oriented in a variety of directions and intertwine.

The material localized in the subendothelial zone of the inner membrane is characterized by high electron density. The density of fibrillar elements in different areas is uneven and takes on the appearance of a heterogeneous spotted structure. Collagen fibers, elastic elements, and smooth muscle cells are also often found here. The elastic membrane is not a continuous formation. It contains numerous fenestrae through which the processes of endothelial cells can penetrate.

This membrane structure helps make the vessel more flexible.

The tunica media of the arteriolar wall (Fig. 1) is made up of two or three layers of smooth muscle cells, which are mostly arranged in a circular pattern. Smooth myocytes are spindle-shaped, with uneven serrated edges.

Cell sizes are not constant within the wall of the same vessel. The nuclei are round or oval in shape, depending on the projection of the section.

Often, folds form on their surface, giving the nucleus an irregular shape.

In areas of the cytoplasm free of myofilaments, mitochondria are well defined, located around the nucleus, but they often form abundant clusters at the periphery of the cell. Some of them are associated with elements of a well-developed endoplasmic reticulum.

Canaliculi of the cytoplasmic network are identified, which are sometimes lost between myofibrils. Membrane structures of the endoplasmic network are observed in the central areas of the cell, where the lamellar complex, ribosomes, and polysomes are located. Micropinocytic vesicles are often found.

Myofibrils occupy most of the cytoplasm, except for the perinuclear zone and the most peripheral areas of the cell. They are located along the length of the myocyte without a strict orientation. Adjacent smooth muscle cells are separated from each other by elastic membranes and layers of connective tissue, whose collagen and elastic fibers form a well-defined framework for these cells.

Contacts between them can be quite complex and are established by means of marginal cytoplasmic protrusions that pass through the basement membrane of both cells to the surface of adjacent cells, where, in particular, corresponding indentations are formed.

These connections are formed by the plasma membranes of muscle cells and the basal processes of endothelial cells, which are adjacent to the surface of the myocyte with their expanded part.

The plasma membrane of smooth muscle cells often has osmiophilic formations that are relatively evenly

distributed around the periphery of the myocyte. In some places, it can be seen that these bodies are in close contact with myofibrils.

The fibrous elements of connective tissue in the muscle membrane are represented by separate collagen fibers and microfilaments in the spaces between the basement membranes.

Myoendothelial contacts in arterioles are not pronounced due to the presence of a well-defined basement membrane of the endothelium, which clearly separates the inner layer of the arteriole from the middle and muscular layers, and are a random finding in those areas where the elastic membrane is almost completely absent (transition of arterioles into terminal branches).

As arterioles branch and become smaller, their walls become thinner and the lumen narrower. In small arterioles, the inner and outer elastic membranes become very thin or may be absent. The number of layers of smooth muscle cells decreases to one, and their size also decreases.

The outer layer of small arterioles is super thin and is mostly made up of delicate bundles of collagen fibers.

Meanwhile, the wall of the arteriole can be really close to the basement membrane and the basal labyrinth of the ependyma. True capillaries are located nearby. A layer of epithelium covers the bulging surface of the villi where the arterioles pass through the marginal zone.

Morphometric data indicate rapid growth in the diameter of the arterioles of the vascular plexus of the third ventricle in newborns and a sharp decrease in its diameter by early childhood, which cannot be said about the diameter of the arterioles in the vascular plexuses of the fourth and lateral ventricles. Further, the arterioles of the third ventricle lag behind in diameter until adulthood, when their diameters equal those of the arterioles of the vascular plexuses of the lateral and fourth ventricles. With the onset of old age and senility, their diameter decreases sharply.

The diameter of the arterioles of the vascular plexuses of the lateral ventricles gradually increases until early childhood, followed by a sharp increase until adolescence and reaching a maximum in adulthood. The arterioles of the vascular plexuses of the fourth ventricle repeat the diameters of those of the lateral ventricles in their development, but with the smallest values.

There is also a sharp increase in their diameters during adolescence and a sharp decrease in old age.

This is obviously associated with age-related and sclerotic phenomena that occur in the walls of blood vessels.

The diameter of the precapillaries of the vascular plexus of the fourth ventricle lags behind the diameter of the precapillaries of the third and lateral ventricles until infancy.

From early childhood to late childhood, there is a noticeable rapid increase in their diameter. Then, their diameter decreases again in adulthood. During these periods, the smallest diameter remains in the precapillaries of the vascular plexus of the third ventricle.

In children during their first year of life, there is mainly an intensive process of differentiation and growth of the diameters of the vessels of the vascular plexus of the cerebral ventricle.

At the same time, qualitative changes in the vessels also occur. In the vascular plexus, the ependymal epithelium is represented by columnar ependymocytes. The height of the cells varies from 15 (cubic cells) to 30 μm (prismatic).

Most ependymocytes (Fig. 2) have cilia. In terms of their structure and configuration, they are true epithelial cells.

Both the endothelium of the vascular plexus and the epithelium of the ventricles are elements of the blood-cerebrospinal fluid barrier.

The cytoplasm of the epithelial cell contains a large number of round or oval-shaped mitochondria. The endoplasmic reticulum is well developed, and a moderate number of electron-dense vesicles occupy the apical part of the cell cytoplasm. The cytomembrane of the apical part contains numerous microvilli in the form of finger-like protrusions.

This refers to the aforementioned cilia that form the cell border of the apical surface of ependymocytes, as well as the richly branched processes on their basal surface: together they form the so-called labyrinth, because the cell processes branch in the basement membrane and form a system of gaps between the processes filled with lipoproteins. A wide layer of the basal labyrinth is in contact with the connective tissue cells of the soft membrane and with the basement membrane of the blood capillaries.

DISCUSSION

In the literature, the vascular plexus is defined as a special vascular organ of the central nervous system that develops from the pia mater.

According to textbooks [1, 7], the tela chorioidea of the fourth ventricle is represented by a triangular section of the soft meninges covered with epithelium and penetrating the transverse fissure of the cerebellum. The base of the triangle points upward and forward, and the apex points backward toward the posterior end of the fourth ventricle. It is attached to the lower lateral edges of the rhomboid fossa and to the edge of the inferior cerebral velum. The vascular plexus of the fourth ventricle is enclosed between two layers

of the base: the upper and lower, facing the ventricle cavity. The latter has villous formations along the entire length of the base. They end in a thickening at the back. Through the median aperture, the processes of the plexus emerge onto the lower surface of the cerebellar vermis. Similar vascular plexuses deviate laterally, where they thin and enter the subarachnoid space through the lateral apertures.

The vascular plexus is an unpaired continuation of the soft meninges, forming a villous covering of the ventricle and a vascular protrusion covered by the ependyma. The vascular base is triangular in shape and located between the ventral surface of the corpus callosum, the fornix, and the dorsal surface of the diencephalon. Its protrusion extends into the sagittal fissure between the optic thalamus and is connected to the vascular plexuses of the lateral ventricles. The structure of the vascular base of the third ventricle includes a duplication of the soft meninges. One of its layers first extends forward as the upper layer along the lower surface of the corpus callosum. In the area of the interventricular openings, it turns back and follows as the lower layer to the rear, where it fuses with the ependymal plate of the third ventricle, covering most of the dorsal surface of the thalamus on both sides. Between these two layers of the vascular covering, in loose connective tissue, there are two internal and unpaired veins of the brain. On the lower layer of the covering, there are villi, which are designated as components of the vascular plexus.

The vascular plexus of the third ventricle participates in the formation of the vascular plexuses of the lateral ventricles, directing villous protrusions with a huge number of blood vessels into the cavities of the lateral ventricles. These formations are the vascular plexuses of the lateral ventricles. They are located in the central part and in the lower horn of each lateral ventricle.

They are located in the central part and in the lower horn of each lateral ventricle.

The plexuses cover the upper surface of the optic thalamus and part of the vault that is not fused with the corpus callosum. The main mass of the vascular plexus is located in the lateral horn and forms a thickening at the level of the central part of the lateral ventricle – the glomus. Here, the vascular plexus is located above the hippocampus, covering it.

The vascular plexuses of the lateral ventricles of the human brain communicate with the plexus of the third ventricle through the interventricular openings [10, 11, 12].

They vary greatly in size and shape, and their outgrowths form additional villi on the surface of the vascular plexuses.

The vascular plexuses of the cerebral ventricles receive the necessary amount of blood from two main systems: 1) through special branches of the internal carotid artery and 2) the basilar artery.

The vascular plexus and vascular base of the fourth ventricle are supplied with blood by branches of the posterior inferior cerebellar artery. Branches from the anterior inferior cerebellar artery, posterior spinal artery, and sometimes the vertebral artery also enter the vascular plexus of the fourth ventricle.

Blood flows into the vascular plexus of the lateral ventricles from the anterior choroidal artery and the choroidal branches of the posterior cerebral artery. They penetrate through the pedicle of the vascular plexus along the lateral and medial edges, then divide into arterioles that spread along the entire length of the vascular plexus.

The anterior choroidal artery branches off from the internal carotid artery and, penetrating the lower horn of the lateral ventricle, occupies the outer edge of the lateral vascular plexus, supplying two-thirds of the plexus and the ependyma covering the posterior part of the caudate nucleus. Along its path, the anterior choroidal artery gives rise to numerous arterial branches that penetrate the thickness of the plexus in parallel. They anastomose with each other and with the branches of the posterior choroidal artery, simultaneously supplying the vascular base of the third ventricle [13].

According to some authors [14], it can also branch directly from the bifurcation of the internal carotid artery (10%) or from the posterior communicating artery (20%) in some cases. In such variants of the anterior villous artery, aneurysms often occur [15], which require surgical intervention to prevent rupture of the aneurysm [16, 17] or various types of complications [18, 19] in the form of ischemic or infarct manifestations.

The posterior villous artery branches off from the posterior cerebral artery, the outer branch of which penetrates the vascular plexus of the lateral ventricle along the inner edge and gives 4-5 pairs of branches, running parallel in the thickness of the plexus. They anastomose with the branches of the anterior choroidal artery, supplying blood mainly to the villi of the racemose part of the plexus.

The arteries of the vascular plexuses of the cerebral ventricles anastomose with each other, forming loops. Arterioles and then capillaries branch off from each of the arteries.

The microcirculatory bed of the plexuses has a complex structure and is inextricably linked to the peculiarities of the macroscopic and microscopic organization of these organs. The essential components of this system are arterioles, precapillaries, capillaries, postcapillaries, venules, and an undifferentiated vascular-capillary network. Some capillaries are directly adjacent to the

choroidal epithelium – these are functional capillaries, while others do not come into contact with epithelial cells and do not form villi—these are trophic capillaries. Capillaries with a wide lumen are the main capillaries, while narrow, short capillaries are the connectives of wide, loop-shaped capillary vessels.

Blood from the capillaries collects in the venules and veins of the plexuses, then in the internal cerebral veins, which are located between the layers of the third ventricle, and from there into the great cerebral vein, forming a unique drainage system [20, 21]. Until now, researchers have focused on studying the blood supply and innervation of the blood vessels of the brain and its membranes, without taking into account the vascular plexuses, despite the fact that they are derivatives of the soft meninges and an integral part of the brain components, morphologically and functionally connected to them.

Knowledge of individual anatomical variations in the blood supply system of vascular plexuses may be particularly important in neurosurgery.

We have established that the density of the capillary bed per 1 mm^2 of the vascular plexus area varies significantly: the largest number of capillaries are found in the vascular plexuses of the lateral ventricles, and the smallest per 1 mm^2 in the plexus of the fourth ventricle. The microcirculatory bed of the plexus makes up most of its volume and basically determines its functions.

The vessels have a tortuous course, forming loops along their path, especially at the edges of the plexus. The arches and tortuosity of the capillaries not only change the direction of blood flow, but also the force of the pulse, which once again points to the important role of precapillaries in peripheral vascular resistance. Along the course and at the points of division of the arterioles, clusters of smooth muscle cells have been identified, the presence of which has been reported earlier by a number of authors [14, 22, 23].

It can be assumed that the presence of smooth muscle couplings has some effect on the regulation of blood volume in the vascular plexus.

It is believed that the strategic position of precapillary sphincters determines their participation in the selective distribution of blood between the metabolic links of the microcirculatory bed. The contraction of smooth muscle cells throughout the precapillaries allows individual capillary links to be shut off. In this way, the amount of blood flowing to different parts of the capillary network of the plexus is regulated. Muscle sphincters regulate blood flow in the microcirculatory bed.

It is an indisputable fact that vascular motor reactions occur as a result of myocyte excitation, which can be achieved by direct or indirect exposure to various metabolites, neurotransmitters, or other vasoactive substances. Myoendothelial connections serve as pathways for conducting excitation from the endothelium to the myocytes.

The action of these factors is directed and cumulative and is superimposed on the spontaneous activity of myocytes, whose sensitivity and rhythm of work in different parts of the vascular system are not the same. In this way, blood factors act on the vascular wall.

We have established that the arterioles and venules of the vascular plexuses of the lateral, third, and fourth ventricles are characterized by typical features of their wall structure: monolayer endothelial cells varying in length and thickness, with a well-defined basement membrane throughout. It separates a layer of myocytes with a circular arrangement. No noticeable differences in the structure of these cells were found.

Their unequal number and localization indicate a repeated functional load performed by these cells in the processes of hemodynamic regulation in vascular plexuses.

The ultrastructural organization of myocytes in the muscle layer of arteries and arterioles, with the presence of special contacts between myocytes, can ensure a synchronous generalized response of the vessel to irritation even from a small group of cells. Myoendothelial contacts serve to conduct excitation from the endothelium to the myocytes of the intima. These connections between the inner and middle layers facilitate the action of biologically active substances in the blood on the vascular wall through their reception by receptor proteins of endothelial cells.

The outer layer of arterioles is made up of loose connective tissue, which contains fibroblasts and collagen fibers.

In precapillary arterioles, the distance between smooth muscle cells increases. The nuclei of endothelial cells in precapillary arterioles and capillaries protrude into the lumen of the vessel, causing it to narrow. This is extremely important in regulating blood flow.

Studies show that the contacting surfaces of two adjacent endothelial cells are highly diverse, complex, and dynamic in their organization. They can range from simple, tile-like overlaps of endothelial cells to complex structures formed by the invagination of one cell surface into another or interdigitating insertions of cytoplasmic processes of adjacent endothelial cells.

The complex organization and heterogeneity of intercellular connections between endothelial cells of the arteries of the vascular plexuses of the ventricles of the brain make it likely that these contacts perform a variety of functions.

Thus, as a result of this study of the vascular plexuses of the cerebral ventricles, along with typical morphological features, characteristics of organ specificity were identified.

CONCLUSSIONS

The basis of the vascular plexuses of the cerebral ventricles is formed by a set of blood vessels, ranging from muscular arteries to capillaries. The microcirculatory bed is adapted to its connective tissue environment and is closely functionally related to the epithelium of the vascular organ. Capillaries with polar arrangement of fenestrated endothelial cells on the side of the vascular plexus epithelium predominate, indicating their active transport function and participation in the function of the blood-cerebrospinal fluid barrier as part of the blood-brain barrier. Microvessels undergo changes during ontogenesis that correlate with changes in the plexus itself, which occur simultaneously with the development of the brain and reflect the functional loads of the vascular plexus.

REFERENCES

1. Chinery HR, Ruitenberg NJ, McMenamin PG. Novel characterization of monocite-derived cell populations in the meninges and choroid plexus and their rates of replenishment in bone marrow chimeric mice. J. Neuropathol. Exp. Neurol. 2010;69(9):896-909.
2. Scala G, Corona M, Pavone LM [et al.] Structural and funcţional features of choroid epithelium from bufalo brain. Anat. Rec. 2007;290(11):1399-1412.
3. Swetloff A, Ferretti P. Changes in E2F5 intracellular localization in mouse and human choroid plexus epithelium with development. Int. J. Dev. Biol. 2005;49(7):859–865.
4. Berthiaume AA, Grant RI, McDowell K, Underly RG, Hartman DA, Levy M, Shih AY. (). Dynamic remodeling of pericytes in vivo maintains capillary coverage in the adult mouse brain. Cell Reports. 2018;22:8–16.
5. Banizs B, Komlosi P, Bevensee MO [et al.] Altered pH regulation and Na –HCO3 transporter activity in choroid plexus. Am. J. Physiol. Cell Physiol. 2007;292(4):1409-1416.
6. Tirrapelli DR, Lopes LdaS, Lachat JJ [et al.] Ultrastructural study of the lateral ventricle choroid plexus in experimental hydrocefalus in Wistar rats. Arg. Neuropsiquiatr. 2007;65(4A):974– 977.
7. Skinner SJ, Geanev MS, Lin H [et al.] Encapsulated living choroid plexus cells: potenţial long-term treatmens for central nervous system disease and trauma. J. Neural. Ehg. 2009;6(6):P. 650-657.
8. Thanos CG. Bintz B, Emerich DF. Microencapsulated choroid plexus epithelial cell transplants for repair of the brain. Adv. Exp. Med. Biol. 2010;670:80–91.
9. Matsumoto N, Taguchi A, Kitayama H [et al.] Transplantation of cultured choroid plexus epitelial cells via cerebrospinal fluid cells via cerebrospinal fluid shows prominent neuroprotectiv effects against acute ischemic brain injury in the rat. Neurossci Lett. 2010;469(3):283-288.
10. Chen J, Luo Y, Hui H, Cai T, Huang H, Yang F, Yan X. CD146 coordinates brain endothelial cell-pericyte communication for blood-brain barrier development. Proceedings of the National Academy of Sciences of the United States of America. 2017;114:E7622–E7631.
11. Kubotera H, Ikeshima-Kataoka H, Hatashita Y, Allegra Mascaro AL, Pavone FS & Inoue T. Astrocytic endfeet re-cover blood vessels after removal by laser ablation. Scientific Reports. 2019:9:1263.
12. Coelho-Santos V, Shih AY. Postnatal development of cerebrovascular structure and the neurogliovascular unit. Wiley Interdiscip Rev Dev Biol. 2020;9(2):e363. doi: 10.1002/wdev.363. DOI
13. Zagorska-Swiezy K, Litwin JA, Gorczyca J [et al.] The microvascular architecture of the choroid plexus in fetal human brain lateral ventricle: a scanning electron microscopy study of corrosion casts. J. Anat. 2008;213(3):259–265.
14. Acar A, Sengul G, Aydin IH. The variations of the anterior choroidal artery. An intraoperativ study. Turc. Neurosurg. 2009;19(4):349-352.
15. Senturk C, Bandeira A, Bruneau M [et al.] Endovascular treatment of anterior chorioidal artery aneurysms. J. Neuroradiol. 2009;36(4):228-232.
16. Shibata K, Nishimura Y, Kondo H [et al.] Isolated homonimous hemianopsia due to lateral posterior choroidal artery region infarction: a case report. Clin. Neurol. Neurosurg. 2009;111(8):713-716.
17. Yurt A, Turan Y, Ucar K [et al.] Ruptured distal anterior choroidal artery aneurism. J. Clin. Neurosci. 2009;16(1):132-134.
18. Red-Horse K, Siekmann AF. Veins and arteries build hierarchical branching patterns differently: Bottom-up versus top-down. BioEssays. 2019;41:e1800198.
19. Furtado SV, Venkatesh PK, Hegde AS. Neurological complications and surgical outcome in patients with anterior choroidal segment aneurysms. Int. J. Neurosci. 2010;120(4):291-297.
20. Zagórska-Swiezy K, Litwin JA, Gorczyca J [et al.] Arterial supply and venous drainage of the choroid plexus of the human lateral ventricle in the prenatal period as revealed by vascular corrosion casts and SEM. Folia Morphol. 2008;67(3):209-213.

21. Payne LB, Zhao H, James CC, Darden J, McGuire D, TaylorS, Chappell JC. The Pericyte microenvironment during vascular development. Microcirculation (Epub ahead of print). 2019.
22. Darii A. Unele date experimentale despre sursele de inervatie ale plexurilor coroide din ventriculele creierului. Anale Stiintifice. 2005;6(1):78-81.
23. Darii A. Microscopia plexului coroid al ventriculului III din creierul uman. Anale Stiintifice. 2006; 7(1):60-63.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Larysa Ya. Fedoniuk
Medical Biology Department,
I. Horbachevsky Ternopil National Medical University,
9 Valova St., 46000 Ternopil, Ukraine
e-mail: fedonyuk22larisa@gmail.com

ORCID AND CONTRIBUTIONSHIP
Larysa Ya. Fedoniuk: 0000-0003-4910-6888 A B C E F
Yaroslav O. Bilyk: 0000-0001-8971-1420 B D
Oksana M. Matolinets: 0009-0008-6521-8867 A B
Nataliia V. Porokhovska: 0000-0001-5221-8544 C D
Oksana H. Popadynets: 0000-0002-2093-5984 B C F
Nadiya S. Tokaruk: 0000-0003-1404-7857 D E
Olha M. Herman: 0000-0002-3864-8588 A F

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

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ORIGINAL ARTICLE

CONTENTS

Anticancer and apoptotic effect of alogliptin on A549 cancer cell line

Safa Hussien Radhi Abbas, Ahsan F. Bairam
DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY, FACULTY OF PHARMACY, UNIVERSITY OF KUFA, NAJAF, IRAQ

ABSTRACT

Aim: To evaluate the anticancer, apoptotic, and antioxidant effects of Alo on A549 cells, both alone and in combination with CP, and to elucidate the molecular mechanisms underlying the death of cancer cells.

Materials and Methods: The American Type Culture Collection's (ATCC) normal HBL100 cells and human lung A549 cells were used in the investigation. The cells were split into four groups. Following a 72-hour incubation period, ELISA assays were used to quantify the levels of the DPP-4 enzyme, apoptotic regulators (Bax and caspase-3), and oxidative stress marker (malondialdehyde) in lung cancer cell and normal cell lines. One-way ANOVA with significance set at $P < 0.05$ were used in the statistical analysis.

Results: The findings showed that Alo reduced the activity of the DPP-4 enzyme in both cell lines ($P < 0.0001$). Molecular analysis showed a considerable increase in pro-apoptotic markers (BAX, Caspase-3). Higher amounts of malondialdehyde were indicative of increased oxidative stress in both monotherapy and combination. But in HBL 100 cells, Alo decreased BAX, caspase-3, and MDA levels.

ConclusionS: Alo has caused cancer cell death through a variety of mechanisms, such as DPP4 inhibition, apoptotic pathway activation, and oxidative stress enhancement based on DPP-4, BAX, caspase-3, and MDA measurements.

KEY WORDS: A549 cell line, alogliptin, BAX, caspase-3, DPP4 inhibitor, lung cancer, MDA

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INTRODUCTION

One of the most common and deadly types of cancer worldwide, lung cancer is caused by the overgrowth of epithelial cells in the pulmonary system, which impairs respiratory function [1]. It frequently spreads to distant organs such the brain, bones, liver, and adrenal glands as well as regional lymph nodes and pleural tissues due to its aggressive clinical nature [2]. As a result, the prognosis is typically dismal, with median survival spans for localized disease estimated at 13 months and for metastatic cases at roughly five months [3]. More than 2.2 million new cases of lung cancer were diagnosed worldwide in 2020, according to new statistics from the Global Cancer Observatory (GLOBOCAN) [4]. It continues to be the second most deadly cancer in women, behind breast cancer, and the primary cause of cancer-related fatalities in males [5]. Small cell lung cancer (SCLC), which makes up around 15% of cases, and non-small cell lung cancer (NSCLC), which makes up the remaining 85% of cases, are the two main histological subtypes of lung cancer [6]. Surgery, radiation, immunotherapy, and chemotherapy—most notably with cisplatin (CP), an intravenous platinum-based chemotherapeutic agent—are common treatment approaches [7]. Despite Cis's widespread use and clinical efficacy, systemic toxicity, tumor cell resistance, and unfavorable side effects usually restrict its use [8]. Adjunctive medicines that potentially improve efficacy and lower toxicity profiles have drawn attention as a result [9]. In a number of cancer models, the selective dipeptidyl peptidase-4 (DPP4) inhibitor alogliptin (Alo), which is mainly used to treat type 2 diabetes, has demonstrated apoptotic properties [10]. DPP4 inhibitors improve glycemic control and may alter pathways linked to tumor growth by increasing the activity of incretin hormones including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide GIP [11]. In cancer, the DPP4 enzyme CD26 has a context-dependent role: under expression has been connected to tumor suppression in cancers including breast and endometrial cancers, while overexpression has been linked to carcinogenesis in lung, colon, and renal cancers [12]. The significance of this duality lies in the fact that it has the

potential to serve as a therapeutic target in oncology [13]. Comprehensive studies assessing the specific cytotoxic and antioxidant effects of alogliptin against lung cancer cells, namely A549, are scarce, despite earlier research documenting the overall anticancer effects of DPP4 inhibitors [14]. Furthermore, little is known about the precise mechanisms by which alogliptin affects oxidative stress and lung cancer cell survival [15].

AIM

The purpose of this study is to evaluate the apoptotic and anticancer effects of alogliptin both by itself and in conjunction with cisplatin on the A549 lung cancer cell line. The results could help create better treatment plans and shed light on how DPP4 inhibition functions in the treatment of lung cancer.

MATERIALS AND METHODS

CHEMICALS AND CELL LINE

In order to evaluate the anticancer and apoptotic effects of alogliptin, both alone and in combination with cisplatin, on the A549 lung cancer cell line, this study was conducted. The results could guide the creation of better treatment plans and shed light on the function of DPP4 inhibition in the treatment of lung cancer.

HBL100 CELL LINE

In cancer research studies, the human breast epithelial cell line HBL100 is used as a standard cell control. HBL100 cells, which were initially isolated from healthy breast tissue, offer an essential comparison model for evaluating how well anticancer therapies distinguish between cancerous and healthy cells. For the study, Sigma in the United States of America provided the RIPA lysis buffer, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide dye powder, and dimethyl sulfoxide (DMSO). Fetal bovine serum (10% FBS), phosphate-buffered saline (PBS), and RPMI-1640 medium containing fetal bovine serum were supplied by Gibco (USA). Trypsin-EDTA and trypan blue stain were purchased from Capricorn in the United States of America and Flow Laboratories in the United Kingdom, respectively. Troge, based in Germany, provided the streptomycin and benzylpenicillin antibiotics, respectively. Alogliptin (Alo) and Cisplatin (CP) both target Moi (USA). The amounts of MDA, caspase-3, and BAX in the sample were determined using a human-specific ELISA kit developed by the Bioassay Technology Laboratory in China. An ELISA kit for human caspase-3, BAX, and MDA was acquired from Elabscience under the name "UNITED STATES".

CELL CULTURE

Fetal bovine serum (FBS) was used to neutralize the A549 cells after they had been cultured with trypsin-EDTA for enzymatic detachment and washed with phosphate-buffered saline (PBS). After that, the cells were plated on 96-well culture plates using RPMI-1640 medium that had been treated with 100 µg/mL of streptomycin and 100 units/mL of penicillin until they reached the desired concentration. To achieve about 80% confluence and create a monolayer, cultures were cultured for 24 hours at 37 °C in a humidified environment with 5% CO_2. Trypan blue exclusion was used to calculate the number of viable cells. After the incubation period, the medium was either left untreated for control or replaced with 200 µL of new media containing the test medicines. The four experimental groups consisted of control cells, (cells that had not been treated), cells that had been treated with CP, cells that had been treated with Alo, and cells that had been treated with a combination of CP and Alo. By assessing DPP-4, BAX, caspase-3, and MDA levels after 72 hours, the apoptotic effect, oxidative stress, and DPP-4 expression were evaluated.

STATISTICAL ANALYSIS

We used Microsoft Excel 2019 and GraphPad Prism 10 to analyze all of the experimental data. For statistical comparisons, Tukey's post hoc test was used after a one-way Graph Prism ANOVA. The results were considered to be statistically significant if the p-value was lower than 0.05 percentage points.

RESULTS

Dipeptidyl Peptidase 4 expression in A549 and HBL100 cells

The expression of DPP4 in A549 and HBL100 cells is presented in Table 1

HUMAN DIPEPTIDYL PEPTIDASE 4 DETERMINATION

Upon exposure to the IC50 of CP, the findings of the CP used showed that the concentration of DPP4 did not significantly alter ($P > 0.05$) in the A549 and HBL100 cells when compared to the control group. However, as illustrated in figure (1), after A549 and HBL100 cells were treated with the IC50 of Alo ($P < 0.0001$), the concentration of DPP4 significantly decreased in contrast to the control group.

Table 1: The expression of DPP4 in A549 and HBL100 cells

Cell line	DPP4 level (ng/ml)	Total protein (ng/ml)	DPP4 expression %
A549 control	7.547±0.89	1385059±174348	5.45×10^{-4} **±0.09
HBL100 control	7.792±0.45	1477358±412460	5.27×10^{-4} **±0.10

** (P<0.01) n=4

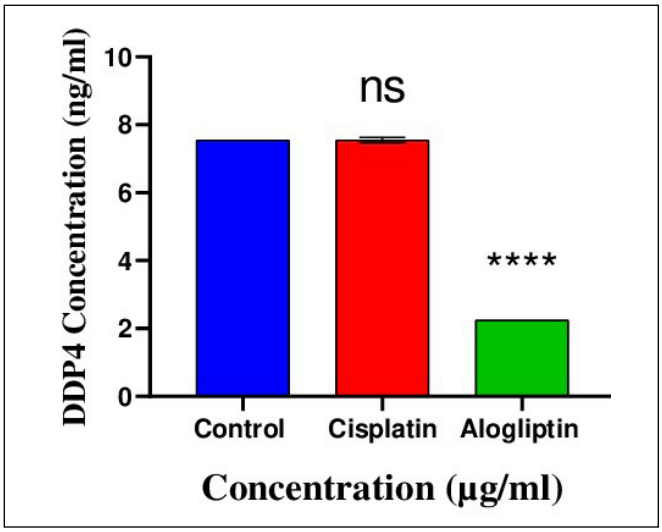

Fig. 1. Cisplatin and alogliptin's effects on the A549 cell line's DPP4 levels
A one-way ANOVA was used in the study, and the results are displayed as mean ± SD with ns = P > 0.05 and **** P < 0.0001 in comparison to the control, figure (2)
Source: Own materials

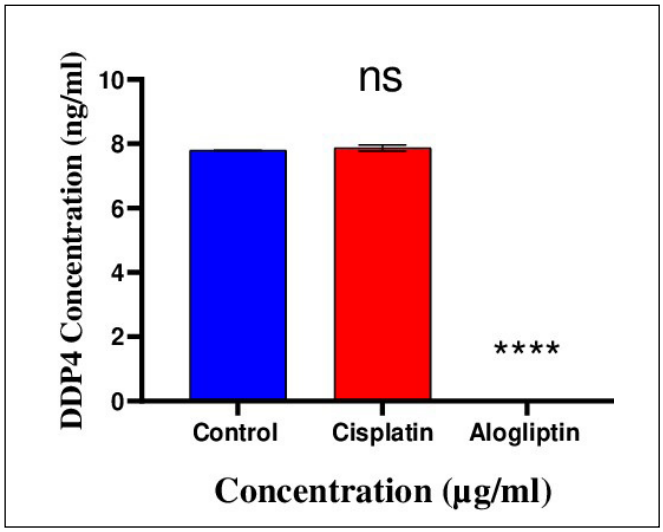

Fig. 2. Cisplatin and alogliptin's effects on the HBL100 cell line's DPP4 values
The analysis was conducted using a one-way ANOVA. In contrast to the control, the data are shown as mean ± SD. ns = P < 0.05, **** P < 0.0001
Source: Own materials

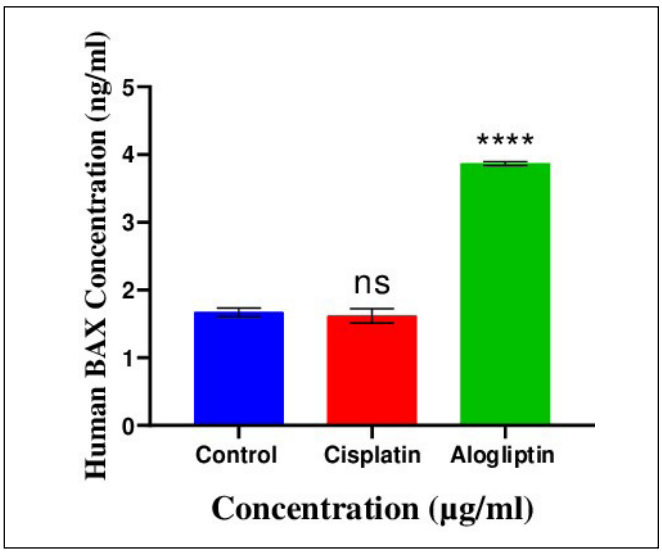

Fig. 3. Changes in human BAX levels in the A549 cell line caused by cisplatin and alogliptin
A one-way ANOVA was used for the analysis. In relation to the control, the results are displayed as mean ± SD. ns indicate P > 0.05, **** P < 0.0001, respectively, figure (4)
Source: Own materials

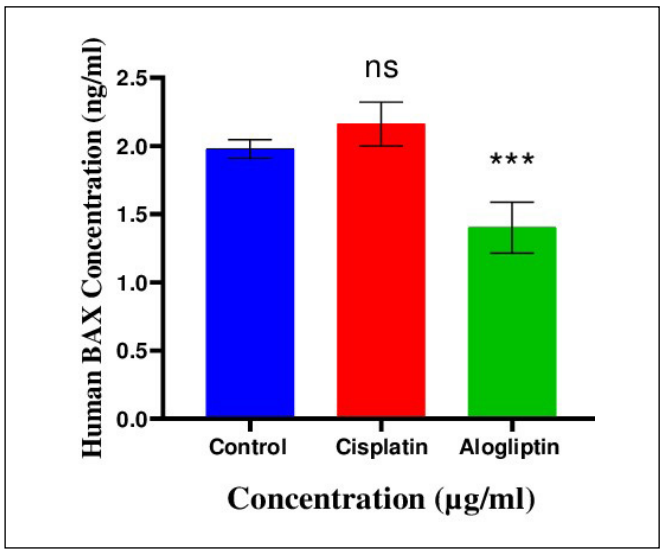

Fig 4. Changes in human BAX levels in the HBL100 cell line caused by cisplatin and alogliptin
The analysis was conducted using a one-way ANOVA. In contrast to the control, the data are shown as mean ± SD. ns = P > 0.05, *** P < 0.001
Source: Own materials

DETERMINATION OF HUMAN APOPTOSIS REGULATOR (BAX)

When compared to the control group, the CP did not significantly increase BAX levels after giving the IC50 to A549 and HBL100 cells (P > 0.05). However, as Figure 3 illustrates, BAX levels dramatically rose (P < 0.0001) in comparison to the control group following treatment of A549 cells with Alo's IC50. On the other hand, Figure 4 illustrates that Alo

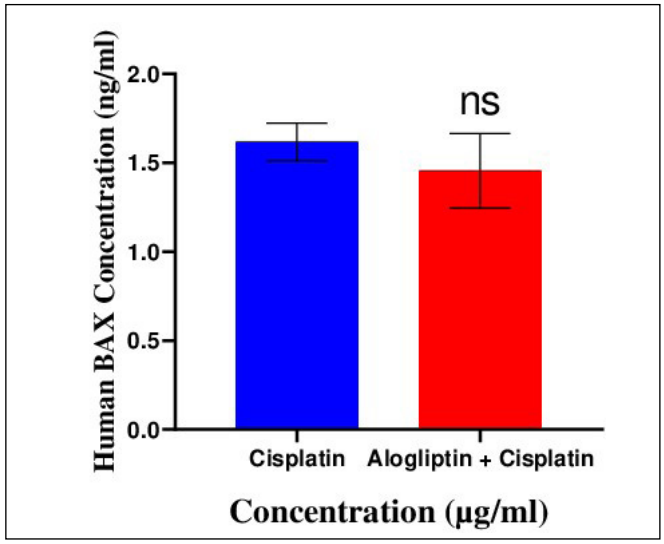

Fig. 5. In the A549 cell line, the effects of cisplatin alone and cisplatin + alogliptin combos on the human BAX level are compared
An ANOVA in one direction was used in the analysis. Mean ± SD is used to illustrate the results, *P is less than 0.05
Source: Own materials

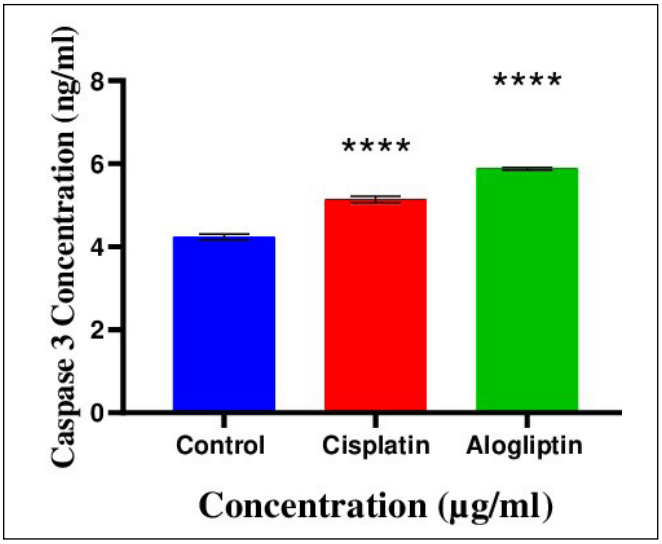

Fig. 6. Caspases 3 levels in the A549 cell line are affected by cisplatin and alogliptin
An ANOVA in one direction was used for the analysis. The mean ± SD is used to display the data. **** P < 0.0001 in comparison to the control
Source: Own materials

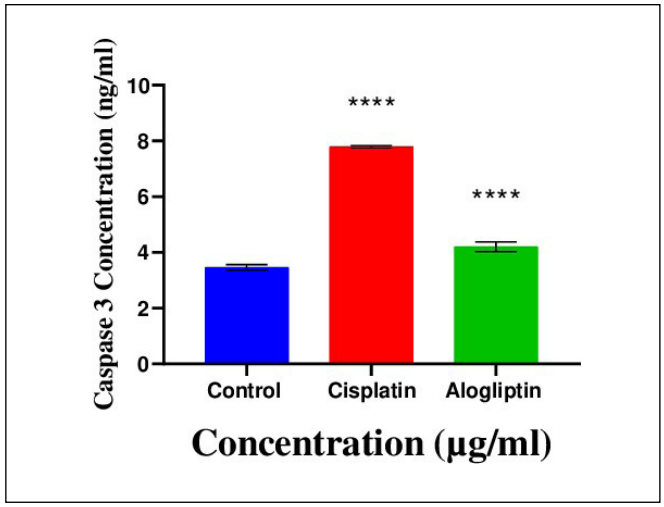

Fig. 7. Alogliptin and cisplatin's effects on the HBL100 cell line's caspase 3 levels
An ANOVA in one direction was used for the analysis. It displays the data as mean ± SD. **** P < 0.0001, in comparison to the control group
Source: Own materials

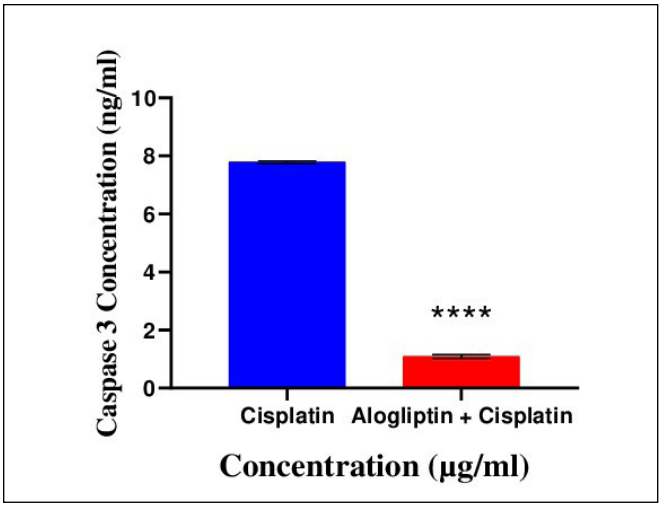

Fig. 8. The impact of cisplatin alone and cisplatin + alogliptin combos on the HBL100 cell line's Caspase 3 level is compared
In the analysis, a one-way analysis of variance was used. Presented in the form of mean ± standard deviation, the data are utilized. **** The value of P is lower than 0.0001
Source: Own materials

significantly reduced the concentration of BAX (P > 0.001) after administering the IC50 of Alo to HBL100 cells in comparison to the control group, figure (3).

COMPARISON BETWEEN THE ACTIVITY OF ALOGLIPTIN PLUS CISPLATIN VERSUS CISPLATIN ALONE ON BAX CONCENTRATION

As illustrated in figures (5-6), the Alo plus CP combination did not significantly raise the BAX concentration in A549 cells (P > 0.05), but the HBL100 cells' BAX level significantly decreased (P < 0.01) when exposed to the combination's IC50 as opposed to cells exposed to CP alone.

HUMAN CASPASE-3 DETERMINATION

Following treatment with the IC50 of CP, the quantity of Caspase-3 increased significantly (P < 0.0001) in A549 and HBL100 cells compared to the control group. Comparing A549 and HBL100 cells to the control group, the amount of Caspase-3 rose dramatically (P < 0.0001) when the IC50 of CP was administered. In contrast to

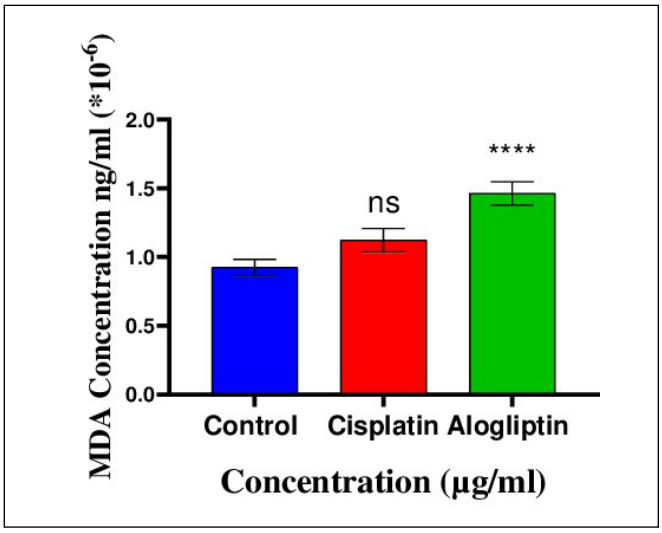

Fig. 9. MDA levels in the A549 cell line are affected by cisplatin and alogliptin To conduct the analysis, a one-way analysis of variance was utilized. The data is presented in the form of mean ± standard deviation. The significance level (ns) is set at P > 0.05, and the significance level (****) is set at P < 0.0001 in comparison to the control
Source: Own materials

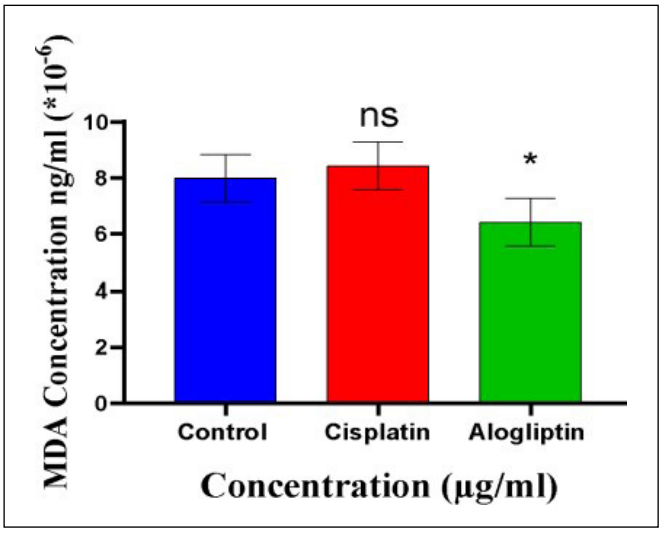

Fig. 10. Alogliptin and cisplatin's effects on the HBL100 cell line's MDA levels An ANOVA in one direction was used for the analysis. It displays the data as mean ± SD. * P <0.05, in comparison to the control group
Source: Own materials

the control group, figures (6-8) show that the quantity of caspase-3 in HBL100 cells treated with the IC50 of Alo dropped dramatically, P<0.0001.

DETERMINATION OF HUMAN MALONDIALDEHYDE LEVEL

After exposing the A549 and HBL100 cells to CP's IC50 in contrast to the control group, CP did not significantly raise the MDA concentration (P > 0.05), as Figures 9 and 10 demonstrate. After treating A549 cells with Alo's IC50, Alo demonstrated a significant rise in MDA concentration (P < 0.0001) compared to the control group, as shown in figure (9). After treating HBL100 cells with the IC50 of Alo, MDA levels significantly decreased (P < 0.05) compared to the control group, figure (10).

COMPARISON BETWEEN ALOGLIPTIN AND CISPLATIN VERSUS CISPLATIN ALONE ON MDA CONCENTRATION

In contrast to cells exposed to the IC50 of CP alone, figure (11) demonstrated that the Alo plus CP combination significantly increases MDA concentration after treating the A549 and HBL100 cells with the combination's IC50 (P < 0.0001 and P < 0.001, respectively).

DISCUSSION

One of the primary causes of cancer-related death globally is still lung cancer, and the effectiveness of current treatments is sometimes hampered by serious side effects and medication resistance [16]. Thus, novel therapy strategies are desperately needed [17]. Given their known safety profiles and well-characterized pharmacokinetics, repurposing currently approved FDA medications, such as DPP-4 inhibitors, may be a viable approach [18].

DPP-4 ENZYME INHIBITION AND MOLECULAR TARGETS

DPP-4 EXPRESSION AND INHIBITION

The findings validate Alo's main mode of action by showing that it effectively reduced DPP-4 enzyme activity in both cell lines [19]. DPP-4 levels were not significantly impacted by CP. This outcome is consistent with our understanding of Alo, a potent and precise DPP-4 inhibitor. We found that A549 lung carcinoma had an overexpression of the DPP-4 enzyme. This suggests that DPP-4 is essential for lung cancer cell survival and proliferation, as evidenced by the substantial decrease in DPP-4 activity caused by Alo. According to literature, DPP-4's expression varies according to the type of cancer and is implicated in immunological control, glucose metabolism, signaling, and apoptosis [20]. The significant decrease in DPP-4 activity after Alo therapy raises the possibility that this enzyme could be used as a therapeutic target and biomarker in the treatment of lung cancer. Furthermore, studies identified DPP-4 as a unique possible diagnostic marker in gastric cancer, and showed that DPP-4/CD26 expression patterns can function as prognostic markers in a variety of hemato-

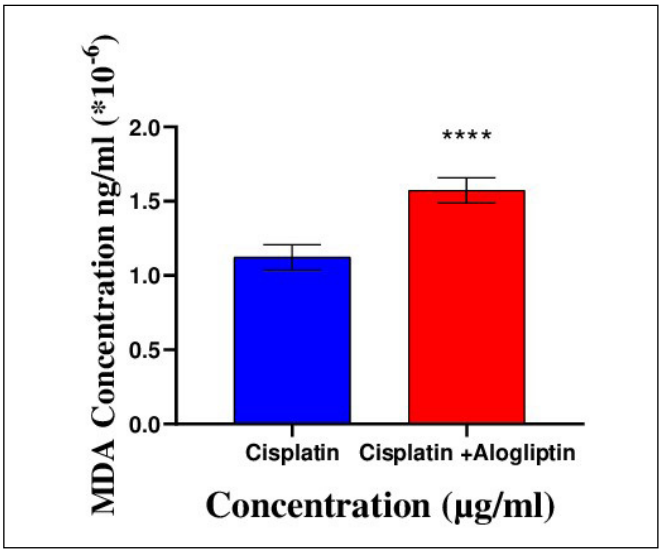

Fig. 11. In the A549 cell line, the effects of cisplatin alone and cisplatin plus alogliptin combos on the MDA level are compared
In the analysis, a one-way analysis of variance was used. Presented in the form of mean ± standard deviation, the data are utilized. **** The value of P is lower than 0.0001
Source: Own materials

logical malignancies [21]. As demonstrated by researchers, who demonstrated that long-term DPP-4 inhibitor treatment decreased colon carcinogenesis in animal models, the therapeutic implications of DPP-4 inhibition go beyond glucose homeostasis [22]. Hypoxia-inducible factor-1a (HIF-1a) VEGFA production was increased by overexpression of DPP-4, according to another endometrial cancer investigation [23]. Both in vitro and in vivo, DPP-4 overexpression caused a change in the morphology of the cell as well as an acceleration of invasion, proliferation, and carcinogenesis. These effects were mitigated by DPP-4 knockdown or pharmacological suppression with sitagliptin [24]. The possibility of dipeptidyl peptidase-4 (DPP-4) inhibitors as anticancer drugs has been investigated in recent research. DPP-4 inhibitors, which were first developed to treat type 2 diabetes, have shown promising results in improving progression-free survival in advanced colorectal and airway malignancies and lowering the growth of cancer cells [25]. In another study of urothelial carcinoma (UC), DPP-4 overexpression and destructive tumor features were seen. Overexpression of DPP-4 stimulates cell invasion, migration, proliferation, and growth [26]. Reduced UC aggressiveness and increased apoptosis were linked to DPP-4 inhibition. Based on particular biomarkers such as DPP-4, a new study implies that targeting senescent cells in cancer for the purpose of addressing tumor dormancy, recurrences, and resistance to conventional chemotherapy and radiation therapies offers a novel therapeutic approach [27]. Senescent cells multiply as people get older, which can result in inflammation and tissue dysfunction. Additionally, senescent cells can cause cell cycle arrest in response to stress, which can make DNA more vulnerable to damage [28]. STX-1 is a first-in-class antibody-drug combination (ADC) that can be used to treat a number of cancer indications, according to the outcomes of the inquiry.

APOPTOTIC PATHWAY MODULATION

PRO-APOPTOTIC BAX EXPRESSION

The study illustrates how Alo has varying impacts on the production of BAX, a crucial pro-apoptotic protein in cancerous cells [29]. Apoptotic pathways were selectively activated in malignant cells while normal HBL100 cells were protected by Alo treatment, which markedly elevated BAX expression in A549 cancer cells while lowering it in mammary epithelial cells (HBL100). This differential effect supports the idea of taking advantage of cancer cells' weaknesses while preserving healthy tissues, which is essential for therapeutic selectivity [30]. The molecular mechanism put out by researchers, in which pro-apoptotic members of the BCL-2 family promote mitochondrial outer membrane permeabilization, resulting in cytochrome c release and BAX activation, is supported by the observed increase in BAX expression in cancer cells after Alo therapy. This effect's selectivity supports Alo's promise as a tailored treatment with better safety records than traditional chemotherapeutics [31]. Studies who detailed the intrinsic apoptotic route involving BAX-mediated mitochondrial malfunction, provides more support for this mechanism. Furthermore, research highlighted the significance of BAX as a therapeutic target by underscoring its crucial role in developmental and therapeutic apoptosis [32]. The idea of cancer-selective apoptosis induction is consistent with the distinct impacts seen in cancer and healthy cells. These results are consistent with another study that demonstrated that the percentage of thyroid cancer cells that undergo apoptosis rises as the ratio of BAX to BCl-2 increases due to an increase in BAX protein levels while Bcl2 levels stay unchanged following gemigliptin treatment of the cells. By increasing cell survival and modifying the BAX/BCl-2 ratio, Huang et al.'s parallel studies found that pretreatment of SH-SY5Y neuroblastoma cells with the DPP4 inhibitor teneligliptin reduces MPP+-induced cytotoxicity [33].

CASPASE-3 ACTIVATION

The study illustrated the impact on caspase-3, the primary apoptotic effector caspase [34]. Alo administration

caused down-regulation effects on normal cells and markedly increased caspase-3 levels in A549 carcinoma cells, suggesting that the apoptotic machinery was selectively activated in malignant lung tissues. The discovery that DPP4 inhibitors may enhance Caspase-3 activity in hepatocellular carcinoma cells lends credence to this conclusion [35]. Alo's anticancer action is largely due to the specific activation of Caspase-3 in cancer cells. DNA fragmentation and cellular shrinkage are two hallmarks of programmed cell death that result from the activation of Caspase-3, the primary protein regulating apoptosis. The results also concur with observations made by researchers, who explained that executioner caspases, such as caspase-3, are crucial targets for cancer treatment since they constitute the last common pathway in apoptotic cell death [36]. But when A549 and HBL100 cells were treated with CP, their caspase-3 levels significantly increased in comparison to the control group. In one study, Niazmand investigated the effects of sitagliptin, either alone or in conjunction with paclitaxel, on the growth and metastasis of EOCs, or epithelial ovarian cancer cells.

MALONDIALDEHYDE (MDA) LEVELS

The MDA test was used in this study to assess the levels of oxidative stress that the monotherapy drugs and their combinations induced in tumor and normal cells [37]. Alo raised MDA levels in A549 cancer cells while lowering them in normal HBL100 cells, according to the evaluation of oxidative stress by MDA assay. Another way that Alo affects cancer cells specifically is through its differential action on oxidative stress [38]. According to research, increased oxidative stress in cancer cells might overwhelm their antioxidant defenses and cause apoptosis. As evidenced by the significantly lower levels of MDA in the group that was treated with alogliptin in comparison to the group that was not treated with the drug and the significantly higher levels of GSH in the group that was treated with the drug, alo had a nephroprotective effect in diabetic rats and may be considered a promising treatment for diabetic kidney disease. Alo may use the cancer cells' existing high levels of oxidative stress to cause selective cytotoxicity, as evidenced by the enhanced MDA levels after therapy [39]. This process is corroborated by researchers, who found that it is possible to control oxidative stress in the tumor microenvironment to encourage the death of cancer cells while preserving healthy tissues. Measuring oxidative stress biomarkers like MDA also provide important information about cellular damage and the effectiveness of treatments. According to a different study, pretreating human neuroblastoma cell line SH-SY5Y cells with the DPP4 inhibitor teneligliptin improved cell survival and decreased MPP+-induced cellular damage. Teneligliptin also prevented reactive oxygen species (ROS) from forming, restored glutathione (GSH), decreased malondialdehyde (MDA) levels, and prevented the neuroblastoma cells' mitochondrial membrane potential from degrading.

CONCLUSIONS

As a monotherapy, Alo exhibits a potent anticancer impact on A549 lung adenocarcinoma cells and selectively selects malignant cells over healthy HBL100 cells, according to the findings of this in vitro investigation. Numerous mechanisms, such as DPP4 inhibition, apoptotic pathway modulation, and increased oxidative stress levels, contribute to Alo's anticancer action. Alo demonstrated preferential toxicity against cancer by significantly increasing the levels of oxidative stress in the A549 cells both when used alone and in conjunction with CP. Alo showed antioxidant activity by dramatically lowering oxidative stress levels in HBL100 normal cells. Furthermore, apoptotic modulators (caspase-3 and BAX) were significantly reduced in Alo.

REFERENCES

1. Brown S, Banfill K, Aznar MC, Whitehurst P, Finn FC. The evolving role of radiotherapy in non-small cell lung cancer. Br J Radiol. 2019;92(1104): 20190524. doi:10.1259/bjr.20190524. DOI
2. Tchounwou PB, Dasari S, Noubissi FK, Ray P, Kumar S. Advances in our understanding of the molecular mechanisms of action of cisplatin in cancer therapy. J Experiment Pharmacol. 2021;13:303-328. doi:10.2147/JEP.S267383. DOI
3. Aldossary SA. Review on the pharmacology of cisplatin: clinical use, toxicity and mechanism of resistance of cisplatin. Biomed Pharmacol J. 2019;12(1):7-15. doi:10.13005/bpj/1608. DOI
4. Almagthali AG, Barakat H, Iyer A, Humboldt J, Khandelwal P. Dipeptidyl peptidase-4 inhibitors: Anti-diabetic drugs with potential effects on cancer. Diabetes & Metabolic Syndrome: Clin Res Rev. 2019;13(1):36-39. doi:10.1016/j.dsx.2018.08.012. DOI
5. Wilson AL, Saunders SP, Phyu S, Judd LM, Giraud AS. DPP4 inhibitor sitagliptin enhances lymphocyte recruitment and prolongs survival in a syngeneic ovarian cancer mouse model. Cancers. 2021;13(3):487. doi:10.3390/cancers13030487. DOI
6. Gilbert MP, Pratley RE. GLP-1 Analogs and DPP-4 Inhibitors in Type 2 Diabetes Therapy: Review of Head-to-Head Clinical Trials. Front Endocrinol. 2020;11:178. doi:10.3389/fendo.2020.00178. DOI

7. Holst JJ, Gasbjerg LS, Rosenkilde MM. The role of incretins on insulin function and glucose homeostasis. Endocrinology. 2021;162(7):bqab065. doi:10.1210/endocr/bqab065 DOI
8. Bishnoi R, Hong L, Hassan R, Mukherje S, Patel K, Dang LH, Lyman GH. Dipeptidyl peptidase 4 inhibitors as novel agents in improving survival in diabetic patients with colorectal cancer and lung cancer: A Surveillance Epidemiology and Endpoint Research Medicare study. Cancer Med. 2019;8(8):3918-3927. doi:10.1002/cam4.2278. DOI
9. Salama MM, Zaghloul RA, Khalil RM, El-Shishtawy MM. Sitagliptin potentiates the anti-neoplastic activity of doxorubicin in experimentally-induced mammary adenocarcinoma in mice: implication of oxidative stress, inflammation, angiogenesis, and apoptosis. Sci Pharm. 2022;90(3):42. doi:10.3390/scipharm90030042. DOI
10. Sun X, Cao Z, Mao K, Wu C, Chen H, Wang J, Wang X. Recent advances in access to overcome cancer drug resistance by nanocarrier drug delivery system. Cancer Drug Resist. 2023;6(2):390-415. doi:10.20517/cdr.2023.16 DOI
11. Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, Bray F. Cancer statistics for the year 2020: An overview. Int J Cancer. 2021;149(4):778-789. doi:10.1002/ijc.33588. DOI
12. Gilbert MP, Pratley RE. GLP-1 Analogs and DPP-4 Inhibitors in Type 2 Diabetes Therapy: Review of Head-to-Head Clinical Trials. Front Endocrinol. 2020;11:178. doi:10.3389/fendo.2020.00178. DOI
13. Ng L, Tao Y, Liu Y, Chen J, Yu J, Chan AB, Liu L. CD26 induces colorectal cancer angiogenesis and metastasis through CAV1/MMP1 signaling. Int J Mol Sci. 2022;23(3):1181. doi:10.3390/ijms23031181. DOI
14. Sicuranza A, Raspadori D, Bocchia M. CD26/DPP-4 in chronic myeloid leukemia. Cancers. 2024;14(4):891. doi:10.3390/cancers14040891. DOI
15. Lo C, Toyama T, Wang Y, Lin J, et al. Insulin and glucose-lowering agents for treating people with diabetes and chronic kidney disease. Cochrane Database Syst Rev. 2018 Sep 24;9(9):CD011798. doi: 10.1002/14651858.CD011798.pub2. DOI
16. Liaqat S, Fatima B, Hussain D, Imran M, Batool R, Majeed S, Najam-ul-Haq M. Evaluation of antiproliferative effect of doxorubicin loaded zinc selenium quantum dots to MCF-7 cell lines by linagliptin functionalized lignin nanoparticles. Eur Polymer J. 2024;208:112867. doi:10.1016/j.eurpolymj.2024.112867. DOI
17. Le Calvé B, Dayde D, Lelarge V, Jouffroy G, Choeur J, Angevin E. 14P Dipeptidyl peptidase 4 (DPP4) as a new senescence-associated target to eliminate cancer cells. ESMO Open. 2025;10. doi:10.1016/j.esmoop.2025.104170. DOI
18. Escriche-Navarro B, Garrido E, Escudero A, Montoya-Méndez I, et al. Targeting the senescent surfaceome through DPP4 antibody-functionalized nanoparticles. An application to cancer therapy. Biomaterials. 2025;123461. doi:10.1016/j.biomaterials.2025.123461. DOI
19. Ali N, Yadav UP, Tyagi Y, Babu MA, et al. A drug repurposing-based investigation of USFDA-approved anticancer drugs towards dipeptidyl-peptidase 4 (DPP4) receptor inhibition: An in silico and in vitro investigation to treat diabetes and associated conditions. J Ind Chem Soci. 2025;101826. doi:10.1016/j.jics.2025.101826. DOI
20. Lossi L. The concept of intrinsic versus extrinsic apoptosis. Biochem J. 2022;479(3):357-384. doi:10.1042/BCJ20210854. DOI
21. Voss AK, Strasser A. The essentials of developmental apoptosis. F1000Research. 2020;9. doi:10.12688/f1000research.21571.1. DOI
22. Carneiro BA, El-Deiry WS. Targeting apoptosis in cancer therapy. Nature Rev Clin Oncol. 2020;17(7):395-417. doi:10.1038/s41571-020-0341-y. DOI
23. Huang L, Pi J, Gu L, Liao Z, Wang W. Teneligliptin, a DPP4 Inhibitor Protects Dopaminergic Neurons in PD Models via Inhibiting of Oxidative Stress and Ferroptosis. Eur J Pharmacol. 2025;177782. doi:10.1016/j.ejphar.2025.177782. DOI
24. Selim SM, El Fayoumi HM, El-Sayed NM, Mehanna ET, Hazem RM. Alogliptin attenuates STZ-induced diabetic nephropathy in rats through the modulation of autophagy, apoptosis, and inflammation pathways: Targeting NF-κB and AMPK/mTOR pathway. Life Sciences. 2025; 361: 123307. doi:10.1016/j.lfs.2024.123307. bn DOI
25. Mani RJ, Mettu VS, Srinivas B, Banothu AK, Narsim NOx D, Hathiram SK. A systematic review of molecular pathway analysis of drugs for potential use in liver cancer treatment. Drugs Drug Candid. 2023; 2(2):210-231. doi:10.3390/ddc2020013. DOI
26. Asadi M, Taghizadeh S, Kaviani E, Vakili O, Taheri-Anganeh M, Tahamtan A, Savardashtaki A. Caspase-3: structure, function, and biotechnological aspects. Biotechnol Appl Biochem. 2022;69(4):1633-1645. doi:10.1002/bab.2233. DOI
27. Hussar P. Apoptosis Regulators Bcl-2 and Caspase-3. Encyclopedia. 2022;1624-1636. doi:10.3390/encyclopedia2040111. DOI
28. Niazmand A, Nedaeinia R, Vatandoost N, Jafarpour S, Safabakhsh S, Kolahdouz M, Salehi R. The impacts of dipeptidyl-peptidase 4 (DPP-4) inhibitors on common female malignancies: A systematic review. Gene. 2024;148659. doi:10.1016/j.gene.2024.148659. DOI
29. Chang YT, Chang CC, Chang MJ, Shieh JJ, Wu MJ. Targeting the NRF2 pathway with linagliptin to inhibit human hepatocellular carcinoma growth. Free Radic Biol Med. 2025. doi:10.1016/j.freeradbiomed.2025.06.048. DOI
30. Pethanasamy M, Dhanabalan K, Gunasekaran K, Muniyandi J, Natesan S, Palaniswamy K. In vitro evaluation of the antioxidant and anticancer activities of chromogenic acid on human colon cancer (HT-29) cells. Tropic J Natur Prod Res. 2024;8(3):6582-6588. doi:10.26538/tjnpr/v8i3.16 DOI
31. Hayes JD, Dinkova-Kostova AT, Tew KD. Oxidative stress in cancer. Cancer Cell. 2020;38(2):167-197. doi:10.1016/j.ccell.2020.06.001. DOI

32. Ye YY, Chen Y, Yang J, Wu J, Wang P. Dapagliflozin restores autophagy and attenuates apoptosis via the AMPK/mTOR pathway in diabetic nephropathy rats and high glucose-induced HK-2 cells. Int Urol Nephrol. 2025 Jan;57(1): 249-261. doi: 10.1007/s11255-024-04172-9. DOI
33. Dharshini LCP, Rasmi RR, Kathirvelan C, Karunakar P, Sakthivel KM. Regulatory components of oxidative stress and inflammation and their complex interplay in carcinogenesis. Appl Biochem Biotechnol. 2023;195(5):2893-2916. doi:10.1007/s12010-022-04266-z. DOI
34. Chavda V, Chaurasia B, Garg K, Deora H, Sarkar B, Raval J, Lu B. Molecular mechanisms of oxidative stress in stroke and cancer. Brain Disorders. 2022;5:100029. doi:10.1016/j.dscb.2021.100029. DOI
35. Caturano A, D'Angelo M, Mormone A, Russo V, Mollica MP, Salvatore T, Sasso FC. Oxidative stress in type 2 diabetes: impacts from pathogenesis to lifestyle modifications. Curr Iss Molec Biol. 2023;45(8):6651-6666. doi:10.3390/cimb45080420. DOI
36. Huang L, Pi J, Gu L, Liao Z, Wang W. Teneligliptin, a DPP4 Inhibitor Protects Dopaminergic Neurons in PD Models via Inhibiting of Oxidative Stress and Ferroptosis. Eur J Pharmacol. 2025;177782. doi:10.1016/j.ejphar.2025.177782. DOI
37. Majano BS, Ellis L, Rachet B. Epidemiology of Lung Cancer. Encyclop Respiratory Medicine, Second Edition. 2022; 4:663-672. https://doi.org/10.1016/b978-0-08-102723-3.00034-2
38. Thandra KC, Barsouk A, Saginala K, Aluru JS, Barsouk A. Epidemiology of lung cancer. Contemp Oncol. 2021;25(1):45. doi:10.5114/wo.2021.103829. DOI
39. Schabath MB, Cote ML. Cancer progress and priorities: lung cancer. Cancer Epidemiol, Biomark Prevent. 2019;28(10):1563-1579. doi:10.1158/1055-9965.epi-19-0221. DOI

Ethical considerations

As this study utilized established human cancer cell lines (A549 lung adenocarcinoma) and normal cell lines (HBL100 breast epithelial cells) obtained from the Iraq Biotech Cell Bank Unit in Basrah and involved only in vitro experiments without human subjects or animals, ethical approval was not required according to institutional guidelines at the University of Kufa, Najaf, Iraq.

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AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and design of the study, data collection and analysis, interpretation of the results, and drafting of the manuscript. Each author approved the final version of the manuscript for submission.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

CORRESPONDING AUTHOR

Safa Hussien Radhi Abbas
Department of Pharmacology and Toxicology,
Faculty of Pharmacy, University of Kufa, Najaf, Iraq
e-mail: smuh73710@gmail.com

ORCID AND CONTRIBUTIONSHIP

Safa Hussien Radhi Abbas: 0009-0009-5264-1372 A B C D E F
Ahsan F. Bairam: 0000-0002-0832-6502 A F

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

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ORIGINAL ARTICLE

CONTENTS

Legal regulation of health care management based on forensic medical assessment of early in-hospital mortality

Alina O. Pletenetska[1], Anzhela B. Berzina[1], Ruslan A. Volynets[2], Oksana O. Cherniak[3], Oleksandr V. Felyk[3], Valentyn V. Halunko[3]

[1]BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE
[2]TARAS SHEVCHENKO NATIONAL UNIVERSITY OF KYIV, KYIV, UKRAINE
[3]KYIV UNIVERSITY OF INTELLECTUAL PROPERTY AND LAW, KYIV, UKRAINE

ABSTRACT

Aim: To analyze the legal regulation of health care management based on the analysis of forensic medical examinations concerning the quality of medical care in cases of early in-hospital mortality.

Materials and Methods: A retrospective forensic medical examination was conducted of 51 cases of patient mortality occurring within the first 24 hours after hospital admission in a multidisciplinary hospital in Kyiv. Organizational and legal aspects of medical care delivery were analyzed for compliance with legislation and clinical protocols.

Results: Systemic deficiencies included the absence of electrocardiographic examination in 90,2 ± 4,2% of cases, regardless of diagnostic accuracy. Significant trends were identified as the hospital stay duration increased: the absence of oxygen therapy showed a downward trend (from 86,7 ± 8,8% to 38,5 ± 13,5%; $p_{trend} = 0,006$), while diagnostic discrepancies demonstrated an upward trend (from 6,7 ± 6,4% to 46,2 ± 13,8%; $p_{trend} = 0,016$). The median number of deficiencies doubled from 2 (1–3) in the <3 h group to 4 (3–4) in the 12–24 h group ($p = 0,002$). A moderate direct correlation was established between patient age and the total number of deficiencies ($\rho = 0,462$; $p < 0,001$).

Conclusions: The identified deficiencies correlate with non-compliance with mandatory regulatory requirements. The accumulation of medical errors with increasing length of stay and patient age indicates systemic violations of organizational and legal standards, necessitating stricter public administration control over medical care quality.

KEY WORDS: forensic medicine, public administration, medical care

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INTRODUCTION

Early in-hospital mortality (death occurring within the first 24 hours after hospital admission) is an integral indicator of the quality, effectiveness, and organization of medical care [1]. It reflects not only the severity of the patient's clinical condition but also the timeliness of diagnosis, the adequacy of the pre-hospital care pathway, and adherence to clinical protocols.

International guidelines of the European Society of Cardiology (ESC) and the American Heart Association (AHA) identify early diagnosis, particularly electrocardiography (ECG), risk stratification, and timely initiation of therapy as important factors in reducing in-hospital mortality in acute conditions [2–5]. Contemporary studies employing machine learning methods further confirm the critical role of the completeness of early diagnostic data in predicting mortality outcomes [6–9]. Non-compliance with these requirements significantly worsens prognosis and constitutes a defect in the provision of medical care.

Early in-hospital mortality serves as an indicator of public administration and regulatory frameworks in health care and as a marker of the quality of medical care delivery within the system of state governance in the health sector. Health care management is a component of public administration, a normatively regulated process based on a system of standards in the health care sector, which, in accordance with the Law of Ukraine "Fundamentals of the Legislation of Ukraine on Health Care" (1993), includes medical standards, clinical protocols, drug formularies, and related instruments. These standards are mandatory for all health care institutions [10]. Forensic medical examination is a main tool for assessing the quality of medical care, enabling

the establishment of a causal relationship between the actions (or omissions) of medical professionals and a fatal outcome [11,12].

AIM

The aim of the study was to analyze the legal regulation of health care management based on the analysis of forensic medical examinations concerning the quality of medical care in cases of early in-hospital mortality; to identify associations between violations of regulatory requirements and adverse medical outcomes; and to determine directions for improving the legal regulation of health care management, including in the context of ensuring quality control of medical care.

MATERIALS AND METHODS

A retrospective forensic medical examination was conducted of 51 cases of patient mortality occurring within the first 24 hours after hospital admission to a multidisciplinary hospital in Kyiv during the pre-war period. Patients were divided into four groups according to the length of hospital stay: up to 3 hours (n = 15), 3–6 hours (n = 7), 6–12 hours (n = 16), and 12–24 hours (n = 13).

The forensic medical assessment of compliance of the provided medical care was performed based on applicable regulatory legal acts, including the Law of Ukraine "Fundamentals of the Legislation of Ukraine on Health Care" (1993) [10], the Criminal Code of Ukraine (2002) [13], and multiple orders of the Ministry of Health of Ukraine.

Statistical analysis. Data were processed using IBM SPSS Statistics v. 27.0 (Armonk, NY: IBM Corp., USA). Quantitative variables are presented as M ± SE (mean ± standard error of the mean) or median (Me) with interquartile range (IQR [Q_1-Q_3]), and qualitative indicators as percentages with standard error of the percentage (SE%). Normality of distribution was assessed using the Shapiro–Wilk test. The comparisons of two independent groups were performed using Student's t-test (for normally distributed quantitative variables) or the Mann–Whitney U-test (for non-normally distributed data). Four independent groups were compared by the use of ANOVA with the post hoc Tukey HSD test (for normally distributed quantitative variables), or Kruskal-Wallis test with the post hoc Mann–Whitney U-test (for non-normally distributed data) (considering the Bonferroni correction). The Associations between categorical variables were evaluated using the Fisher's exact test. The strength of association between binary variables was assessed using the phi-coefficient (φ). The binary qualitative data in four independent groups were compared by the use of χ^2 test (including the χ^2 test for trend), with the post hoc Marascuilo-Liakh-Gurianov procedure. The correlation analysis was performed by the use of Spearman's rank correlation coefficient (ρ). Differences were considered statistically significant at $p<0,05$ (considering the multiple comparisons correction).

ETHICS

The study was conducted in accordance with provisions of Helsinki Declaration of World Medical Association, Council of Europe Convention on Human Rights and Biomedicine, and Ukrainian legislation. Aggregated statistical data without disclosure of personal information were used. Analysis of clinical cases was conducted in generalized form, without possibility of identifying specific persons.

FRAMEWORK

The study was performed within framework of initiative research work "Expert-diagnostic system for objectification of forensic medical examination of traumatic brain injury" (state registration number 0123U101528; term 2023-2026), as a fragment of the complex scientific project of the Educational and Scientific Institute of Law Taras Shevchenko National University of Kyiv «Legal support for the sustainable development of the economy of Ukraine in the context of European integration» (state registration number 0124U003297; term: 2024-2029).

RESULTS

The general characteristics of the cases subjected to forensic medical examination are presented in Table 1. The mean age of the deceased patients was 67,5 ± 1,7 years. Sex distribution was as follows: males – 49,0% (25 persons), females – 51,0% (26 persons). The mean age of males was 66,2 ± 2,6 years, being comparable to that of females (68,6 ± 2,3 years).

The cardiovascular diseases were the leading cause of death identified by forensic medical examination, accounting for 37 cases (72,6 ± 6,2%).

Deficiencies in the provision of medical care identified during the forensic medical examination are presented in Table 2.

The most critical deficiency identified during the forensic medical examination was the failure to perform ECG in 90,2 ± 4,2% of cases.

ANALYSIS OF THE ASSOCIATION BETWEEN DEFICIENCIES AND DIAGNOSTIC ERRORS

An analysis of the association between the identified deficiencies and adverse outcomes was conducted

Table 1. General characteristics of cases subjected to forensic medical examination of early in-hospital mortality

Indicator	Absolute number (n = 51)	Frequency (% ± SE%)	Forensic medical assessment
Demographic structure			
Men	25	49.0 ± 7.0	-
Women	26	51.0 ± 7.0	-
Mean age (years. M ± SE)	-	67.5 ± 1.7	Consistent with the epidemiology of ACD*
Nosological structure			
Diseases of the cardiovascular system	37	72.6 ± 6.2	Typical structure for EIHM**
Other diseases	14	27.4 ± 6.2	-
Path of hospitalization			
Emergency medical service	43	84.3 ± 5.1	Meets the standard (> 80%)
Outpatient and polyclinic facilities	8	15.7 ± 5.1	Requires optimization***
Quality of diagnostics			
Diagnostic concordance	40	78.4 ± 5.8	Below standard (standard >88%)
Diagnosis discrepancy	11	21.6 ± 5.8	Violation of quality standard

Notes: * ACD – acute cardiovascular diseases; ** EIHM – early in-hospital mortality; *** According to the results of the forensic medical examination, some patients required hospitalization by the emergency medical service (EMS)
Source: compiled by the authors of this study

Table 2. Deficiencies in medical care identified by forensic medical examination

Diagnostic and Therapeutic Intervention	Performed n (% ± SE%)	Deficiency Identified n (% ± SE%)	Regulatory Requirement, %	Legal Qualification of the Deficiency
Electrocardiography (ECG)	5 (9.8 ± 4.2)	46 (90.2 ± 4.2)	100	Gross violation of the CC* (Article 140)
Venous access	32 (62.7 ± 6.8)	19 (37.3 ± 6.8)	95	Violation of the EMC** standard
Pharmacotherapy	39 (76.5 ± 5.9)	12 (23.5 ± 5.9)	90	Incomplete scope of care
Oxygen therapy	16 (31.4 ± 6.5)	35 (68.6 ± 6.5)	85	Violation of the clinical protocol despite medical indications

Notes: * CC – Criminal Code; ** EMC – emergency medical care
Source: compiled by the authors of this study

within the framework of the forensic medical examination (Table 3).

The analysis showed that ECG was not performed in 90,9% of cases with diagnostic discrepancies and in 90,0% of cases with diagnostic concordance. The lack of a statistically significant difference indicates that the failure to perform an ECG was a universal systemic deficiency in medical care delivery, rather than a factor specific only to diagnostic errors.

A statistically significant moderate association was identified between the absence of pharmacotherapy and diagnostic discrepancies ($\varphi = 0,38$; $p = 0,013$). Moreover, the absence of venous access tended to be more frequent in the diagnostic discrepancy group. The association between the absence of oxygen therapy and diagnostic discrepancies did not reach statistical significance (Table 4).

ANALYSIS OF DEFICIENCIES ACCORDING TO THE DURATION OF HOSPITAL STAY

The analysis revealed significant temporal variations in medical care quality (Table 5). A trend in age distribution was observed ($p = 0,052$), with the oldest patients in the 3–6 h group (75,6 ± 1,8 years) and the youngest in the 12–24 h group (62,9 ± 3,1 years). The absence of oxygen therapy showed a significant downward trend (from 86,7 ± 8,8% to 38,5 ± 13,5%; $p_{trend} = 0,006$), whereas diagnostic discrepancies demonstrated a significant upward trend as the duration of stay increased, reaching 46,2 ± 13,8% in the 12–24 h group ($p_{trend} = 0,016$). The median number of deficiencies per patient significantly increased with the duration of stay, doubling from 2 (1–3) in the earliest group to 4 (3–4) in the 12–24 h one (Table 5). Furthermore, a moderate direct correlation

Table 3. Association between the absence of ecg examination and diagnostic discrepancies

Parameters	Diagnostic Discrepancy	Diagnostic Concordance	Total
ECG not performed	10 (90.9 ± 8.7%)	36 (90.0 ± 4.7%)	46
ECG performed	1 (9.1 ± 8.7%)	4 (10.0 ± 4.7%)	5
Total	11	40	51

Source: compiled by the authors of this study

Table 4. Association Between Other Deficiencies and Diagnostic Discrepancies

Deficiency	With Diagnostic Discrepancy (n = 11)	With Diagnostic Concordance (n = 40)	Fisher's Exact Test	φ
Absence of venous access, n (% ± SE%)	7 (63.6 ± 14.5 %)	12 (30.0 ± 7.2 %)	p = 0.075	0.29
Absence of pharmacotherapy, n (% ± SE%)	6 (54.5 ± 15.0 %)	6 (15.0 ± 5.6 %)	p = 0.013	0.38
Absence of oxygen therapy, n (% ± SE%)	9 (81.8 ± 11.6 %)	26 (65.0 ± 7.5 %)	p = 0.466	0.15

Source: compiled by the authors of this study

Table 5. Deficiencies According to the Duration of Hospital Stay

Indicator		Up to 3 h (n = 15)	3-6 h (n = 7)	6-12 h (n = 16)	12-24 h (n = 13)	p
Age, years, M ± SE		68.7 ± 2.8	75.6 ± 1.8	72.3 ± 2.1	62.9 ± 3.1	0.052
Age group, years, n (% ± SE)	<60	4 (26.7 ± 11.4)	0	2 (12.5 ± 8.3)	6 (46.1 ± 13.8)	0,071
	60-75	6 (40.0 ± 12.6)	4 (57.1 ± 18.7)	9 (56.3 ± 12.4)	4 (30.8 ± 12.8)	0,484
	>75	5 (33.3 ± 12.2)	3 (42.9 ± 18.7)	5 (31.3 ± 11.6)	3 (23.1 ± 11.7)	0,832
Females, n (% ± SE%)		9 (60.0 ± 12.6)	1 (14.3 ± 13.2)	10 (62.5 ± 12.1)	6 (46.2 ± 13.8)	0.156
Absence of ECG, n (% ± SE%)		13 (86.7 ± 8.8)	6 (85.7 ± 13.2)	14 (87.5 ± 8.3)	13 (100)	0.590
Absence of venous access, n (% ± SE%)		5 (33.3 ± 12.2)	4 (57.1 ± 18.7)	4 (25.0 ± 10.8)	6 (46.2 ± 13.8)	0.432
Absence of pharmacotherapy, n (% ± SE%)		2 (13.3 ± 8.8)	2 (28.6 ± 17.1)	3 (18.8 ± 9.8)	5 (38.5 ± 13.5)	0.427
Absence of oxygen therapy, n (% ± SE%)		13 (86.7 ± 8.8)	6 (85.7 ± 13.2)	11 (68.8 ± 11.6)	5 (38.5 ± 13.5)	0.033*
Diagnostic discrepancy, n (% ± SE%)		1 (6.7 ± 6.4)	1 (14.3 ± 13.2)	3 (18.8 ± 9.8)	6 (46.2 ± 13.8)	0.075**
Total deficiencies count per patient, Me (IQR)		2 (1-3)	2 (2-4)	2 (1-3***)	4 (3-4)	$p_{1-4} = 0.002$ $p_{3-4} < 0.001$

Notes: * $p_{trend} = 0{,}006$; ** $p_{trend} = 0{,}016$; *** Q_3 rounded from 2,5 (an average value between 2 and 3) to 3; p_{1-4} – the significance of difference between «up to 3 h» and «12-24 h» groups; p_{3-4} – the significance of difference between «6-12 h» and «12-24 h» groups
Source: compiled by the authors of this study

was established between patient age and the total number of deficiencies ($\rho = 0{,}462$; $p < 0{,}001$), suggesting that older age was associated with a higher accumulation of organizational and diagnostic shortcomings.

DISCUSSION

Early in-hospital mortality, as an integral indicator of the quality of medical care, enables the identification of systemic organizational and legal gaps regardless of the nosological structure [1]. In the context of this study, early in-hospital mortality is considered not only a statistical indicator but also a marker of the quality of medical care delivery. The identified deficiencies indicate the absence of effective public administration in the health care sector. The lack of managerial decisions regarding interaction between the stages of medical care, insufficient regulatory specification of the scope and sequence of diagnostic and therapeutic interventions, violations of medical documentation requirements, as well as ineffective quality control and accountability of medical professionals, require in-depth analysis.

Medeiros et al. (2020) demonstrated that early mortality is a sensitive indicator of organizational problems within the health care system [1]. Our findings are consistent with these conclusions. Hernesniemi et al. (2019), Li et al. (2021), Soleimani et al. (2025), and Liu

et al. (2024) showed that the accuracy of mortality prediction critically depends on the completeness of early diagnostic data [6–9].

Statistical Interpretation of Diagnostic Omissions. The ubiquitous absence of ECG examination (90,2% of all cases) resulted in the lack of a statistically significant association with diagnostic discrepancies. However, this does not diminish its clinical importance. According to the systematic analysis, such a mass omission of a fundamental diagnostic standard across all study groups indicates a "ceiling effect" of organizational failure. Rather than being a variable factor, the absence of ECG has become a constant systemic deficiency that fundamentally undermines diagnostic reliability. The fact that ECG was not performed in 90,9% of cases with diagnostic discrepancies confirms that this omission is a foundational barrier to accurate clinical assessment, regardless of the length of hospital stay.

Ukrainian studies have substantiated that forensic medical examination is an important instrument for identifying systemic gaps in legal regulation [11,12]. Our study confirms these findings: the identified gross violations of clinical protocols qualify as actions containing elements of offenses stipulated in Articles 139 and 140 of the Criminal Code of Ukraine.

LEGAL LEVERS FOR IMPROVING HEALTH CARE MANAGEMENT

International guidelines mandate ECG performance within 10 minutes in 100% of cases [2–5]. In developed countries, this indicator reaches 95–98%. Our data (9,8%) demonstrate a critical gap, necessitating the improvement of public administration mechanisms in health care through the legal establishment of mandatory ECG performance within a defined time frame as a managerial quality indicator integrated into systems of state control, accreditation, and accountability of health care institution managers.

ORGANIZATIONAL AND MANAGERIAL ASPECTS

The Ministry of Health of Ukraine should legislatively mandate 100% equipment of emergency medical service teams with ECG devices with autonomous power supply. Digitalization through the Electronic Health Care System (EHCS) should include automated monitoring of compliance with mandatory procedures and automatic notification of supervisory authorities in case of violations.

REGULATION OF MEDICAL PRACTICE

Licensing conditions for conducting medical practice should include mandatory availability of ECG equipment as a prerequisite for obtaining and renewing a medical practice license. Legislative establishment of mandatory forensic medical examination of all cases of early in-hospital mortality is required.

PATIENT RIGHTS PROTECTION AND FINANCIAL MECHANISMS

The National Health Service of Ukraine should monitor quality and safety indicators of medical care and apply financial and organizational sanctions to health care institutions with high mortality rates. Wang et al. (2021) demonstrated the economic feasibility of investments in diagnostic methods [14], while Ho et al. (2023) showed the critical impact of diagnostic timeliness on mortality [15]. In our study, 74,5% of patients sought medical care later than the optimal therapeutic window, indicating the need for legal regulation of public information campaigns.

LEGAL REGULATION UNDER MARTIAL LAW

Novakivskyy et al. (2023) analyzed the impact of the war in Ukraine on the health care system, including infrastructure destruction, power outages, logistical disruptions, shortages of medications, and personnel deficits [16]. Galea et al. (2020) and Daniels et al. (2021) demonstrated that emergencies exacerbate organizational deficiencies [17, 18].

If, in the pre-war period, the identified deficiencies resulted in mortality within 24 hours in 100% of cases, under wartime conditions these deficiencies become critically aggravated. Infrastructure destruction renders equipment non-operational; power outages transform the deficit in ECG performance (90,2%) into an almost complete absence of diagnostics; logistical disruptions prolong transport times, increasing the number of deficiencies; medication shortages exacerbate inadequate pharmacotherapy; and staff shortages prevent the use of available equipment.

Required Special Legal Regulation:

1. *Protected infrastructure*. Amendments to the Code of Civil Protection of Ukraine [19] mandating placement of diagnostic equipment in underground facilities with autonomous power supply, particularly operating rooms. For Kyiv, the obligation is to establish at least 10 underground diagnostic centers.
2. *System redundancy*. Amendments to licensing conditions require reserve diagnostic equipment, generators with fuel supply for 72 hours, and medication reserves for 21 days.
3. *Evacuation priority*. Amendments to traffic regulations [20] and the Law of Ukraine "On the Legal Re-

gime of Martial Law" [21] establish absolute priority passage for ambulances, even during air raid alerts, with predefined alternative routes.

4. *Decentralization*. Amendments to building regulations to create a network of small protected diagnostic units in populated areas.
5. *Enhanced control*. Legislative establishment of an automated system for analysis of all cases of early in-hospital mortality.

Future research perspectives include expanding the sample to a multicenter level, implementing a prospective design with continuous monitoring through the EHCS, and evaluating the effectiveness of the proposed legal and public administration mechanisms in reducing early in-hospital mortality both in peacetime and during armed conflict.

The study is limited by its single-center design and pre-war data. However, statistical power was sufficient to detect significant associations, and the identified patterns reflect systemic problems of the entire health care system of Ukraine.

CONCLUSIONS

1. Critical organizational and legal gaps were identified in early in-hospital mortality cases, characterized by systemic non-compliance with clinical protocols (ECG, venous access, oxygen therapy). These gross violations of medical standards qualify as actions containing elements of offenses stipulated in Articles 139 and 140 of the Criminal Code of Ukraine.
2. The absence of ECG examination (90,2 ± 4,2%) is a universal systemic failure that persists regardless of the diagnostic outcome or length of stay. This fundamental breach of health care legislation reflects profound gaps in the existing quality control and legal regulation of medical management.
3. Diagnostic discrepancies (21,6 ± 5,8%) significantly exceed acceptable medical standards, showing a significant upward trend as the length of hospital stay increases. The cumulative effect of deficiencies, and their moderate correlation with patient age, confirm that the longer a patient remains in the facility, the higher the risk of legal and clinical omissions.
4. Legal mechanisms for health care quality must be strengthened through the legislative establishment of 100% diagnostic equipment coverage, automated monitoring via the Electronic Health Care System (EHCS), and the implementation of stringent financial sanctions by the National Health Service of Ukraine for protocol violations.
5. Under wartime conditions, identified systemic deficiencies are critically exacerbated. Special legal regulation is required to ensure medical resilience, including the legislative establishment of autonomous underground diagnostic centers with 100% redundancy of critical systems, which is projected to reduce preventable mortality during large-scale infrastructure disruptions.

REFERENCES

1. Medeiros RP, Pereira R, Teixeira C. Mortality in the first 24 hours after admission in the intensive care unit. Rev Bras Ter Intensiva. 2020;32(4):537-546. doi:10.1590/0103-507x-2020-0082. DOI
2. Collet JP, Thiele H, Barbato E et al. 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. Eur Heart J. 2021;42(14):1289-1367. doi:10.1093/eurheartj/ehaa575. DOI
3. Byrne RA, Rossello X, Coughlan JJ et al. 2023 ESC Guidelines for the management of acute coronary syndromes. Eur Heart J. 2023;44(38):3720-3826. doi:10.1093/eurheartj/ehad191. DOI
4. Gulati M, Levy PD, Mukherjee D et al. 2021 AHA/ACC/ASE/CHEST/SAEM/SCCT/SCMR Guideline for the Evaluation and Diagnosis of Chest Pain. Circulation. 2021;144(22):e368-e454. doi:10.1161/CIR.0000000000001029. DOI
5. Lawton JS, Tamis-Holland JE, Bangalore S et al. 2021 ACC/AHA/SCAI Guideline for Coronary Artery Revascularization. Circulation. 2022;145(3):e18-e114. doi/10.1161/CIR.0000000000001038.
6. Hernesniemi JA, Mahdiani S, Tynkkynen JA et al. Extensive phenotype data and machine learning in prediction of mortality in acute coronary syndrome – the MADDEC study. Ann Med. 2019;51(2):156-163. doi:10.1080/07853890.2019.1596302. DOI
7. Li F, Xin H, Zhang J et al. Prediction model of in-hospital mortality in intensive care unit patients with heart failure: machine learning-based retrospective analysis of the MIMIC-III database. BMJ Open. 2021;11(7):e044779. doi:10.1136/bmjopen-2020-044779. DOI
8. Soleimani H, Najdaghi S, Narimani Davani D et al. Predicting in-hospital mortality in patients with acute myocardial infarction: a comparison of machine learning approaches. Clin Cardiol. 2025;48(3):e70124. doi:10.1002/clc.70124. DOI
9. Liu X, Xie Z, Zhang Y et al. Machine learning for predicting in-hospital mortality in elderly patients with heart failure combined with hypertension. Cardiovasc Diabetol. 2024;23(1):407. doi:10.1186/s12933-024-02503-9. DOI
10. Osnovy zakonodavstva Ukrayiny pro okhoronu zdorov'ya: Zakon Ukrayiny vid 19.11.1992 № 2801-KHII. [Fundamentals of the Legislation of Ukraine on Health Care: Law of Ukraine from 19.11.1992 № 2801-XII]. https://zakon.rada.gov.ua/laws/anot/en/2801-12 [Accessed 19 January 2026] (Ukrainian)

11. Pletenetska A, Demchenko I, Varfolomeiev Y. Expert assessment of diagnostic defects in traumatic brain injury with daily fatality rates depending on the duration of treatment and patients' hospital stay. AD ALTA: J Int Res. 2023;13(1):217–220. doi:10.33543/j.130235.217220. DOI
12. Berzina A, Pletenetska A. Forensic medical and legal analysis of cases of improper performance of professional duties by medical employees. Pol Merkur Lekarski. 2025;53(1):94-99. doi:10.36740/Merkur202501113. DOI
13. Kryminal'nyy kodeks Ukrayiny. Kodeks Ukrayiny vid 05.04.2001 № 2341-III. [The Criminal Code of Ukraine. Code of Ukraine from 05.04.2001 № 2341-III]. https://zakon.rada.gov.ua/laws/show/2341-14?lang=en#Text [Accessed 19 January 2026] (Ukrainian)
14. Wang CL, Chan YH, Wu VC et al. Incremental prognostic value of global myocardial work over ejection fraction and global longitudinal strain in patients with heart failure and reduced ejection fraction. Eur Heart J Cardiovasc Imaging. 2021;22(3):348-356. doi: 10.1093/ehjci/jeaa162. DOI
15. Ho YH, Lim CT, Chua CZF et al. Factors associated with in-hospital mortality among infective endocarditis patients. Med J Malaysia. 2023;78(6):743-750.
16. Novakivskyy V, Shurduk R, Grin I et al. War in Ukraine and dialysis treatment: human suffering and organizational challenges. Clin Kidney J. 2023;16(4):676-683. doi:10.1093/ckj/sfad003. DOI
17. Galea S, Merchant RM, Lurie N. The mental health consequences of COVID-19 and physical distancing. JAMA Intern Med. 2020;180(6):817-818. doi:10.1001/jamainternmed.2020.1562. DOI
18. Daniels LB, Ren X, Kumar K et al. Relation of prior statin and antihypertensive use to severity of disease among patients hospitalized with COVID-19. PLoS One. 2021;16(7):e0254635. doi:10.1371/journal.pone.0254635. DOI
19. Kodeks tsyvil'noho zakhystu Ukrayiny: Kodeks Ukrayiny vid 02.10.2012 № 5403-VI. [Code of Civil Protection of Ukraine: Code of Ukraine from 02.10.2012 № 5403-VI]. https://zakon.rada.gov.ua/laws/show/5403-17?lang=en#Text [Accessed 19 January 2026] (Ukrainian)
20. Pro Pravyla dorozhn'oho rukhu: Postanova Kabinetu Ministriv Ukrayiny vid 10.10.2001 № 1306. [On traffic rules. Resolution of the Cabinet of Ministers of Ukraine from 10.10.2001 № 1306]. https://zakon.rada.gov.ua/laws/show/1306-2001-%D0%BF#Text [Accessed 19 January 2026]. (Ukrainian)
21. Zakon Ukrainy «Pro pravovyi rezhym voiennoho stanu» vid 14.05.2025 № 389-VIII. [On Legal Regime of Martial Law: Law of Ukraine from 14.05.2025 № 389-VIII]. https://zakon.rada.gov.ua/laws/show/en/389-19?lang=uk [Accessed 19 January 2026]. (Ukrainian)

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Alina O. Pletenetska
Bogomolets National Medical University
5 Mechnikov St., 01133 Kyiv, Ukraine
e-mail: fantasyalinka@gmail.com

ORCID AND CONTRIBUTIONSHIP

Alina O. Pletenetska: 0000-0002-7029-3377 A B C D E
Anzhela B. Berzina: 0000-0002-9885-309X A B D E F
Ruslan A. Volynets: 0000-0002-8134-8572 A D F
Oksana O. Cherniak: 0000-0003-0991-4477 A E F
Oleksandr V. Felyk: 0000-0003-0862-1453 A E F
Valentyn V. Halunko: 0000-0002-8133-6766 A D F

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

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ORIGINAL ARTICLE

CONTENTS

Dose adjustment of oral thyroxine in patients consuming dairy products: A cohort retrospective study

Zahraa Al-Isawi[1], Ammar Azzam[2], Mohammed Sh. Sachit[3], Fadhil Abdulameer Wdaah Alsailawi[4], Salim Kadhim[5]

[1]DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY, FACULTY OF PHARMACY, UNIVERSITY OF KUFA, NAJAF, IRAQ
[2]COLLEGE OF PHARMACY, UNIVERSITY OF BABYLON, HILLAH, IRAQ
[3]COLLEGE OF PHARMACY, AL-ZAHRAWI UNIVERSITY, KARBALA, IRAQ
[4]COLLEGE OF PHARMACY, UNIVERSITY OF ALTOOSI, NAJAF , IRAQ
[5]COLLEGE OF PHARMACY, UNIVERSITY OF ALKAFEEL, NAJAF, IRAQ

ABSTRACT

Aim: Hypothyroidism management with levothyroxine might be affected by a range of factors, including diet. Dairy sources are rich in calcium and proteins which may interfere with the absorption of thyroxine so dose adaptability might be required. This study assesses the effect of dairy intake on levothyroxine dose and clinical outcomes.

Materials and Methods: Retrospective cohort study over 14 months in an endocrinology center in Najaf, included 150 adult patients with primary hypothyroidism on stable oral levothyroxine therapy. We classified individuals into high, medium and low dairy consumers. A comparative approach was performed between these groups on terms of the dose, time to stabilization, dose response, clinical outcome, and economic outcome.

Results: After multivariable analysis, we found that participants who consumed one or more servings of dairy products per day were 6.8 times more likely to report taking their medications at least once with inadequate preparation $p<0.001$. These findings suggest that dietary interventions targeting dairy intake could stabilize treatment and reduce healthcare costs.

Conclusions: Dietary intake of dairy has a major impact on both the absorption and dosing of levothyroxine in hypothyroid patients. Given the healthcare burden arising from dose adjustments, clinicians should consider dietary counselling and timing strategies to improve therapy.

KEY WORDS: thyroxine, hypothyroidism, dairy products, dose adjustment

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INTRODUCTION

Hypothyroidism is a metabolic disorder that occurs when there is reduced secretion of the thyroid hormones triiodothyronine (T3) and thyroxine (T4). Low thyroid hormone is detected by the pituitary gland, which responds by producing more TSH [1]. The dysfunctional thyroid gland itself is a hallmark of primary hypothyroidism [2]. TSH secretion elevates and serum TSH levels rise in relation to thyroid hormone deficiency. Inadequate stimulation of a structurally normal gland due to diminution in the release of pituitary TSH (secondary hypothyroidism) or primary failure of hypothalamic thyrotropin-releasing hormone (TRH) stimulation can also lead to diminished thyroidal secretion of thyroid hormone [3]. Practically clinical discrimination between secondary (pituitary origin) and tertiary (hypothalamic) hypothyroidism is not always possible and, therefore, they are referred to as 'central hypothyroidism' [1]. Generally speaking, a thyroid gland that is under-producing thyroid hormone is referred to as hypothyroidism. It may be of primary etiology (due to an abnormality in the thyroid gland per se) or secondary/central (due to hypothalamic or pituitary dysfunction). Subclinical hypothyroidism is a term referring to mild forms of primary hypothyroidism with normal free thyroxine (FT4) and total or free T3 levels but an elevated serum TSH level [4]. The incidence of progress from subclinical to manifest hypothyroidism is 2-5% per year [5]. Hypothyroidism has a prevalence of 3.8-4.6% in the general population. Four-point-one new cases of hypothyroidism per 1000 women and 0.6 new cases per 1000 men per year were found in the Whickham survey [6]. Most cases are a result of primary thyroid gland failure due to long-standing thyroid autoimmune (Hashimoto's) thyroiditis, radioactive iodine (I^{131}) therapy, or surgery.

Levothyroxine is mainly absorbed in the small intestines and absorption is known to be influenced by gastric pH, food intake and some minerals and compounds [7]. Calcium, abundant in milk, is known to form an insoluble complex with thyroxine in the gut, and may decrease the

oral bioavailability of thyroxine by 20–25% [8]. Moreover, the protein in dairy products might also compromise the absorption, possibly due to the effects on gastric emptying and intestinal transit time [9]. Clinicians usually advise levothyroxine in a fasted state, preferably 30-60 min before breakfast, to maximize its absorption. Yet, actual patient compliance with these time recommendations is highly variable in practice, with many patients unintentionally ingesting dairy products shortly before and after their thyroxine dose. This poses a considerable clinical problem as continued poor absorption results in continued hypothyroid symptoms, requiring dose increases that may not solve the underlying absorption problem. Conflicting evidence surrounding the clinical impact of dairy product intake on thyroxine therapy has been reported by previous studies. Although there is evidence from a few trials that absorption of thyroxine is reduced by a measurable degree when thyroxine is taken with calcium-containing compounds [10-12], the long-term consequences of this and the most appropriate management strategy are not well established. In addition, the extent to which systematic dose correction can compensate absorption impairment due to dairy in the "real world" of clinical practice, based on the available evidence, is unclear. This interaction is complicated by patient-specific factors including timing and amount of dairy intake, baseline thyroid function, and any co-administered medications. The knowledge of these factors is important for establishing evidence-based recommendations which might aid clinicians in optimizing thyroxine therapy for dairy-product consuming patients.

AIM

This is a retrospective cohort study to assess the variation in dosing strategies for oral thyroxine in the presence of regular dietary dairy intake as measured by its effect on both biochemical and clinical end points. In examining real-world clinical data, the aim of this study is to offer clinically-relevant information on how to approach this frequently encountered therapeutic dilemma and inform evidence-based recommendations for thyroxine dosing in the setting of dairy intake.

MATERIALS AND METHODS

STUDY DESIGN

The objective of this cohort study was to assess the relationship between intake of dairy products and the necessity to adjust the dose of oral thyroxine replacement in hypothyroid patients. It was a longitudinal study with retrospective review of data retrieved from the electronic health record in the endocrinology center in Najaf over 14 months (September 2023- November 2024). All participating sites have standardized electronic health record systems and practices for monitoring thyroid hormone replacement therapy.

STUDY POPULATION

INCLUSION CRITERIA

Inclusion criteria included adult patients aged above 18 years with a diagnosis of primary hypothyroidism, continues on oral levothyroxine treatment within the study period, at least 3 TSH measurements and 12 months after treatment at least, full medical recall and dosage available and dietary intake information obtained from clinical notes or structured questionnaires.

EXCLUSION CRITERIA

Exclusion criteria included those that have secondary or tertiary hypothyroidism, pregnant or lactating women at the time of the study, malabsorption diseases (coeliac disease, inflammatory bowel disease, short gut syndrome) patients, concomitant treatment with substances which interfere with the absorption of levothyroxine (eg, antacids, proton pump inhibitors, calcium supplements, iron supplements or sucralfate), missing chart records or follow-up less than 12 months, patients with large comorbidities that disturbed the pleiotropic effects predicated on thyroxine metabolism (severe liver or kidney impairment) and patient with poor adherence and dietary system. Those patients who were characterized as "non-consumers" of dairy products were also excluded because of their small sample size.

DATA COLLECTION

DATA SOURCES

Patients meeting eligibility criteria were identified by searching electronic health records. Data collection was carried out by trained research staff using data collection forms designed for the study, which included quality control mechanisms.

VARIABLES COLLECTED

PRIMARY EXPOSURE VARIABLE

Dairy Products intake: Categorized according to intake record as:

Table 1. Baseline characteristics by dairy consumption groups

Characteristic	Group 1 (n=45)	Group 2 (n=55)	Group 3 (n=50)	P-value
Demographics				
Age (years, mean ± SEM)	38 ± 6	37 ± 4	39 ± 7	0.80
Female sex (%)	33	34	33	0.99
BMI [kg/m^2, mean ± SEM]	29 ± 4	30.5 ± 6	29.5 ± 3	0.78
Clinical parameters				
Initial TSH [mIU/L, median (IQR)]	9.33 (7.45-15.35)	9.58 (8.15-16.22)	8.99 (7.11-16.22)	0.99
Initial T4 [µg/dL, mean ± SEM]	0.55 ± 0.02	0.48 ± 0.11	0.51 ± 0.14	0.85
TPO antibodies positive [%]	82	83	81	0.98
Medication factors				
Initial levothyroxine dose [mcg, mean ± SEM]	110 ± 13	115.5 ± 12.5	112 ± 18	0.44
Comorbidities				
Diabetes mellitus [%]	8	7	9	0.99
Cardiovascular disease [%]	2	3	3	0.98
Lifestyle factors				
Current smoker [%]	33	35	32	0.99
Alcohol consumption [%]	NA	NA	NA	NA

Group 1: High Consumers, Group 2: Moderate Consumers, Group 3: Low Consumers

Group 1: High consumers (> 3 serving of dairy/day, n=45)
Group 2: Moderate (1-3 serving of dairy/day, n=55)
Group 3: Low consumers (< 1 serving of dairy/day, n=50)

PRIMARY OUTCOME VARIABLE

Dose Adjustment: Increase in levothyroxine dose by ≥25 mcg during follow-up to normalize TSH levels (target 0.4-4.0 mIU/L in most sick patients or goal agreed between the patient and the clinician).

SECONDARY OUTCOME VARIABLES

Time to stable TSH (defined as experience of 2 consecutive TSH measurements in target range at least 6 weeks apart)

Total number of dose adjustments needed

Final stable dose of levothyroxine (mcg/kg body weight)

Percentage of patients who need a dose increase >50% than the initial dose

COVARIATES AND CONFOUNDING VARIABLES

Demographics: Age, gender, ethnicity, body mass index

Clinical variables: TSH at onset, thyroid autoimmunity, cause of hypothyroidism

ETHICAL CONSIDERATIONS

Approval for this study was obtained from College of Pharmacy, University of Alkafeel. Because this was a retrospective study that used de-identified health records, the need for informed consent was waived.

SAMPLE SIZE CALCULATION

Power calculation was performed based on the primary efficacy outcome of dose adjustment needs. Considering a 40% as a baseline dose adjustment rate in those with low dairy intake, with a clinically relevant 20% difference between groups (relative risk=1.5), 80% power, and α=0.05, assuming potential confounding in multivariable analyses. A total of 150 patients were chosen as the target sample size.

STATISTICAL ANALYSIS

Data for 150 patients were presented as Mean ± SEM. All analyses and graphs were conducted using GraphPad Prism 9.3.1. Descriptive statistics were used to describe baseline characteristics. Continuous variables were expressed as means ± standard error of mean (SEM) according to data distribution normality (the Shapiro-Wilk test). Categorical variables were reported as frequencies and percentages. One Way ANOVA was performed to test differences and p value less than 0.05 was considered significantly different.

RESULTS

All groups were comparable with respect of baseline characteristics with no significant differences concern-

Table 2. Characteristics of dairy consumption

Parameter	Group 1 (n=45)	Group 2 (n=55)	Group 3 (n=50)
Dairy servings/day, mean ± SEM	6 ± 2	3 ± 1	1 ± 0.5
Timing of consumption			
< 2 hours [%]	44	51	48
> 2 hours [%]	56	49	52
Primary dairy products consumed			
Milk [%]	30	31	34
Yogurt (%)	34	32	34
Cheese [%]	36	37	32

Group 1: High Consumers, Group 2: Moderate Consumers, Group 3: Low Consumers

Table 3. Primary outcome - dose adjustment requirements

Outcome	Group 1 (n=45)	Group 2 (n=55)	Group 3 (n=50)	P-value
Primary Outcome				
Dose adjustment ≥25 mcg [%]	63.1	37.3	15.8	0.001
Dose Adjustment Magnitude				
Mean dose increase [mcg]	31.5 ± 12.4	17.8 ± 11.3	9.8 ± 4.6	0.001
Dose increase >50% [%]	21.3	7.5	2.1	0.001
Final Stabilized Dose				
Final levothyroxine dose [mcg]	136.5 ± 24.5	122.7 ± 13.6	106 ± 7.2	0.04
Final dose per kg body weight [mcg/kg]	1.4 ± 0.7	1.6 ± 0.55	1.5 ± 0.2	0.022

Group 1: High Consumers, Group 2: Moderate Consumers, Group 3: Low Consumers

Table 4. Association between dairy consumption and dose adjustment requirements

Variable	Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
Dairy Consumption				
Low consumers	Reference	-	Reference	-
Moderate consumers	2.74 (1.30–6.25)	0.009	2.60 (1.10–6.00)	0.028
High consumers	7.42 (3.20–17.50)	<0.001	6.80 (2.90–15.80)	<0.001

Table 5. Time to achieve stable TSH levels

Group	Median time to stabilization (weeks)	95% CI (weeks)	Log-rank P-value
Low consumers	5.5	7.4 – 9.3	
Moderate consumers	6.5	5.6 – 7.9	0.022
High consumers	9	4.3 – 6.9	

ing age, sex, BMI, initial thyroid function and antibody status, comorbidities or the initial levothyroxine dose, all P>0.05, table (1).

Daily dairy intake was as expected significantly different among the groups: High consumers reported 6 daily servings on average, while moderate and low consumers reported consuming 3 and 1 daily servings respectively (Table 2). Group assignment did not systematically influence the type or timing of dairy products.

Dairy intake is the single most influential dietary factor related to higher levothyroxine dose requirements. High consumers were more likely than low consumers to enroll with ≥25 mcg dose increases (63.1% *vs.* 15.8%), required greater mean dose escalation at time of entry (31.5 mcg vs 9.8 mcg), and needed >50% dose increases more frequently (21.3% *vs.* 2.1%, P < 0.001), table (3). Multivariable analysis demonstrated a dose-response relationship, with moderate and high consumption associated with 2.6- and 6.8-fold larger odds of adjustment required, table (4).

Table 6. Dosage modifications based on dairy intake

Number of adjustments	Group 1 n (%)	Group 2 n(%)	Group 3 n (%)	P-value
0	31 (68.9%)	26 (47.3%)	11 (20.0%)	
1	9 (20%)	14 (25.5%)	9 (20.0%)	0.001
2	4 (8.9%)	11 (20%)	16 (30.0%)	
≥3	1 (2.2%)	4 (7.2%)	14 (30.0%)	

Group 1: High Consumers, Group 2: Moderate Consumers, Group 3: Low Consumers

Table 7. TSH range distribution

TSH range	Group 1 n (%)	Group 2 n (%)	Group 3 n (%)	P-value
Within target (0.4-4.0 mIU/L)	36 (80%)	41 (74.5%)	32 (64%)	0.045
Below target (<0.4 mIU/L)	4 (8.8%)	8 (14.5%)	4 (8%)	
Above target (>4.0 mIU/L)	5 (11.1%)	6 (11%)	14 (28%)	

Group 1: High Consumers, Group 2: Moderate Consumers, Group 3: Low Consumers

Table 8. Clinical impact of follow-up

Comparison	Absolute risk difference [%]	Number needed to treat	95% CI
Group 1 vs. group 2	13	8	5-21
Group 1 vs. group 3	29	5	19-43

Table 9. Economic impact follow-up utilization measures

Measure	Group 1 (n=45)	Group 2 (n=55)	Group 3 (n=50)	P-value
No. of follow-up visits (mean ± SEM)	8 ± 1.3	4 ± 1.2	2 ± 0.8	0.045
No. of TSH tests	6 ± 1.1	4.4 ± 0.8	4.2 ± 0.6	0.36
Duration for stabilization (weeks)	12 ± 2.1	7.3 ± 1.7	3.4 ± 1.01	0.034

Group 1: High Consumers, Group 2: Moderate Consumers, Group 3: Low Consumers

In addition to the fact that intake of dairy delayed normalization of TSH. Time to stabilization Median time to stabilization in low consumers: 5.5 weeks (95%CI 3–8) median time to stabilization in moderate consumers: 6.5 weeks (95%CI, 4–18) median time to stabilization in high consumers:9 weeks (95%CI,6–26), P=0.022, log-rank test, table (5).

High consumers were more likely to have ≥3 dose adjustments (30% *vs*. 2.2%, P = 0.001), table (6) and less likely to achieve target TSH by study end (64% *vs.* 80%, P=0.045) compared with low consumers, table (7). At the clinical level these absolute risk reductions corresponded to numbers-needed-to-treat of 5–8 to prevent one case of PD in participants asked to reduce their intake of dairy, table (8). Additionally, stabilizing the diet for high consumers required more frequent follow-up visits and a longer sustained period (12 weeks vs. 3.4 weeks, P=0.034), demonstrating the healthcare burden of dietary interference from an economic perspective, table (9).

DISCUSSION

Our main finding in this study is the robust and meaningful correlation between dairy products' consumption and the dose of levothyroxine such that it becomes clinically significant among subjects with primary hypothyroidism. Baseline demographic and clinical parameters were similar across groups, but subjects with greater dairy intake experienced more dose escalation, later attainment of stable TSH levels, more frequent adjustments in treatment, and higher healthcare utilization. These results highlight the relevance of dietary factors in management of levothyroxine replacement therapy. Our findings are consistent with previous pharmacokinetic data when taken regarding the known impact that calcium and other dairy components have on levothyroxine absorption in the gastrointestinal tract [8, 13-15]. Dairy products, especially milk and yogurt, are rich in calcium that may chelate levothyroxine, lowering its absorption [16-17]. Protein and fats content, fat also affect gastric emptying time as well as intestinal transport that can further lead to an impaired absorption [9]. Paradoxically,

while the effect on time of dairy intake relative to medication ingestion failed to be demonstrated we did find that, in absolute terms, dairy timing was sufficiently influential as a whole to differentiate thyroxine preparation requirements between groups. A robust dose–response pattern was observed in our study. High-dairy consumers were nearly 7 times more likely than low consumers to require significant dose escalation, and each additional daily dairy serving independently predicted a larger final levothyroxine dose. The associated therefore not only argument is also used for causal inference and to inform clinicians about what level of tax could guide dietary advice. In high dairy consumers, stabilization was 4 weeks later and many required ≥2 dose adjustments to maintain TSH target by follow-up. In clinical terms, this translates into extremely low numbers needed to treat (in the area of 5–8) suggesting that even small reductions in dairy should have a significant effect on outcomes. Co-ingestion of 2% cow's milk (12 oz) with levothyroxine significantly lowered the area under the curve (AUC) for serum total T4 levels compared to levothyroxine taken with water — Further, ingesting cow's milk or eating within 30 minutes of taking levothyroxine resulted in lower peak concentrations, compared to fasting [16]. This is consistent with your observation that increased absolute dairy intake is associated with higher dose responses. Another study found that concurrent use of calcium carbonate (1,200 mg/day elemental calcium) with levothyroxine reduced serum total T4 and free T4 levels and increased TSH in a 20-patient cohort (e.g., >30% increase from baseline; P <0.001) [8]. In healthy volunteers whole-body retention of T4 was studied during a 24 h period, which showed significant decreases in absorption of T4: Taking levothyroxine with calcium carbonate (2 g) versus without calcium carbonate p=0.022, 84% *vs.* 58% [18]. This works perfectly with the dose–dependent interference effect your studies revealed - more calcium (from dairy) - more likely to need a higher dose escalation. A recently published review on the topic established that both calcium and iron (di- and tri-valent elements) decrease levothyroxine absorption, probably as a consequence of nonspecific adsorption in the gastrointestinal tract leading to insoluble complexes. These parallel phenomena with other nutritional compounds — coffee, soy, supplements & medications etc. are known to decrease levothyroxine bioavailability as well [16]. The general consensus from expert opinion panels as well as important sources of information advises that levothyroxine should be given at least 2-4 hours apart from dietary products containing dairy and calcium to prevent the two agents from interacting [19]. The FDA advises waiting 4 hours between levothyroxine and dairy consumption [20]. Our finding that even modest reductions in daily dairy or more careful dietary timing could be meaningfully altering the efficacy of thyroid hormone treatment support such a conclusion. In addition to clinical end-points, our study also underscores the economic costs associated with uncontrolled dietary interference. Highs required more than twice as many additional visit follow up plus a significantly longer cadre stabilization period. It concluded that such unnecessary health care utilization is a cost-effective reason for simple dietary approaches. Thus, an unrestrained dietary consumption further paves the way for chronic diseases like type 2 diabetes, obesity and cardiovascular diseases. The victims of these diseases usually require frequent doctor visits, hospitalization and long term care, making healthcare expensive. For example, one study demonstrated that individuals with food insecurity and chronic diseases had greater health care costs than their food-secure counterparts [21]. There is a large economic burden due to poor nutrition, and dietary interventions could be an economical way to address this. Outcomes of many studies have established that intervention programs having a focus on healthy dietary and physical activity behaviors are effective to provide better health results at an affordable cost. For instance, a study based on an adolescent dietary and physical activity intervention yielded health improvements at an extra cost to providers of £123 per participant [22]. There is a high economic cost of malnutrition Unhealthy eating is associated with an excess of $50 billion in healthcare costs related to heart disease, stroke, and diabetes annually in the US alone. The figure includes out-of-pocket medical expenses as well as indirect costs, such as lost productivity [23]. Addressing such dietary interventions in healthcare strategy may be an economical means of downgrading the economic burden related to malnutrition and chronic diseases. Yet thoughtful planning is needed to ensure that these interventions are implemented effectively and in a sustainable manner. Our study further emphasizes the clinical burden and economic price of dietary noncompliance as high consumers necessitated > twofold greater follow-up and additional time to achieve stabilization. This underscores that basic dietary changes could offer a low-cost solution for reducing superfluous healthcare consumption. We also need more work on the long-term effects of dietary patterns and related interventions on healthcare costs, effectiveness of personalized nutrition interventions, integration with digital health tools, and public-health policy that reduces the burden of diet on national economies.

CONCLUSIONS

Dietary intake of dairy has a major impact on both the absorption and dosing of levothyroxine in hypothyroid patients. Given the healthcare burden arising from dose adjustments, clinicians should consider dietary counselling and timing strategies to improve therapy.

REFERENCES

1. Bereda G. Definition, causes, pathophysiology, and management of hypothyroidism. Mathews J Pharm Sci. 2023;7(1):1-5. doi:https://doi.org/10.30654/MJPS.10014. DOI
2. Hegedüs L, Bianco AC, Jonklaas J, Pearce SH, Weetman AP, Perros P. Primary hypothyroidism and quality of life. Nat Rev Endocrinol. 2022;18(4):230-242. doi:10.1038/s41574-021-00625-8. DOI
3. Zamwar UM, Muneshwar KN. Epidemiology, types, causes, clinical presentation, diagnosis, and treatment of hypothyroidism. Cureus. 2023;15(9). doi:10.7759/cureus.46241. DOI
4. Biondi B, Cappola AR. Subclinical hypothyroidism in older individuals. Lancet Diabetes Endocrinol. 2022;10(2):129-141. doi:10.1016/S2213-8587(21)00285-0. DOI
5. Yoo WS, Chung HK. Subclinical Hypothyroidism: Prevalence, Health Impact, and Treatment Landscape. Endocrinol Metab (Seoul). 2021;36(3):500-513. doi:10.3803/EnM.2021.1066. DOI
6. Siskind SM, Lee SY, Pearce EN. Investigating hypothyroidism. BMJ. 2021;373:n993. doi:10.1136/bmj.n993. DOI
7. Lipp HP. Administration and Pharmacokinetics of Levothyroxine BT - 70 Years of Levothyroxine. In: Kahaly GJ (ed.). Springer International Publishing. 2021, pp. 13-22. doi:10.1007/978-3-030-63277-9_2. DOI
8. Wiesner A, Gajewska D, Paśko P. Levothyroxine Interactions with Food and Dietary Supplements - A Systematic Review. Pharmaceuticals. 2021;14(3). doi:10.3390/ph14030206. DOI
9. Loveday SM. Protein digestion and absorption: the influence of food processing. Nutr Res Rev. 2023;36(2):544-559. doi:10.1017/S0954422422000245. DOI
10. Vuong M, González Aragón C, Montarroyos SS. Common Food and Drug Interactions. Pediatr Rev. 2023;44(2):68-80. doi:10.1542/pir.2022-005641. DOI
11. Wongdee K, Chanpaisaeng K, Teerapornpuntakit J, Charoenphandhu N. Intestinal Calcium Absorption. Compr Physiol. 2021;11(3):2047-2073. doi:10.1002/j.2040-4603.2021.tb00173.x. DOI
12. Gatta E, Bambini F, Buoso C, et al. Liquid levothyroxine formulations in patients taking drugs interfering with L-T4 absorption. Front Endocrinol (Lausanne). 2022;13:1080108. doi:10.3389/fendo.2022.1080108. DOI
13. Skelin M, Lucijanić T, Amidžić Klarić D, et al. Factors Affecting Gastrointestinal Absorption of Levothyroxine: A Review. Clin Ther. 2017;39(2):378-403. doi:10.1016/j.clinthera.2017.01.005. DOI
14. Liu H, Lu M, Hu J, et al. Medications and Food Interfering with the Bioavailability of Levothyroxine: A Systematic Review. Ther Clin Risk Manag. 2023;19(null):503-523. doi:10.2147/TCRM.S414460. DOI
15. Virili C, Antonelli A, Santaguida MG, Benvenga S, Centanni M. Gastrointestinal Malabsorption of Thyroxine. Endocr Rev. 2019;40(1):118-136. doi:10.1210/er.2018-00168. DOI
16. Chon DA, Reisman T, Weinreb JE, Hershman JM, Leung AM. Concurrent Milk Ingestion Decreases Absorption of Levothyroxine. Thyroid®. 2018;28(4):454-457. doi:10.1089/thy.2017.0428. DOI
17. Chandan RC. Dairy Ingredients for Food Processing: An Overview. In: Chandan RC, Kilara A (eds). Dairy Ingredients for Food Processing. 2011; 3-33. doi: 10.1002/9780470959169.ch1. DOI
18. Miccoli P, Materazzi G, Rossi L. Levothyroxine Therapy in Thyrodectomized Patients. Front Endocrinol (Lausanne). 2021 Jan 29;11:626268. doi: 10.3389/fendo.2020.626268. DOI
19. Liu H, Li W, Zhang W, Sun S, Chen C. Levothyroxine: Conventional and Novel Drug Delivery Formulations. Endocr Rev. 2023 May 8;44(3):393-416. doi: 10.1210/endrev/bnac030. DOI
20. Garcia SP, Haddix A, Barnett K. Incremental health care costs associated with food insecurity and chronic conditions among older adults. Prev Chronic Dis. 2018;15(8):1-11. doi:10.5888/pcd15.180058. DOI
21. Kalita N, Cooper K, Baird J, et al. Cost-effectiveness of a dietary and physical activity intervention in adolescents: a prototype modelling study based on the Engaging Adolescents in Changing Behaviour (EACH-B) programme. BMJ Open. 2022; 12(8): e052611. doi:10.1136/bmjopen-2021-052611. DOI
22. Jardim TV, Mozaffarian D, Abrahams-Gessel S, et al. Cardiometabolic disease costs associated with suboptimal diet in the United States: A cost analysis based on a microsimulation model. PLOS Med. 2019; 16(12): e1002981. doi: 10.1371/journal.pmed.1002981. DOI
23. Gatta E, Bambini F, Buoso C, Gava M, Maltese V, Anelli V, Delbarba A, Pirola I, Cappelli C. Liquid levothyroxine formulations in patients taking drugs interfering with L-T4 absorption. Front Endocrinol (Lausanne). 2022 Dec 6;13:1080108. doi: 10.3389/fendo.2022.1080108. DOI

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Salim Kadhim
College of Pharmacy, University of Alkafeel, Najaf, Iraq
e-mail: Sfk9@alkafeel.edu.iq

ORCID AND CONTRIBUTIONSHIP
Zahraa Al-Isawi: 0000-0001-7413-7278 B C
Ammar Azzam: 0009-0006-0760-7309 C D
Mohammed Sh. Sachit: 0009-0009-2428-5876 C D E
Fadhil Abdulameer Wdaah Alsailawi: 0000-0003-1880-1335 D E
Salim Kadhim: 0000-0003-0015-2218 A F

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

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REVIEW ARTICLE

CONTENTS

The surgical *versus* non-surgical approach to the management of acute cholelithiasis within the elderly population: A narrative review

Ehtesam A. Chowdhury[1], Olivia C. Jadeja[2], Surajit Sinha[3]

[1]DEPARTMENT OF GENERAL SURGERY, HEREFORD COUNTY HOSPITAL, WYE VALLEY NHS TRUST, UNITED KINGDOM

[2]DEPARTMENT OF GENERAL SURGERY, SOUTHMEAD HOSPITAL, NORTH BRISTOL NHS TRUST, UNITED KINGDOM

[3]DEPARTMENT OF GENERAL SURGERY, TORBAY HOSPITAL, TORBAY & SOUTH DEVON NHS FOUNDATION TRUST, UNITED KINGDOM

ABSTRACT

Aim: This narrative review aims to critically evaluate current evidence comparing surgical and non-surgical management strategies for acute cholelithiasis in elderly patients, focusing on outcomes, risks, and decision-making factors unique to this population.

Materials and Methods: A comprehensive literature search was performed in MEDLINE®, Embase™, PubMed®, and Google Scholar™ using the terms: "acute cholecystitis," "cholelithiasis," "elderly," "surgical management," "laparoscopic cholecystectomy," "non-surgical," and "percutaneous cholecystostomy." Studies published between 2005 and 2025 were included if they evaluated outcomes such as morbidity, mortality, recurrence, and hospital stay in elderly patients. Both surgical and non-operative management strategies were compared, including antibiotic therapy and cholecystostomy. Articles were selected in accordance with PRISMA principles.

Conclusions: Laparoscopic cholecystectomy remains the gold standard for acute gallstone disease but carries higher morbidity and mortality in elderly patients due to comorbidities and frailty. Non-operative approaches such as percutaneous cholecystostomy, or antibiotic therapy may reduce immediate surgical risk but are associated with higher recurrence and readmission rates. Optimal management requires an individualised, multidisciplinary approach considering physiological reserve, inflammatory markers, and patient preference. More prospective studies are needed to standardise risk stratification and management pathways specific to geriatric patients with acute cholelithiasis.

KEY WORDS: cholelithiasis, laparoscopic cholecystectomy, elderly, non-operative management, percutaneous cholecystostomy

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INTRODUCTION

Cholelithiasis, defined as the formation of gallstones within the gallbladder, affects approximately 10–20% of adults in developed nations, with prevalence increasing sharply with age [1]. It is estimated that over 30% of individuals above 70 years develop gallstones, and women are affected nearly twice as often as men, particularly between the fifth and seventh decades of life due to hormonal and metabolic factors [2, 3].

The aging population has led to a corresponding rise in gallstone-related complications, which commonly include acute cholecystitis, choledocholithiasis, biliary pancreatitis, and cholangitis [4]. In elderly patients, these complications occur more frequently and can present atypically, resulting in delayed diagnosis and increased morbidity [5]. Among those older than 65 years, the incidence of acute cholecystitis is estimated at 6–11 cases per 1,000 persons annually, with associated mortality rates up to 5–10% in complicated cases [6, 7].

Management of acute gallstone disease in older adults presents unique challenges due to altered physiology, frailty, and the higher prevalence of cardiovascular and pulmonary comorbidities [8]. These factors amplify surgical risk and often necessitate consideration of conservative or minimally invasive options. Therefore, balancing the benefits of definitive surgical management against the risks of operative morbidity forms a critical part of clinical decision-making in this group.

AIM

The aim of this review is to compare and critically analyse surgical and non-surgical approaches to the management of acute cholelithiasis in the elderly population, assessing evidence regarding safety, efficacy, recurrence, and overall outcomes to guide optimal, individualised patient care.

Table 1. Inclusion and exclusion criteria for study eligibility

Criteria	Inclusion	Exclusion
Study focus	Acute gall stone disease/cholelithiasis and related complications	Other non-biliary stone disease
Population	Elderly (≥65 years) or studies identifying "geriatric" cohorts	Non-elderly populations
Study type	Original research, clinical trials, meta-analyses, or reviews	Commentaries, abstracts, conference abstracts, letters to the editor
Language and methods	Quantitative/qualitative/mixed methodology in English	Non-English
Timescale	Literature published between 2025 and 2005 (past 20 years)	Literature published before 2005

MATERIALS AND METHODS

This narrative review was conducted following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) framework to ensure transparency and reproducibility.

The search strategy was largely through electronic databases such as MEDLINE®, Embase™, PubMed®, and Google Scholar™. These were systematically searched using combinations of the following Boolean operators: ("surgical" OR "operative" OR "invasive") AND ("non-surgical" OR "non-operative" OR "conservative") AND ("management" OR "treatment") AND ("gall stones" OR "cholelithiasis") AND ("elderly" OR "geriatric")

Search limits included articles published in the English language, original research, reviews, meta-analyses and studies published between January 2005 and January 2025. Additional manual screening of bibliographies was performed to identify relevant studies not indexed by database algorithms.

INCLUSION AND EXCLUSION CRITERIA

Studies were included if they:

1. Focused on elderly patients (≥65 years) diagnosed with acute cholelithiasis or cholecystitis.
2. Reported outcomes of surgical versus non-surgical management.
3. Included quantitative or qualitative assessment of morbidity, mortality, or recurrence.

Studies were excluded if they:

- Focused on non-biliary pathology (e.g., renal or pancreatic stones).
- Were commentaries, letters, or conference abstracts without full data.

Data were extracted from each eligible study regarding:

- Study design and population characteristics
- Age distribution of participants
- Type of management (laparoscopic cholecystectomy, open cholecystectomy, percutaneous cholecystostomy, or antibiotic therapy)
- Key outcomes (mortality, morbidity, recurrence, length of stay, and conversion rate)

No new patient data were collected; therefore, ethical approval was not required. A summary of inclusion and exclusion criteria is presented in Table 1.

REVIEW

The search identified approximately 62 potentially relevant studies. After screening this was narrowed down to 55 records which further reduced to 32 articles after eligibility assessment. After further exclusion, eight studies were assessed and included in this narrative review (fig.1). These were a mixture of cohort studies, systematic reviews, and randomised controlled trials (RCTs) focusing on the management of acute cholecystitis or cholelithiasis in elderly patients. The breakdown of study selection is outline in Figure 1.

PATIENT DEMOGRAPHICS

Across studies, the mean patient age ranged from 67 to 85 years, with most defining "elderly" as ≥65 years. Female predominance was consistent, with women comprising approximately 60–70% of cases [1–3, 8]. The prevalence of comorbid conditions (particularly cardiovascular disease, diabetes, and chronic kidney disease) was reported in up to 75% of elderly cohorts [9].

SURGICAL MANAGEMENT

Laparoscopic cholecystectomy remains the definitive treatment for symptomatic gallstone disease. Conversion to open surgery occurs in 5–15% of elderly cases, often due to dense adhesions, inflammation, or unclear biliary anatomy [10,11]. Conversion to open cholecystectomy can take place with different incisions, however, regardless of how it is achieved, it ultimately provides safe exposure of the gallbladder in difficult cases.

Mortality following laparoscopic cholecystectomy is generally low (0.3–1.5%), but significantly higher in octogenarians or frail patients (up to 5%) [12]. Morbidity is often related to cardiopulmonary complications and wound infection. Studies demonstrate that age, ASA (American Society of

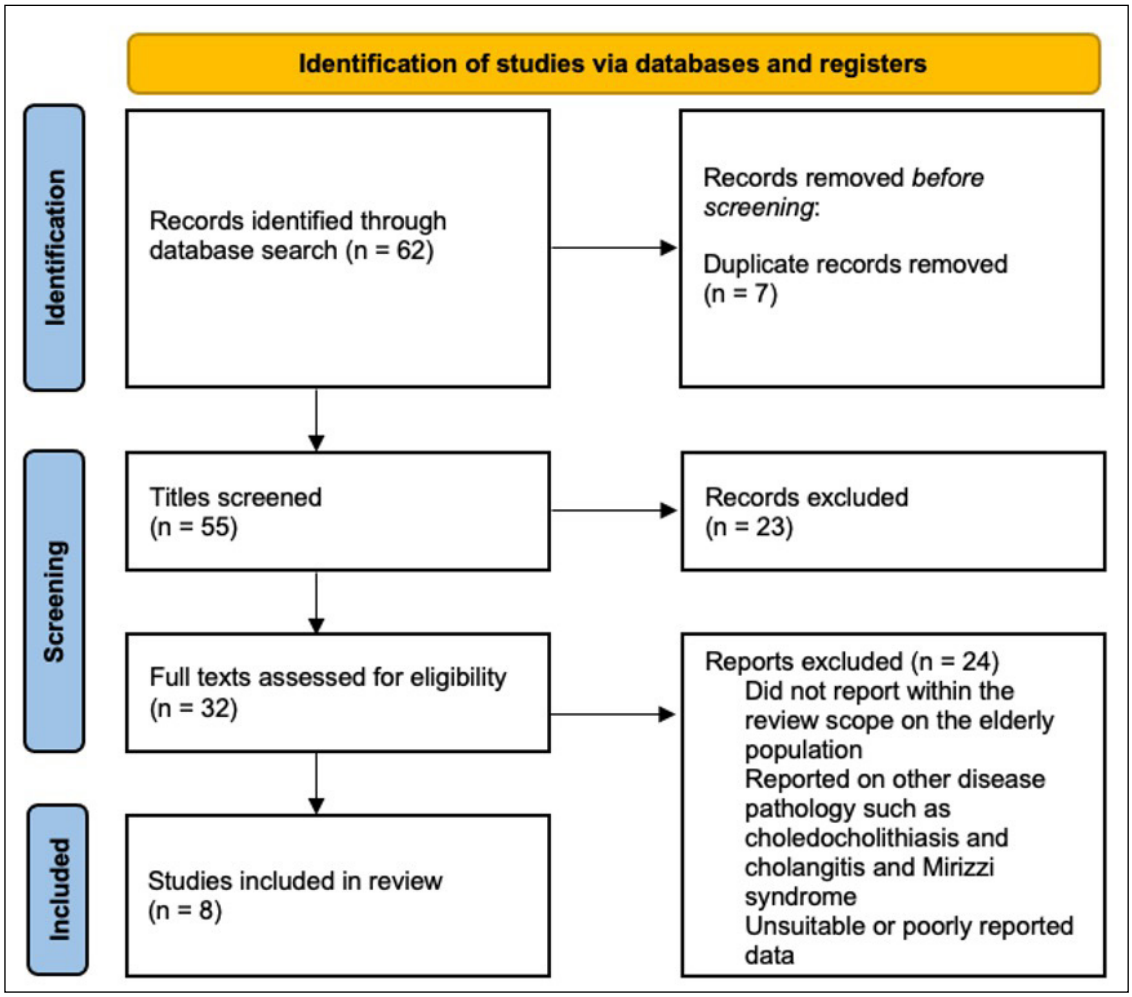

Fig. 1. Flowchart of study selection
Source: Own materials

Anaesthesiologists) score, and elevated inflammatory markers—notably C-reactive protein (CRP) and procalcitonin—predict the likelihood of conversion to open surgery or postoperative complications [13,14].

Laparoscopic techniques require intra-abdominal insufflation pressures of 12–15 mmHg, which can cause hemodynamic instability, reduced venous return, and compromised pulmonary function in the elderly [15]. Thus, reduced-pressure pneumoperitoneum and meticulous anaesthetic monitoring are recommended in high-risk patients.

HOSPITAL STAY AND OUTCOMES

Elective laparoscopic cholecystectomy results in shorter hospital stays (average 2–4 days) compared with urgent or emergency procedures (average 6–10 days) [16]. Studies consistently report that emergency cases have higher morbidity, whereas early elective surgery yields improved outcomes [17].

In elderly populations, prolonged hospitalisation is linked to increased nosocomial infections and deconditioning. Figure 2 summarises trends in length of stay across age groups.

NON-SURGICAL MANAGEMENT

Non-operative approaches include percutaneous cholecystostomy and antibiotic therapy.

Percutaneous cholecystostomy is typically performed under ultrasound or CT guidance using either a transhepatic or transperitoneal approach. Both methods are valid, though the transhepatic route may reduce bile leakage risk, while the transperitoneal approach is preferred when gallbladder distension limits access [18, 19].

Mortality after cholecystostomy ranges from 5–10%, often reflecting underlying illness rather than procedural complications [20]. Readmission and recurrence of biliary symptoms occur in up to 30% of elderly patients treated non-operatively [21].

Antibiotic-only therapy may be suitable for mild to moderate cases, particularly when surgery is contraindicated. However, recurrence rates of 20–25% and mortality up to 27% in severe disease have been reported [22, 23].

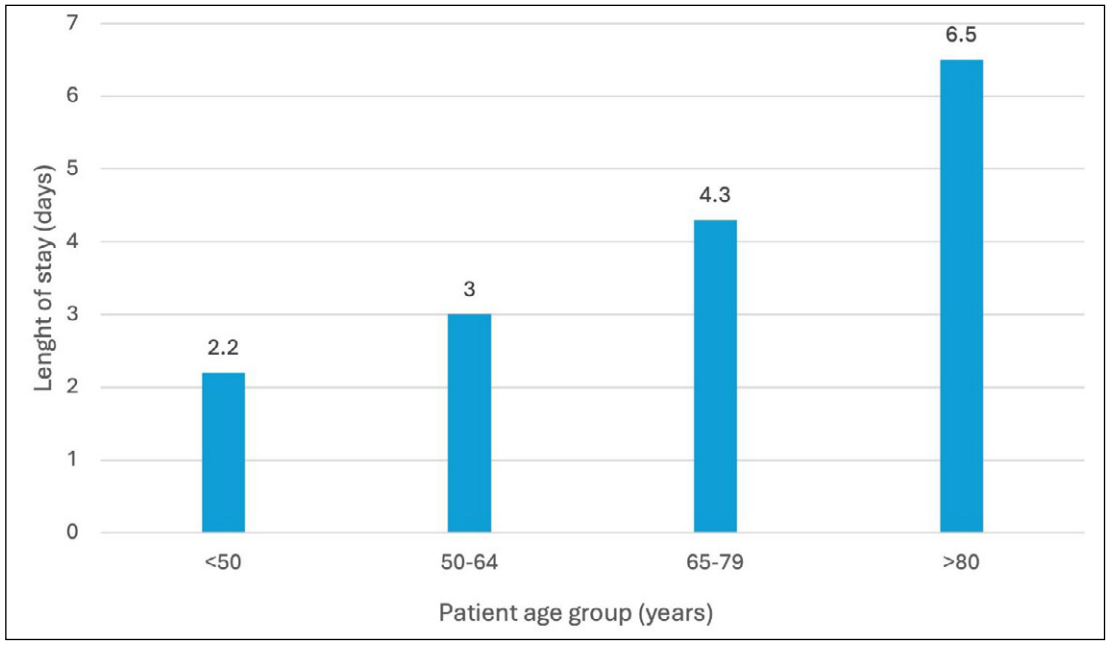

Fig. 2. Average post-operative length of hospital stay (LOS) following laparoscopic cholecystectomy for acute cholelithiasis by age group.
Data derived from Nassar & Richter [9], Lord et al. [10], Lupinacci et al. [11], Rees et al. [16], and Lai et al. [17]. LOS increases progressively with advancing age, reflecting higher comorbidity burden and delayed postoperative recovery among elderly patients

Escartín et al. specifically compared elderly patients managed surgically versus conservatively: Group A (surgical) underwent cholecystectomy or cholecystostomy, and Group B (conservative) received antibiotics alone. The study found lower recurrence and readmission rates in Group A, confirming the superiority of definitive management [22].

DISCUSSION

Acute cholelithiasis and its complications, such as acute cholecystitis, cholangitis, mechanical jaundice, and biliary pancreatitis, represent a significant source of morbidity in the elderly population. The management of these conditions requires a balance between the benefits of early definitive surgical treatment and the risks associated with frailty, comorbidity, and physiological decline.

SURGICAL MANAGEMENT

Laparoscopic cholecystectomy (LC) remains the preferred treatment for acute gallstone disease, supported by international guidelines including NICE and the World Society of Emergency Surgery [7, 8, 12]. Early LC within the first week of symptom onset reduces recurrence and complications, with lower overall cost and improved patient satisfaction compared to delayed or conservative management [16, 17].

Despite its advantages, LC in the elderly carries increased risks due to reduced physiological reserve and higher rates of cardiopulmonary disease. The elderly are more likely to experience perioperative instability related to pneumoperitoneum, as increased intra-abdominal pressure can reduce venous return, compromise cardiac output, and elevate pulmonary pressures [15]. Adjustments such as low-pressure laparoscopy, meticulous anaesthesia, and preoperative optimisation are therefore essential.

Conversion from laparoscopic to open cholecystectomy occurs more frequently in older adults, with rates reaching up to 15% [10]. Predictors include male sex, high body mass index, elevated C-reactive protein (CRP) and procalcitonin levels, thickened gallbladder wall, and severe inflammation [13,14]. These parameters may serve as early warning indicators, facilitating preoperative risk stratification.

NON-SURGICAL MANAGEMENT

When surgery poses unacceptable risk, non-operative strategies become vital alternatives. Percutaneous cholecystostomy (PC) provides effective decompression of the inflamed gallbladder under local anaesthesia. Both transhepatic and transperitoneal routes are widely used. The transhepatic approach offers reduced risk of bile leakage, while the transperitoneal route provides easier access when the gallbladder is markedly distended or hepatomegaly is present [18,19]. Mortality associated with PC is largely reflective of comorbidity rather than procedural complication, ranging between 5–10% [20].

Antibiotic-only therapy may be appropriate for mild cases or as a temporising measure before definitive surgery. However, recurrence and readmission rates remain high, particularly in frail or comorbid patients. Escartín et al. demonstrated that elderly patients managed conservatively with antibiotics alone had higher recurrence (22%) and mortality (27% in severe disease) compared to those undergoing surgical or interventional procedures [22]. These findings underscore that while conservative management may stabilise acute inflammation, it is rarely curative.

LENGTH OF STAY, READMISSION, AND RECURRENCE

Hospital length of stay (LOS) remains a key determinant of outcome and cost-effectiveness. Lupinacci et al. showed that elective LC is associated with the shortest LOS, while urgent and emergency procedures result in longer stays and higher intensive care unit admissions [11]. Elderly patients are particularly vulnerable to hospital-related complications, including infections, delirium, and deconditioning, all of which can prolong recovery [17].

Non-operative management, while initially reducing surgical risk, is frequently followed by readmission due to recurrent biliary symptoms. Studies by Bergman et al. and Pisano et al. reported recurrence rates of 25–31% after conservative therapy, with most episodes occurring within three months [19,20]. Recurrent admissions increase healthcare costs and often lead to delayed, higher-risk surgery, compounding overall morbidity.

PATIENT'S PERSPECTIVE

Patient-reported outcomes (PROs) are increasingly recognised as central to evaluating healthcare quality. Studies assessing LC outcomes have shown that both elderly and younger patients value the experience of the surgeon, avoidance of complications, and rapid recovery as their primary concerns [22, 23]. Elderly patients, in particular, place greater emphasis on continuity of care, communication, and rehabilitation support. A patient-centred approach—focusing on shared decision-making and expectation management—is therefore critical in geriatric surgical care.

CLINICAL IMPLICATIONS

The findings from this review reinforce that chronological age alone should not preclude surgery. Instead, decision-making should be individualised using comprehensive geriatric assessment, frailty scoring, and multidisciplinary input. Early LC should remain the default where feasible, while PC or antibiotic therapy may serve as temporising measures in unstable or high-risk patients. Optimisation before surgery—including correction of fluid and electrolyte imbalance, cardiopulmonary evaluation, and infection control—is key to minimising complications.

LIMITATIONS

This review was limited by heterogeneity among included studies regarding definitions of "elderly," outcome measures, and follow-up durations. Additionally, few randomised controlled trials exist specifically targeting geriatric populations, and most evidence derives from retrospective data. Further research is warranted to establish standardised protocols and predictive models for treatment selection in older adults.

CONCLUSIONS

Surgical and non-surgical management strategies for acute cholelithiasis in the elderly must be individualised, balancing procedural risk against disease recurrence and overall prognosis. Laparoscopic cholecystectomy remains the gold-standard treatment when tolerated, offering definitive resolution and reduced long-term complications. Non-operative management, including percutaneous cholecystostomy and antibiotic therapy, has a role in frail or unstable patients but is associated with higher recurrence and readmission rates.

A multidisciplinary, patient-centred approach is essential to optimise outcomes. Future studies should focus on refining risk stratification models, integrating biomarkers such as CRP and procalcitonin, and developing consensus-based guidelines tailored to the elderly population.

REFERENCES

1. Wilkins T, Agabin E, Varghese J, Talukder A. Gallbladder dysfunction: cholecystitis, choledocholithiasis, cholangitis, and biliary dyskinesia. Prim Care. 2017;44(4):575–597. doi: 10.1016/j.pop.2017.07.002. DOI
2. Shaffer EA. Gallstone disease: Epidemiology of gallbladder stone disease. Best Pract Res Clin Gastroenterol. 2006;20(6):981–996. doi: 10.1016/j.bpg.2006.05.004. DOI
3. Méndez-Sánchez N, et al. Epidemiology of gallstones in aging populations. Curr Opin Gastroenterol. 2020;36(2):71–77. doi: 10.1016/j.gtc.2010.02.003. DOI

4. Kimura Y, Takada T, Kawarada Y, et al. Definitions, pathophysiology, and epidemiology of acute cholangitis and cholecystitis: Tokyo Guidelines. J Hepatobiliary Pancreat Surg. 2007;14(1):15–26. doi: 10.1007/s00534-006-1152-y. DOI
5. Chhoda A, Mukewar S, Mahadev S. Managing gallstone disease in the elderly. Clin Geriatr Med. 2021;37(1):43–69. doi: 10.1016/j.cger.2020.08.005. DOI
6. Riall TS. Evidence-based management of gallstone disease in older adults. JAMA Surg. 2018;153(6):551–552. doi: 10.1177/0885066614554192. DOI
7. NICE. Gallstone disease: diagnosis and management. NICE guideline NG104. 2021. https://www.nice.org.uk/guidance/cg188 (Access: January 2025)
8. Pisano M, Ceresoli M, Cimbanassi S, et al. 2017 WSES and SICG guidelines on acute calculous cholecystitis in the elderly population. World J Emerg Surg. 2019;14:10. doi: 10.1186/s13017-019-0224-7. DOI
9. Nassar Y, Richter S. Management of complicated gallstones in the elderly: comparing surgical and non-surgical treatment options. Gastroenterol Rep. 2019;7(3):205–211. doi: 10.1093/gastro/goy046. DOI
10. Lord AC, Hicks G, Pearce B, Tanno L, Pucher PH. Safety and outcomes of laparoscopic cholecystectomy in the extremely elderly: a systematic review and meta-analysis. Acta Chir Belg. 2019;119(6):349–356. doi: 10.1080/00015458.2019.1658356. DOI
11. Lupinacci RM, Nadal L, Rego R, et al. Surgical management of gallbladder disease in the very elderly. Eur J Gastroenterol Hepatol. 2013;25(3):380–384. doi: 10.1097/MEG.0b013e32835b7124. DOI
12. Kim S, Donahue TR. Laparoscopic cholecystectomy. JAMA. 2018;319(17):1834. doi: 10.1001/jama.2018.3438. DOI
13. Magnano San Lio RM, Barchitta M, Maugeri A, et al. Preoperative risk factors for conversion from laparoscopic to open cholecystectomy: a systematic review and meta-analysis. Int J Environ Res Public Health. 2023;20(1):408. doi:10.3390/ijerph20010408. DOI
14. Yaow CYL, Chong RIH, Chan KS, et al. Should procalcitonin be included in acute cholecystitis guidelines? A systematic review. Medicina (Kaunas). 2023;59(4):805. doi:10.3390/medicina59040805. DOI
15. Zhu Q, Mao Z, Yu B, et al. The safety of carbon dioxide pneumoperitoneum for elderly patients during laparoscopic surgery. Surg Laparosc Endosc Percutan Tech. 2010;20(1):54–57. doi:10.1097/SLE.0b013e3181ce1462. DOI
16. Gurusamy KS, Samraj K, Gluud C, et al. Meta-analysis of randomized controlled trials on the safety and effectiveness of early versus delayed laparoscopic cholecystectomy for acute cholecystitis. Br J Surg. 2010;97(2):141–150. doi:10.1002/bjs.6870. DOI
17. Barka M, Khalil J, Mouna B, et al. Early laparoscopic cholecystectomy for acute cholecystitis: does age matter? Geriatr Gerontol Int. 2023;23(9):671–675. doi:10.1111/ggi.14643. DOI
18. Papis D, Khalifa E, Bhogal R, et al. Is percutaneous cholecystostomy a good alternative treatment for acute cholecystitis in high-risk patients? Am Surg. 2017;83(6):623–627.
19. Bergman S, Al-Bader M, Sourial N, et al. Recurrence of biliary disease following non-operative management in elderly patients. Surg Endosc. 2015;29(12):3485–3490. doi: 10.1007/s00464-015-4098-9. DOI
20. Garcés-Albir M, Martín-Gorgojo V, Perdomo R, et al. Acute cholecystitis in elderly and high-risk surgical patients: is percutaneous cholecystostomy preferable to emergency cholecystectomy? J Gastrointest Surg. 2019;24(12):2579–2586. doi: 10.1007/s11605-019-04424-5. DOI
21. Mora-Guzmán I, Di Martino M, Bonito A, et al. Conservative management of gallstone disease in the elderly population: outcomes and recurrence. Scand J Surg. 2019;108(3):205–210. doi: 10.1177/1457496919832147. DOI
22. Escartín A, González M, Cuello E, et al. Acute cholecystitis in very elderly patients: disease management, outcomes, and risk factors for complications. Surg Res Pract. 2019;2019:9709242. doi: 10.1155/2019/9709242. DOI
23. Parkin E, Stott M, Brockbank J, et al. Patient-reported outcomes for acute gallstone pathology. World J Surg. 2017;41(5):1234–1238. doi: 10.1007/s00268-016-3854-x.

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Ehtesam Ahmed Chowdhury
General Surgery, Hereford County Hospital,
Wye Valley NHS Trust,
Herford, United Kingdom
e-mail: ehtesam.chowdhury@gmail.com

ORCID AND CONTRIBUTIONSHIP
Ehtesam Ahmed Chowdhury: 0009-0008-7820-4966 A B D E F
Olivia C Jadeja: 0009-0007-3139-4371 B D
Surajit Sinha E F

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

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REVIEW ARTICLE

CONTENTS

Diet therapy as a component of ancient medicine

Marta J. Petryshyn[1], Halina M. Zahaiska[2], Oxana V. Liubimova[2]
[1]VASYL STEFANYK CARPATHIAN NATIONAL UNIVERSITY, IVANO-FRANKIVSK, UKRAINE
[2]YURIY FEDKOVYCH CHERNIVTSI NATIONAL UNIVERSITY, CHERNIVTSI, UKRAINE

ABSTRACT

Aim: To analyze of dietotherapy as employed by Roman physicians in the treatment of therapeutic diseases since one of the important methods of successful treatment and recovery is a properly formulated diet.

Materials and Methods: The subject of this study is *De Medicina*, the work of Aulus Cornelius Celsus, the renowned Roman scholar and encyclopedist, the author of an eight-volume medical treatise, which summarizes the experience of ancient medicine in the fields of therapy, surgery, pathology, and dietetics. The application of analytical and synthetic methods, contextual and comparative analysis, and the descriptive method enabled the investigation of the specifics of diet therapy as a key treatment modality in ancient medical practice

Conclusions: Critically summarizing the achievements of medicine in therapy, surgery, and pathology, Celsus consistently emphasized the importance of diet therapy in the treatment and prevention of diseases. As a result of the research, it has been established that even in ancient times, the nutrition of a sick person was considered the fundamental basis upon which other therapeutic measures should be applied. Dietary nutrition was aimed at reducing the risk of complications, restoring the body, and preventing diseases. The main principles of ancient diet therapy were based on the following: aligning the diet with the physiological needs of the body during illness, adapting food processing methods for specific diseases, determining the duration of the diet depending on the characteristics of the disease's progression, maintaining water balance, and applying principles of adjustment, substitution, and physical or mechanical protection of the affected system or organ.

KEY WORDS: history of medicine, Aulus Cornelius Celsus, diet and nutrition, diet therapy, internal diseases

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INTRODUCTION

The connection between nutrition and health has been recognized since ancient times. Food has always been not only a crucial factor in ensuring human vitality, growth, and development of the organism but also a means of preventing and treating many diseases, a source of preserving health, enhancing resistance to harmful environmental factors, maintaining high work capacity, and promoting active longevity. Ancient physicians accumulated significant experience regarding the role of nutrition in the rehabilitation of patients, understanding that diet therapy is not only an effective means of comprehensive treatment for many diseases but also contributes to strengthening and preserving health. In light of this, it is important to study the experience of ancient medicine in historical retrospect, analyze it, and adapt their contributions to the history of the development of modern dietetics.

AIM

The aim of the present article is to analyze of dietotherapy as employed by Roman physicians in the treatment of therapeutic diseases since one of the important methods of successful treatment and recovery is a properly formulated diet.

MATERIALS AND METHODS

The research material is based on *De Medicina*, the work by the Roman encyclopedist, physician, and medical theorist of the era of Emperors Augustus and Tiberius, Aulus Cornelius Celsus (25 BCE – 50 CE) [1], who is considered the founder of Latin anatomical terminology. Due to his clear and comprehensible style of presentation, contemporaries rightly referred to him as the 'Cicero of Medicine' or the 'Roman Hippocrates.' For centuries, Celsus' work was regarded as one of the best medical manuals in Europe.

The methodological framework for studying the role of dietary nutrition in treatment and rapid rehabilitation in ancient medicine involves a range of general and specialized scientific methods. The method of analysis and synthesis allowed for the systematization of scientific developments on the mentioned issues. The method of text analysis and interpretation was employed to identify key terms and concepts of dietary nutrition in ancient medicine, the therapeutic properties of food, and to discern dietary recommendations for the prevention and treatment of internal diseases within the text. Contextual analysis elucidated the link between treatment efficacy for internal organ disorders and patient diet, and situated Celsus' diet therapy in ancient medical traditions. Comparative analysis facilitated the interpretation of ancient diet therapy principles relative to modern medical practice. The descriptive method was used to classify and interpret the research material.

ETHICS

All sources used in this literature review are publicly available.

REVIEW AND DISCUSSION

Nutrition is the foundation of all vital processes in the organism, encompassing intake, digestion, absorption, and assimilation of proteins, fats, carbohydrates, vitamins, minerals, and water. Rational nutrition supports growth and development, maintains immunity, helps protect the human body from adverse conditions, aids in recovery and disease prevention, and positively impacts the restoration of work capacity and the extension of life expectancy.

Over the years, a number of works have emerged dedicated to the study of the clinical-physiological and clinical-biochemical foundations of nutrition for both healthy and ill individuals. In the book *Health on Your Table* by Ilina S., the experience of generations regarding the medicinal properties of the plant world and their use to overcome ailments is summarized [2]. In the work *The ABCs of Nutrition* by Stolmakova G. and Martyniuk I., the organization of therapeutic nutrition in hospital settings is thoroughly described, along with the principles of nutrition at home for diseases of the digestive, circulatory, respiratory, urinary systems, metabolic disorders, and more [3]. The monographic study by Telezhenko L., Dziuba N., and Kashkano M. is devoted to characterizing the impact of alimentary factors on the functioning of the main systems of the human body, and outlining the principles of nutrition for diabetes, nervous system disorders, vision problems, and more [4]. In the book by the renowned doctor of medicine M. Greger, the specifics of various diseases are thoroughly examined, and the corresponding dietary regimens are determined [5]. The research by Cherevko O. and others is related to the study of modern approaches to dietary nutrition for various diseases of organs and systems [6]. The scientific investigation by Downer S. and her colleagues addresses the issue of properly tailored nutrition for patients for prevention, treatment, or recovery after severe illness [7]. In the article by Khasanova G. and others, the recommendations of the World Health Organization and leading European dietitians and cardiologists regarding the alimentary specifics of hypertension are summarized [8]. The scientific inquiries of Demydenko M. and Zakharova I. are related to the study of the impact of diet therapy and controlled physical exercise on weight reduction and the prevention of obesity-related complications [9]. In the research by Hovorun D. and Horoshko V., the results of a study on the influence of diet therapy on the recovery of brain function and the overall health of patients after ischemic stroke are presented. [10]. A thorough analysis of the literature on the use of neuroprotective diets, various food, vitamin and mineral supplements in the treatment and rehabilitation of patients with acute stroke can be found in the article *Nutrition, Energy Expenditure, Dysphagia, and Self-Efficacy in Stroke Rehabilitation: A Review of the Literature* [11]. The scientific study by Kolasinski S. highlights the specifics of dietary regimens for patients with gout, particularly: limiting the consumption of purine-rich foods, restricting alcohol intake, and diets that lead to weight loss by reducing calorie and carbohydrate intake, which can be effective in lowering serum urate levels and the risk of gout [12]. The analysis of scientific sources demonstrates that researchers are focusing on a wide range of issues related to the modern application of specially designed dietary regimens and nutrition plans for therapeutic or preventive purposes for patients with various pathologies and across different age groups. However, the origins of diet therapy as a system of treatment and disease prevention date back to antiquity. Certain studies are dedicated to examining the contributions of ancient Greek physicians to the development and popularization of diet therapy. For example, in the article *Synergism of Health-Preserving Ideas of Hippocrates in the Context of the Development of Dietetics*, based on the works of Hippocrates, the founder of dietetics as a distinct field of health preservation and strengthening, the emergence of the term *dietetics* is explored, its essence in antiquity is explained, and an analysis of the ancient Greek physician's recommendations regarding dietary principles and regimen according to the seasons

is presented [13]. The influence of Hippocratic ideas on the development and dissemination of dietetics has been the subject of scholarly investigations by Greek researchers such as Kritikos A., Bekiari A., and others [14]. In the context of our study, among recent publications, the research by Maca-Sánchez M. stands out, as it examines the development of dietary therapy in ancient medicine based on the *Corpus Hippocraticum* [15]. A comprehensive analysis of the impact of Hippocratic medicine on the development of modern perspectives in disease prevention is presented in the article *Impact of Hippocratic Medicine on the Development of Disease Observation and Prevention in Modern Medical Practice* [16]. The description of medieval dietary based on *Regimen Sanitatis Salerni* by Arnold of Villanova, are provided in the study by Zahaiska H. and others [17]. The issue of dietary nutrition and its role as a preventive and therapeutic measure, based on Cornelius Celsus' work *De Medicina*, is partially addressed in the research of Earl LeV. Crum [18]. However, Celsus' views on the impact of healthy nutrition on overall health, treatment, and the prevention of therapeutic-profile diseases require further study.

Therapeutic nutrition refers to the diet of a patient that fully meets their needs for nutrients and energy while also taking into account individual characteristics, the nature of the disease, and its stage. Even in ancient times, physicians observed that certain types of food and methods of preparation had a positive effect on the course of illnesses. The experience of ancient medical practitioners in using food for therapeutic purposes was summarized in the work of Aulus Cornelius Celsus, *De Medicina*, the first printed edition of which was published in Florence in 1478. In his work**,** Celsus not only summarized the views of his predecessors, whose original works were irretrievably lost, but also sought, examined, and critically analyzed certain concepts, drawing on his own practical experience in treating patients at a valetudinarium for slaves. The first book of his medical treatise is dedicated to issues of hygiene and dietetics for a healthy person. This section of the medical encyclopedia contains valuable insights not only on the effects of heat and cold, sleep and wakefulness, prolonged walking, and sea voyages on health but also includes generalizations and recommendations on dietary patterns across different seasons, drinking regimens according to both season and illness, and dietary restrictions in cases of vomiting, diarrhea, stomach pain, and plague. For instance, a healthy person was advised to eat twice a day: *Bis die potius, quam semel cibum capere* (I, 1:2)[1]. The primary factor in preventing alimentary-dependent diseases was considered to be the consumption of vegetables and pickled foods at the beginning of a meal, followed by meat dishes: *Cibus a salsamentis, holeribus similibusque rebus melius incipit; tum caro adsumenda est*. (I, 1:8). Ancient physicians recommended limiting the consumption of spices. Such a diet increased the load on the gastrointestinal tract, causing discomfort, a feeling of heaviness, and constipation: *plus propter dulcedinem adsumitur, et quod modo par est, tamen aegrius concoquitur* (I, 1:9). However, modern clinical studies indicate that the inclusion of herbs and spices in the diet has a positive impact on the prevention and treatment of metabolic syndrome and related diseases [19]. Certain recommendations in the first book of the work *De Medicina* are devoted to the issue of body weight correction. In ancient society, the problem of underweight was relevant due to insufficient and unbalanced nutrition with low amounts of fats and carbohydrates, which led to health issues such as weakened immunity, gastrointestinal diseases, decreased bone density, and increased bone fragility, and so on. To achieve success in weight gain, Celsus recommended frequently consuming fatty and sweet foods and drinks in the most digestible amounts: *adsumpta per cibos et potiones maxime dulcia et pinguia* (I, 3:15). Conversely, for excess body weight and obesity, he advised combining baths, hot saltwater baths, and physical activity with eating once a day, consuming sour and astringent foods, and drinking cold wine on an empty stomach: *acidae res et austerae; et semel die adsumptae epulae; et vini non praefrigidi ieiuno potio in consuetudinem adducta* (I, 3:16). Ancient physicians repeatedly emphasized the benefits of water for the body, understanding that water is a fundamental and vital element necessary for the course of any biochemical process in the human body. In particular, insufficient fluid intake contributed to constipation: *alvum adstringit … exigua potio* (I, 3:30), while drinking beverages during meals accelerated the passage of food through the intestines, stimulated the motility of the digestive system, and had a laxative effect: *contra solvit aucta ambulatio atque esca potusque, motus, qui post cibum est, subinde potiones cibo inmixtae* (I, 3:31). For example, Celsus recommended drinking two or three cyathi[2] of cold water for intestinal atony: *duos tresve cyathos per tenuem fistulam bibant* (I, 8: 3), and to maintain water balance during diarrhea, he advised drinking cold water after each bowel movement: *quotiens alvus ei constiterit, frigida potione potissimum utatur* (I, 8:4). According to Celsus, the choice of diet should depend on the season, which not only influenced changes in the body's needs for

[1] *The Roman numeral stands for the book, the Arabic numeral – for the chapter; after the colon – the paragraph.*

[2] *Cyathus (pl. cyathi; Lat., from Gk. κύαθος, kyathos) – Roman measure of liquids and bulk solids, approximately equal to 0.045 liters.*

nutrients but also determined the availability of dietary components (I, 3: 34). The summer diet was to consist of foods that promote body cooling, boiled meat, a large amount of vegetables, and fluids: *Ei tempori aptissima sunt et caro et holus, potio quam dilutissima, ut et sitim tollat nec corpus* (I, 3: 36). In contrast, the winter diet was to provide the body with energy, strengthen immunity, and consist of calorie-dense and hearty meals. In particular, ancient physicians recommended drinking less fluid in winter, consuming more roasted meat with a small amount of vegetables, and eating foods that promote body warming: *Hieme plus esse convenit, minus sed meracius bibere; multo pane uti, carne potius elixa, modice holeribus* (I, 3: 34). In autumn, preference was to be given to fruits, while reducing calorie-dense foods in the diet: *Poma nocere … quae inmodice toto die plerumque sic adsumuntur, ne quid ex densiore cibo remittatur* (I, 3: 34). Confirmation of this theory can also be found in the prescriptions of modern diet therapy. As researchers note, food products should be consumed not only according to the place of residence but also according to the season [6, 20].

The second book of Celsus' medical treatise is dedicated to the characteristics of the causes and symptoms of diseases, as well as recommendations for the treatment of various pathologies. Additionally, in this book, the author provides information on the nutritional value of foods, since an appropriate balance of proteins, fats, carbohydrates, vitamins, and minerals ensured energy, strengthened immunity, and improved the overall condition of the human body. Thus, all types of legumes, bread, meat from cattle and wild animals, large poultry, honey, and cheese were classified as highly nutritious foods: *omnia legumina, quaeque ex frumentis panificia sunt, generis valentissimi esse … item omne animal quadrupes domi natum; omnem grandem feram, … omnem grandem avem, … item mel et caseum* (II, 18:2). On the other hand, low-calorie foods included pumpkin, cucumber, capers, tree fruits, snails, oysters, and the like: *cucurbita et cucumis et capparis, omnia poma, oleas, cochleas, itemque conculia* (II, 18:3). The frequency and regimen of food intake were of great importance in therapeutic nutrition. In particular, at the onset of an illness, Celsus recommended complete abstinence from food: *Abstinentiae vero duo genera sunt, alterum ubi nihil adsumit aeger* (II, 16:1), as this helped to alleviate inflammatory processes in the human body. These recommendations from ancient medicine are also supported by modern nutritionists and dietitians [9-11]. Even in ancient times, physicians understood that foods had different effects on the human body: some foods contributed to the formation of mucus in the body, others caused flatulence, had a diuretic or laxative effect, and so on: … *aliae lenes, aliae acres; aliae crassiorem pituitam in nobis faciunt, aliae tenuiorem; aliae idoneae stomacho, aliae alienae sunt; itemque aliae inflant, aliae ab hoc absunt; aliae calfaciunt, aliae refrigerant* (II, 19:1). They referred to these as foods of good or bad juice. For example, the consumption of raw eggs, rice, molasses, pearl barley, milk, bulbous plants, and glutinous dishes contributed to the formation and accumulation of mucus in the body: *Crassiorem autem pituitam faciunt ova sorbilia, halica, oriza, amulum, tisana, lac, bulbi, omniaque fere glutinosa* (II, 23:1). One of the most common causes of flatulence was considered to be the consumption of foods that stimulate gas formation. These included fatty and sweet dishes, all legumes, garlic, onions, cabbage, young wine, and the like: *Inflant autem omnia fere legumina, omnia pinguia, omnia dulcia, omnia iurulenta, mustum, … ex holeribus alium, cepa, brassica, omnesque radices, excepto sisere et pastinaca* (II, 26:1). To cleanse the intestines, it was recommended to increase the consumption of fiber, which was found in vegetables (lettuce, undercooked cabbage, lettuce, purslane, radish, beetroot, pumpkin), fruits (cherries, mulberries, fresh grapes), and grains (barley). These foods helped cleanse the intestines and improve their motility: *At alvum movent … brassica, … lactuca, anteum, nasturcium, ocimum, urtica, portulaca, radicula, capparis, alium, cepa, malva, lapatium, beta, asparagus, cucurbita, cerasia, mora, uva ex olla, omnia mitia, ficus etiam arida, sed magis viridis, uvae recentes* (II, 29:1). To achieve a diuretic effect, foods such as celery, parsnip, dill, fennel, radish, basil, mint, and hare meat were included in the diet: *Urinam autem movent … ut apium, ruta, anetum, ocimum, menta, hysopum, anesum, coriandrum, nasturcium, eruca, feniculum; praeter haec asparagus, capparis, nepeta, thymum, satureia, lapsanum, pastinaca, magisque agrestis, radicula, siser, cepa; ex venatione maxime lepus; vinum tenue, piper et rotundum et longum, sinapi, absinthium, nuclei pinei* (II, 31:1). Sedative, sleep-inducing, and analgesic effects were attributed to poppy, lettuce, mulberries, and leeks: *Somno vero aptum est papaver, lactuca, … morum, porrus* (II, 32:1). During fever, it was recommended to include basil, poppy leaves, coriander, grapevine tendrils, cabbage, mashed pears and apples, quince, and wine in the diet: … *reprimunt et refrigerant herba muralis … serpullum, puleium, ocimum, herba sanguinalis, … portulaca, papaveris folium, capriolique vitium, coriandrum, folia hyocimum, muscus, siser, apium, solanum,… brassicae folia, intubus, plantago, feniculi semen; contrita pira vel mala, praecipueque Cotonea, lenticula; aqua frigida, maximeque pluvialis, vinum, acetum* (II, 33:2-3). Some principles of the influence of various foods on the human body have been adopted by modern diet therapy [3, 7-9].

The third book of Aulus Cornelius Celsus' medical treatise is dedicated to elucidating methods of treating acute and chronic diseases of a therapeutic nature. Alongside the description of traditional treatment methods in antiquity for fever, dropsy, cachexia, respiratory diseases, tuberculosis, gastrointestinal pathologies, and epilepsy, Celsus emphasizes the role of diet therapy as an essential component of the comprehensive treatment of acute and chronic diseases. This is because, where there is no therapeutic nutrition, there is no rational treatment. Thus, even ancient physicians observed that recovery occurred more quickly when certain foods were consumed compared to conventional treatment. In the acute phase of an illness, according to Celsus, one should drink water, introduce vegetables and liquid dishes into the diet, avoid consuming meat and bread, or even abstain from food and wine altogether: *facile sit non aquam tantum bibere sed etiam cibo carnem subtrahere, interdum panis quoque minus quam pro consuetudine adsumere, umidoque cibo esse contentos et holere potissimum, satisque sit tum ex toto a cibo, a vino* (III, 2:7). Thus, in the treatment of fever, one of the best remedies was considered to be a liquid diet with the addition of honey to strengthen the body: *Cibus autem febricitantibus umidus est aptissimus … Mel quoque despumatum huic recte adicitur, quo corpus magis nutriatur* (III, 6:10). When the dynamics of the disease began to decline, it was recommended to consume small amounts of warm liquid food, namely vegetables, soups made from shellfish and lobster, and boiled meat: *uti cibo serius sunt rarius, tenui, simplici, molli, calido, exiguo, maximeque holeribus, qualia sunt lapatium, urtica, malva, vel iure etiam concharum musculorumve aut lucustarum* (III, 6:14), along with plenty of fluids before, during, or after meals: *At potio esse debet magis liberalis, et ante cibum et post hunc et cum hoc ultra quam sitis coget* (III, 6:14). If the fever was accompanied by vomiting or diarrhea, the patient was to be given nourishing food, such as toasted bread, roasted meat, and astringent wine served warm for diarrhea and cold for vomiting: *panis tostus, caro assa, vinum austerum vel certe subausterum; si venter profluit, calidum, si sudores nocent vomitusve sunt, frigidum* (III, 6:17). In cases of mental disorders arising from fever, physicians recommended prioritizing soups and honeyed drinks: *Opus est cibo infirmo maximeque sorbitione, potione aquae mulsae* (III, 18:16). Modern principles of diet therapy following fever are partially based on Celsus› theory. As researchers note, after the normalization of body temperature, the patient is allowed to transition to regular meals with frequent consumption of warm food in small portions [3]. For gastrointestinal spasms caused by improper nutrition and accompanying stomach and intestinal diseases, a mechanically gentle diet was prescribed, and it was recommended to limit or exclude the consumption of wine: *Cibus non multus quidem, sed saepe tamen noce ac die dandus est, ut nutriat, neque oneret* (III, 19:3). In the treatment of ascites, Celsus preferred dietary measures over medicinal therapy: *Sed id ipsum tamen moliri cibo quam medicamento melius est* (III, 21:7). He recommended consuming moderately nutritious foods, limiting fluid intake, and incorporating diuretic products into the diet: *Cibus esse debet ex media quidem materia … potio non ultra danda est quam ut vitam sustineat, optimaque est, quae urinam movet* (III, 21:6). Ancient medicine took a comprehensive approach to treating bodily exhaustion. In cases of cachexia caused by starvation, insufficient caloric intake, or digestive disorders, physicians recommended a wholesome diet rich in beneficial substances and the inclusion of astringent wine: *Cibis vero opus est copiosis, variis, boni suci, … vino austero* (III, 22:7). In cases of bodily exhaustion due to tuberculosis accompanied by fever, physicians limited food intake. During the remission phase, alongside moderate physical activity and massage, they initially included spicy dishes made from onions, garlic, chicory, and basil, prepared with vinegar, to enhance the body's resistance to infection and reduce intoxication. Later, they introduced barley soups or spelt boiled with milk, rice porridge, and similar foods: *Cibus esse debet primo acer, ut alium, porrum, idque ipsum ex aceto, vel ex eodem intubus, ocimum, lactuca, dein lenis, ut sorbitio ex tisana vel ex halica vel ex amulo, lacte adiecto. Idem oriza quoque et, … far praestat* (III, 22:11). Additionally, to enhance the regenerative properties of the affected organs, they recommended consuming dishes made from brain tissue, small fish, and flour roasted with goat or sheep fat: … *adiciendaque quaedam ex media materia, praecipueque vel ex pruna cerebellum vel pisciculus … Farina etiam cum sebo ovillo caprinove mixta, deinde incocta pro medicamento est* (III, 22:11). In cases of epileptic seizures, ancient physicians applied dehydration therapy and cleansing of the intestinal contents with an enema containing black hellebore. Moderately nutritious food was introduced into the diet only on the third day after the seizure, with restrictions on flour-based dishes and pork: *cibum post diem tertium … Neque sorbitiones autem his aliique molles et faciles cibi neque caro, minimeque suilla, convenit* (III, 23:3). For jaundice, after cleansing the stomach, a light diet and salted Greek wine were prioritized for the first three days. In the following three days, calorie-rich foods and meat were introduced, and the body's water balance was maintained: *triduo primo modice cibum oportet adsumere ex media materia, et vinum bibere Graecum salsum … tum altero triduo validiores cibos, et carnis quoque aliquid esse, intra aquam manere* (III, 24:4). For elephantiasis, it was recommended to consume

low-fat, gluten-free foods that did not cause bloating, along with wine: *cibus sine pinguibus, sine glutinosis, sine inflantibus* (III, 25:3). The diet of individuals suffering from paralysis was to consist of moderately calorie-rich foods, particularly game, with limited wine consumption: *Cibus esse debet ex materia media, maximeque ex venatione: potio sine vino aquae calidae* (III, 27:1).

CONCLUSIONS

Ancient medicine accumulated significant experience in treating various diseases through dietary nutrition, which included specific types of foods and methods of food preparation. As a result of analyzing the text of Celsus' work *De Medicina*, it has been established that even in ancient times, the nutrition of a sick person was considered the foundational basis upon which other therapeutic measures should be applied. Ancient physicians understood that where there is no therapeutic nutrition, there is no rational treatment. The principles of using food for therapeutic purposes were based on the physiological needs of the patient's body, taking into account the stage of the disease, the season, and other factors. Dietary nutrition was aimed at reducing the risk of complications, restoring the body, and preventing diseases. The fundamental principles of ancient diet therapy were based on aligning the dietary regimen with the physiological needs of the body during illness, appropriate food processing for specific diseases, the duration of the diet depending on the characteristics of the disease's progression, maintaining water balance, and the principles of adjustment, substitution, and physical or mechanical protection of the affected system or organ. Studying the experience of past medicine sheds light on the development of diet therapy as a means of preventing and treating diseases in antiquity.

REFERENCES

1. Aulus Cornelius Celsus. De medicina. [On medicine.]. https://penelope.uchicago.edu/Thayer/E/Roman/Texts/Celsus/home.html [Accessed 20 April 2025] (Latin)
2. Ilina S. Zdorov'ia na vashomu stoli : nauk.-popul. vyd. [Health on your table : popular science publication]. Kyiv: VSV «Medytsyna». 2018. (Ukrainian)
3. Stolmakova H, Martyniuk I et al. Azbuka kharchuvannia. Likuvalne kharchuvannia: Dovidnyk. [The ABCs of Nutrition. Therapeutic nutrition: Reference book]. Lviv: Svit; 1991, p.202. (Ukrainian)
4. Telezhenko LM, Dziuba NA, Kashkano MA. Zdorove kharchuvannia: praktychni rekomendatsii: monohrafia. [Healthy eating: practical recommendations: a monograph]. Kherson: Oldi-plius. 2018.(Ukrainian)
5. Greger M. Yak ne pomerty peredchasno : yizha, yaka vidvertaie ta likuie khvoroby. [How Not to Die: Discover the Foods Scientifically Proven to Prevent and Reverse Disease]. Kyiv: KM-Buks. 2018, p.512. (Ukrainian)
6. Cherevko OI, Dudenko NV, Pavlotska LF et al. Diietychne kharchuvannia : pidruchnyk. [Dietary nutrition : Study guide]. Kharkiv: Svit knyh. 2019. (Ukrainian)
7. Downer S, Berkowitz SA, Harlan TS et al. Food is medicine: actions to integrate food and nutrition into healthcare. BMJ. 2020;369:2482. doi:10.1136/bmj.m2482. DOI
8. Khasanova GH, Tukhtaeva NK, Saidov BM, Zhulkevych IV. Suchasni pidkhody do diietoterapii pry hipertonichnii khvorobi. [Modern Approaches to Dietotherapy in Hypertensive Disease]. Visnyk Naukovyh Doslidzhen. 2019;(1):11-4. doi: 10.11603/2415-8798.2019.1.9964. (Ukrainian) DOI
9. Demydenko M. Diietoterapiia ta fizychne vykhovannia u studentiv SMH pry ozhyrinni 1-2 stupeniv. [Diettherapy and physical education in students of the special medical group with 1-2 degree obesity]. Naukovy Chasopys Natsionalnoho Pedahohichnoho Universytetu im MP Drahomanova. 2023;1(159):63-7. doi:10.31392/NPU-nc.series15.2023.1(159).16. (Ukrainian) DOI
10. Hovorun D, Horoshko V. Kompleksne vidnovlennia pislia ishemichnoho insultu: manualna terapiia ta diietoterapiia. [Complex Recovery after Ischemic Stroke: Manual Therapy and Diet Therapy]. Rehabilitation & Recreation. 2022;12:10-4. doi:10.32782/2522-1795.2022.12. (Ukrainian) DOI
11. Lieber AC, Hong E, Putrino D et al. Nutrition, Energy Expenditure, Dysphagia, and Self-Efficacy in Stroke Rehabilitation: A Review of the Literature. Brain Sci. 2018;8(12):218. doi:10.3390/brainsci8120218. DOI
12. Kolasinski SL. Food, drink, and herbs: alternative therapies and gout. Curr Rheumatol Rep. 2014;16(4):409. doi:10.1007/s11926-014-0409-8. DOI
13. Shukatka O, Kryvoruchko I, Horlova L et al. Synerhizm zdoroviazberezhuvalnykh idei hippokrata v konteksti rozvytku diietetyky [Synergism of health-preserving ideas of hippocrates in the context of the development of dietetics]. Osvita. Innovatyka. Praktyka. 2024;12(4):59-63. doi:10.31110/2616-650X-vol12i4-010. (Ukrainian) DOI
14. Kritikos A, Bekiari A, Nikitaras N et al. Hippocrates' counselling with regard to physical exercise, gymnastics, dietetics and health. Ir J Med Sci. 2009;178(3):377–383. doi:10.1007/s11845-009-0323-7. DOI

15. Maca-Sánchez MP. La dietética como una elección de vida: una mirada desde la medicina hipocrática. [Dietetics as a lifestyle choice: a perspective from Hippocratic medicine]. Rev. Guillermo de Ockham. 2019;17(2):29-37. doi:10.21500/22563202.4148 (Spanish) DOI
16. Cedeño D, Chimbo Á, Zambrano P. Impact of Hippocratic medicine on the development of disease observation and prevention in modern medical practice. Revista Gregoriana De Ciencias De La Salud, 2024; 1(1): 109–119. doi:10.36097/rgcs.v1i1.3107. DOI
17. Zahaiska HM, Petryshyn MJ, Liubimova OV. Suggestions and prescriptions for maintaining good health in «Regimen sanitatis Salerni» by Arnold of Villanova. Wiad lek. 2022;75(1):117–122. doi:10.36740/wlek202201122. DOI
18. Earl LeV. Crum. Diet in Ancient Medical Practice as Shown by Celsus in His De Medicina. The Classical Weekly. 1932;25(20):153–159. doi:10.2307/4339084. DOI
19. Mackonochie M, Rodriguez-Mateos A, Mills S, Rolfe V. A scoping review of the clinical evidence for the health benefits of culinary doses of herbs and spices for the prevention and treatment of metabolic syndrome. Nutrients. 2023;15:4867. doi:10.3390/nu15234867. DOI
20. Mikheienko O. Kultura kharchuvannia yak skladova kultury zdorovia liudyny (valeolohichnyi aspekt). [Nutrition culture as a component of human health culture (valeological aspect)]. Pedahohika Psykholohiia Ta Medyko Biolohichni Probl Fiz Vykhovannia I Sportu. 2011;3:116-21. (Ukrainian)

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Oxana V. Liubimova
Yuriy Fedkovych Chernivtsi National University
2 Kotsyubynsky st., 58012 Chernivtsi, Ukraine
e-mail: o.liubimova@chnu.edu.ua

ORCID AND CONTRIBUTIONSHIP

Marta J. Petryshyn: 0000-0003-4060-7440 A B D E
Halina M. Zahaiska: 0000-0003-1449-0269 A B D
Oxana V. Liubimova: 0000-0003-0413-309X A E F

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

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REVIEW ARTICLE

CONTENTS

Effectiveness of kidney transplantation in lurus nephritis: Literature review

Jaroslav Tolstyak[1,2,3]
[1]DEPARTMENT OF CLINICAL IMMUNOLOGY AND ALLERGOLOGY, LVIV, UKRAINE
[2]DANYLO HALYTSKY LVIV NATIONAL MEDICAL UNIVERSITY, LVIV, UKRAINE
[3]LVIV REGIONAL CLINICAL HOSPITAL, LVIV, UKRAINE

ABSTRACT

Aim: Conducting a scientific analysis of domestic and foreign sources of information regarding modern conceptual views diagnostic and effectiveness of kidney transplantation in Lupus Nephritis.

Materials and Methods: Used methods of historical-bibliographic and systematic approach. The search for literary sources was carried out in four main scientific databases: Google Scholar, PubMed, The review included original articles, research, and official recommendations from medical associations. The search query used in both platforms was: («lupus nephritis» AND «kidney transplantation»). In Google Scholar, the results were limited to publications from 2017 to 2024. As a result of the search, Google Scholar provided 25 relevant articles, while PubMed yielded 57.

Conclusions: Scientific analysis confirmed better survival rates and a lower complication rate compared to hemodialysis and peritoneal dialysis. Survival rates for kidney transplantation over 5 to 10 years are comparable to those for patients with other diseases. Identifying new serological and immunological markers is crucial for the early diagnosis and prevention of acute kidney damage and subsequent graft loss.

KEY WORDS: effectiveness, lupus-nephritis, kidney transplantation, modern biomarkers

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a connective tissue disease with a high presence of autoantibodies, autoreactive T-lymphocytes or B-lymphocytes, and elevated levels of pro-inflammatory cytokine in the blood, leading to damage in multiple internal systems. The incidence of SLE in the USA averages 5.14 cases per 100,000 population per year, though this rate varies significantly by region [1].

In the last two decade, big progreses has been made in understanding the etiopathogenesis of systemic lupus erythematosus (SLE), particularly through the study of various forms of apoptosis.

However, SLE remains incompletely understood and manifests through systemic symptoms due to the involvement of multiple organs and tissues [2]. Lupus nephritis (LN) is considered a poor prognostic factor in SLE.

AIM

Conducting a scientific analysis of domestic and foreign sources of information regarding modern conceptual views effectiveness of kidney transplantation in Lupus Nephritis.

MATERIALS AND METHODS

The search for scientific sources related to the research topic was conducted using Google Scholar (Google Academy) and PubMed. This choice is justified because Google Scholar allows access to a wide range of full-text versions of scientific publications across all disciplines, while the specialized PubMed database focuses specifically on medical and biological sciences. The combined use of both platforms significantly expands the search results, capturing publications that may not be indexed in one of these services. This approach greatly enhances the quality of the subsequent review and critical analysis.

The search query used in both platforms was: («lupus nephritis» AND «kidney transplantation»). In Google Scholar, the results were limited to publications from 2017 to 2024. This timeframe was chosen to focus on recent research in the field. The following filters were applied for searching in PubMed: publication date within the last five years, and full-text availability.

As a result of the search, Google Scholar provided 25 relevant articles, while PubMed yielded 57. The subsequent processing of these publications followed

the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. This approach was chosen because the PRISMA framework ensures a structured, transparent, and reproducible process for systematic reviews and meta-analyses. It helps minimize bias, promotes comprehensive searching by including various sources, and enhances the quality of research by ensuring clear and standardized reporting. Adhering to PRISMA improves the scientific credibility of the results, reduces the risk of omitting important information, and ensures that the publications maintain a high reputation, which in turn increases their citation potential and future use in research and practice.

Thus, in this study, during the identification stage of scientific sources, which is the first and most critical step according to the PRISMA guidelines, the retrieved articles were compiled into a single document, and duplicates from overlapping indexing in both platforms were removed. Following this step, 55 articles were included in the review. During the screening and eligibility stages, several articles were excluded for being only tangentially related to the research topic or lacking full-text access. Consequently, at the inclusion stage, 21 articles that fully met the search criteria were selected for further review and analysis.

ETHICS

All sources used in this literature review are publicly available.

REVIEW AND DISCUSSION

The histological classification of LN includes six classes of glomerulonephritis, which were last revised by the International Society of Nephrology (ISN) and the Renal Pathology Society (RPS) in 2016, in Leiden, the Netherlands. During this revision, definitions were clarified, and indices of chronic activity of lupus nephritis were modified. This classification system is critical for guiding treatment and predicting outcomes in sick man with LN [3]. In the pathogenesis of LN, activation of B-lymphocytes and interferon production is observed in kidney tissues. B-lymphocytes contribute to the formation of autoantibodies, while interferons, particularly type I interferon, have a important role in driving the inflammatory and tissue damage. Knowledge these mechanisms is essential for progress targeted therapies to manage treat lupus nephritis effectively [4].

Recent studies have determined that several types of non-hematopoietic kidney cells express various immune molecules, contributing to the progression of the inflammatory in the kidneys. These connections suggest that not only immune cells but also proper kidney cells, such as tubular epithelial and mesangial cells, play active roles in the pathogenesis of LN by promoting local inflammation and immune responses [5].

In recent years, the insights of the geneticopathogenetic base of LN has significantly improved, thanks to extensive studies on various aspects of its pathogenesis. Apoptosis and secondary necrosis of leukocytes play a leading role in this process. Despite these advancements, kidney damage stay alone of the primary occasion of lethality in sick man with SLE, highlighting the need for ongoing research and improved therapeutic strategies [6].

In 2019, a group of scientists applied single-cell RNA sequencing to kidney biopsies of sick man with lupus nephritis and analyzed keratinocyte sequencing in skin biopsies of case with proliferative LN, comparing these results to a control group. Their findings revealed that high levels of interferon and fibrosis in kidney glomeruli were associated with treatment ineffectiveness [7]. Additionally, B-lymphocyte IFN-β correlated with the severe course of LN and depositions of small immune complexes (IK) in basal membrane of kidney glomeruli. In recent years, the frequency of kidney transplantation has increased as a strategy to improve the treatment of relapses and to alleviate complications associated with chronic kidney disease. This approach aims to provide better long-term outcomes and character of life for case suffering from refractory LN.

The results of our latest studies (2021) align with those of Taiwanese scientists from 2019. However, while we compared to effectiveness of kidney transplantation (KT) one month after surgery in a group of patients with terminal stage kidney disease, the Taiwanese researchers specifically examined the consequence and survival rate of case with end-stage renal failure caused by LN. Their study evaluated three distinctive renal subsititution therapies: peritoneal dialysis, hemodialysis and kidney transplantation. Scientists from Taiwan retrospective analyzed 94 patients with stage V chronic kidney disease caused by disease kidney. Among them, 42 (44.6%) get hemodialysis, 12 (12.7%) accepted peritoneal dialysis, 40 (42.5%) have KT. The study recorded potential bad events, survival rate, and accident of death. The general survival were higher in the KT compared to the peritoneal dialysis or hemodialysis groups. This indicates that KT is the most efficacious treatment therapy for sick man with V stage kidney failure caused by lupus nephritis [8].

Patients with SLE who require kidney transplantation need additional screening tests before surgery. These include tests for autoantibodies to dsDNA, ANA, and antiphospholipid antibodies (aCL, AFA), as well as a com-

prehensive thrombophilia screening. Special evaluation tests for immunological compatibility and radiological assessments for avascular necrosis of the femoral neck and coronary artery diseases are also necessary.

Screening for thrombophilia in SLE candidates for kidney transplantation includes testing for antiphospholipid antibodies (lupus anticoagulant, antiphospholipid antibodies), and other hemostatic markers of coagulation (protein C and S, antithrombin III, Leiden factor V). Additionally, a detailed history of vascular thrombosis, spontaneous abortions, and premature births should be obtained to ensure thorough assessment and optimal management of these patients [9].

Before kidney transplantation, patients must undergo tests for immunological compatibility. In patients with lupus nephritis, these tests have unique considerations. For example, complement-dependent cytotoxicity (CDC) cross-match test can yield pseudo-positive results due to the presence of autoantibodies or other autoimmune conditions, as SLE can cause cell lysis. To ensure reliable results, an autocross-match is performed using the recipient's serum and lymphocytes. Additionally, the solid-phase cross-match on a flow cytometr (FCXM) is used for greater accuracy and reliability in assessing immunological compatibility [10].

Clinical features of transplantation in lupus nephritis indicate the following: most patients with SLE who undergo hemodialysis do not exhibit active SLE. However, in some cases, SLE activity may persist despite hemodialysis. Many transplant centers recommend pre-transplant hemodialysis to reduce disease activity. While this approach can be beneficial in managing SLE, it may moderately worsen the long-term prognosis of kidney graft survival. With peritoneal dialysis, graft survival tends to fluctuate in tandem with the activity of SLE. This suggests that the effectiveness of peritoneal dialysis is closely linked to the disease's activity levels. Extended waiting periods for transplantation may be associated with outcomes that are equivalent to or worse than those of patients who receive a transplant sooner. This highlights the need for careful management and timely transplantation to optimize outcomes for patients with lupus nephritis [11].

Results from other studies indicate that kidney transplantation reduces the incidence of cardiological and vascular complications in sick man with LN. To further enhance the benefits of preventive kidney transplantation in SLE, it is essential to address socio-demographic difference and the extraordinaty challenges angular by these case. Improving access to transplantation and tailoring care to the specific needs of SLE patients can help optimize outcomes and mitigate complications associated with the disease [12].

After kidney transplantation, 2-9% sick man with SLE may experience recurrent LN. Risk factors for recurrence include female sex, young age (under 33 years), African-American race, and the attendance of anticardiolipin antibodies in the recipient. Diagnosis of recurrent LN is confirmed by several indicators: a rapid increase in previously existing proteinuria, the new appearance of protein and erythrocytes in the urine, and a transplant biopsy. Management of recurrent lupus nephritis typically involves modifying the immunosuppressive regimen, increasing the dose of glucocorticosteroids, and administering cytotoxic agents to control the disease and protect the transplanted kidney.

The literature on the prognosis and effectiveness of KT in LN over a 15-years period reveals encouraging results. In studies conducted during this time frame, recipients did not experience arterial or venous thrombosis. Standard immunosuppressive protocols were employed in the treatment, and incidente of acute rejection transplantan were reported to be low, ranging from 1% to 2%. Furthermore, kidney transplant function was deemed satisfactory in 90% of recipients, highlighting the long-term success and stability of transplantation for lupus nephritis patients. Further studies assessed the survival rates of 185 kidney transplant recipients with lupus nephritis, revealing the following outcomes: 88% survival at one year, 82% at three years, 78% at five years, and 67% at ten years. Graft survival rates, excluding deaths, were 93% at one year, 89% at three years, 87% at five years, and 80% at ten years. Recurrent lupus nephritis was diagnosed in 2 patients (1.08%), but no grafts were lost due to this condition. During the follow-up period, 39 patients (21.1%) died, and 65 patients (35.1%) experienced graft loss. Multivariable Cox analysis identified that older recipients age and a glomerular filtration rate (GFR) of less than 45 mL/min/1.73 m^2 one month post-transplant were associated with poorer patients survival and an increased risk of graft loss. Conversely, induction immunosuppressive therapy was found to have a protective effect on patient survival [13].

Our studies on predicting kidney transplant loss in a cohort of 152 recipients, including five with SLE, confirmed these findings. Exploitation Kaplan-Meier method, Cox multivariate analysis, and advanced mathematical prediction techniques, we found that biochemical and immunological indicators—such as leukocyte counts, creatinine levels, proteinuria, and HLA antigen compatibility—played a crucial role. Additionally, the impact of different immunosuppression protocols was also significant in determining transplant outcomes one month after transplantation [14-16].

Elevated levels of both biomarkers were observed in two of the three recipients, who subsequently

experienced acute rejection crises and loss of graft function. NGAL is a promissing biomarker for detecting proximal tubular damage and predicting retarded graft function in KT recipients, yet, its prognostic value varies between studies. One meta-analysis comparing the presageful values of urine NGAL and blood NGAL found that urine NGAL had superior predictive value for delayed graft function [17]. Another important biomarker of acute tubular dysfunction is uCystatin C, a 13 kDa protein from the cystatin family that inhibits cysteine proteases. Synthesized by fully nucleated cells at a persevering rate, Cystatin C is fully reabsorbed and metabolized in the renal tubules, resulting in complete clearance from the body. Cystatin C levels in urine and plasma are largely independent of body weight, age, and sex [18].

Several serological tests are effective in predicting acute kidney injury and transplant rejection: interleukin-18 (IL-18): this pro-inflammatory cytokine, excreted by macrophages, epithelial cells, and activated T lymphocytes, plays a basic role in inflammation and immune responses. KIM-1 (Kidney Injury Molecule-1): biomarker indicating kidney damage. L-FABP (Liver Fatty Acid-Binding Protein): a liver protein that binds adipose acids and is used to assess renal injury. Additionally, two genes are known to predict acute rejection: GOT2 (Glutamic-Oxaloacetic Transaminase 2): An enzyme involved in amino acid metabolism. STXBP3 (Syntaxin Binding Protein 3): A protein that interacts with syntaxin 3, influencing cellular processes. Interleukin-21 (IL-21): This cytokine promotes type 1 activation and cytotoxicity of CD56+ dim CD16+ bright natural killer (NK) cells during antibody-mediated rejection of renal allografts, highlighting a novel connection between adaptive allo-immunity and innate humoral allo-immunity. Importantly, interleukin-6 (IL-6) and its receptor can be effectively inhibited by administering anti-IL-6/IL-6R monoclonal antibodies. This inhibition induces T regulatory (Treg) and B regulatory (Breg) lymphocytes in vivo, which may be crucial for preventing the cultivated of donor-specific antibodies (DSA) and mitigating allograft rejection. Preformed donor-specific DSA are a significant risk factor for acute rejection crises and graft loss. Even recipients who undergo desensitization regimens to address these antibodies face a statistically significant increased danger of acute rejection and graft loss after KT. Consequently, many kidney transplants ultimately fail after several years, typically due to chronic graft rejection and other undetected injuries [19, 20].

To address the need for early discovery of circulating pathology in transplantation, specific biomarker known as donor-derived cell-free DNA (ddcfDNA) was developed in the USA. This biomarker offers a proactive beginning to monitoring by enabling first eduction of transplant-related issues. CcfDNA is present in plasma and can be measured using various methods. Advances now allow for the differentiation of ddcfDNA from recipient cfDNA in the plasma or urine of solid organ transplant recipients, even without prior genotyping case of donor and recipient.

The ddcfDNA assay is gaining attention as a non-invasive tool for determine acute AR in solid organ transplantation, including subclinical rejection crises, infections, or acute tubular damage. However, it can too be detected in clinically stable sick-man with normal histology. The assay shows higher sensitivity for detecting AMR compared to CMR. Unfortunately, normal ddcfDNA levels do not rule out significant cellular rejection. Therefore, while ddcfDNA measurement provides valuable information, it cannot replace biopsy, which last the basic standard for diagnosing AR [21].

Regulatory B-lymphocytes (Bregs) acting a crucial role in modulating the immune response across various diseases, including transplantation. Interleukin-10 (IL-10) expression is the most commonly used to marker for identifying Bregs. Although this review primarily focuses on transplantation efficacy in lupus nephritis, the role of Bregs in clinical transplantation is also significant. The overall impact B-lymphocytes on the immune responses and clinical exist results from a balance between Bregs and effector B-lymphocytes. Evidence suggests that the IL-10 in B-lymphocytes can predict immunological reactivity or clinical outcomes in kidney transplantation, offering valuable mechanistic insights into potential therapeutic strategies. Establishing immune tolerance and monitoring recipient immune status to improve allograft survival remain primary objectives in kidney transplantation [22].

Additionally, recent studies highlight that inhibiting activin can reduce the innate immune response in the early stages of KT in animals models. This inhibition also limits fibroblast accumulation in the graft, propose that activins may play a role in early fibrogenic signaling post-transplant. Serum activin A has been implicated in promoting vascular calcification and renal fibrosis in chronic kidney failure, with post-transplant activin levels independently associated with both allograft function and coronary artery calcification in renal transplant patients [23].

CONCLUSIONS

Based on the above findings, we can conclude that KT is a favorable choice for patients with LN and V stage chronic kidney disease. It offers better survival rates and a lower complication rate compared to hemodialysis

and peritoneal dialysis. Survival rates for kidney transplantation over 5 to 10 years are comparable to those for patients with other diseases. The risk of recurrent lupus nephritis (2-9%) is lower compared to other glomerular diseases. While recurrent lupus nephritis can occur, particularly when assessed by treatment protocols and biopsy results, it rarely progresses to graft failure in most patients. However, serological markers such as dsDNA titers and complement levels are not always reliable for predicting disease recurrence. Therefore, thrombophilia screening is recommended for transplant candidates with SLE, and anticoagulants should be prescribed for patients with antiphospholipid antibodies. Identifying new immunological markers is crucial for the early diagnosis and prevention of acute kidney damage and subsequent graft loss.

REFERENCES

1. 1. Alduraibi FK, Tsokos GC. Lupus Nephritis Biomarkers: A Critical Review. Int J Mol Sci. 2024;25(2):805. doi:10.3390/ijms25020805. DOI
2. Kril I, Tolstyak J, Havrylyuk A et al. Relationship of apoptosis and secondary necrosis of leucocytes with activity of disease and clinical manifestation in patients with systemic lupus erythematosus. Proceeding of the Shevchenko Scientific Society. Medical Sciences. 2022;69(2):10-17. doi:10.25040/ntsh2022.02.01. DOI
3. Bajema IM, Wilhelmus S, Alpers CE et al. Revision of the International Society of Nephrology/Renal Pathology Society classification for lupus nephritis: Clarification of definitions, and modified National Institutes of Health activity and chronicity indices. Kidney Int. 2018;93(4):789–796. doi: 10.1016/j.kint.2017.11.023. DOI
4. Chopyak VV, Tolstyak YaF, Synenkyi OV. Systemnyy chervonyy vovchak, diahnostyka, likuvannya ta profilaktyka. [Systemic lupus erythematosus, diagnosis, treatment and prevention]. Lviv 2019, p.264. (Ukrainian)
5. Tsokos G, Boulougoura A, Kasinath V et al. The immunoregulatory roles of non-haematopoietic cells in the kidney. Nature. Reviews. Nephrology. 2023;20(4). doi: 10.1038/s41581-023-00786-x. DOI
6. Anders H.-J, Saxena R, Zhao M.-H, Parodis I, Salmon JE., Mohan C. Lupus nephritis. Nat. Rev. Dis. Primers. 2020; 23; 6(1):7. doi: 10.1038/s41572-019-0141-9. DOI
7. Der E, Suryawanshi H, Morozov P et al. Tubular cell and keratinocyte single-cell transcriptomics applied to lupus nephritis reveal type I IFN and fibrosis relevant pathways. Nat Immunol. 2019;20(7):915-927. doi: 10.1038/s41590-019-0386-1. DOI
8. Tolstyak Ya, Zhuk R, Yakovlev I et al. The Ensembles of Machine Learning Methods for Survival Predicting after Kidney Transplantation. Applied Sciences 2021;11(21):10380. doi: 10.3390/app112110380. DOI
9. Kotzen SE, Roy S, Jain K. Antiphospholipid Syndrome Nephropathy and Other Thrombotic Microangiopathies Among Patients With Systemic Lupus Erythematosus. Adv Chronic Kidney Dis. 2019;26(5):376–386. doi: 10.1053/j.ackd.2019.08.012.
10. Mohanka R, Kosi EM, Jin J et al. Careful Interpretation of HLA Typing and Cross-Match Tests in Kidney Transplant. JOJ Urology & Nephrology. 2017;3(5):555625. doi: 10.19080/JOJUN.2017.03.555625. DOI
11. Alduraibi FK, Sullivan KA, Chatham WW et al. Interrelation of T cell cytokines and autoantibodies in systemic lupus erythematosus: A cross-sectional study. Clin. Immunol. 2023;247:109239. doi: 10.1016/j.clim.2023.109239. DOI
12. Jorge A, Fu X, Cook C et al. Kidney Transplantation and Cardiovascular Events Among Patients With End-Stage Renal Disease Due to Lupus Nephritis: A Nationwide Cohort Study.Arthritis Care Res (Hoboken). 2022;74(11):1829-1834. doi: 10.1002/acr.24725. DOI
13. Zahab M, Elhendy YA, Elokey AM et al. Outcome of lupus nephritis after live-donor renal transplantation: single-center experience. Journal of The Egyptian Society of Nephrology and Transplantation 2016;16(3):73 doi:104103/1110-9165.197380. DOI
14. Izonin I, Tkachenko R, Horbal N et al. An Approach Toward Numerical Data Augmentation and Regression Modeling Using Polynomial-Kernel-Based SVR. Proceedings of International Conference on Data Science and Applications. 2021. doi: 10.1007/978-981-16-5120-558. DOI
15. Tolstyak Ya, Havryliuk M. An assessment of the transplant`s survival level for Recipients after Kidney Transplantations using Cox Proportional-Hazards Model. The 5th International Conference on Informatics& Data-Driven Medicine (IDDM 18-20 November 2022, Lyon, France) CEUR Workshop Proceedings. https://ceur-ws.org/Vol-3302/short12.pdf [Accessed 05 May 2025]
16. Tolstyak Ya, Chopyak V, Havryliuk M. An investigation of the primary immunosuppressive therapy's influence on kidney transplant survival at one month after transplantation. Transplant Immunol. 2023;78:101832. doi: 10.1016/j.trim.2023.101832. DOI
17. Ya Mei Li, Yi Li, Lin Yan et al.Comparison of urine and blood NGAL for early prediction of delayed graft function in aduld kidney transplant recipsents: a meta-analysis of observational studies. BMC Nephrol. 2019;20:291. doi:10.1186/s 12882-019-1491-y. DOI
18. Yaffe HC, von Ahrens D, Urioste A et al. Impact of Deceased-donor acute kidney Injury on kidney Transplantation. Transplantation: 2024;108(6):1283-1295. doi: 10.1097/TP.0000000000004848. DOI
19. Bailly E, Macedo C, Ossart J et al. Interleukin-21 promotes Type-1 activation and cytotoxity of CD56dim CD16bright natural killer cells during kidney allograft antibody-mediated rejection showing a new link between adaptive and innate humoral allo-immunity. Kidney Int. 2023;104(4):707-723. doi: 10.1016/j.kint.2023.04.024. DOI

20. Jordan SC, Ammerman N, Huang E, Vo A. Importance of IL-6 inhibition in prevention and treatment of antibody-mediated rejection in kidney allografts. Clinical Trial Am J Transplant. 2022;22(4):28-37. doi: 10.1111/ajt.17207. DOI
21. Graver AS, Lee D, Power DA, Whitlam JB. Understanding Donor-derived Cell-free DNA in Kidney Transplantation: An Overview and Case-based Guide for Clinicians Transplantation. 2023;107(8):1675-1686. doi: 10.1097/TP.0000000000004482. DOI
22. Elias C, Chen C, Cherekuri A. Regulatory B Cells in Solid Organ Transplantation: From immune Monitoring to Immunotherapy. Transplantation 2024;108(5):1080-1089. doi: 10.1097/TP.0000000000004798. DOI
23. Jung HY, Ryu JH, Kim MG et al. Association of serum activin levels with allograft outcomes in patients with kidney transplant: Results from the KNOW-KT. J Am J Nephrol. 2024:55(2):245-254. doi: 10.1159/000536198. DOI

CONFLICT OF INTEREST

The Author declare no conflict of interest

CORRESPONDING AUTHOR

Jaroslav Tolstyak
Danylo Halytsky Lviv National Medical University
69 Pekarska St., 79010 Lviv, Ukraine
e-mail:tolstyakyaroslav@gmail.com

ORCID AND CONTRIBUTIONSHIP

Jaroslav Tolstyak: 0000-0002-5990-5977 A B D E F

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

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REVIEW ARTICLE

CONTENTS

The role of the MIND diet in prevention and treatment of Alzheimer's disease: A literature review

Jan Krupa[1], Maciej Malinowski[1], Michał Krasowski[2], Alicja Kalinowska[1], Wiktoria Pietras[1], Adrian Kozieł[1], Zofia Kurek[1], Aleksander Jentkiewicz[1], Esmail Haj Obeid[1], Jakub Ulrych[1]

[1]STUDENT OF THE FACULTY OF MEDICINE, MEDICAL UNIVERSITY OF WARSAW, WARSAW, POLAND

[2]STUDENT OF THE FACULTY OF MEDICINE, POZNAN UNIVERSITY OF MEDICAL SCIENCES, POZNAN, POLAND

ABSTRACT

Aim: Recent research increasingly point to modifiable risk factors, especially dietary patterns, as potential tools to prevent or delay neurodegeneration. This review evaluates the impact of the MIND diet on the prevention and progression of AD and compares it with other dietary interventions.

Materials and Methods: A literature search was conducted using the PubMed and Google Scholar databases for articles published from January 2015 to January 2025, focusing on the influence of the MIND diet, as well as other dietary patterns, on AD progression and cognitive performance.

Conclusions: While the MIND diet shows promise as a feasible non-pharmacological strategy, current evidence is largely observational and limited by population heterogeneity and inconsistent adherence definitions. Short-term randomized controlled trials are less conclusive. Long-term clinical trials are needed to establish causality. Despite these limitations, the MIND diet remains a practical and potentially effective approach to reducing cognitive decline and delaying the onset of AD.

KEY WORDS: Alzheimer disease, neuroprotection, dietary patterns, neurodegenerative diseases, diet therapy

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INTRODUCTION

Alzheimer's Disease (AD) represents one of the most serious health problems facing aging societies, affecting millions of people worldwide. The number of patients affected by AD in the United States alone is estimated to increase to 13.8 million by 2060, compared to approximately 6.9 million cases in 2024, indicating a significant increase from the current number [1]. Pathological changes in AD, such as the accumulation of beta-amyloid (Aβ) plaques, the formation of neurofibrillary tangles and chronic inflammation within the central nervous system , disrupt neuronal function, resulting in synaptic dysfunction and impaired neurotransmission [2]. Disease progression increases susceptibility of neurons to degeneration, leading to widespread neuronal loss, especially in areas critical for memory processing and cognitive function, such as the hippocampus and cerebral cortex. As a consequence, the quality of life of patients deteriorates and the burden on healthcare system increases [3]. The increase in life expectancy and changing demographic structures make it essential to search for effective preventive and therapeutic strategies [4].

Anti-inflammatory dietary patterns, such as the Mediterranean diet (MedDiet) and the Dietary Approaches to Stop Hypertension (DASH) diet, characterized by a high intake of foods that help reduce inflammation and oxidative stress, may exert neuroprotective effects by inhibiting neuroinflammation associated with AD [5]. The Mediterranean-DASH Diet Intervention for Neurodegenerative Delay (MIND) diet incorporates key elements from both diets, making it one of the most promising dietary approaches [6].

Since the introduction of the MIND diet pattern in 2015, it has been consistently linked to reduced cognitive decline and favorable brain outcomes. Studies suggests it may play a significant role in reducing oxidative stress and modulating inflammatory processes within the brain [7]. Appropriate nutritional strategies may support cognitive function and brain health [8], which may be particularly important in genetically predisposed populations.

AIM

The primary aim of this review is to evaluate the potential of the MIND diet in the prevention and progression of AD, including an assessment of its impact on genetically predisposed populations. The review also explores the proposed neuroprotective mechanisms, compares the MIND diet with other dietary strategies, and identifies areas requiring further research.

MATERIALS AND METHODS

We conducted a literature search for relevant papers in PubMed and Google Scholar databases. The search strategy incorporated combinations of the following keywords: "MIND diet", "Alzheimer's disease", "cognitive decline", "cognitive impairment", "nutritional intervention", "diet", "Mediterranean diet", "Ketogenic diet" and "APOE 4". Inclusion criteria were restricted to peer-reviewed publications published between January 2015 and January 2025. Exceptions were made for earlier foundational studies where necessary to define core concepts. Studies were considered eligible if they focused on human subjects, were written in English, and were available in full text. After title and abstract screening, 67 studies meeting the initial criteria were retained for review. Full-text articles were then assessed for eligibility. In total, 35 studies met all inclusion criteria and were selected for detailed analysis.

REVIEW

MIND DIET OVERVIEW

The MIND diet represents a dietary pattern that combines elements of the Mediterranean and DASH diets, with modifications based on the most significant findings in nutrition and dementia, to improve brain health and mitigate age-related cognitive decline. The basic principle of the MIND diet is to encourage the consumption of ten food groups, each with proven cognitive benefits, while limiting the consumption of five food groups that may promote neurodegenerative processes (Table 1) [6].

Table 1. Core principles of the MIND diet [6]

Food group	Recommendation
Green leafy vegetables	≥6 servings per week
Other vegetables	at least once daily
Berries	≥2 times per week
Nuts	≥5 times per week
Olive oil	main source of fat
Whole grains	≥3 servings per day
Fish	at least once per week
Beans	≥4 times per week
Poultry (not fried)	≥2 times per week
Wine	1 glass per day
Butter, margarine	<1 tablespoon/day
Cheese	<1 serving/week
Red Meat and products	<4 times/week
Fast fried foods	<1 time/week
Pastries and sweets	<5 times/week

The MIND diet recommends regularly including leafy green vegetables, such as spinach, kale, and lettuce, which are abundant in folate, vitamin E, carotenoids, and flavonoids-nutrients associated with reduced risk of dementia and cognitive decline. Another key component of the MIND diet is berries eaten at least twice a week, which has been linked to improved cognitive function and better performance on cognitive tests [6]. Similarly, research suggests that the frequent consumption of nuts, another component of the diet, may support cognitive function and slow its age-related decline. The bioactive compounds found in nuts, including omega-3 fatty acids and polyphenols, play a role in reducing oxidative stress and enhancing neuroprotection [9].

The MIND diet promotes the regular consumption of whole grain products, which are a source of polyphenols, that protect the brain against oxidative stress and neurodegeneration [10]. They also contain various phytochemicals that may influence cognitive outcomes, with brown rice specifically containing higher levels of γ-amino butyric acid, which, in combination with ferulic acid, has been suggested to enhance spatial learning in some model of AD [11]. Legumes, such as beans, peas, lentils, pulses, peanuts, and chickpeas, are an important component of the diet [8] due to their high content of plant-based protein [12], as well as polyphenols and folates [10]. These nutrients may help reduce oxidative stress and lower homocysteine levels [10], which, when elevated, can compromise the blood-brain barrier, cause neuronal damage, alter Aβ production, and further promote oxidative stress. The MIND diet also includes the consumption of fish rich in omega-3 fatty acids, recommended at least once a week [6]. Consuming fatty fish more than twice a week is linked to a 41% lower risk of AD compared with eating fish less than once a month [8]. Olive oil is another key dietary component. Biophenols present in olive oil act as potent scavengers, capturing free radicals by combining with peroxide and alkoxyl radicals and chelating trace metals [13]. Oleuropein and oleocanthal, two biophenols found in olive oil, may have neuroprotective potential,

Table 2. Bioactive components associated with foods in the MIND diet and their impact on AD risk reduction

Bioactive component	Food group	Mechanisms of action
Folate	Leafy green vegetables, legumes	Reduce homocysteine level [12]
Vitamin E	Leafy green vegetables, whole grain products	Inhibition of Aβ plaque deposition [7]
Carotenoids	leafy green vegetables, other vegetables whole grain, legumes	Reduce oxidative stress [12]; strong antioxidants
Omega-3 Fatty Acids	Fish Nuts	Anti-inflammator effects; reduce oxidative stress, enhance neuroprotection
Polyphenols (general)	Legumes, nuts	Reduce oxidative stress, enhance neuroprotection [11]; brain protection against oxidative stress and neurodegeneration [12]
Flavonoids	Berries, leafy green vegetables, other vegetables, whole grain products	Suppression of microglia-induced inflammation, oxidative stress [11]
Oleuropein	Olive oil	Significant antioxidant properties, protecting nerve cells from neurotoxin-induced apoptosis; potential to lower Aβ levels; prevent its aggregation, and decrease the expression of glutaminyl cyclase, an enzyme associated with Aβ synthesis [12];
Oleocanthal	Olive oil	Anti-inflammatory activity through cyclooxygenase inhibition; may possess neuroprotective potential against AD by reducing tau protein polymerization, inhibiting Aβ aggregation, and enhancing Aβ clearance from the brain [18].
Plant-based protein	Legumes	A source of protein in the diet that allows to limit the consumption of animal products containing saturated fatty acids [14]

particularly in protecting against neuroinflammation and Aβ aggregation [14]. Eating a diet rich in vegetables, fruits, legumes, nuts, and seeds, while using olive oil as the primary culinary fat, is an effective strategy for reducing the risk of developing AD, particularly as a preventative approach rather than a treatment after the disease has occurred [15]. The bioactive compounds and their mechanisms of action responsible for the neuroprotective effect as a key element of the MIND diet are summarized in Table 2.

At the same time, the MIND diet emphasizes the elimination of products high in saturated and trans fats, which negatively impact brain health. This includes limiting red meat and meat products, butter and stick margarine, full-fat cheese, pastries, sweets, and fried or fast foods. Diets rich in saturated and trans fats, and low in polyunsaturated and monounsaturated fats can impair blood-brain barrier function [6], promote the accumulation of Aβ plaques, and contribute to cognitive decline [9]. Excessive meat consumption, particularly red meat, common in developed countries has been linked to dementia and AD [16]. The typical Western diet, characterized by low dietary fiber and high levels of animal protein and saturated fat, is associated with gut dysbiosis. This gut imbalance can trigger both local and systemic immune-mediated inflammation, leading to neuroinflammation, a well-documented contributor to neurodegenerative diseases [17]. Chronic inflammation, driven by pro-inflammatory cytokines and bacterial components, facilitates the entry of neurotoxic substances into the brain, exacerbating neurodegenerative processes [18]. Additionally, the consumption of added sugars, particularly in ultra-processed foods (UPFs) and sugar-sweetened beverages, has been associated with cognitive decline. Studies suggest that diets high in glucose, fructose, high-fructose corn syrup, high-glycemic-index carbohydrates, and salt contribute to brain atrophy, neuronal loss, and may ultimately increase the risk of developing AD [19].

Moreover, this dietary pattern has been shown to provide protection against several cardiovascular risk factors, such as hypertension and elevated LDL cholesterol, which also contribute to the development of dementia and AD. These protective effects are achieved, at least in part, by modulating key pathological processes underlying AD, including oxidative stress, inflammation, and insulin resistance [17].

ASSOCIATIONS BETWEEN THE MIND DIET AND ALZHEIMER'S DISEASE RISK

Empirical evidence supporting the effectiveness of the MIND diet comes mainly from large, multicenter cohort studies. In the Rush Memory and Aging Project (MAP),

involving 923 individuals aged 58 to 98 years and followed for an average of 4.5 years, those in the highest tertile of MIND diet adherence had a 53% lower risk of developing AD [20]. Notably, even moderate adherence conferred significant benefits, distinguishing the MIND diet from other dietary patterns such as DASH or the MedDiet. However, it is important to note that participants with the highest MIND scores generally had more favorable demographic and health profiles, including higher levels of education, lower BMI, a lower incidence of diabetes, and higher levels of physical and cognitive activity. Nevertheless, even after adjusting for these variables, the protective effect of the MIND diet remained statistically significant after multivariate adjustment, although somewhat attenuated [20]. Similar findings have been reported in other populations. An analysis combining three large cohorts (Whitehall II in the UK, and the Health and Retirement Study and Framingham Offspring Study in the US) comprising a total of 18,136 participants, showed that every 3-point increase in the MIND score was associated with a 17% relative reduction in dementia risk. In this analysis, the protective effect of the MIND diet was particularly evident among non-smokers, while no significant effect was observed in smokers [21].

In the European Three-City Bordeaux cohort, which included 1,412 participants followed for almost 10 years, each one-point increase in MIND adherence was associated with a 12% lower risk of developing AD [22]. This effect was independent of demographic and health factors. Furthermore, neuroimaging data from a subgroup of 175 participants revealed an association between higher MIND scores and greater microstructural integrity of the brain's white matter. Notably, no significant differences were observed in grey matter volume (based on VBM analysis), suggesting that the MIND diet may help preserve the integrity of neural connections rather than increase brain volume [22].

Data from the Chinese CHNS study (n = 4,066) indicated that a 3-point increase in the MIND score corresponded to a 0.110 z-score improvement in cognitive function, roughly equivalent to one year of younger cognitive age [7]. Benefits were also evident in long-term lifestyle analyses. In the CHAP study (Chicago Health and Aging Project), individuals aged 65 who adhered to 4–5 healthy lifestyle habits lived an average of 4.5 years longer (women) and 6.4 years longer (men) without dementia compared to those with one or no healthy habits [23].

In a systematic review of 40 studies (32 cohort studies), van Soest et al. reported that 70% confirmed a significant inverse association between MIND diet adherence and dementia risk, particularly in North American populations [24]. Observational analyses, including both cross-sectional and longitudinal studies, support a strong relationship between higher MIND diet adherence and better cognitive performance. Among 960 American adults, slower cognitive decline was observed across five cognitive domains: episodic memory, semantic memory, spatial memory, processing speed, and working memory [6].

However, not all studies are fully consistent with each other. One larger study (n = 16,058) did not confirm a significant association between the MIND diet and cognitive function [25], highlighting the need for further research.

CLINICAL APPLICATIONS OF THE MIND DIET

Despite promising findings from cohort studies, data from randomized controlled trials remain more inconclusive. The largest intervention study to date was a three-year randomized trial conducted at two centers in the US (Chicago and Boston), involving 604 individuals at risk of AD (ages 65–84, with family history of AD, BMI > 25), but without clinically significant cognitive impairment. It compared the effects of a calorie-restricted MIND diet to a standard calorie-restricted control diet. Although both groups showed cognitive improvement, the difference between them was not statistically significant (0.035 units; p = 0.23), and MRI outcomes (hippocampal, white matter, and grey matter volumes) did not differ between the groups [26]. The authors suggest that improvement in both groups may have been partially attributable to practice effects resulting from repeated cognitive testing, as well as to the similar levels of support provided to both groups. They also note that the full potential effects of the MIND diet may require a longer intervention period to fully manifest.

In a review by Devranis et al., a small randomized trial (n = 37) was included, in which a three-month MIND diet intervention in obese women (BMI = 32) led to statistically significant improvements in working memory, verbal memory, short-term memory, attention, visual scanning, and, to some extent, executive function [27].

BENEFITS FOR GENETICALLY PREDISPOSED INDIVIDUALS

In the context of AD prevention, attention is being paid to the effectiveness of diets among individuals with the APOE ε4 allele - the main genetic risk factor for sporadic AD [28]. Studies indicate that carriers of this allele not only have an increased risk of developing AD but may also respond differently to dietary interventions [29]. In a sample of 389 older adults (52% women, mean

Table 3. Comparison of the MIND, DASH, mediterranean, and ketogenic diets in the context of Alzheimer's disease

Feature	MIND	DASH	Mediterranean	Ketogenic
General dietary recommendations	Emphasis on 10 groups of food, limits red meat, butter, sweets, fried food, cheese [6]	Low sodium, reduced saturated fats and refined sugars, high intake of vegetables and fruits [13]	High intake of vegetables, fruits, legumes, olive oil, nuts; moderate alcohol; limited red meat [20]	Very low carbohydrate (<30–50 g/day), high fat intake (up to 90% of energy) [31]
Mechanism of action	Antioxidant, anti-inflammatory, vascular protective effects [8, 10]	Blood pressure reduction; improved vascular health [13]	Antioxidant, anti-inflammatory; improved lipid profile and endothelial function [27, 32]	Ketosis; ketone bodies as alternative energy substrate [31]
Effects in AD	Associated with slower cognitive decline and reduced AD risk [20, 22, 23]	Some protective effects, mainly at very high adherence to the diet [27]	18% lower risk of cognitive decline, 30% lower risk of AD; reduced amyloid burden [32]	Potential cognitive benefits; limited data; reduced ROS and neuroinflammation [27, 31]; ambiguous results in APOE4 carriers [13, 27]

age 69 years), including individuals with mild cognitive impairment (MCI) and siblings of patients with AD, a significant association was found between MIND diet adherence and improved memory performance [30]. After adjustment for covariates such as age, education, physical activity, BMI, and APOE ε4 status, regression models confirmed that this effect was independent of genetic risk. Although the analysis did not test for interaction effects between diet and genotype, the inclusion of APOE ε4 as a covariate contributes valuable insights into the potential efficacy of the MIND diet in populations at elevated risk of AD.

COMPARISON OF THE MIND DIET WITH OTHER DIETARY INTERVENTIONS

Other dietary interventions, including the MedDiet, the DASH diet, and the ketogenic diet, may affect inflammatory pathways involved in the development of AD, although their underlying principles and mechanisms of action differ (Table 3).

MIND VS. KETOGENIC DIET

The ketogenic diet (KD), characterized by a low-carbohydrate, high-fat diet, induces ketosis. In this state ketone bodies become the brain's primary energy source in the absence of glucose [31]. Reduced production of reactive oxygen species (ROS) in the brain has been observed in this state, potentially beneficially impacting brain function by reducing neuroinflammation associated with neurodegenerative diseases. In AD, where brain glucose metabolism is impaired, ketone bodies - particularly β-hydroxybutyrate (βHB) - may help compensate for energy deficits [31]. βHB has been shown to reduce ROS production by modulating mitochondrial complex I, which may reduce neurotoxicity in AD. The KD also increases uncoupling proteins (UCPs), enhancing oxidative phosphorylation - often impaired in AD - and elevates the NAD+/NADH ratio, thereby protecting neurons from damage [31].

Both clinical and preclinical studies indicate that the KD may improve cognitive function in patients with MCI. In one study, administration of medium-chain triglycerides (MCTs) to individuals with AD resulted in better performance on the ADAS-cog scale and higher blood ketone levels [13]. A six-week low-carbohydrate diet (20 g per day) also significantly improved verbal memory in older adults with MCI. However, some findings suggest that the effectiveness of such interventions may be limited in carriers of the ApoE4 allele [13].

While the KD may benefit those with impaired glucose regulation, it has some practical limitations. Strict carbohydrate restriction can potentially lead to the so-called "ketogenic flu" (symptoms such as nausea, constipation, dizziness) [31], as well as the risk of high intake of saturated fat if not well-balanced. This can potentially contribute to inflammation and adversely affect cardiovascular health. Given these limitations, the MIND diet appears to offer a safer and more practical option for long-term, daily use, especially in older adults.

MIND VS. DASH DIET

Originally developed to reduce hypertension, the DASH diet has also been shown to demonstrate beneficial effects on cognitive functions [27]. Its core principles—reducing sodium, simple sugars, and saturated fats while promoting high intake of vegetables and fruits- partially align with MIND diet recommendations. Evidence from clinical trials indicates that adherence to

the DASH diet significantly improves executive functions and psychomotor speed compared to control groups. Observational data further suggest that it may slow overall cognitive decline [13].

While the MIND diet incorporates components of both the DASH diet and MedDiet, it introduces more targeted recommendations with a greater emphasis on specific brain-healthy foods not present in other dietary patterns [20]. Both the DASH and MIND diets show improvements in executive function and psychomotor speed; however, the MIND diet appears more promising in terms of dementia prevention. Existing findings indicate that the protective effect of the DASH diet is primarily observed with very high adherence level to the diet, whereas the MIND diet shows a linear relationship between the degree of adherence and reduced risk of dementia [27].

MIND VS. MEDITERRANEAN DIET

The MedDiet forms the conceptual basis of the MIND diet model, promoting a high intake of vegetables, fruits, legumes, and unsaturated fats - primarily from olive oil [27]. Both diets provide numerous antioxidants - including flavonoids and phenolic substances - which have been shown to play a role in reducing neuroinflammatory processes associated with the pathogenesis of AD [27, 32]. Differences between the two diets include the extent of restriction of foods potentially harmful to brain health and the inclusion of specific ingredients, such as berries and green leafy vegetables, as key components of the MIND diet.

A meta-analysis of multiple studies demonstrated that adherence to the MedDiet is significantly associated with lower risk of cognitive decline (by 18%), dementia (by 11%), and AD (by 30%) [32]. Studies examining the relationship between the MedDiet and cerebral Aβ accumulation, using positron emission tomography (PET) imaging in older adults, have demonstrated that higher adherence to the diet is associated with reduced Aβ plaque formation, lower cerebral Aβ accumulation, and an improved neuroimaging biomarker profile [27]. Additionally, MedDiet has been shown to positively impact lipid parameters, blood pressure, endothelial function, and reduce inflammatory markers such as C-reactive protein (CRP) and interleukin-6 (IL-6) [33]. Furthermore, MedDiet adherence has been linked to specific alterations in gut microbiota composition. The NU-AGE one-year dietary intervention revealed that MedDiet adherence was associated with favorable changes in gut flora, reduced frailty, enhanced cognitive function, and a decrease in inflammatory markers, including CRP and interleukin-17 (IL-17) [27]. Evidence from over 250 studies suggests that the MedDiet may reduce the risk of cognitive decline among APOE ε4 carriers [29]. In some analyses, this effect was even more pronounced among APOE ε4 carriers. However, results were not consistent across all studies.

Observational studies also reveal that individuals with AD are less likely to adhere to MedDiet recommendations - only 1.4% of AD patients demonstrated high adherence, according to the MEDAS scale, compared to 5.5% in the control group [34]. This may suggest a potential relationship between cognitive decline and deviation from a healthy dietary pattern, though further confirmation is needed.

DISCUSSION

Although the scientific evidence supporting the effectiveness of the MIND diet is promising, it requires critical interpretation to assess its consistency, limitations, and validity. Its effectiveness in practice depends on a number of factors related to daily use, which can significantly influence adherence and health outcomes. Analysis of existing research reveals both barriers and the potential for adaptation, suggesting directions for further research and clinical interventions.

One such barrier is factors related to nutrition education - low levels of nutritional knowledge in at-risk populations may limit the effectiveness of interventions in the absence of adequate educational support. In addition, practical barriers such as food cost and availability may reduce adherence. Notably, it has been observed that the MIND diet can be difficult to maintain due to the high price of key ingredients such as olive oil, nuts, berries, and fish—especially during off-season and in low- and middle-income countries, where the lack of local production limits availability and reduces adherence to the diet [27]. However, there are ways to overcome this limitation. The MIND diet can be culturally adapted, as exemplified by the MIND-NL version developed for the Dutch population, which incorporates local products and a modified adherence scale [35]. Such adaptations may improve the generalizability and effectiveness of the diet across different cultural groups.

Beyond issues of adherence, the timing of dietary intervention may also influence its effectiveness. From a preventive medicine perspective, implementing the MIND diet already in midlife - before the onset of clinically manifest cognitive decline - appears particularly promising. In the MAP study, which involved participants with an average age 81, significant reductions in cognitive decline rates were observed. Participants with the highest adherence exhibited cognitive aging

trajectories comparable to those of individuals approximately 7.5 years younger [6]. Similarly, positive effects were observed in the CHNS cohort (mean age: 62), where the MIND diet was associated with better cognitive performance [7]. These findings emphasize that the MIND diet may be effective not only for secondary prevention in older adults but also for primary prevention in middle-aged individuals.

However, while potential of the diet is encouraging, it must be interpreted in light of important limitations within the current body of evidence. Although observational data continue to accumulate, high-quality confirmation from randomized controlled trials (RCTs) remains sparse. Existing RCTs - though methodologically sound - have failed to demonstrate clear differences between intervention and control groups. This may be due to factors such as the relatively short duration of interventions, test–retest bias, or unexpectedly high adherence in the control groups. Nevertheless, current study provides valuable insights into the feasibility of dietary interventions and their potential impact in populations at elevated risk of cognitive decline. These findings collectively provide a foundation for future trials that integrate longer follow-up, biomarker-based endpoints, and genetic stratification - ultimately confirming that diet is a central component of dementia prevention strategies.

CONCLUSIONS

The accumulated evidence - from both cohort and clinical studies - demonstrates the considerable potential of the MIND diet as a promising preventive approach to AD. Although large-scale interventional trials remain limited, the consistency of observational findings and their alignment with known neurodegenerative mechanisms support the recognition of the MIND diet as one of the most promising dietary interventions. Its design combines targeted neuroprotective components with practical feasibility in clinical settings. Future research should incorporate long-term follow-up, diverse populations, and the evaluation of neurodegeneration biomarkers. Particularly important is the adaptation of the MIND model to local contexts, along with the integration of dietary interventions into broader health education initiatives. Such an approach may represent a tangible step toward effective population-level dementia prevention.

REFERENCES

1. 2024 Alzheimer's disease facts and figures. Alzheimers Dement. 2024 May;20(5):3708-3821. doi: 10.1002/alz.13809 DOI
2. Liu E, Zhang Y, Wang JZ. Updates in Alzheimer's disease: from basic research to diagnosis and therapies. Transl Neurodegener. 2024 Sep 4;13(1):45. Doi: 10.1186/s40035-024-00432-x.
3. Tenchov R, Sasso JM, Zhou QA. Alzheimer's Disease: Exploring the Landscape of Cognitive Decline. ACS Chem Neurosci. 2024 Nov 6;15(21):3800-3827. doi: 10.1021/acschemneuro.4c00339. DOI
4. Breijyeh Z, Karaman R. Comprehensive Review on Alzheimer's Disease: Causes and Treatment. Molecules. 2020 Dec 8;25(24):5789. Doi: 10.3390/molecules25245789.
5. McGrattan AM, McGuinness B, McKinley MC, Kee F, Passmore P, Woodside JV, McEvoy CT. Diet and Inflammation in Cognitive Ageing and Alzheimer's Disease. Curr Nutr Rep. 2019 Jun;8(2):53-65. doi: 10.1007/s13668-019-0271-4. DOI
6. Morris MC, Tangney CC, Wang Y, Sacks FM, Barnes LL, Bennett DA, Aggarwal NT. MIND diet slows cognitive decline with aging. Alzheimers Dement. 2015 Sep;11(9):1015-22. doi: 10.1016/j.jalz.2015.04.011 DOI
7. Huang L, Tao Y, Chen H, Chen X, Shen J, Zhao C, Xu X, He M, Zhu D, Zhang R, Yang M, Zheng Y, Yuan C. Mediterranean-Dietary Approaches to Stop Hypertension Intervention for Neurodegenerative Delay (MIND) Diet and Cognitive Function and its Decline: A Prospective Study and Meta-analysis of Cohort Studies. Am J Clin Nutr. 2023 Jul;118(1):174-182. doi: 10.1016/j.ajcnut.2023.04.025
8. Bhuiyan NZ, Hasan MK, Mahmud Z, Hossain MS, Rahman A. Prevention of Alzheimer's disease through diet: An exploratory review. Metabol Open. 2023 Sep 21;20:100257. doi: 10.1016/j.metop.2023.100257. DOI
9. Rajaram S, Jones J, Lee GJ. Plant-Based Dietary Patterns, Plant Foods, and Age-Related Cognitive Decline. Adv Nutr. 2019 Nov 1;10(Suppl_4):S422-S436. doi: 10.1093/advances/nmz081. DOI
10. Grant WB, Blake SM. Diet's Role in Modifying Risk of Alzheimer's Disease: History and Present Understanding. J Alzheimers Dis. 2023;96(4):1353-1382. doi: 10.3233/JAD-230418. DOI
11. Ross AB, Shertukde SP, Livingston Staffier K, Chung M, Jacques PF, McKeown NM. The Relationship between Whole-Grain Intake and Measures of Cognitive Decline, Mood, and Anxiety-A Systematic Review. Adv Nutr. 2023 Jul;14(4):652-670. doi: 10.1016/j.advnut.2023.04.003. DOI
12. Cena H, Calder PC. Defining a Healthy Diet: Evidence for The Role of Contemporary Dietary Patterns in Health and Disease. Nutrients. 2020 Jan 27;12(2):334. doi: 10.3390/nu12020334. DOI
13. Omar SH. Mediterranean and MIND Diets Containing Olive Biophenols Reduces the Prevalence of Alzheimer's Disease. Int J Mol Sci. 2019 Jun 7;20(11):2797. doi: 10.3390/ijms20112797. DOI

14. Rodríguez-Morató J, Xicota L, Fitó M, Farré M, Dierssen M, de la Torre R. Potential role of olive oil phenolic compounds in the prevention of neurodegenerative diseases. Molecules. 2015 Mar 13;20(3):4655-80. Doi: 10.3390/molecules20034655.
15. Pinto-Hernandez P, Castilla-Silgado J, Coto-Vilcapoma A, et al. Modulation of microRNAs through Lifestyle Changes in Alzheimer's Disease. Nutrients. 2023 Aug 23;15(17):3688. doi: 10.3390/nu15173688. DOI
16. Warren A. The relationship between gender differences in dietary habits, neuroinflammation, and Alzheimer's disease. Front Aging Neurosci. 2024 Apr 17;16:1395825. doi: 10.3389/fnagi.2024.1395825. DOI
17. Cremonini AL, Caffa I, Cea M, Nencioni A, Odetti P, Monacelli F. Nutrients in the Prevention of Alzheimer's Disease. Oxid Med Cell Longev. 2019 Sep 4;2019:9874159. doi: 10.1155/2019/9874159. DOI
18. Park KJ, Gao Y. Gut-brain axis and neurodegeneration: mechanisms and therapeutic potentials. Front Neurosci. 2024 Oct 23;18:1481390. Doi: 10.3389/fnins.2024.1481390.
19. Arora S, Santiago JA, Bernstein M, Potashkin JA. Diet and lifestyle impact the development and progression of Alzheimer's dementia. Front Nutr. 2023 Jun 29;10:1213223. doi: 10.3389/fnut.2023.1213223. DOI
20. Morris MC, Tangney CC, Wang Y, Sacks FM, Bennett DA, Aggarwal NT. MIND diet associated with reduced incidence of Alzheimer's disease. Alzheimers Dement. 2015 Sep;11(9):1007-14. Doi: 10.1016/j.jalz.2014.11.009
21. Chen H, Dhana K, Huang Y, Huang L, Tao Y, Liu X, Melo van Lent D, Zheng Y, Ascherio A, Willett W, Yuan C. Association of the Mediterranean Dietary Approaches to Stop Hypertension Intervention for Neurodegenerative Delay (MIND) Diet With the Risk of Dementia. JAMA Psychiatry. 2023 Jun 1;80(6):630-638. Doi: 10.1001/jamapsychiatry.2023.0800.
22. Thomas A, Lefèvre-Arbogast S, Féart C, Foubert-Samier A, Helmer C, Catheline G, Samieri C. Association of a MIND Diet with Brain Structure and Dementia in a French Population. J Prev Alzheimers Dis. 2022;9(4):655-664. doi: 10.14283/jpad.2022.67. DOI
23. Dhana K, Franco O H, Ritz E M, Ford C N, et al. Healthy lifestyle and life expectancy with and without Alzheimer's dementia: population based cohort study. BMJ 2022;377:e068390. doi: 10.1136/bmj-2021-068390. DOI
24. van Soest AP, Beers S, van de Rest O, de Groot LC. The Mediterranean-Dietary Approaches to Stop Hypertension Intervention for Neurodegenerative Delay (MIND) Diet for the Aging Brain: A Systematic Review. Adv Nutr. 2024 Mar;15(3):100184. doi: 10.1016/j.advnut.2024.100184. DOI
25. van den Brink AC, Brouwer-Brolsma EM, Berendsen AAM, van de Rest O. The Mediterranean, Dietary Approaches to Stop Hypertension (DASH), and Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND) Diets Are Associated with Less Cognitive Decline and a Lower Risk of Alzheimer's Disease-A Review. Adv Nutr. 2019 Nov 1;10(6):1040-1065. Doi: 10.1093/advances/nmz054.
26. Barnes LL, Dhana K, Liu X, Carey VJ, et al. Trial of the MIND Diet for Prevention of Cognitive Decline in Older Persons. N Engl J Med. 2023 Aug 17;389(7):602-611. doi: 10.1056/NEJMoa2302368. DOI
27. Devranis P, Vassilopoulou E, Tsironis V, Sotiriadis PM, Chourdakis M, Aivaliotis M, Tsolaki M. Mediterranean Diet, Ketogenic Diet or MIND Diet for Aging Populations with Cognitive Decline: A Systematic Review. Life (Basel). 2023 Jan 6;13(1):173. doi: 10.3390/life13010173. DOI
28. Norwitz NG, Saif N, Ariza IE, Isaacson RS. Precision Nutrition for Alzheimer's Prevention in ApoE4 Carriers. Nutrients. 2021 Apr 19;13(4):1362. doi: 10.3390/nu13041362. DOI
29. Fote GM, Geller NR, Reyes-Ortiz AM, Thompson LM, Steffan JS, Grill JD. A Scoping Review of Dietary Factors Conferring Risk or Protection for Cognitive Decline in APOE ε4 Carriers. J Nutr Health Aging. 2021;25(10):1167-1178. doi: 10.1007/s12603-021-1705-4. DOI
30. Wesselman LMP, van Lent DM, Schröder A, van de Rest O, et al. patterns are related to cognitive functioning in elderly enriched with individuals at increased risk for Alzheimer's disease. Eur J Nutr. 2021 Mar;60(2):849-860. Doi: 10.1007/s00394-020-02257-6.
31. Hersant H, Grossberg G. The Ketogenic Diet and Alzheimer's Disease. J Nutr Health Aging. 2022;26(6):606-614. doi: 10.1007/s12603-022-1807-7. DOI
32. Fekete M, Varga P, Ungvari Z, Fekete JT, et al. The role of the Mediterranean diet in reducing the risk of cognitive impairement, dementia, and Alzheimer's disease: a meta-analysis. Geroscience. 2025 Jun;47(3):3111-3130. Doi: 10.1007/s11357-024-01488-3.
33. Guasch-Ferré M, Willett WC. The Mediterranean diet and health: a comprehensive overview. J Intern Med. 2021 Sep;290(3):549-566. doi: 10.1111/joim.13333. DOI
34. Dominguez LJ, Veronese N, Parisi A, Seminara F, Vernuccio L, Catanese G, Barbagallo M. Mediterranean Diet and Lifestyle in Persons with Mild to Moderate Alzheimer's Disease. Nutrients. 2024 Oct 9;16(19):3421. Doi: 10.3390/nu16193421.
35. Beers S, van Houdt S, de Jong HBT, de Vries JHM, van de Rest O, de Groot LCPGM, de van der Schueren MAE. Development of the Dutch Mediterranean-Dietary Approaches to Stop Hypertension Intervention for Neurodegenerative Delay (MIND) Diet and its scoring system, alongside the modification of a brief FFQ for assessing dietary adherence. Br J Nutr. 2024 Nov 12:1-9. doi: 10.1017/S0007114524001892. DOI

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Jan Krupa
Student of the Faculty of Medicine, Medical University of Warsaw,
Warsaw, Poland
e-mail: janeg.krupa@gmail.com

ORCID AND CONTRIBITIONSHIP
Jan Krupa: 0009-0001-2175-806X B C D E
Maciej Malinowski: 0009-0003-7637-6290 A B E
Michał Krasowski: 0009-0006-6243-1246 E F
Alicja Kalinowska: 0009-0000-9011-843X B E
Wiktoria Pietras: 0009-0003-2887-8755 A D
Adrian Kozieł: 0009-0006-6096-5850 B D
Zofia Kurek: 0009-0002-4156-8666 A B
Aleksander Jentkiewicz: 0009-0008-4224-4069 A F
Esmail Haj Obeid: 0009-0008-5165-1221 B D
Jakub Ulrych: 0009-0004-7460-965X E

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REVIEW ARTICLE

CONTENTS

Organization of secondary level medical institution operations in the context of implementing the Medical Guarantees Program

Ihor V. Hushchuk[1], Vladyslav A. Smiianov[2], Nataliia P. Topishko[3], Oleh M. Vivsyannyk[4], Vitalii I. Boiko[5], Sergii M. Galetskyi[6], Tetiana I. Galetska[3]

[1]DEPARTMENT OF PUBLIC HEALTH AND PHYSICAL EDUCATION, NATIONAL UNIVERSITY OF OSTROH ACADEMY, OSTROH, UKRAINE
[2]DEPARTMENT OF PUBLIC HEALTH, SUMY STATE UNIVERSITY, SUMY, UKRAINE
[3]DEPARTMENT OF MANAGEMENT AND MARKETING, NATIONAL UNIVERSITY OF OSTROH ACADEMY, OSTROH, UKRAINE
[4]DEPARTMENT OF CIVIL PROTECTION AND PUBLIC HEALTH, RIVNE REGIONAL STATE ADMINISTRATION, RIVNE, UKRAINE
[5]MUNICIPAL NON-COMMERCIAL ENTERPRISE (MNCE) "OSTROH MULTISPECIALTY HOSPITAL," OSTROH CITY COUNCIL, OSTROH, UKRAINE
[6]DEPARTMENT OF LANGUAGE MEDIATION, NATIONAL UNIVERSITY OF OSTROH ACADEMY, OSTROH, UKRAINE

ABSTRACT

Aim: The study aims to investigate the organization of work in a secondary-level medical institution regarding the implementation of the MGP.

Materials and Methods: The assessment of MGP implementation at the secondary level was conducted using observation, analysis, synthesis, grouping, and generalization methods.

Conclusions: Organizing the work of secondary-level healthcare institutions for MGP implementation requires strict adherence to the program's provisions and standards regarding patient care within state-guaranteed services. This is achieved through contracts with the National Health Service of Ukraine (NHSU), ensuring patient access to relevant services, compliance with NHSU-established tariffs and service packages, and maintaining electronic documentation in accordance with MGP requirements.

KEY WORDS: healthcare system reform, secondary (specialized) level of medical care, Medical Guarantee Program

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Medical Care (MC)
Medical Guarantee Program (MGP)
Healthcare System (HCS)
National Health Service of Ukraine (NHSU)
Municipal Non-Commercial Enterprise (MNCE)
Center for Disease Control and Prevention (CDCP)

INTRODUCTION

The differentiation of MC into emergency, primary, secondary, tertiary, palliative, and medical rehabilitation levels—based on specialization, complexity and severity of patient health conditions, technical sophistication, and cost—has enabled a more effective redistribution of financial resources among healthcare institutions [1]. This stratification has facilitated the implementation of a state-guaranteed MGP package, introduced new financial mechanisms at each level of care, and supported the modernization of material, technical, human, and informational resources in medical institutions [2].

AIM

The study aims to investigate the organization of work in a secondary-level medical institution regarding the implementation of the MGP.

MATERIALS AND METHODS

The assessment of MGP implementation at the secondary level was conducted using observation, analysis, synthesis, grouping, and generalization methods.

REVIEW AND DISCUSSION

Primary care, based on family medicine, has become the cornerstone of the HCS. General practitioners provide the bulk of services to the population, including preventive

care. The majority of patient requests for healthcare are addressed at the primary care level. When further treatment is required, patients are referred by their physician to secondary or tertiary care institutions. Specialized medical care is needed by no more than 20% of patients.

Secondary (specialized) medical care includes multispecialty hospitals, medical facilities for rehabilitation and planned treatment, hospices, and specialized medical centers of various profiles. It is provided either on a planned basis or in urgent cases by state and municipal healthcare institutions when a patient's condition requires specialized diagnostic and treatment methods delivered by narrow-profile specialists in hospitals or outpatient settings.

Secondary care is provided free of charge, provided that state and municipal healthcare institutions have concluded a contract with the NHSU for medical care provision, ensure patient access to appropriate services, maintain electronic medical records in accordance with MGP requirements, and comply with the tariffs and service packages established by the NHSU.

Since January 1, 2020, healthcare institutions at this level have transitioned to a new financing model based on the volume of services provided. The cost of services is determined according to the applicable medical service tariffs [3]. The 2021 MGP included 35 service packages; in 2022, the list was expanded to 38 packages. In 2023, the MGP was adapted to wartime conditions. By 2024, the MGP included 44 service packages. In 2024, the total payment under the MGP amounted to UAH 151.3 billion, covering medical services provided to 24.4 million patients. In the Rivne region, total payments under the MGP reached UAH 5,526.1 million, with 910530 patients receiving services [4]. The volume of payments to MC providers by type of service in Ukraine and the Rivne region is presented in Table 1.

The implementation of the MGP in 2025 provided funding of UAH 175.5 billion and included 44 medical service packages [5]. More than UAH 25 billion was allocated for primary care services, nearly UAH 11 billion for emergency medical care, over UAH 122 billion for specialized and palliative care, and more than UAH 6 billion for medical rehabilitation [5].

The activities of a healthcare institution are aimed at providing high-quality and effective medical care to the population. They are based on key principles, namely: accessibility for all segments of the population, adherence to modern quality standards, continuity of care at all stages, transparency and accountability of institutional operations, and efficient use of resources.

The MGP for secondary-level medical care includes five priority areas: treatment of acute myocardial infarction, acute stroke, neonatal care, obstetric care, and endoscopic and screening examinations for early cancer detection. These types of medical services are reimbursed by NHSU at elevated tariffs.

The organization of a healthcare institution's work encompasses activities related to planning, management, coordination, control, and evaluation of the achievement of established goals and objectives [6, p. 29]. The main aspects of organizing the work of a healthcare institution are presented in Table 2.

The functioning of a healthcare institution is influenced by both external and internal factors. External economic factors include GDP growth rate, increasing inequality in the distribution of material goods and access to services, the volume of domestic and foreign investments, inflation, and the exchange rate. Political factors encompass levels of bureaucracy and corruption, as well as national and global political conditions. Social factors include the population's educational level, their adoption of new technologies, and patterns of internal and international migration [9]. Economic and demographic aspects also play a critical role. The contracting of medical service packages depends on population size, while the availability and variety of services are influenced by the number of displaced persons.

Internal factors affecting the operation of a healthcare institution can be divided into:

- *Medical-technological factors:* the level of material and technical support and medical technologies, the cost of medical materials and equipment from suppliers, the qualification level of medical staff, and optimization of patient pathways;
- *Economic-managerial factors:* the cost of medical materials and equipment, the financial potential of the institution, the level of management and marketing, and the cost of medical services;
- *Organizational-legal factors:* licensing requirements, standards for medical office equipment, requirements for medical facilities, and public attitudes toward paid medical services [9, p. 12].

Granting autonomy to medical institutions has heightened the importance of addressing economic and managerial factors. Key economic aspects of organizational activity include the efficiency of using material and financial resources. Managerial factors involve the personal qualities of leaders, organizational culture, communication systems, staff motivation and values, availability of human and material resources, information technologies and their effective

Table 1. Payment volumes to healthcare providers under the Medical Guarantee Program in Ukraine and the Rivne region in 2024

	Amount of payment (UAH)	including in Rivne region	Number of providers	including in Rivne region
By type of assistance:				
emergency	1498683336	344298705	25	1
primary	23514364474	838137409	2600	124
specialized	117293454504	4343628798	2399	97
By form of ownership:				
state	1190360923	-	13	
municipal	143692546353	5416315824	2163	84
Private (without sole proprietorship)	4937012564	51215825	523	16
Individual entrepreneur	1486582475	58533263	926	55

Compiled from: [4, pp. 49, 116]

Table 2. Key areas of organization of a healthcare institution's work

Areas	Components
Management of the institution	Strategic planning and operational management, development and improvement of organizational and staffing structures; formation and use of financial resources, ensuring financial stability and efficiency; staff motivation and control of their activities; management decision-making.
Organization of medical care	Implementation of modern methods of diagnosis and treatment; rational use of equipment; organization of the work of medical personnel; coordination of the work of the institution's departments.
Interaction with patients	Ensuring the availability of medical care, providing information to patients, resolving conflict situations.
Quality control of medical care	Implementation of a system for monitoring and evaluating the quality of medical services.
Financing of the medical institution	Ensuring stable financing of the facility; optimizing costs and effective use of funds; cost control; attracting additional sources of financing; implementing a financial accounting and reporting system.
Human resources policy	Recruitment, training, and development of medical personnel; creation of favorable working conditions; motivation and control of personnel work.
Patient and staff safety	Compliance with sanitation and hygiene rules, ensuring the safety of medical equipment and premises.
Information and communication technologies	Implementation of electronic document management, use of medical information systems; ensuring the confidentiality of medical information.

Compiled from: [6; 7; 8]

use, and marketing strategies for promoting medical services to the population.

Managing these factors is a complex, multidimensional process that requires consideration of situational variables, potential risks, and effective decision-making. In healthcare provision, several risks are present, including: provider risk related to staff qualifications, physical, financial, psychological, social risks, and the risk of time loss.

The features of organizing the work of a secondary-level healthcare institution are illustrated by the example of the Municipal Non-Commercial Enterprise (MNCE) "Ostroh Multispecialty Hospital" under the Ostroh City Council, Rivne District, Rivne Region.

The hospital provides specialized secondary-level medical care to all individuals in accordance with Ukrainian healthcare legislation and the institution's Statute; it also implements disease prevention and public health measures. The hospital is owned jointly by the local communities of villages and settlements within the Ostroh territorial community.

The hospital includes the following units: a consultative polyclinic and departments (surgical, cardio-neurological, therapeutic, infectious, palliative, obstetric-gynecological, pathological, and emergency care with intensive care beds); a clinical-diagnostic laboratory; women's consultation; and a consultative polyclinic. The hospital operates 176 beds and

Table 3. Sources of financial resources for the MNC "Ostroh Multispecialty Hospital," Ostroh City Council, Rivne District, Rivne Region, 2022

Source of financial resources	Amount, million UAH	Share, %
NHSU	62	79
Local budget	5	6
Extrabudgetary	12	15
Total	79	100

Source: reports of the Ostroh Multidisciplinary Hospital, a municipal non-profit enterprise of the Ostroh City Council, Rivne District, Rivne Region

Table 4. Trends in key performance indicators of the MNPE "Ostroh Multispecialty Hospital," Ostroh District, Rivne Region, (for the first 9 months of 2021 and 2022)

N	Indicators	2021	2022	Trend
1	Maternal mortality	0	0	-
2.	Infant mortality	18.6(4)	9.3 (2)	
3.	Stillbirths per per 1000 live births	4.6	4.6	-
4	Birth rate	5.1	5.06	
5	Mortality rate from major causes (per 10000 population):	11.1	10.05	
6	Morbidity per 10000 population, %	559.6	781.06	
7	Cancer cases detected during medical examinations	13	19,1	
8	Neglected forms of tuberculosis in v/v	20	38.1	
9	destructive forms of tuberculosis in v/v	33.4	61.9	
10	Bed availability per 10000 people.	41.6	41.6	-
11	Births per 1000 women of childbearing age (15-49 years old)	12.3	11.3	
12	Number of operations in hospitals per 10000 people.	165.8	137.5	
13	Number of outpatient operations per 10000 people.	121.5	142.4	
14	Hospitalization rate	78.5	90.3	
15	Bed occupancy rate	158.5	164.5	
16	Mortality	2.7	1.7	

Source: reports of the Ostroh Multidisciplinary Hospital of the Ostroh City Council of the Rivne District, Rivne Region, for the first nine months of 2021 and the first nine months of 2022

employs 62 doctors and 123 nursing staff, serving 42,263 residents of the Ostroh territorial community and temporarily displaced persons [10].

Secondary-level healthcare institutions receive funding from the NHSU based on contracting medical service packages [11; 12]. The MNCE "Ostroh Multispecialty Hospital" provides medical care to residents of the Ostroh territorial community through 14 service packages, including:

1. Surgical operations for adults and children in inpatient settings.
2. Inpatient care for adults and children without surgical operations.
3. Maternity care.
4. Prevention, diagnostics, observation, treatment, and rehabilitation in outpatient settings.
5. Hysteroscopy.
6. Esophagogastroduodenoscopy.
7. Colonoscopy.
8. Cystoscopy.
9. Diagnosis, treatment, and management of individuals with HIV.
10. Inpatient palliative care for adults and children.
11. Mobile palliative care for adults and children.
12. Management of pregnancy in outpatient settings.
13. Day-surgery operations for adults and children.
14. Dental care for adults and children.

The hospital uses the medical information system "MEDIX," which allows patients to book appointments with their chosen physician online.

The hospital functions as a medical enterprise that transforms input resources into medical services. In this process, physical, human, financial, technological, informational, and other resources are consumed. In 2022, the hospital utilized financial resources totaling UAH 79 million. Of these, 79% were state funds (NHSU), 6% were local budget funds, and 15% were extrabudgetary resources (Table 3; Figure 1).

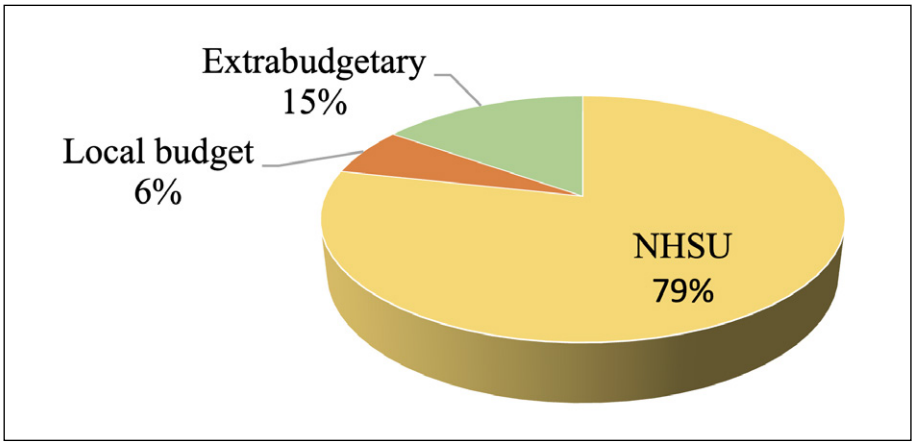

Fig. 1. Structure of financial resources of the Ostroh Multidisciplinary Hospital, a non-profit enterprise of the Ostroh City Council, Rivne District, Rivne Region, 2022. *Source: reports of the Ostroh Multidisciplinary Hospital of the Ostroh City Council of the Rivne District, Rivne Region*

Table 5. Work plan of the inpatient care service of the MNPE "Ostroh Multispecialty Hospital," Ostroh City Council, Rivne Region, 2022

No	Name of event	Date of implementation	Implementation
1.	Achieve the following hospitalization rates: a) urban – 140.0 b) rural – 159.5 c) total – 155.5	by 31/12	Deputy Chief Medical Officer, department heads
2.	Ensure average bed occupancy: BL – 340	-//-	-//-
3.	While maintaining the quality of treatment, ensure the average duration of treatment, days: according to BL – 8.0 In order to ensure the rational use of bed capacity, monitor compliance with the average length of treatment in the hospital by department: - therapeutic – 8.0 - cardiology and neurology – 6.7 - surgical – 8.7 - palliative care – 12.0 - infectious diseases –10.0 - obstetrics and gynecology – 7.0	-//-	-//-
4.	Ensure surgical activity indicators: - surgery – 55.1 - obstetrics and gynecology department – 40.1	By the end of the year	Deputy Chief Medical Officer, department heads
5.	Ensure the fulfillment of auxiliary services indicators: Physiotherapy service: a) implementation of treatment methods for 1 inpatient – 6.7 b) number of procedures per inpatient who has completed physiotherapy treatment – 9.9 c) number of massage procedures per inpatient who has completed treatment – 2.0 d) number of laboratory tests per inpatient – 44.2 e) number of functional examinations per inpatient – 1.0	-//-	-//-
6.	In order to improve the coordination between the polyclinic and the hospital, conduct an analysis of: - the completeness of examinations during planned hospitalisation; - the quality of medical record keeping in the hospital; - conduct medical examinations by day and week; for the rational use of bed capacity, develop average terms of treatment in the hospital by nosological units.	Once per quarter	Deputy Chief Medical Officer
7.	Regularly conduct inspections on the prevention of hospital infections.	Once per quarter	Infectious Disease Control Department
8.	Conduct classes among inpatients to actively promote sanitary and hygienic knowledge.	Continuously according to plan	Heads of departments
9.	Analyze the consultative assistance provided by regional specialists in the hospital.	By 30/12	Deputy Chief Medical Officer

Source: reports from the Ostroh Multidisciplinary Hospital, Ostroh City Council, Rivne District, Rivne Region

Table 6. Composition, structure, and staffing levels of the MNPE "Ostroh Multispecialty Hospital," Ostroh City Council, Rivne Region, 2022

No	Personnel category	Number of persons	Actual, %	Difference between full and actual occupancy, %
1	Doctors	54	77,1	-22.9
2	Pharmacist	1	100	-
3	Specialists with higher non-medical education (biologists)	2	88.9	-11.1
4	Junior specialists with medical and pharmaceutical education	124	95.2	-4.8
5	Junior medical staff	71	95.3	-4.7
6	Other (non-medical) staff	54	84.4	-15.6
	TOTAL	306	89.5	-10.5

Source: reports of the Ostroh Multidisciplinary Hospital, Ostroh City Council, Rivne District, Rivne Region

Extrabudgetary resources are generated from paid services, including professional examinations, laboratory tests, rental fees, and humanitarian aid.

As observed, the hospital received the largest share of its funding from the NHSU under the MGP. The structure of fund utilization in 2022 was as follows: 89% for salaries, 6% for the purchase of medications, 1% for meals, and the remaining portion for other material expenses.

The performance indicators of the hospital for 2021 and 2022, presented in Table 4, reflect trends related to the overall complex social conditions in the country. Especially in the context of the war with the Russian Federation [13]. These include a general increase in population morbidity, particularly for social diseases such as destructive and neglected forms of tuberculosis. The detection of oncological diseases during population screenings also increased. At the same time, overall mortality, including infant mortality, decreased. The number of outpatient surgeries increased, while the number of inpatient surgeries decreased significantly.

The hospital has established an effective management system. Each year, a development plan for the following year is prepared, identifying the main directions of activity based on the assessment of results from the previous period. Data regarding resource provision and expenditure are analyzed.

Based on the evaluation of the hospital's performance, strategic directions for development are determined. These include the implementation of modern technologies, provision of medical care to children and women during pregnancy and childbirth, emergency medical services, epidemiological measures to prevent infectious diseases, participation in mass informational and diagnostic campaigns for early disease detection, improvement of preventive health check-ups through targeted population monitoring, implementation of mass screening programs, digitalization of healthcare, and ensuring compliance with medical ethics by healthcare staff.

The hospital management sets goals and priority tasks for socio-economic development for the planning period with specific performance indicators, namely:

1. **Population health improvement:** reducing infant mortality; preventing maternal mortality (target: zero maternal deaths); reducing the incidence of primary disability among the working-age population and children.
2. **Preventive work:** achieving 100% coverage of pregnant women with two ultrasound screenings before 22 weeks of gestation; preventing cardiovascular, cerebrovascular, oncological diseases, and tuberculosis.
3. **Accessibility and quality of medical care:** postoperative mortality for acute surgical conditions – 0; hospitalization rate – 158.9; bed utilization – 340.0.
4. **Strengthening the hospital's material and technical base:** carrying out current and major repairs of departments to improve patient conditions and service provision (e.g., renovation of the traumatology department).

Planned outpatient and polyclinic activities are preventive in nature. They focus on controlling the quality and effectiveness of monitoring patients under regular or first-time observation, early detection of oncological pathology during preventive examinations (primarily in early stages), organizing comprehensive screenings for specific population groups (agricultural workers, industrial employees in hazardous working conditions, medical staff, Chernobyl disaster cleanup participants, war-disabled persons, combatants, families of fallen soldiers, and schoolchildren), and screening for diabetes using

glycated hemoglobin and blood glucose tests in high-risk groups. Other priorities include early detection, diagnosis, and treatment of infectious diseases, timely reporting to the CDCP of emergency cases, and intervention in cases of patients violating isolation or epidemiological requirements, providing women of reproductive age with cancer screenings and cytological examinations, regular hospital infection prevention audits, and analysis of population mortality structures. A system of measures is being developed to reduce overall mortality among the working-age population.

In planning inpatient care activities, hospital administration follows principles of accessibility, quality, and rational use of bed capacity. Key directions and objectives include:

1. Maximizing the fulfillment of inpatient care needs for the district population, with priority given to specific groups (children, war veterans, designated groups, and monitored patients).
2. Implementing a differentiated approach in the reorganization of inpatient care.
3. Introducing new diagnostic and therapeutic methods and best practices.
4. Organizing sanitary and hygienic regimes.
5. Managing the diagnostic and therapeutic process in inpatient care.
6. Personnel management.
7. Ensuring medical security and patient care.

Planning is carried out with specific performance indicators to be achieved in each department, with designated executors and deadlines (Table 5).

In terms of staff numbers, the MNPE "Ostroh Multispecialty Hospital" is a major enterprise within the Ostroh territorial community. The staff structure is as follows: physicians – 17.6%; biologists and pharmacists – 1%; junior specialists with medical and pharmaceutical education – 40.5%; junior medical staff – 23.2%; non-medical personnel – 17.7% (Table 6).

In the organization of hospital operations, personnel management plays a pivotal role. The primary tool for managing staff is remuneration. Although the average salary at the hospital exceeds regional and national levels, the freedom for personnel to seek employment and better pay in other sectors or countries stimulates the mobility of medical staff both in the healthcare system at large and specifically within the MNPE. The base salary for physicians is 20,000 UAH, and for junior medical personnel – 13,500 UAH [14]. Young doctors are offered dormitory accommodation within the MNPE for full-time employment, with the possibility of receiving land from the city council for housing construction in the future.

The hospital faces a shortage of doctors, as only three-quarters of positions are filled according to the staffing schedule. Some physicians work part-time, while others exceed their workload, further exacerbating staffing issues. Of the total number of doctors, 40% are of retirement or senior retirement age. Personnel aged 70+ for men and 65+ for women account for one-fifth of the staff. This situation necessitates a revision of the institution's human resource policy.

The quality of medical care largely depends on the qualifications of the medical staff. In hospital planning and organization, numerous measures are undertaken to enhance professional training, recruitment, allocation, and utilization of medical personnel. These include participation in clinical-theoretical conferences, specialized seminars for clinic physicians, training programs for junior and mid-level medical staff, patient education through lectures in the inpatient department to promote sanitary and hygienic knowledge, and familiarization of staff with relevant directive documents. During operational meetings in hospital departments, performance indicators are analyzed, staff motivation issues are reviewed, and labor discipline is assessed.

Monitoring and control are implemented to ensure the effective organization and application of scientific achievements, new organizational work forms, scientific, informational, medical-statistical, innovative, and inventive activities; computerized processing of medical-statistical data; and participation of doctors, nurses, and junior medical personnel in educational and scientific events (conferences, seminars, and trainings). According to the established plan, physicians are responsible for public health education through local media, public speeches, and publications to support community health policy development.

Amid the ongoing war with Russia, the hospital adapts to wartime challenges, maintaining uninterrupted operations under limited resource conditions. Medical care is provided to affected individuals and temporarily displaced persons, with psychological support offered to both patients and staff.

On January 1, 2025, medium-term contracts were introduced with medical institutions, transitioning to agreements of up to three years for primary care, emergency care, cluster, and supra-cluster hospitals. This reform aimed to improve financial and operational planning and reduce administrative burdens associated with annual contract renewals. A new monitoring system was implemented to track contract compliance, enhancing data verification processes to improve transparency and the efficient use of budgetary funds [2].

CONCLUSIONS

The organization of secondary (specialized) healthcare facilities under the Medical Guarantees Program (MGP) is focused on strict adherence to program provisions and requirements, ensuring patient access to services guaranteed by the state. This is achieved through contracts with the National Health Service of Ukraine (NHSU), guaranteeing service accessibility for patients, compliance with NHSU-established tariffs and service packages, and maintaining electronic documentation in accordance with MGP requirements.

REFERENCES

1. Hushchuk I, Topishko N, Vivsiannyk O, Stratiuk O, Galetska T. The public health system as a fundamental component of the socio-economic system of the state. In Public Health System in Ukraine and EU Countries: Realities, Transformation, Development Vectors, Perspectives, scientific monograph. Riga, Latvia. Baltiia Publishing. 2024, pp. 2–25. http://baltijapublishing.lv/omp/index.php/bp/catalog/book/532
2. Hushchuk I, Topishko N, Galetskyi S, Dovhuny`k O. Development and financial support of public health programmes at the national and local (regional) levels: A programme-target approach. Public Health Journal. 2024;2(6):61–69. https://journals.ostroh-academy.rv.ua/index.php/publichealth/article/view/82
3. Kornilova OV. Legal regulation of secondary (specialized) medical care. 2019. https://www.vestnik-pravo.mgu.od.ua/archive/juspradenc38/29.pdf
4. National Health Service of Ukraine Report 2024. https://medplatforma.com.ua/images/96374/zvit_nszu_2024.pdf
5. Resolution of the Cabinet of Ministers of Ukraine № 150 dated December 24, 2024 "Certain issues of implementing the State Guarantees Program of medical care in 2025." https://www.kmu.gov.ua/npas/deiaki-pytannia-realizatsii-prohramy-derzhavnykh-garantii-medychnoho-obsluhovuvannia-naselennia-u-2025-rotsi-1503-241224
6. Melnyk A, Adamyk V. Analysis of organizational activity of healthcare institutions amid sectoral reform: theoretical, methodological, and applied aspects. Economic Analysis, 2024;34(1). doi:10.35774/econa2024.01.026. https://www.econa.org.ua/index.php/econa/article/view/5934 DOI
7. Shkilnyak MM, Zhelyuk TL. Organization of healthcare institution activities. Ternopil: Krok. 2021. 516 p.
8. Alkema VG, Sumen OM, Kyrychenko OS. Healthcare management.Krok. 2023. 255 p.
9. Shyshka IV. Analysis of the external environment of the healthcare system of Ukraine. 2023. http://www.psae-jrnl.nau.in.ua/journal/2_91_2023_ukr/27.pdf
10. MNC "Ostroh Multispecialty Hospital, Ostroh City Council." https://ostrog-hospital.itmed.org
11. Resolution of the Cabinet of Ministers of Ukraine № 410 dated 25.04.2018 "On contracts for the provision of medical services under the Medical Guarantees Program." https://zakon.rada.gov.ua/laws/show/410-2018-%D0%BF#Text
12. Resolution of the Cabinet of Ministers of Ukraine № 1464 dated 27.12.2022 "Certain issues of implementing the State Guarantees Program of medical care in 2023." https://document.vobu.ua/doc/16634
13. Guschuk I, Oleksiuk N. Overview of the state of the Ukrainian healthcare system since the start of the war. Zdrowie Publiczne i Zarządzanie. 2022;20 (1): 8-10. doi:10.4467/20842627OZ.22.003.1642
14. Resolution of the Cabinet of Ministers of Ukraine dated January 13, 2023 № 28 "Certain issues of remuneration of medical, pharmaceutical, and rehabilitation specialists of state and municipal healthcare institutions." https://www.kmu.gov.ua/npas/deiaki-pytannia-oplaty-pratsi-medychnykh-farmatsevtychnykh-pratsivnykiv-ta-fakhivtsiv-z-reabilitatsii-t130123

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Igor V. Hushchuk

Department of Public Health and Physical Education
National University of Ostroh Academy,
Ostroh, Ukraine
e-mail: igorgus2014@gmail.com

ORCID AND CONTRIBUTIONSHIP
Igor V. Hushchuk: 0000-0002-8075-9388 A E F
Vladyslav A. Smiianov: 0000-0002-4240-5968 E F
Natalya P. Topishko: 0000-0001-9823-0805 B D
Oleh M. Vivsyanyk: 0000-0003-2441-9992 E F
Vitalii I. Boiko: 0009-0005-8152-7254 B F
Sergii M. Galetskyi: 0000-0001-6532-3108 B F
Tetiana I. Galetska: 0000-0002-0795-008X B D

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REVIEW ARTICLE

CONTENTS

Current landscape of photosensitizers in photodynamic therapy: challenges and future perspectives – a literature review

Barbara Lipka[1], Michał Lipka[2], Mikołaj Rycerski[3], Małgorzata Toroń[1], Antoni Wołoszyn[1], Wojciech Tokarczyk[4], Jakub Mordarski[4]

[1]THE DOCTORAL SCHOOL OF THE MEDICAL UNIVERSITY OF SILESIA IN KATOWICE, MEDICAL UNIVERSITY OF SILESIA, KATOWICE, POLAND
[2]STUDENT SCIENTIFIC ASSOCIATION AT THE DEPARTMENT OF VASCULAR, GENERAL AND TRANSPLANT SURGERY, MEDICAL UNIVERSITY OF WROCLAW, WROCLAW, POLAND
[3]STUDENT SCIENTIFIC ASSOCIATION AT THE DEPARTMENT OF LUNG DISEASES AND TUBERCULOSIS, MEDICAL UNIVERSITY OF SILESIA, KATOWICE, POLAND
[4]PROFESSOR K. GIBIŃSKI UNIVERSITY CLINICAL CENTER OF THE SILESIAN MEDICAL UNIVERSITY IN KATOWICE, MEDICAL UNIVERSITY OF SILESIA, KATOWICE, POLAND

ABSTRACT

Aim: To provide an updated overview of photodynamic therapy (PDT), emphasizing the evolution of photosensitizers, their mechanisms of action, and current challenges and innovations aimed at improving therapeutic outcomes.

Materials and Methods: A structured literature review was conducted using PubMed, Scopus, and Embase databases to identify studies published between 2000 and 2025. Search terms included photodynamic therapy, photosensitizers, nanoparticles, reactive oxygen species, and drug delivery systems. Experimental studies, clinical trials, and review articles focused on photosensitizer development and applications were analyzed.

Photosensitizers have advanced through three generations. First-generation agents such as Photofrin demonstrated clinical success but were hindered by shallow tissue penetration and prolonged photosensitivity. Second-generation compounds achieved stronger absorption within the therapeutic window (650-800 nm) and higher singlet oxygen yields but encountered solubility and delivery limitations. Third-generation systems integrate targeting ligands or nanocarriers, improving selectivity, bioavailability, and pharmacokinetics. Despite these advances, PDT remains limited by insufficient light penetration, tumor hypoxia, and non-specific toxicity, and is therefore used only in select clinical settings. Recent approaches, such as multifunctional theranostic photosensitizers, gene-encoded PSs, and combination therapies with immunotherapy or chemotherapy, aim to overcome these barriers.

Conclusions: Continued innovation in photosensitizer chemistry, nanotechnology-based delivery, and combination strategies promises to enhance PDT's selectivity, depth, and clinical effectiveness across oncology, dermatology, and infectious disease treatment.

KEY WORDS: photodynamic therapy, nanoparticles, reactive oxygen species, drug delivery systems, photosensitizing agents

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INTRODUCTION

Photodynamic therapy (PDT) is an advanced therapeutic approach that combines three essential components: a photosensitizer (PS), light of a specific wavelength, and molecular oxygen present in tissue [1-2]. The process begins with administration of the photosensitizer, which selectively accumulates in target tissues such as tumors or infected areas. Upon illumination with light of an appropriate wavelength, often in the red spectrum to ensure tissue penetration-the photosensitizer absorbs photons and is excited from its ground singlet state to an unstable excited singlet state [3-5]. This excited state quickly undergoes intersystem crossing to a more stable, longer-lived triplet state, which can then transfer energy directly to molecular oxygen to generate reactive oxygen species (ROS), particularly highly cytotoxic singlet oxygen [6-7]. These reactive molecules induce oxidative damage leading to cell membrane disruption, mitochondrial damage, and DNA damage, which ultimately trigger programmed cell death (apoptosis) or necrosis of the targeted cells. Besides direct cytotoxicity, PDT also disrupts tumor vasculature, causing ischemia, and stimulates an immune response that can further assist in destroying abnormal tissues. This multimechanistic action underpins PDT's clinical relevance across a range of applications including oncology, dermatology, and infectious diseases [7-10].

The role of photosensitizers in PDT is central; they must have selective accumulation in diseased tissues, low dark toxicity, and high phototoxicity upon light activation. Different generations of photosensitizers have been developed over the years to improve therapeutic outcomes. First-generation photosensitizers, such as porfimer sodium, showed efficacy but suffered from limited tissue penetration, prolonged photosensitivity, and less specific targeting. Second-generation PSs were designed to have improved absorption properties and shorter skin photosensitivity duration [11-12]. Third-generation photosensitizers enhance targeting further by conjugation with biomolecules (e.g., antibodies or peptides) or encapsulation in nanoparticles to achieve higher selectivity, deeper tissue penetration, and better pharmacokinetics, reducing off-target effects. Understanding these advancements in photosensitizer technology is crucial for clinicians and researchers to optimize PDT protocols.

AIM

The aim of this review is to provide a comprehensive overview of the photodynamic mechanism, emphasizing the critical role and evolution of photosensitizers, the underlying biochemical reactions leading to cytotoxicity, and their clinical applications. It also discusses the advantages and limitations of current photosensitizer generations and outlines future trends and innovations to enhance the specificity and efficacy of PDT, thereby broadening its therapeutic scope in oncology and beyond.

MATERIALS AND METHODS

A structured literature search was conducted using the PubMed, Scopus, and Embase databases to identify peer-reviewed studies published between January 2000 and April 2025. The search strategy included combinations of keywords and MeSH terms such as "photodynamic therapy", "photosensitizing agents", "photosensitizers", "nanoparticles", "drug delivery systems", "reactive oxygen species", "porphyrins", "chlorins", "phthalocyanines", and "bacteriochlorins". Inclusion criteria comprised experimental studies, clinical trials, systematic reviews, and narrative reviews focusing on the development, mechanisms, and clinical applications of photosensitizers in PDT. Articles without English full-text availability or those unrelated to therapeutic PDT applications were excluded. Reference lists of relevant papers were screened manually to identify additional sources. A total of 39 publications meeting the inclusion criteria were selected and analyzed. Data synthesis followed a narrative review methodology, emphasizing the evolution of photosensitizer generations, classification by chemical structure, advances in nanocarrier-based delivery systems, and future research directions aimed at improving PDT efficacy and clinical translation.

REVIEW AND DISCUSSION

MECHANISM OF PDT AND ROLE OF PHOTOSENSITIZERS

The basic photochemical mechanism of PDT involves two primary pathways of ROS generation known as Type I and Type II photoreactions (Figure 1). Upon light activation, the PS transitions to an excited triplet state and can interact with nearby molecules via these two pathways. In the Type I mechanism, the excited PS undergoes electron or hydrogen atom transfer reactions with substrate molecules, leading to the formation of radical species and radical ions such as superoxide anion ($O_2^{\bullet -}$), hydroxyl radicals (HO^-), and hydrogen peroxide (H_2O_2) [13-14]. These reactive radicals cause oxidative damage leading to cell death. In contrast, the Type II mechanism involves direct energy transfer from the excited triplet PS to ground-state molecular oxygen ($^3O_2$), producing highly cytotoxic singlet oxygen ($^1O_2$). Singlet oxygen is considered the main cytotoxic agent in PDT as it rapidly reacts with cellular components like lipids, proteins, and nucleic acids within a very short diffusion distance, inducing cell damage and death. Both pathways can occur simultaneously, but PDT efficacy often relies primarily on the Type II pathway, especially under normoxic conditions [15]. However, Type I pathways can dominate in hypoxic environments, relevant in many tumor tissues, supporting PDT efficacy despite limited oxygen availability.

Key characteristics of an ideal photosensitizer for effective PDT include selective accumulation within target tissues to minimize damage to healthy cells and high quantum yield of singlet oxygen generation to maximize therapeutic effect. Photostability is essential to withstand repeated light exposure without degradation, ensuring consistent ROS formation. Low dark toxicity ensures that the PS is non-toxic in the absence of light, enhancing safety. Additionally, an ideal photosensitizer should absorb light strongly within the therapeutic window of absorption of light at red or near infrared wavelengths 650-800 nm, where tissue penetration of light is optimal, allowing treatment of deeper lesions. These properties collectively enable targeted, efficient, and safe photodynamic treatment. Understanding these mechanistic and molecular

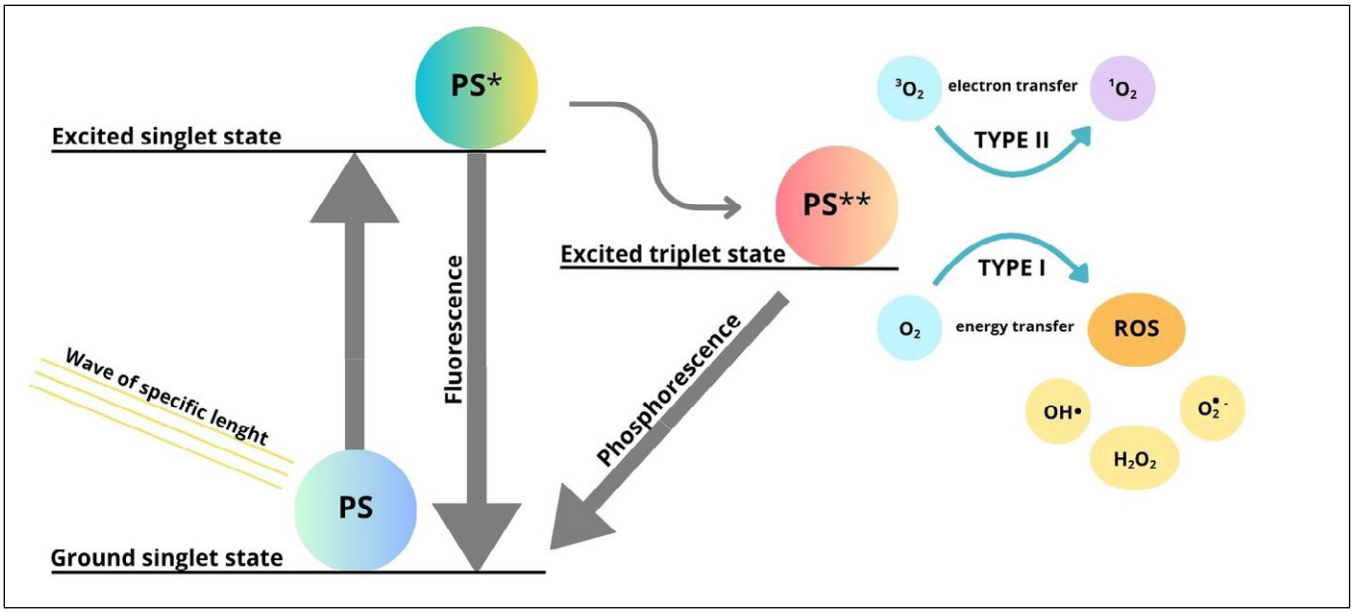

Fig. 1. Photodynamic reaction. PS – photosensitizer
Source: Authors' own work

Table 1. Examples of photosensitizers based on generations.

Photosensitizer generation	Examples
1st generation	• Hematoporphyrin (Hp); • Hematoporphyrin derivative Photofrin® (HpD).
2nd generation	• Phorphyrins: benzoporphyrin derivatives, texapyrin, 5-aminolevulinic acid (5-ALA); • Chlorins: talaporfin sodium, temoporfin - Foscan®; • Phthalocyanines; • Thiopurine derivatives; • Bacteriochlorin analogues.
3rd generation	1st or 2nd generation photosensitizer: • combined with molecules targeting specific cancer cell receptors; • combined with LDL lipoproteins; • conjugated with monoclonal antibodies directed against specific antigen or tumour surface markers (e.g. growth factor receptors, transferrin).

Source: Authors' own work

considerations guides the design and application of photosensitizers to improve PDT outcomes in clinical settings [16-20].

CLASSIFICATION OF PHOTOSENSITIZERS

BASED ON GENERATIONS

PSs used in PDT are classified into three main generations based on their chemical properties, clinical history, and advancements in targeted delivery technologies (Table 1). The 1st generation photosensitizers were naturally occurring porphyrins, such as hematoporphyrin (Hp) and its derivative Photofrin® (HpD), a water-soluble mixture of porphyrin dimers and oligomers, also known as sodium porfimer. HpD and its commercial product Photofrin® was first introduced for clinical use on a commercial scale in the 1970s by Dougherty et al., and remains one of commonly used PS [7, 21]. They offer the advantage of well-documented safety profiles and established clinical protocols; however, their use is limited by prolonged skin photosensitivity resulting from high skin accumulation and the long half-life of the photosensitizer, as well as by suboptimal tissue penetration due to absorption at shorter light wavelengths (~630 nm) and non-specific accumulation in both healthy and diseased tissues [20, 22-24]. These limitations often lead to side effects and restrict treatment depth. Consequently, as early as the 1980s, studies on the next generation of photosensitizers were initiated.

2nd generation photosensitizers include structurally well-defined compounds such as porphyrins (e.g., benzoporphyrin derivatives, texaphyrin, and 5-ami-

nolevulinic acid); chlorins (e.g., talaporfin sodium and temoporfin); phthalocyanines; thiopurine derivatives; and bacteriochlorin analogues [7, 21]. These agents have enhanced photophysical properties, including stronger absorption peaks in the near-infrared region (around 650-800 nm), which allows deeper tissue penetration due to reduced light scattering and absorption by endogenous chromophores [24]. Additionally, they exhibit higher singlet oxygen quantum yields, which improve therapeutic efficacy, and demonstrate fewer side effects due to greater selectivity for cancerous tissues and faster elimination from the body [20]. 5-aminolevulinic acid (5-ALA) is currently one of the most commonly used photosensitizers. It is a prodrug that is endogenously converted into protoporphyrin IX (PpIX), which becomes activated upon stimulation with light at a wavelength of 630-650 nm [21, 25]. PpIX accumulates mainly in mucous membranes, enabling selective destruction of epithelial cells while sparing deeper tissues. Owing to its oral or topical administration and rapid clearance from healthy tissues, 5-ALA minimizes photosensitivity and is widely used in clinical practice [23, 26]. However, many second-generation PSs are hydrophobic, complicating their solubility and systemic delivery, which presents challenges for clinical translation and forces the search for new methods of drug delivery [20, 27].

3rd generation photosensitizers are characterized by enhanced selectivity for cancer cells while minimizing accumulation in healthy tissues, thereby reducing collateral damage. They are build upon prior generations by incorporating targeting moieties such as antibodies, peptides, or ligands that enable selective binding to specific cellular markers expressed preferentially on tumor or pathogenic cells. This generation also utilizes advanced drug delivery systems including nanocarriers like liposomes, micelles, dendrimers, and nanoparticle conjugates to enhance PS solubility, stability, and selective accumulation within diseased tissues. These strategies markedly enhance tumor selectivity, minimize off-target effects, and reduce systemic toxicity [15-24]. Examples include photosensitizer conjugates with monoclonal antibodies targeting specific antigens or tumor surface markers, complexes with molecules that recognize cancer cell receptors, low-density lipoproteins (LDL), or nanoparticle-based delivery systems, all representing the forefront of precision photodynamic therapy (PDT) technologies. This classification highlights the evolution of photosensitizer development aimed at overcoming the limitations of earlier generations by improving selectivity, tissue penetration, and overall therapeutic index in clinical applications [21, 27, 28].

BASED ON CHEMICAL STRUCTURE

Porphyrins and their derivatives are a fundamental class of photosensitizers in PDT, characterized by a tetrapyrrolic macrocycle structure capable of efficient light absorption and reactive ROS generation [20, 24, 29]. Clinically, porphyrins like Photofrin and hematoporphyrin derivatives (HpD) have been extensively used for treating various cancers, including those of the lung, esophagus, skin, and brain. Their mechanism involves selective uptake by tumor cells and vascular tissues, followed by light activation inducing oxidative damage leading to tumor cell death. Case studies have shown effectiveness in improving prognosis in cerebral gliomas and various carcinomas, although challenges such as prolonged skin photosensitivity and limited light penetration necessitate advancements in PS development [22-24].

Chlorins, such as talaporfin sodium, represent a second-generation photosensitizer subclass with enhanced absorption properties in the near-infrared region, around 660-700 nm, which allows for deeper tissue penetration compared to porphyrins. Talaporfin has been used effectively in clinical settings, particularly for lung cancer and brain tumors, due to its favorable photophysical and pharmacokinetic profile [23, 24].

Phthalocyanines exhibit strong absorption in the 670-700 nm range and are promising for deeper tissue treatment due to this property. Their chemical stability and high singlet oxygen quantum yield contribute to their effectiveness in PDT, although their hydrophobic nature often requires delivery strategies to improve solubility and targeting [20, 24, 29].

Bacteriochlorins absorb even further into the near-infrared, enabling deeper tissue penetration, which is advantageous for treating larger or more deeply situated tumors. Their structural modifications have enhanced their photostability and ROS generation capacity [24, 30].

Natural product-based photosensitizers, such as hypericin and curcumin, offer biocompatibility and exhibit photodynamic activity, with ongoing research exploring their therapeutic potential and combinational uses [3, 8, 10, 31].

Novel synthetic photosensitizers, including aza-BODIPY derivatives, are designed to combine high photostability, targeted delivery, and absorption in the therapeutic window, aiming to overcome limitations of earlier generations and expand the clinical utility of PDT through tailored chemical and photophysical properties. This diverse range of photosensitizers reflects the evolving landscape of PDT, where structural modifications and delivery technologies continue to enhance efficacy and safety profiles across various clinical applications [11, 20, 23, 24].

DELIVERY STRATEGIES FOR PHOTOSENSITIZERS

Nanoparticle-based systems have emerged as revolutionary carriers for PS in PDT, addressing challenges associated with PS solubility, stability, and selective delivery [11, 22]. Common nanocarriers include liposomes, polymeric nanoparticles, and gold nanoparticles, each offering unique advantages. Liposomes enhance PS bioavailability and reduce systemic toxicity by encapsulating hydrophobic PSs in a biocompatible lipid bilayer. Polymeric nanoparticles provide controlled release and surface functionalization possibilities, improving pharmacokinetics [20, 22, 23]. Gold nanoparticles offer excellent biocompatibility, surface plasmon resonance properties for enhanced photodynamic effects, and facile conjugation with targeting molecules. Targeted delivery is achieved by functionalizing nanoparticles with ligands or antibodies that specifically recognize tumour biomarkers or microenvironment features, enabling selective accumulation in diseased tissues while sparing healthy cells [23]. This active targeting significantly improves therapeutic efficacy and reduces side effects compared to conventional PS administration. Stimuli-responsive systems are advanced nanoparticle platforms designed to release or activate PSs in response to specific physiological triggers such as acidic pH, redox conditions, or enzyme presence in the tumour microenvironment [11, 18]. This on-demand release enhances spatial and temporal control over PDT, further minimizing off-target effects. Despite these advantages, nanoparticle systems face challenges related to pharmacokinetics and biodistribution, including potential reticuloendothelial system clearance, non-specific uptake by healthy organs, and complex in vivo behaviour that complicates dose optimization [20, 22]. Overcoming these hurdles requires careful design of nanoparticle size, surface charge, and coating to maximize circulation time and tumour accumulation. Overall, nanoparticle-based delivery systems represent a promising frontier in PDT by improving photosensitizer performance, targeting precision, and therapeutic outcomes while addressing inherent limitations of traditional photosensitizer formulations.

CLINICAL INDICATIONS AND BARRIERS TO BROADER IMPLEMENTATION

Although photodynamic therapy is widely recognized in dermatology, its clinical utility extends well beyond cutaneous applications and includes several oncological indications in which PDT has been incorporated into treatment algorithms or acknowledged in clinical recommendations. According to contemporary practice guidelines and expert panels, PDT is used in selected cases of early central lung cancer, superficial esophageal neoplasia, and high-grade gliomas, where the tumor is anatomically accessible and light delivery is feasible [20, 22, 24, 31]. In early central non-small cell lung cancer limited to the bronchial mucosa, Photofrin®-based PDT is an accepted option for patients unsuited to surgery or radiotherapy, and may be used as stand-alone therapy or palliatively to restore airway patency [22, 24]. In esophageal high-grade dysplasia and early superficial cancer, PDT provides local eradication when endoscopic resection is incomplete or contraindicated, functioning primarily as monotherapy in mucosal disease [32, 33]. In neuro-oncology, PDT serves as an adjuvant intraoperative modality: 5-ALA enables fluorescence-guided resection with subsequent photoactivation, while talaporfin sodium PDT is approved in Japan for recurrent gliomas, improving local control in selected patients [7, 23]. Across these clinical settings, PDT offers organ preservation, repeatability, and minimal systemic toxicity, making it a meaningful therapeutic tool when conventional options are limited or contraindicated.

Despite its therapeutic potential, PDT is not widely available in routine oncological practice, even in healthcare systems that could theoretically support its cost. Several barriers limit broader implementation. First, PDT requires specialized equipment, including lasers with specific emission wavelengths and dedicated light-delivery systems such as bronchoscopic or neurosurgical fibers, which increases infrastructure requirements [31, 34]. Second, expertise is center-dependent, as successful PDT relies on precise dosimetry, timing between photosensitizer administration and illumination, and operator experience. Third, the limited penetration depth of light (1–2 cm) restricts its use to tumors located near epithelial surfaces or accessible endoscopically, making many deep-seated lesions unsuitable for treatment [22, 24, 35]. Additionally, tumor hypoxia, common in advanced malignancies, reduces ROS generation and may diminish treatment efficacy, creating another physiological limitation [19, 22]. These factors explain why PDT, although promising, remains reserved for specific clinical scenarios rather than being a universally applied oncologic modality.

ADVERSE EFFECTS AND SAFETY PROFILE

Photodynamic therapy (PDT) is generally considered safe, with adverse effects dependent on the photosensitizer, anatomical site, and illumination parameters. The most frequent systemic reaction is photosensitivity, particularly after first-generation porphyrins such as Photofrin®, which may require prolonged light avoidance

due to slow drug clearance [20, 23, 24]. Newer agents, including 5-ALA, temoporfin, and talaporfin sodium, demonstrate faster elimination and a markedly lower risk of prolonged phototoxicity [21, 25, 26].

Local reactions vary by treatment site: airway PDT may cause transient edema, dyspnea, cough, or necrotic debris retention [22, 24]; esophageal PDT can induce short-lasting odynophagia, chest discomfort, or mucosal ulceration, while strictures remain uncommon with proper dosimetry [32, 33]. In neurosurgical applications, 5-ALA and talaporfin sodium may lead to localized edema or transient neurological worsening, though serious events are rare when illumination is correctly targeted [7, 23].

Pain during or shortly after illumination is common due to ROS-mediated inflammatory responses [15, 20]. Mild erythema, swelling, or superficial necrosis may also occur at the illumination site. Importantly, PDT causes minimal systemic toxicity because its effects are restricted to light-exposed tissues, distinguishing it from chemotherapy and radiotherapy [20, 23, 24, 31].

Overall, PDT has a favorable safety profile, and its adverse effects are typically predictable, self-limiting, and manageable. These considerations guide patient selection and partly explain the selective, rather than widespread, use of PDT across medical fields.

CHALLENGES AND LIMITATIONS

Photodynamic therapy faces several notable challenges and limitations that impact its clinical effectiveness. A primary limitation is the restricted light penetration depth in tissues; visible and near-infrared light used to activate photosensitizers generally penetrates only a few millimetres to about 1-2 cm, depending on tissue type and wavelength [22]. This restricts PDT efficacy in treating deep-seated tumours, as deeper tissues receive insufficient light energy for effective photosensitizer activation [20, 22-24, 35]. Although longer wavelengths near the therapeutic window (650–800 nm) improve penetration, scattering and absorption by endogenous chromophores still limit treatment depth [22]. Tumour hypoxia poses another significant challenge because PDT relies on molecular oxygen to create reactive ROS that mediate cytotoxicity. Hypoxic tumour microenvironments reduce ROS generation, diminishing PDT efficacy, especially in aggressive or late-stage tumours with poor vascularization [19]. Strategies to overcome this include oxygen delivery systems and combining PDT with therapies altering tumor oxygenation [18, 22]. Off-target effects and phototoxicity result from non-specific photosensitizer accumulation in healthy tissues, leading to undesired damage upon light exposure. Patients often require strict light avoidance post-treatment to prevent skin photosensitivity and related adverse effects, impacting quality of life [20, 23]. Finally, cost and scalability issues arise with novel photosensitizers and advanced delivery systems such as nanoparticles. Manufacturing complexity, regulatory hurdles, and high production costs can limit widespread adoption and accessibility in clinical practice [20, 22-24, 29]. Addressing these challenges requires continued innovation in photosensitizer design, light delivery technologies, and combinational therapeutic approaches to enhance PDT specificity, depth, and overall therapeutic index.

FUTURE PERSPECTIVES

Future perspectives in photodynamic therapy focus on developing multifunctional photosensitizers known as theranostic agents, which combine therapeutic and diagnostic capabilities for improved treatment monitoring and personalization [11, 22, 34]. These advanced PSs can simultaneously enable imaging and targeted therapy, enhancing precision in treatment delivery [32]. Combination therapies are also a major frontier. PDT is increasingly integrated with immunotherapy to boost antitumor immune responses by inducing immunogenic cell death and modifying the tumor microenvironment to a more immune-active state [20, 34]. Clinical and preclinical studies show synergistic effects when PDT is combined with immune checkpoint inhibitors, adoptive cell therapies, or immunoadjuvants [33]. Furthermore, PDT combined with chemotherapy or photothermal therapy augments efficacy by attacking tumors through complementary mechanisms, addressing limitations like hypoxia or resistance [36]. Advances in targeted nanotechnology enable precise delivery of PSs and combination agents through stimuli-responsive, ligand-targeted, or biomimetic nanoparticles, offering spatiotemporal control over therapy [11, 18, 23]. Gene-encoded photosensitizers represent a novel approach whereby PSs are produced intracellularly, potentially allowing for highly specific and controlled PDT in targeted cells [37, 38]. Personalized PDT approaches are envisioned through integrating patient-specific tumor biology, molecular imaging, and real-time dosimetry to tailor PS selection, light dose, and combinatorial regimens for maximal efficacy with minimal side effects [11, 22]. Collectively, these developments aim to overcome current PDT limitations and broaden its therapeutic impact by combining modality synergies, enhancing targeting precision, and individualizing treatment paradigms for diverse cancers and other diseases [39].

CONCLUSIONS

In conclusion, photodynamic therapy (PDT) represents a highly versatile and evolving modality that leverages the unique properties of photosensitizers to selectively target diseased tissues through light-activated cytotoxicity mediated by reactive oxygen species. The continuous development from first- to third-generation photosensitizers has markedly enhanced selectivity, tissue penetration, and therapeutic efficacy while reducing adverse effects such as prolonged photosensitivity and off-target toxicity. Advances in nanoparticle-based delivery systems and targeting strategies further optimize PS bioavailability and tumor specificity, overcoming key pharmacokinetic challenges. Despite inherent limitations like light penetration depth and tumor hypoxia, ongoing innovations in multifunctional theranostic agents, combination therapies integrating immunotherapy or chemotherapy, and personalized dosimetry herald a new era of precise, effective PDT treatments. The potential of third-generation photosensitizers and emerging nanotechnologies is particularly promising for expanding PDT's clinical applicability across oncology and beyond, offering hope for improved patient outcomes with minimized side effects. Continued interdisciplinary research and clinical translation are essential to fully harness PDT's capabilities and address current challenges in the rapidly advancing landscape of photodynamic therapy.

REFERENCES

1. Correia JH, Rodrigues JA, Pimenta S, Dong T, Yang Z. Photodynamic therapy review: principles, photosensitizers, applications, and future directions. Pharmaceutics. 2021;13(9):1332. doi:10.3390/pharmaceutics13091332. DOI
2. Alvarez N, Sevilla A. Current advances in photodynamic therapy (PDT) and the future potential of PDT-combinatorial cancer therapies. Int J Mol Sci. 2024;25(2):1023. doi:10.3390/ijms25021023 DOI
3. Łopaciński M, Fiegler-Rudol J, Niemczyk W, Skaba D, Wiench R. Riboflavin- and hypericin-mediated antimicrobial photodynamic therapy as alternative treatments for oral candidiasis: a systematic review. Pharmaceutics. 2024;17(1):33. doi:10.3390/pharmaceutics17010033. DOI
4. Jiang W, Liang M, Lei Q, Li G, Wu S. The current status of photodynamic therapy in cancer treatment. Cancers (Basel). 2023;15(3):585. doi:10.3390/cancers15030585. DOI
5. Ni J, Wang Y, Zhang H, Sun JZ, Tang BZ. Aggregation-induced generation of reactive oxygen species: mechanism and photosensitizer construction. Molecules. 2021;26(2):268. doi:10.3390/molecules26020268. DOI
6. Wiench R, Fiegler-Rudol J, Zięba N, Misiołek M. Laser interventions for intraoral halitosis: a systematic review of randomized controlled trials. Pharmaceutics. 2025;17(8):1046. doi:10.3390/pharmaceutics17081046. DOI
7. Tetard MC, Vermandel M, Mordon S, Lejeune JP, Reyns N. Experimental use of photodynamic therapy in high-grade gliomas: a review focused on 5-aminolevulinic acid. Photodiagn Photodyn Ther. 2014;11(3):319-30. doi:10.1016/j.pdpdt.2014.04.004. DOI
8. Przygoda M, Bartusik-Aebisher D, Dynarowicz K, Cieślar G, Kawczyk-Krupka A, Aebisher D. Cellular mechanisms of singlet oxygen in photodynamic therapy. Int J Mol Sci. 2023;24(23):16890. doi:10.3390/ijms242316890 DOI
9. Wiench R, Fiegler-Rudol J, Latusek K, Brus-Sawczuk K, Fiegler H, Kasperski J, Skaba D. Indocyanine green as a photosensitizer in periodontitis treatment: a systematic review of randomized controlled trials. Life (Basel). 2025;15(7):1015. doi:10.3390/life15071015. DOI
10. Kubizna M, Fiegler-Rudol J, Niemczyk W, Wiench R. The efficacy of curcumin-mediated photodynamic therapy in the treatment of oral squamous cell carcinoma: a systematic review of in vitro studies. Life (Basel). 2025;15(6):924. doi:10.3390/life15060924. DOI
11. Maharjan PS, Bhattarai HK. Singlet oxygen, photodynamic therapy, and mechanisms of cancer cell death. J Oncol. 2022;2022:7211485. doi:10.1155/2022/7211485. DOI
12. Fiegler-Rudol J, Zięba N, Turski R, Misiołek M, Wiench R. Hypericin-mediated photodynamic therapy for head and neck cancers: a systematic review. Biomedicines. 2025;13(1):181. doi:10.3390/biomedicines13010181. DOI
13. Castano AP, Demidova TN, Hamblin MR. Mechanisms in photodynamic therapy: part one-photosensitizers, photochemistry and cellular localization. Photodiagn Photodyn Ther. 2004;1(4):279-93. doi:10.1016/S1572-1000(05)00007-4. DOI
14. Fiegler-Rudol J, Kapłon K, Kotucha K, Moś M, Skaba D, Kawczyk-Krupka A, Wiench R. Hypocrellin-mediated photodynamic therapy: a systematic review of its efficacy, applications, and outcomes. Int J Mol Sci. 2025;26(9):4038. doi:10.3390/ijms26094038. DOI
15. Werłos M, Barzowska-Gogola A, Pucelik B, Repetowski P, Warszyńska M, Dąbrowski JM. One change, many benefits: a glycine-modified bacteriochlorin with NIR absorption and a type I photochemical mechanism for versatile photodynamic therapy. Int J Mol Sci. 2024;25(23):13132. doi:10.3390/ijms252313132. DOI
16. Jomova K, Raptova R, Alomar SY, et al. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. Arch Toxicol. 2023;97(10):2499–2574. doi:10.1007/s00204-023-03562-9. DOI
17. Warakomska A, Fiegler-Rudol J, Kubizna M, Skaba D, Wiench R. The role of photodynamic therapy mediated by natural photosensitizers in the management of peri-implantitis: a systematic review. Pharmaceutics. 2025;17(4):443. doi:10.3390/pharmaceutics17040443. DOI

18. Kruczek-Kazibudzka A, Lipka B, Fiegler-Rudol J, Tkaczyk M, Skaba D, Wiench R. Toluidine blue and chlorin e6-mediated photodynamic therapy in the treatment of oral potentially malignant disorders: a systematic review. Int J Mol Sci. 2025;26(6):2528. doi:10.3390/ijms26062528. DOI
19. Tkaczyk M, Mertas A, Kuśka-Kiełbratowska A, et al. In vitro evaluation of Candida spp. and Staphylococcus aureus sensitivity to 450 nm diode laser-mediated antimicrobial photodynamic therapy with curcumin and riboflavin. Int J Mol Sci. 2025;26(12):5645. doi:10.3390/ijms26125645. DOI
20. Wiench R, Fiegler-Rudol J, Grzech-Leśniak K, Skaba D, Arnabat-Dominguez J. Photodithazine-mediated antimicrobial photodynamic therapy: a systematic review of efficacy and applications. Int J Mol Sci. 2025;26(16):8049. doi:10.3390/ijms26168049. DOI
21. Kwiatkowski S, Knap B, Przystupski D, Saczko J, et al. Photodynamic therapy-mechanisms, photosensitizers and combinations. Biomed Pharmacother. 2018;106:1098-107. doi:10.1016/j.biopha.2018.07.049 DOI
22. Du Y, Han J, Jin F, Du Y. Recent strategies to address hypoxic tumor environments in photodynamic therapy. Pharmaceutics. 2022;14(9):1763. doi:10.3390/pharmaceutics14091763. DOI
23. Shinoda Y, Kato D, Ando R, Endo H, Takahashi T, Tsuneoka Y, Fujiwara Y. Systematic review and meta-analysis of in vitro anti-human cancer experiments investigating the use of 5-aminolevulinic acid (5-ALA) for photodynamic therapy. Pharmaceuticals (Basel). 2021;14(3):229. doi:10.3390/ph14030229. DOI
24. Fiegler-Rudol J, Lipka B, Kapłon K, Moś M, Skaba D, Kawczyk-Krupka A, Wiench R. Evaluating the efficacy of rose bengal as a photosensitizer in antimicrobial photodynamic therapy against Candida albicans: a systematic review. Int J Mol Sci. 2025;26(11):5034. doi:10.3390/ijms26115034. DOI
25. Aebisher D, Rogóż K, Myśliwiec A, Dynarowicz K, Wiench R, Cieślar G, Kawczyk-Krupka A, Bartusik-Aebisher D. The use of photodynamic therapy in medical practice. Front Oncol. 2024;14:1373263. doi:10.3389/fonc.2024.1373263. DOI
26. Morton C. The emerging role of 5-ALA-PDT in dermatology: is PDT superior to standard treatments? J Dermatol Treat. 2002;13(Suppl 1):s25-s29. doi:10.1080/095466302317414672. DOI
27. Josefsen LB, Boyle RW. Photodynamic therapy: novel third-generation photosensitizers one step closer? Br J Pharmacol. 2008;154(1):1-3. doi:10.1038/bjp.2008.98. DOI
28. Liu Z, Zou H, Zhao Z, Zhang P, Shan GG, Kwok RTK, et al. Tuning organelle specificity and photodynamic therapy efficiency by molecular function design. ACS Nano. 2019;13(9):11283-93. doi:10.1021/acsnano.9b04430. DOI
29. Aebisher D, Serafin I, Batóg-Szczęch K, et al. Photodynamic therapy in the treatment of cancer-the selection of synthetic photosensitizers. Pharmaceuticals (Basel). 2024;17(7):932. doi:10.3390/ph17070932. DOI
30. Hayata Y, Kato H, Okitsu H, Kawaguchi M, Konaka C. Photodynamic therapy with hematoporphyrin derivative in cancer of the upper gastrointestinal tract. Semin Surg Oncol. 1985;1(1):1-11. doi:10.1002/ssu.2980010103 DOI
31. Fiegler-Rudol J, Łopaciński M, Los A, Skaba D, Wiench R. Riboflavin-mediated photodynamic therapy in periodontology: a systematic review of applications and outcomes. Pharmaceutics. 2025;17(2):217. doi:10.3390/pharmaceutics17020217. DOI
32. Czarnecka-Czapczyńska M, Czuba Z, Aebisher D, Mytych W, Fiegler-Rudol J, Wiench R, Kawczyk-Krupka A. Hypericin photodynamic therapy induces cytotoxicity and modulates cytokine secretion in MCF-7 breast cancer cells. J Clin Med. 2025;14(21):7514. doi:10.3390/jcm14217514. DOI
33. Aires-Fernandes M, Botelho Costa R, Rochetti do Amaral S, Mussagy CU, Santos-Ebinuma VC, Primo FL. Development of biotechnological photosensitizers for photodynamic therapy: cancer research and treatment from benchtop to clinical practice. Molecules. 2022;27(20):6848. doi:10.3390/molecules27206848 DOI
34. Yoon I, Li JZ, Shim YK. Advances in photosensitizers and light delivery for photodynamic therapy. Clin Endosc. 2013;46(1):7-23. doi:10.5946/ce.2013.46.1.7 DOI
35. Huang Z. A review of progress in clinical photodynamic therapy. Technol Cancer Res Treat. 2005;4(3):283-93. doi:10.1177/153303460500400308. DOI
36. Kou J, Dou D, Yang L. Porphyrin photosensitizers in photodynamic therapy and its applications. Oncotarget. 2017;8(46):81591-603. doi:10.18632/oncotarget.20189 DOI
37. Allison RR, Sibata CH. Oncologic photodynamic therapy photosensitizers: a clinical review. Photodiagn Photodyn Ther. 2010;7(2):61-75. doi:10.1016/j.pdpdt.2010.02.001. DOI
38. Fang S, Zhou Z, Wu Y, Zheng Z, Zhao Y, Sun X, et al. Photodynamic therapy combined with carbon dioxide laser for successful treatment of cutaneous squamous cell carcinoma within a long-standing and huge seborrheic keratosis. Photodiagn Photodyn Ther. 2021;36:102536. doi:10.1016/j.pdpdt.2021.102536. DOI
39. Wu H, Minamide T, Yano T. Role of photodynamic therapy in the treatment of esophageal cancer. Dig Endosc. 2019;31(5):508-16. doi:10.1111/den.13353. DOI

CONFLICT OF INTEREST
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Mikołaj Rycerski
Student Scientific Association at the Department
of Lung Diseases and Tuberculosis,
Medical University of Silesia,
Katowice, Poland
email: miko.ryc99@gmail.com

ORCID AND CONTRIBUTIONSHIP
Barbara Lipka: 0009-0009-8049-0831 A D E F
Michał Lipka: 0009-0001-8597-5688 B D
Mikołaj Rycerski: 0009-0009-4825-9330 D E
Małgorzata Toroń: 0009-0006-8197-2232 D E
Antoni Wołoszyn: 0009-0009-0145-266X B D
Wojciech Tokarczyk: 0009-0000-8905-6833 B D
Jakub Mordarski: 0009-0001-9501-3204 B D

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REVIEW ARTICLE

CONTENTS

The right to psychiatric assistance in places of deprivation of liberty: european standards

Olga Tyshchenko[1], Andrii Hnatiuk[2], Inna Bespalko[1]
[1]YAROSLAV MUDRYI NATIONAL LAW UNIVERSITY, KHARKIV, UKRAINE
[2]POLTAVA LAW INSTITUTE OF YAROSLAV MUDRYI NATIONAL LAW UNIVERSITY, POLTAVA, UKRAINE

ABSTRACT

Aim: The article aims to deepen the scientific discussion on ensuring the right to psychiatric assistance in places of deprivation of liberty.

Materials and Methods: In preparing the article, the following were examined: the provisions of international legal instruments regulating adequate treatment of persons suffering from mental disorders; relevant legal positions of the European Court of Human Rights in this regard; and scholarly research within the outlined vector of scientific inquiry. The methodological basis of the study consists of dialectical, comparative-legal, systemic-structural, analytical, synthetic, and comprehensive methods.

Conclusions: The current case law of the European Court of Human Rights indicates that subparagraph "e" of paragraph 1 of Article 5 of the Convention for the Protection of Human Rights and Fundamental Freedoms (lawful detention of persons of unsound mind) serves two protective functions: a social one (ensuring the protection of society) and a therapeutic one (providing the person with adequate treatment while in detention). The comprehensive concept of "adequate treatment of a person suffering from mental disorders" includes the following components: a) an individual treatment plan; b) an appropriate institution; c) the language in which the treatment is provided. Adequate treatment is an integral part of the concept of an "appropriate institution". The right to adequate treatment becomes illusory if there is a language barrier between the medical staff and the patient suffering from mental disorders.

KEY WORDS: adequate treatment, compulsory medical measures, criminal process, ECHR practice, mental disorder

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INTRODUCTION

Current case law shows that the provision of such care does not always comply with the requirements of Articles 3 and 5 of the European Convention on Human Rights (hereinafter – the Convention), leading to systemic violations of the Convention rights of persons suffering from mental disorders [1]. The authors' scholarly interest in examining this issue is driven by the fact that the Convention does not explicitly guarantee the right to adequate treatment for such persons, and therefore the interpretation of its provisions by the European Court of Human Rights (hereinafter – the ECHR) depends on the specific circumstances of each case.

AIM

The article aims to deepen the scientific discussion on ensuring the right to psychiatric assistance in places of deprivation of liberty.

MATERIALS AND METHODS

In preparing the article, the following sources were examined: the provisions of international legal instruments regulating adequate treatment of persons suffering from mental disorders; relevant legal positions of the ECHR regarding the observance of the Convention rights of persons suffering from mental disorders (an analysis was conducted of 18 judgments in which the ECHR addressed the issue of adequate treatment in the context of Article 3 of the Convention (prohibition of torture) and/or Article 5 of the Convention (right to liberty and personal inviolability)); and scholarly articles by domestic and foreign researchers on the protection of the rights of persons suffering from mental disorders. In addition, one of the co-authors (O. Tyshchenko), in the course of preparing the monograph *Criminal Proceedings Involving Persons Suffering from Mental Disorders*, conducted a survey via the Google Forms platform of

forensic psychiatric experts working in Odesa, Poltava, and Kharkiv branches of the State Institution "Institute of Forensic Psychiatry of the Ministry of Health of Ukraine" (20 respondents were surveyed) [2].

In the course of the study, a set of general scientific and specialized methods of cognition was used, including dialectical, comparative-legal, system-structural, analytical, synthetic, and comprehensive methods.

ETHICS

All sources used in this literature review are publicly available.

REVIEW AND DISCUSSION

The right of a person suffering from a mental disorder to liberty and personal inviolability is a Convention standard, which includes lawful detention of persons of unsound mind as one of the legitimate cases of deprivation of liberty (subparagraph "e" of paragraph 1 of Article 5 of the Convention) [1]. In the case law of the ECHR, three minimum conditions are clearly distinguished, without compliance with which a person cannot be considered "of unsound mind" and deprived of liberty: 1) the presence of a mental disorder must be established by an objective medical examination; 2) the mental disorder must be of a nature or degree warranting the compulsory confinement of the person in a psychiatric hospital; 3) the necessity of continued confinement in a psychiatric hospital depends on the persistence of such a disorder. These minimum conditions are traditionally known as the Winterwerp criteria (named after their original formulation in the Case of Winterwerp v. the Netherlands [3]), which the ECHR has repeatedly reaffirmed in a series of subsequent judgments (Case of Stanev v. Bulgaria [4]; Case of M. W. v. Poland [5]; Case of Akopyan v. Ukraine [6]; Case of Rooman v. Belgium [7]; Case of M. B. v. Spain [8]; Case of Kaganovskyy v. Ukraine [9], etc.). However, the dynamic development of case law introduces new emphases in this outlined field.

At the early stage of the development of case law in the field of protecting the rights of persons suffering from mental disorders, the ECHR held that the right to adequate treatment of a person did not arise from subparagraph "e" of paragraph 1 of Article 5 of the Convention. Thus, in the Case of Winterwerp v. the Netherlands, Mr. Winterwerp claimed that subparagraph "e" of paragraph 1 of Article 5 implied that every person deprived of liberty as "of unsound mind" has the right to necessary treatment. According to the applicant, his meetings with the psychiatrist were too brief and infrequent, and the medication provided to him contained too many tranquilizers. However, the ECHR recognized that the right of a person of unsound mind to treatment appropriate to their condition does not, in itself, arise from subparagraph "e" of paragraph 1 of Article 5 of the Convention [3]. The ECHR repeated a similar position in the Case of Ashingdane v. United Kingdom [10]. Later, the Human Rights Commission indicated that compulsory hospitalization in a psychiatric hospital indeed serves a dual function – therapeutic and social – but the Convention concerns only the social protective function, allowing, under certain conditions, the deprivation of liberty of a person suffering from a mental disorder (see the Human Rights Commission report on Case of Winterwerp v. Netherlands, Case of Ashingdane v. United Kingdom, Case of Dhoest v. Belgium) [7]. Thus, at that stage of case law development, the ECHR did not consider a violation of subparagraph "e" of paragraph 1 of Article 5 of the Convention to occur if a person was not provided with adequate treatment during compulsory hospitalization. Essentially, the key issue for the ECHR was whether the grounds for detaining a person suffering from mental disorders were lawful – that is, such detention must not be arbitrary.

Gradually, the ECHR expanded the scope of subparagraph "e" of paragraph 1 of Article 5 of the Convention, recognizing the close connection between the "lawfulness" of the detention of persons suffering from mental disorders and the appropriate nature of the treatment of their mental disorder (Case of Aerts v. Belgium, § 49 [11]; Case of Hutchison Reid v. the United Kingdom, § 55 [12]; Case of Ilnseher v. Germany, § 141 [13]). For example, in the Case of Rooman v. Belgium, the ECHR stated that any detention of persons suffering from mental disorders must have a therapeutic purpose aimed at treating or improving their mental health condition, including, where necessary, reducing or controlling the degree of danger posed [7]. Therefore, deprivation of liberty under subparagraph "e" of paragraph 1 of Article 5 of the Convention serves a dual function: a social function and a medical one, related to the individual interest of the person suffering from mental disorders in receiving proper and individualized therapy or course of treatment [7].

Thus, the current established position of the ECHR is that subparagraph "e" of paragraph 1 of Article 5 of the Convention (lawful detention of persons of unsound mind) serves two protective functions: a social function (ensuring the protection of society) and a therapeutic function (adequate treatment of the person while in detention). If the social function is fulfilled but the therapeutic function is not, the ECHR recognizes the absence of adequate treatment as a violation of subparagraph "e" of paragraph 1 of Article 5 of the Convention. When ex-

amining complaints under Article 3 of the Convention, the ECHR considers the totality of detention conditions and the inadequacy of medical treatment (Case of Miranda Magro v. Portugal, § 75 [14]; Case of Strazimiri v. Albania, § 158 [15]). It should be emphasized that the criteria distinguishing violations of these Articles are rather conditional. As rightly noted by ECHR Judge Nussberger, there must be a clearly identified aspect of inhuman or degrading treatment, which is not necessarily present in cases of violations of Article 5 of the Convention. Otherwise, the distinction between the protections provided under these two provisions of the Convention becomes blurred [16].

Given the significant importance that the ECHR attaches to the concept of "adequate treatment of a person suffering from mental disorders", it is appropriate to distinguish the following components, in particular: a) an individual treatment plan; b) an appropriate institution; c) the language in which the treatment is provided. Let us consider each of these in more detail.

INDIVIDUAL TREATMENT PLAN

The "Principles for the Protection of Persons with Mental Illness and the Improvement of Mental Health Care", adopted by UN General Assembly Resolution No. 46/119 of 17 December 1991, establish that persons serving prison sentences for criminal offenses, or persons otherwise subject to detention during judicial proceedings or investigations against them on criminal charges, who suffer from a mental illness or may suffer from one, shall receive the best possible mental health care (Principle 20 – Offenders). Care for each patient and their treatment is based on an individually developed plan, which is discussed with the patient, regularly reviewed, modified if necessary, and provided by qualified medical personnel (Principle 9 – Treatment) [17].

Recommendation No. REC(2004)10 of the Committee of Ministers of the Council of Europe to member states on the protection of the rights and dignity of persons suffering from mental disorders provides that states should implement measures to offer a range of quality services according to the mental health needs of persons with mental disorders, taking into account the differences in needs among various groups of such persons, and ensure equal access to these services (Article 10 – Provision of Health Care) [18]. Persons suffering from mental disorders receive treatment and care from qualified personnel based on an appropriate individual treatment plan. Whenever possible, the treatment plan is agreed upon with the individual, taking their opinion into account. The plan is regularly reviewed and updated if necessary (Part 1, Article 12 – General Principles of Treatment of Mental Disorders) [18]. The explanatory memorandum to this recommendation states:

"Paragraph 90: Paragraph one emphasises the importance of an appropriate individualised treatment plan. When a person has a mild mental disorder that is treated by a primary care physician, that plan may be simple and prepared in discussion between the doctor and the patient. In an emergency situation, the initial plan may be directed at resolving that situation, after which the plan will be further developed;

Paragraph 91: When a person is placed in a facility for treatment of his or her mental disorder, the treatment plan will be more complex. The treatment plan may also address behaviour arising as a consequence of the patient's mental disorder. Additional requirements for involuntary treatment plans are provided in Article 19.2. The European Committee for the Prevention of Torture and Inhuman or Degrading Treatment or Punishment (CPT) has highlighted, in the context of involuntary placement, elements that they consider a treatment plan should contain. Such elements are also relevant to voluntary placements; therefore, a treatment plan should contain a wide range of therapeutic and rehabilitative activities, including, where appropriate:

* Pharmacotherapy;
* Occupational therapy;
* Group therapy;
* Individual psychotherapy;
* Rehabilitative activities relevant to daily living, for example concerning personal hygiene, shopping, cooking and use of public services;
* Art and drama;
* Music and sports;

Paragraph 93: Wherever possible, the treatment plan should be prepared in consultation with the person concerned. The aim is to enable the person to make informed decisions about his or her treatment plan in partnership with the clinical team. It may also be helpful to involve those close to the person in the preparation of the plan. If the person has the capacity to consent, and refuses consent to the clinical team contacting those close to him or her, this refusal should be respected. However, if those close to the person contact the clinical team and offer information relevant to the person's condition, this information can be accepted. Even if the person is too ill to be fully involved in the development of the plan, paragraph one makes clear that attempts should be made to establish his or her opinion and to take this into account" [19].

Based on the analysis of the above-mentioned documents, the importance of an individual treatment plan is beyond doubt; at the same time, the cited provisions are of a recommendatory nature. Accordingly, in its judg-

ments, the ECHR, while referring to the need to develop a treatment plan, uses more flexible terms such as "adequate and individual treatment", "whether an individual programme was implemented", "individual treatment proposals", "therapeutic treatment", "treatment plan", and "treatment programme". Some ECHR judges consider that these expressions do not correspond to a full treatment plan or a comprehensive treatment strategy tailored to the situation and needs of an involuntarily hospitalised patient [20]. As follows from the Case of Rooman v. Belgium, the use of synonymous terms does not prevent the ECHR from finding violations of Articles 3 and/or 5 of the Convention where it is established that the treatment provided to a person with a mental disorder was inadequate [7]. For example, in the Case of Miranda Magro v. Portugal, the ECHR emphasised that the level of care provided must go beyond basic assistance. Mere access to medical staff, consultations, and medication is insufficient for treatment to be considered adequate and satisfactory under Article 5 of the Convention. No therapeutic plan was provided for the applicant, nor were there any documents to that effect. Furthermore, given the applicant's state of health and particular vulnerability, the Court took into account the impact of detention on him, namely the aggravation of his state of confusion and fear due to the restrictive and anti-therapeutic environment of the prison [14]. The absence of a comprehensive programme of therapeutic measures aimed at treating an inmate suffering from mental disorders may be considered a "denial of treatment", which constitutes a violation of Article 3 of the Convention (Case of Strazimiri v. Albania, §§ 108–112) [15]. At the same time, in the Case of Rooman v. Belgium, the ECHR stressed that its role is not to analyse the content of the treatment proposed or provided. What is important is whether an individual treatment programme was implemented, taking into account specific information about the detainee's mental health, with the aim of preparing him or her for possible future reintegration into society. In this field, the Court grants national authorities an appropriate margin of appreciation regarding both the form and the content of medical care or treatment programmes [7]. The ECHR has repeatedly emphasised that the obligation to provide treatment is equally important in situations where the condition of the persons concerned may be considered incurable [7], particularly in cases involving the State's positive obligations under Article 2 of the Convention (right to life), which includes taking measures to protect the lives of those within its jurisdiction.

A PROPER INSTITUTION

In Recommendation No. R(83) 2 of 22 February 1983 concerning the legal protection of persons suffering from mental disorders and subject to involuntary placement, it is stated that involuntary placement means hospitalisation and detention for the treatment of a person suffering from a mental disorder in a hospital, other medical institution, or appropriate place; such detention is not voluntary on the part of the patient (part 2 of Article 1) [21]. The ECHR has also stressed that, regardless of the type of institution in which a person is held, they are entitled to adequate medical conditions accompanied by genuine therapeutic measures aimed at preparing them for possible release. The assessment of whether a particular facility is "adequate" must include an examination of the specific conditions of detention in that facility, particularly the treatment provided to persons with mental disorders. A facility that is *a priori* unsuitable (e.g., a structure within a prison) may still be considered satisfactory if it provides sufficient medical supervision. Conversely, a specialized psychiatric institution, which by definition should be appropriate, may fail to deliver the necessary treatment (Case of Rooman v. Belgium, §§ 199, 203) [7]. In a number of cases against Belgium, the ECHR found that the psychiatric wings of Belgian prisons were inadequate for the long-term detention of mentally ill persons, as they did not receive the care and treatment they required in those conditions. This lack of appropriate care deprived detainees of any realistic prospect of rehabilitation, breaking the necessary link with the purpose – and thus the lawfulness – of detention, which resulted in a violation of Article 5 § 1 of the Convention [22]. Addressing a similar issue in the Case of Proshkin v. Russia, the ECHR noted that, before being transferred to a psychiatric hospital, the applicant had been held in a "cell for the mentally ill" in a pre-trial detention center. The authorities did not explain how the conditions in that cell differed from those in an ordinary detention cell. Furthermore, they did not assert that the applicant had been receiving ongoing medical care for his illness or that the detention conditions created a therapeutic environment. The Court found that the cell in which the applicant was held was not an appropriate facility for the detention of mentally ill persons [23].

The ECHR recognizes that it may be permissible to place a person temporarily in a facility not intended for patients with mental disorders, pending transfer to an appropriate institution, provided that the period of stay is not excessively long (Case of Pankiewicz v. Poland, §§ 44–45; Case of Brand v. the Netherlands, §§ 64–66). At the same time, the Court emphasises that significant delays in admission to a psychiatric hospital are linked to the untimely commencement of treatment, which can affect the eventual treatment outcome. It has found a six-month delay in placing a

person in a psychiatric hospital to be unacceptable in the absence of exceptional circumstances. In Case of Romanov v. Ukraine, the ECHR found no justification for the applicant's prolonged detention in a pre-trial detention center (over two months) before his transfer to a specialized psychiatric facility, holding that this did not comply with the requirements of Article 5 § 1 of the Convention [24]. This finding aligns with the view of 100% of forensic psychiatric experts surveyed, who stated that delays in initiating treatment for mental disorders can negatively impact future treatment outcomes [2]. At the same time, decisions to transfer a person from a specialized facility for individuals with mental disorders to a regular prison environment must be made with great caution. For example, in Case of Haugen v. Norway, the ECHR noted that such a person was moved to a standard prison block where they were no longer under close supervision and had unrestricted access to objects suitable for suicide. Ultimately, they used these means to take their own life [25].

Thus, it can be concluded that, in the ECHR's understanding, adequate treatment is an integral part of the concept of an "appropriate institution". When determining the lawfulness of the detention of persons with mental disorders, the decisive factor for the Court is the level of adequate individual assistance provided to the person in the given facility, while the specific type of institution in which such assistance is delivered is of secondary importance.

THE LANGUAGE IN WHICH THE TREATMENT IS PROVIDED

The right to use a language understandable to the individual is explicitly provided for in Article 5(2) of the Convention (everyone who is arrested must be informed promptly, in a language they understand, of the reasons for their arrest and of any charges against them) and in Article 6(3) "e" of the Convention (everyone charged with a criminal offence who does not understand or speak the language used in court has the right to the free assistance of an interpreter) [1]. At the same time, the Convention contains no provision guaranteeing a person with a mental disorder who is in detention the right to receive treatment in their native language. In the Explanatory Memorandum to Article 7 ("Protection of vulnerable persons with mental disorders") of Recommendation No. REC(2004)10 of the Committee of Ministers of the Council of Europe to member states on the protection of the human rights and dignity of persons with mental disorders, attention is drawn to the linguistic factor as a means of conveying information related to treatment [19]. As noted above, an individual treatment plan includes consultations between the doctor and the patient, as well as individual and group therapy. Wherever possible, such a plan should be agreed with the patient during consultations. It is therefore evident that a language barrier effectively prevents the patient from receiving adequate treatment. In Rooman v. Belgium, the ECHR concluded that, despite repeated findings by health and social welfare authorities regarding the need for the applicant to undergo psychiatric treatment in German to improve his condition and facilitate social reintegration, no such measures were taken. The Court thus found that the absence of individual treatment for 13 years hindered the applicant's potential for positive change – assuming such potential existed. Moreover, the ECHR stressed that overcoming the language barrier was feasible since the patient spoke German, one of Belgium's official languages [7]. By contrast, ECHR Judge Nussberger took the view that the status of a language (whether an official language or a protected minority language) cannot be a factor in assessing the absolute right guaranteed by Article 3 of the Convention. What matters are the individual's suffering; if the applicant had spoken Swahili or Pashto, the judgment would have been the same [17]. Thus, the right to adequate treatment becomes illusory when a language barrier exists between medical personnel and a patient with a mental disorder.

The issue of safeguarding the rights of persons with mental disorders has attracted the scholarly attention of both legal and medical researchers. In criminal procedural scholarship, the most thoroughly developed area concerning the participation of persons with mental disorders in criminal proceedings is the procedure for applying compulsory medical measures to them (V. Kyrychenko [26], D. Kozariichuk [27], V. Pechko [28], H. Teteriatnyk [29], S. Sharenko [30], etc.). Certain aspects of this topic have been addressed in the academic works of I. Hloviuk, V. Hryniuk, and S. Kovalchuk, who examined the application of compulsory hospitalization of persons with mental illnesses in Ukraine's criminal proceedings in the context of ECHR case law [31]. The legal positions of the ECHR regarding the lawfulness of depriving persons with mental disorders of their liberty have been analyzed by V. Zavtur [32]. An analytical study of ECHR practice in the protection, safeguarding, and realization of human rights in the field of psychiatry has been conducted by I. Seniuta [33]. The issues related to ensuring the rights of persons suffering from mental disorders have been examined by various foreign scholars, including Marie Claire Van Hout, Ruth Kaima, Victor Mhango, Stephanie Kewley, Triestino Mariniello [34]; Bălăşoiu, Adriana-Florina [35]; Peter Verbeke, Gert Vermeulen, Tom Vander Beken, Michaël Meysman [36]; Anna Nilsson [37]; Forrester A., Till A., Simpson A., Shaw

J. [38]; Hopkin G., Evans-Lacko S., Thornicroft G. [39]; Picazo, Miriam Fernández [40]; Nathan, Rajan (Taj); Taylor, Paul; Powell, Jason; Morley, Sharon [40]; etc. [41, 42].

Despite the significant volume of work, some aspects of the appropriate treatment of persons suffering from mental disorders and participating in criminal proceedings remain debatable. This article, through the prism of international standards and the case law of the ECHR, examines certain components of the concept of "appropriate treatment" of persons suffering from mental disorders and participating in criminal proceedings under conditions of restriction of freedom (individual treatment plan; appropriate institution; language in which treatment is provided). However, this list is not exhaustive, which indicates the prospects for further research in this area.

CONCLUSIONS

1. The current practice of the ECHR shows that subparagraph "e" of paragraph 1 of Article 5 of the Convention (lawful detention of persons of unsound mind) serves two protective functions: a social function (ensuring the protection of society) and a therapeutic function (providing proper treatment to the person while in detention). If the social function is fulfilled but the therapeutic one is not, the ECHR considers the absence of proper treatment to be a violation of subparagraph "e" of paragraph 1 of Article 5 of the Convention.
2. The concept of "proper treatment" is a complex one, which includes, in particular, the following components:
   a) an individual treatment plan;
   b) an appropriate facility;
   c) the language in which the treatment is provided.
3. An individual treatment plan is an important component of proper treatment for a person suffering from mental disorders and deprived of liberty. The absence of a comprehensive plan of therapeutic measures aimed at treating a detainee with mental disorders may be considered a "refusal of treatment", which constitutes a violation of Article 3 and/or Article 5 of the Convention. The obligation to provide treatment is equally important in situations where the condition of the individuals concerned may be regarded as incurable.
4. Proper treatment is an integral part of the concept of an "appropriate facility". When deciding on the lawfulness of the detention of persons with mental disorders, the ECHR considers the level of proper individual assistance provided in the relevant facility as the key factor, and the specific type of facility where such assistance is provided as secondary.
5. The right to proper treatment is illusory if there is a language barrier between medical staff and a patient suffering from mental disorders. The function of dialogue between the patient and their doctor in a language they both understand becomes of crucial importance.

REFERENCES

1. Konventsiia pro zakhyst prav liudyny i osnovopolozhnykh svobod : vid 04 lystop. 1950 r. (zi zminamy ta dopov., vnes. Protokolamy) [Convention for the Protection of Human Rights and Fundamental Freedoms: from 04 Nov. 1950 (with amendments and addenda, introduced by Protocols)]. Official Gazette of Ukraine. 2006;32(2371). (Ukrainian)
2. Tyshchenko O. Kryminalne provadzhennia za uchastiu osib, yaki strazhdaiut na psykhichni rozlady [Criminal proceedings with the participation of persons suffering from mental disorders]. 2024, p.688. (Ukrainian)
3. Case of Winterwerp v. the Netherlands, application no. 6301/73, judgment of the ECHR of 24 Oct. 1979. http://hudoc.echr.coe.int/eng?i=001–57597 [Accessed 04 June 2025]
4. Case of Stanev v. Bulgaria, application no. 36760/06, judgment of the ECHR of 17 Jan. 2012. https://hudoc.echr.coe.int/eng?i=001–108690 [Accessed 04 June 2025]
5. Case of M. B. v. Poland, application no. 34091/96, judgment of the ECHR of 27 Apr. 2004. https://ips.ligazakon.net/document/ES003721 [Accessed 04 June 2025]
6. Case of Akopyan v. Ukraine, application no. 12317/06, judgment of the ECHR of 5 June, 2014, final 05/09/2014. https://hudoc.echr.coe.int/rus?i=001-144358 [Accessed 04 June 2025]
7. Case of Rooman v. Belgium, application no. 18052/11, judgment of the ECHR of 31 Jan. 2019. https://hudoc.echr.coe.int/eng?i=001-189902 [Accessed 04 June 2025]
8. Case of M. B. v. Spain, application no. 38239/22, judgment of the ECHR of 6 Feb. 2025. https://hudoc.echr.coe.int/eng?i=001-241578 [Accessed 04 June 2025]
9. Case of Kaganovskyy v. Ukraine, application no. 2809/18, judgment of the ECHR of 15 Sep. 2022. https://hudoc.echr.coe.int/eng#{%22itemid%22:[%22001-219200%22]} [Accessed 04 June 2025]
10. Case of Ashingdane v. United Kingdom, application no. 8225/78, judgment of the ECHR of 28 May 1985. https://hudoc.echr.coe.int/ukr?i=001-57425 [Accessed 04 June 2025]

11. Case of Aerts v. Belgium, application no. 8225/78, judgment of the ECHR of 30 July 1998. URL: https://hudoc.echr.coe.int/ukr?i=001-57425 [Accessed 04 June 2025]
12. Case of Hutchison Reid v. the United Kingdom, application no. 50272/99, judgment of the ECHR of 20 Feb. 2003, final 20/05/2023. https://hudoc.echr.coe.int/fre?i=001-60954 [Accessed 04 June 2025]
13. Case of Ilnseher v. Germany, applications nos. 10211/12 and 27505/14, judgment of the ECHR of 04 Dec. 2018. https://hudoc.echr.coe.int/eng?i=001-187540 [Accessed 04 June 2025]
14. Case of Miranda Magro v. Portugal, application no. 30138/21), judgment of the ECHR of 09 Jan. 2024. https://hudoc.echr.coe.int/eng?i=001-229894 [Accessed 04 June 2025]
15. Case of Strazimiri v. Albania, application no. 34602/16, judgment of the ECHR of 21 Jan. 2020, final 21/05/2020. https://hudoc.echr.coe.int/eng?i=001-200452 [Accessed 04 June 2025]
16. Case of Rooman v. Belgium, application no. 18052/11, judgment of the ECHR of 31 Jan. 2019. https://hudoc.echr.coe.int/eng?i=001-189902 [Accessed 04 June 2025]
17. Principles for the Protection of Persons with Mental Illness and the Improvement of Mental Health Care: adopted by UNGA resolution 46/119 on 17 Dec. 1991. Refworld. https://www.refworld.org/docid/3ae6b3920.html [Accessed 04 June 2025]
18. Recommendation No. Rec (2004)10 Concerning the protection of the human rights and dignity of persons with mental disorder and its Explanatory Memorandum: adopted by Committee of Ministers on 22 Sept. 2004. Council of Europe. https://rm.coe.int/rec-2004–10-eme/168066c7e1 [Accessed 04 June 2025]
19. Explanatory Memorandum to Recommendation Rec (2004)10 of the Committee of Minister to member states concerning the protection of the human rights and dignity of persons with mental disorder. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://rm.coe.int/rec-2004-10-em-e/168066c7e1 [Accessed 04 June 2025]
20. Joint partly dissenting opinion of judges Turković, Dedov, Motoc, Ranzoni, Bošnjak and Chanturia. Case of Rooman v. Belgium, application no. 18052/11, judgment of the ECHR of 31 Jan. 2019. https://hudoc.echr.coe.int/eng?i=001-189902 [Accessed 04 June 2025]
21. Recommendation of the Committee of Ministers to the member states concerning the legal protection of persons suffering from mental disorder placed as involuntary patients. R(83)2 of 22 February 1983. https://hrlibrary.umn.edu/instree/coerecr83.html [Accessed 04 June 2025]
22. Annual Report. European Court of Human Rights 2019. Chapter 2 Case-law overview. 2019; 83.
23. Case of Proshkin v. Russia, application no. 28869/03, judgment of the ECHR of 7 Febr. 2012, final 09/07/2012. http://hudoc.echr.coe.int/eng?i=001–108961 [Accessed 04 June 2025]
24. Case of Romanov v. Ukraine, application no. 76273/11, judgment of the ECHR of 28 May 2020. https://zakon.rada.gov.ua/laws/show/974_f10#Text [Accessed 04 June 2025]
25. Case of Haugen v. Norway, application no. 59476/21, judgment of the ECHR of 15 Oct. 2024, final 17/03/2025. https://hudoc.echr.coe.int/eng?i=001-237292 [Accessed 04 June 2025]
26. Kyrychenko V.L. Kryminalne provadzhennia shchodo zastosuvannia prymusovykh zakhodiv medychnoho kharakteru [Criminal proceedings regarding the application of compulsory measures of a medical nature]: dissertation. ... Dnipro. 2020, p.314. (Ukrainian)
27. Kozariichuk DV. Zabezpechennia prav osoby u kryminalnomu provadzhenni shchodo zastosuvannia prymusovykh zakhodiv medychnoho kharakteru [Ensuring the rights of individuals in criminal proceedings regarding the application of compulsory medical measures]. Dissertation. Candidate of Legal Sciences. Kyiv. 2021, p.211. (Ukrainian)
28. Pechko VV. Instytut zastosuvannia prymusovykh zakhodiv medychnoho kharakteru v kryminalnomu protsesi Ukrainy [Institute of application of compulsory medical measures in the criminal procedure of Ukraine]. Dissertation. Doctor of Philosophy. Mariupol. 2021, p.207. (Ukrainian)
29. Teteriatnyk HK. Okhorona prav i zakonnykh interesiv neosudnykh i obmezheno osudnykh na stadii dosudovoho rozsliduvannia u kryminalnomu protsesi Ukrainy [Protection of the rights and legitimate interests of unconvicted and limitedly convicted persons at the stage of pre-trial investigation in the criminal process of Ukraine]. Dissertation. Candidate of Legal Sciences. Donetsk. 2012, p.226. (Ukrainian)
30. Sharenko SL. Kryminalno-protsesualni problemy zastosuvannia prymusovykh zakhodiv medychnoho kharakteru [Criminal procedural problems of applying coercive measures of a medical nature]. Kharkiv. 2002, p.208. (Ukrainian)
31. Hloviuk IV, Hryniuk VO, Kovalchuk SO. Zastosuvannia prymusovoi hospitalizatsii psykhichno khvorykh osib u kryminalnomu provadzhenni Ukrainy v konteksti praktyky Yevropeiskoho sudu z prav liudyny [The use of forced hospitalization of mentally ill persons in criminal proceedings in Ukraine in the context of the practice of the European Court of Human Rights]. Porivnyal'ne ta analitychne pravo. 2020;1:524–528. doi: 10.32782/2524–0390/2020.1.131. (Ukrainian) DOI
32. Zavtur VA. Normatyvne zabezpechennia prava na svobodu ta osobystu nedotorkannist u kryminalnomu provadzhenni shchodo zastosuvannia prymusovykh zakhodiv medychnoho kharakteru (chastyna 1) [Normative provision of the right to freedom and personal integrity in criminal proceedings regarding the application of compulsory medical measures (part 1)]. Elektronnyy naukovo-pravovyy zhurnal. 2022;7:374–378. doi: 10.32782/2524–0374/2022–7/90. (Ukrainian) DOI

33. Seniuta I. Yurydychne zabezpechennia prav osib z psykhichnymy rozladamy v mistsiakh nesvobody: analit. doslidzh. z urakhuvanniam reformy okhorony psykhichnoho zdorovia [Legal protection of the rights of persons with mental disorders in places of detention: analytical research taking into account the reform of mental health care]. Kyiv. 2022, p.97. https://rm.coe.int/report-psychiatry-2022-web/1680a63357 [Accessed 04 June 2025] (Ukrainian)
34. Van Hout MC, Kaima R, Mhango V et al. Judiciarisation of the mentally ill and/or mentally incapacitated in the Malawi criminal justice system: Gaps and flaws of human rights protection. Forensic Science International: Mind and Law. 2023;4:100121. doi:10.1016/j.fsiml.2023.100121. DOI
35. Bălăşoiu A-F. Some clarifications regarding the protection of the natural person by judicial interdict and the protection of persons with mental disorders in the context of the legislative changes brought by Law no. 140/2022 from the perspective of the right to respect for private and family life, provided by the European Convention on Human Rights. Revista de Stiinte Politice. 2024;82(06):47.
36. Verbeke P, Vermeulen G, Vander Beken T, Meysman M. Protecting the fair trial rights of mentally disordered defendants in criminal proceedings: exploring the need for further EU action. Int J Law Psychiatry. 2015;41:67-75. doi: 10.1016/j.ijlp.2015.03.009. DOI
37. Nilsson A. Compulsory mental health interventions and the CRPD: Minding Equality. 2021, p.186.
38. Forrester A, Till A, Simpson A, Shaw J. Mental illness and the provision of mental health services in prisons. Br Med Bull. 2018;127(1):101–109. doi: 10.1093/bmb/ldy027. DOI
39. Hopkin G, Evans-Lacko S, Forrester A et al. Interventions at the transition from prison to the community for prisoners with mental illness: a systematic review. Adm Policy Ment Health. 2018;45(4):623-634. doi: 10.1007/s10488-018-0848-z. DOI
40. Picazo M.F. Theory of positive state obligations under article 2.1 of the convention to protect the lives of persons with mental disorders in state psychiatric institutions. analysis of ecthr case-law. Revista de derecho político. 2024;01(119):291–314.
41. Rajan (Taj) N, Taylor P, Powell J, Morley S. Mental health and the criminal courts: Fitness to plead, culpability and the defence of insanity. Mental Health and Punishments. 2021, pp. 19–32.
42. Studies from University of Warsaw Yield New Data on Mental Disorders. The Notion of 'a Person of Unsound Mind' Under Article 5 /n 1(E) of the European Convention On Human Rights. Mental Health Weekly Digest. 2021, p.826.

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Olga Tyshchenko
Yaroslav Mudryi National Law University
77 Hryhoriya Skovorody St., 61024 Kharkiv, Ukraine
e-mail: Tysholga79@gmail.com

ORCID AND CONTRIBUTIONSHIP

Olga Tyshchenko: 0000-0003-1551-1367 A B D F
Andrii Hnatiuk: 0000-0002-0222-3545 D E
Inna Bespalko: 0000-0001-7161-1720 D E

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

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REVIEW ARTICLE

CONTENTS

Dynamics of the prevalence of thyroid gland diseases among the adult population

Larysa Ya. Fedoniuk[1], Yaroslav O. Bilyk[1], Nataliia V. Porokhovska[2], Oksana M. Matolinets[2], Mariia I. Kulitska[1]
[1]I. HORBACHEVSKY TERNOPIL NATIONAL MEDICAL UNIVERSITY, TERNOPIL, UKRAINE
[2]DANYLO GALYTSKY LVIV NATIONAL MEDICAL UNIVERSITY, LVIV, UKRAINE

ABSTRACT

Aim: To content analysis the problem of thyroid disorders and thyroid gland pathology among adults in Ukraine and Ternopil region.

Materials and Methods: The research was conducted during 2021–2025 in several stages on the base of scientific literature about endocrine system disorders. At the theoretical stage, the state of problem development in the scientific literature of oiodine deficiency was studied. 75 scientific papers were used for the analysis, which were searched for using the following keywords: goitre, hypothyroidism, thyrotoxicosis, thyroiditis, iodine deficiency. Statistical data was also collected and analyzed on the incidence of iodine deficiency disorders among the adult population of Ukraine, particularly in the Ternopil region. International manuscripts and articles on the problem of oiodine deficiency diseases were studied also, such as: Diffuse goitre, Nodular goitre, Hypothyroidism, Postoperative hypothyroidism, Thyrotoxicosis, Thyroiditis; Ukraininan experience was analyzed and generalized.

Conclusions: The reason for iodine deficiency is the consequences of the Chernobyl accident and negative state of the environment due to a natural deficiency of iodine and other elements. The scientific report shows the need to inform about preventive and health-improving measures among the adult population, depending on regional characteristics.

KEY WORDS: thyroid gland, goitre, hypothyroidism, thyrotoxicosis, thyroiditis, iodine deficiency, metabolic disorders, postnatal ontogenesis

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NTRODUCTION

Endocrine system disorders occupy a leading place in the structure of the overall morbidity of the population. In recent years, the incidence of endocrine diseases has been on the rise both worldwide and in our country [1-3]. There has been an increasing of the number of patients with various endocrinopathies, the most common of which are thyroid disease and diabetes mellitus.

According to WHO data, over the past half-century there has been an increase in thyroid pathology, which ranks first among endocrinopathies after diabetes [4, 5]. Almost a third of the world's population is at risk of developing thyroid disorders [6]. This problem is becoming increasingly relevant due to the deterioration of the environmental situation in Ukraine. According to the Ministry of Health of Ukraine, the overall incidence of thyroid disorders has increased fivefold over the past five years, and statistical indicators vary among different regions of Ukraine depending on various factors (iodine-deficient regions, chronic stress, unbalanced nutrition, the presence of comorbidities, etc.) [7, 8].

The thyroid gland is an important part of the neuroendocrine system. Its main function is to maintain normal metabolism in the cells, organs and whole organism by supplying the body with such thyroid hormones as: triiodothyronine (T3) and thyroxine (T4), which contain iodine as an essential structural component [9]. Iodine deficiency conditions lead to decreasing of the production of the hormones of the thyroid gland and the development of iodine deficiency disorders, which can cause miscarriage, reproductive disorders in women, and numerous negative consequences for the fetus [10-12]. Iodine deficiency disorders are all pathological conditions caused by iodine deficiency, the development of which can be completely prevented through the implementation of individual, group, and mass prevention methods [13, 14].

Ukrainian and foreign studies have shown that the most common cause of thyroid disease is a deficiency of iodine intake from the environment into the body. Prolonged iodine deficiency disrupts adaptive processes, leading to iodine deficiency disorders, which

manifest themselves in various pathologies at different ages [15].

The problem of iodine deficiency remains unresolved for Ukraine, with almost the entire territory of Ukraine experiencing iodine deficiency of varying severity [16-18] as there are no regions where the risk of iodine deficiency disorders for the population has not been recorded [19]. In regions with insufficient iodine supply, the most common cause of thyroid dysfunction is endemic goiter, which leads to gland hypofunction. Its annual incidence reaches 3.5 per 1,000 women and 0.6 per 1,000 men [17, 18].

AIM

To content analysis of scientific literature in the problem of thyroid disorders and thyroid gland pathology among adults in Ukraine and Ternopil region.

MATERIALS AND METHODS

The research was conducted during 2021–2025 in several stages on the base of scientific literature about endocrine system disorders. At the theoretical stage, the state of problem development in the scientific literature of oiodine deficiency was studied. 75 scientific papers were used for the analysis, which were searched for using the following keywords: goitre, hypothyroidism, thyrotoxicosis, thyroiditis, iodine deficiency. Statistical data was also collected and analyzed on the incidence of iodine deficiency disorders among the adult population of Ukraine, particularly in the Ternopil region. International manuscripts and articles on the problem of oiodine deficiency diseases were studied also, such as: Diffuse goitre, Nodular goitre, Hypothyroidism, Postoperative hypothyroidism, Thyrotoxicosis, Thyroiditis; Ukraininan experience was analyzed and generalized.

ETHICS

All sources used in this literature review are publicly available.

FRAMEWORK

The work is carried out within the framework of the initiative research work of the I. Horbachevsky Ternopil National Medical University (Ternopil, Ukraine) "0116 U003390 Systemic and organ disorders due to the action of extraordinary factors on the body, the mechanisms of their development and pathogenetic correction".

REVIEW AND DISCUSSION

Diffuse and nodular goiter remain the most common thyroid disorders in Ukraine today. The number of registered goiter patients in Ukraine is measured in hundreds of thousands. The main cause of goiter is iodine deficiency in the population's diet, as well as the direct or indirect influence of various negative factors, including environmental ones [8, 19]. It is precisely the imbalance between micronutrients and vitamins in patients' diets, combined with the deteriorating environmental situation in the region and insufficiently effective preventive measures, that contributes to the development of thyroid diseases.

When assessing the total number of registered patients with goiter in Ukraine, there is a downward trend in the incidence of grade I diffuse goiter. The number of new cases in 2020 was 48,649, and in 2023 – 34,861. Over the course of five years, diffuse goiter of grades II-III also showed a slight downward trend, with a prevalence of 156,613 cases in 2020 and in 2023, 153,556 cases, which translates to 31.5 and 28.6 new cases per 100,000 population. These data truly reflect the positive dynamics of preventive work and educational and informational awareness of the population in Ukraine regarding the reduction of thyroid diseases.

It should be noted that the level of goitre in western region is higher when to compare with the national average and the indicators for the north-eastern regions of Ukraine. In the Ternopil region, there has been a trend towards an increase in the prevalence of grade I goitre in recent years – 2,218 (2019) and 2,225 (2023), which translates into 256.5 and 259.0 newly diagnosed cases per 100,000 population. This is due to an increase in the incidence of thyroid disease among the population of the western regions as a result of iodine deficiency endemic in these regions. An analysis of the prevalence of nodular goitre among the adult population of Ukraine shows an increase in this pathology every year. According to statistical data, since 2019, there has been a trend towards an increase in nodular goitre among the population of Ukraine and the Ternopil region in particular. The number of patients increases by almost 10% annually. The reason for this increase in the incidence of nodular goitre in Ukraine is the consequences of the Chernobyl accident, as well as the negative state of the environment due to a natural deficiency of iodine, selenium and other trace elements. Given the high incidence of benign thyroid neoplasms in Ukraine, in 2019, 28,850 patients with nodular goitre were diagnosed for the first time (the incidence rate was 81.7 per 100,000 people), in 2021 – 29,982 patients (83.6 per 100,000 people), in 2022 – 30,012 patients (87.5 per

100,000 people), and in 2023 – 33,721 patients (90.2 per 100,000 people). The diagnosis of nodular goitre has improved significantly in recent years thanks to modern ultrasound examinations, indicating an overall increase in the disease.

The prevalence of nodular goitre among the adult population in the Ternopil region during 2019-2023 showed a tendency towards progression of the disease. Thus, in 2019, 4,540 patients with nodular goitre were detected for the first time (the incidence rate was 49.6 per 100,000 population), in 2021 – 5,001 patients (56.7 per 100,000 population), in 2022 – 5,544 patients (57.2 per 100,000 population), and in 2023 – 5,712 patients (58.6 per 100,000 population).

Ukrainian and foreign studies have shown that the most common cause of thyroid disease is a deficiency of iodine intake from the environment into the body [6,9, 20]. Today, the problem of iodine deficiency remains unresolved for Ukraine, as there are no regions where the risk of iodine deficiency diseases for the population has not been recorded. Numerous studies conducted by employees of the V.P. Komissarenko Institute of Endocrinology and Metabolism of the National Academy of Medical Sciences of Ukraine have confirmed that almost the entire territory of Ukraine suffers from iodine deficiency of varying severity [17, 21]. In particular, the Ternopil region is classified as a region with a moderate degree of iodine deficiency. In addition, the situation is complicated by the fact that Ukraine has not yet adopted a document at the legislative level that would regulate the prevention of iodine deficiency.

Compared to other thyroid disorders, thyrotoxicosis is much less common, but there are still tens of thousands of cases in Ukraine. The prevalence of thyrotoxicosis in 2019 was 144.9 per 100,000 population, and the incidence of thyrotoxicosis was 13.8 cases per 100,000 population. In 2021, the prevalence was 150.5 per 100,000 population, and the incidence was diagnosed in 16.4 cases per 100,000 population. In 2023, the prevalence of thyrotoxicosis was 154.4, and in 2019, it was 158.1 per 100,000 population. The incidence of this nosology in Ukraine is also gradually increasing, with the number of new cases of thyrotoxicosis in 2019 amounting to 4,999 and in 2023 – 6,027 per 100,000 population. An increase in the prevalence and incidence of hyperthyroidism was also observed in the Ternopil region.

In recent years, the prevalence of thyroiditis has been growing rapidly in Ukraine. This diagnosis includes acute, subacute, chronic thyroiditis and autoimmune thyroiditis. It should be noted that in 2019, the prevalence was 16,330 cases of thyroiditis, and in 2023, it was 18,403 cases, which translates to 37.0 and 49.2 new cases per 100,000 population. An analysis of the prevalence and incidence of thyroiditis in the Ternopil region also showed an upward trend. Thus, in 2019, the prevalence was 26.1, and in 2023, it was 40.6 new cases.

In the population, the prevalence of clinically significant hypothyroidism ranges from 0.5% to 2%, and subclinical hypothyroidism from 5% to 10%. In iodine-deficient regions, hypothyroidism is found in 80% of women during menopause.

Hypothyroidism is a thyroid gland disorder characterised by a hormone deficiency and affecting up to 5% of the general population. It is one of the most common disorders of the endocrine system, caused by a deficiency of thyroid hormones or a reduction in their biological effect at the tissue level. Iodine deficiency is the most common cause of all thyroid disorders worldwide, including hypothyroidism. Subclinical hypothyroidism is a pathological condition in which serum thyroid hormone levels are within the reference range, but serum thyrotropin levels are persistently elevated (at least twice) beyond the control range of more than 4.5 mIU/L. The diagnosis of subclinical hypothyroidism is based solely on the analysis of thyroid function using laboratory diagnostics. In populations with adequate iodine intake, subclinical hypothyroidism affects up to 10% of the population, with the highest prevalence among women and the elderly [22].

In Ukraine, the prevalence of subclinical hypothyroidism among the adult population ranges from 4 to 20%, depending on gender (higher percentage among women), age (over 60 years), body mass index, race, smoking, iodine intake, etc. [23]. The most common cause of subclinical hypothyroidism is autoimmune thyroiditis. Other causes of subclinical hypothyroidism may include postpartum thyroiditis (hypothyroid phase), thyroid surgery, iodine deficiency, and laboratory abnormalities (e.g., heterophilic antibodies in serum) [23, 24].

The prevalence of subclinical hypothyroidism is five to six times higher than that of overt hypothyroidism [25]. Subclinical hypothyroidism is the mildest form of thyroid gland insufficiency with minimal clinical symptoms, which may be masked by the initial manifestations of climacteric disorders. Some studies have found that subclinical hypothyroidism, like overt hypothyroidism, is associated with hypoestrogenemia in perimenopausal women [26]. Therefore, in women of this age group, hypothyroidism may go unnoticed or its symptoms may be misinterpreted as menopausal manifestations.

In the elderly population, hypothyroidism is more common in women during menopause. The annual incidence of primary hypothyroidism is 3.5 per 1,000 women and 0.6 per 1,000 men [2, 6].

The prevalence of hypothyroidism among the population of the Ternopil region is constantly increasing: the prevalence of acquired hypothyroidism in 2019 was 209.7 per 100,000 population. In 2021, it was 254.3 per 100,000 population. In 2023, it will be 292.8.

Hypothyroidism is divided into primary, which develops as a result of damage to the thyroid gland itself, and secondary, which occurs as a result of a deficiency in the synthesis of TSH by the pituitary gland or TRH by the hypothalamus. In the works of some researchers, women without thyroid pathology with a complicated course of menopause showed an increase in TSH, unlike women with a physiological course. A test with 200 mg of thyrotropin showed that the increase in TSH levels is compensatory and characteristic of a euthyroid state [27]. The compensatory increase in TSH levels may be associated with a decrease in the stimulating effect of oestrogens on the thyroid gland. As a result, its reserves are reduced and the risk of developing hypothyroidism increases.

Hypothyroidism progresses slowly and often develops unnoticed by patients and those around them. Diagnosis of this pathology is complicated and is due to its multi-symptomatic nature and similarity of symptoms to the manifestations of ageing [28]. As is known, the course of hypothyroidism is often accompanied by manifestations of goitre in iodine-deficient regions and, according to the literature [27, 28], it is more often detected in women aged 40-60 years. In 2022, the prevalence of hypothyroidism was 292.8 per 100,000 population, and in 2023, it was 348.6 per 100,000 population, while the incidence was diagnosed in 30.9 cases and increased to 32.6 cases per 100,000 population.

Another frequently diagnosed thyroid disease is autoimmune thyroiditis, a genetically determined autoimmune disease that develops under the influence of environmental factors [12]. The genetic nature of autoimmune thyroiditis is confirmed by certain HLA antigens (HLA DR3, HLA DR5). Autoimmune thyroiditis is often associated with other systemic and organ diseases (type 1 diabetes mellitus, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, etc.). In addition to age and gender, environmental factors such as sun exposure, infectious diseases, and excessive iodine intake can contribute to the development of autoimmune thyroiditis. Thus, according to some authors, iodine consumption is accompanied by an increase in the prevalence of autoimmune thyroiditis, while other authors report varying prevalence rates of both thyrotoxicosis and hypothyroidism depending on iodine supply [29, 30]. There are studies indicating that excessive iodine absorption in the presence of genetic predisposition contributes to the formation of active radicals that destroy proteins and lipids in the epithelial cells of the thyroid gland, leading to its lymphoid infiltration.

The course of autoimmune thyroiditis is often accompanied by an hypotrophy of the thyroid gland (Hashimoto's goitre). The atrophic form of thyroiditis is characterised by a reduction in its size (atrophic thyroiditis). The histological picture of autoimmune thyroiditis in thyroid tissue is represented by lymphoid and plasmacytic infiltration, destruction of the follicular apparatus, and fibrosis [14].

Autoimmune thyroiditis is caused by a defect in the immunological response associated with a deficiency of T-suppressors. In the course of various immunological processes under the influence of T-helpers, lymphocytes are transformed into plasma cells and form antibodies to thyroglobulin and peroxidase. Interaction with T-killers has a cytotoxic effect on the follicular cells of the thyroid gland. This eventually leads to destruction, reduction in mass and decreased function of the thyroid gland. Autoantibodies to peroxidase bind to the C1/3 fraction of complement, forming immune complexes that have a cytotoxic effect on thyroid epithelial cells [30, 31]. T-lymphocytes sensitised to specific antigens are capable of secreting lymphokines that are cytotoxic to target cells. Lymphokines include chemotactic factor, macrophage migration inhibitory factor, tumour necrosis factor, and lymphotoxin. Damage to the thyroid epithelium leads to thyroid hypertrophy, a reaction to maintain euthyroid status, and in rare cases, to destructive thyrotoxicosis with thyroid hyperfunction [31]. Subsequently, there is a decrease in the functional activity of the thyroid gland and overt hypothyroidism develops.

One of the main goals of the healthcare system in most countries around the world is to take preventive measures against pathological conditions that can develop as a result of iodine deficiency and contribute to the development of iodine deficiency disorders and diseases. Thyroid pathology is one of the most common reasons for patients seeking medical help, and this is particularly noticeable in iodine-deficient and polluted areas of certain regions.

According to the WHO, about 2 billion people live in conditions of chronic iodine deficiency, with nodular goitre, diffuse goitre, hypothyroidism, thyrotoxicosis and thyroiditis being the most common thyroid disorders. Analysis of official statistics has made it possible to determine the significant prevalence of iodine deficiency and endocrine diseases in many regions. Traditionally, the regions of Ukraine with a pronounced

Table 1. Prevalence of thyroid gland pathology among adults (per 100,000 population) in Ukraine and Ternopil region

Diseases	Country/Region	2020	2021	2022	2023
Diffuse goitre, grade I	Ukraine	1268,8	1285,3	1348,7	1381,5
	Ternopil	1175,4	1326,1	1307,1	1291,3
Diffuse goitre, grade II	Ukraine	434,2	457,3	445,3	441,3
	Ternopil	608,4	645,9	649,0	627,5
Nodular goitre	Ukraine	766,4	817,6	855,3	891,5
	Ternopil	523,4	579,1	612,0	647,3
Hypothyroidism	Ukraine	251,6	268,2	282,3	302,8
	Ternopil	254,3	292,8	318,4	348,6
Postoperative hypothyroidism	Ukraine	213,1	234,5	234,3	246,9
	Ternopil	130,6	120,0	137,9	147,5
Thyrotoxicosis	Ukraine	144,9	150,5	154,4	158,1
	Ternopil	165,6	174,1	177,2	177,9
Thyroiditis	Ukraine	465,3	493,2	543,7	520,7
	Ternopil	140,8	193,4	269,2	226,7

Source: compiled by the authors of this study

iodine deficiency include Ternopil, Ivano-Frankivsk, Chernivtsi, Rivne, Zakarpattia, Volyn, and Lviv regions. An analysis of the prevalence of thyroid diseases in the Ternopil region and in Ukraine as a whole shows that from 2020 to 2023, not only did the overall incidence increase, but the overall structure of thyroid pathology also changed (Table 1).

Iodine, as a trace element, is essential for the synthesis of the necessary amount of thyroid hormones for its functioning. The daily requirement for iodine depends on a person's age and physiological state; it ranges from 90 to 250 mcg/day [3, 32]. Thyroid hormones are necessary for regulating energy metabolism, growth and reproduction. Insufficient iodine intake leads to the development of processes aimed at adapting and maintaining hormone secretion by the gland. Increasing of the interest to the problems of thyroid pathology in Ukraine in recent years is caused by its growing prevalence among the adult population, the high frequency of temporary and permanent disability [7, 33].

Iodine deficiency is a significant risk factor in the development of iodine deficiency disorders and thyroid diseases, but smoking, genetic predisposition, ethnicity, and various endocrine disorders also contribute to their development.

Diffuse and nodular goitre remains the most common thyroid disorder in Ukraine today. The number of registered goitre patients in Ukraine is measured in hundreds of thousands. The main cause of goitre is iodine deficiency in the population's diet, as well as the direct or indirect influence of various negative factors, including environmental ones [34]. It is the imbalance of microelements and vitamins in the population's diet, against the backdrop of a deteriorating environmental situation and insufficiently effective preventive measures at the state level, that causes the development of thyroid diseases.

The endemic nature of thyroid damage contributes to earlier menstrual dysfunction and the onset of menopause [27]. There is a close relationship between menopause and thyroid pathology. In a number of studies, in 30% of cases, the manifestation of thyroid diseases occurs during menopause [9]. Prolonged residence in an iodine-deficient area also gradually leads to nodule formation and the development of thyroid autonomy. Gradually, the number of thyrocytes that have become autonomous and lost sensitivity to the regulatory influence of TSH increases [19]. The production of excessive amounts of thyroid hormones by autonomous tissue leads to the development of thyrotoxicosis even in the absence of additional iodine intake. Taking iodised salt or iodine-containing pharmaceuticals only accelerates this process [10, 11].

Over the past 10 years, the absolute number of thyroid gland diseases in Ukraine has increased from 689,000 to 1,846,000, which translates into a prevalence of 941.6 and 4,210.4 cases per 100,000 population, respectively.

According to WHO data, iodine deficiency is one of the most common causes of mental retardation in children, which is quite dangerous for them in childhood, because the endocrine hormones of the gland are necessary for regulating the development,

differentiation and formation of tissues of all organs and systems of the child's body, primarily the brain.

In Ukraine, no document regulating the prevention of iodine deficiency has yet been approved at the legislative level [18]. Scientists from the National Academy of Medical Sciences of Ukraine, together with experts from the United States, have calculated the economic consequences of prolonged iodine deficiency for our country. Due to reduced working capacity caused by iodine deficiency in Ukraine, losses exceed UAH 1.2 billion. Meanwhile, investing in an IDD prevention programme would reduce expenditures from the State Budget of Ukraine by up to 80% of the funds potentially lost due to reduced labour productivity [21, 34].

CONCLUSSIONS

The presented results of the incidence and prevalence of thyroid diseases indicate a tendency towards an increase in thyroid pathology among the adult population of Ukraine and the Ternopil region. The statistical report dictates the need to make informed decisions at the state level regarding the development of preventive and health-improving measures among the adult population, depending on regional characteristics.

REFERENCES

1. Tkachenko VI, Maksymets YaA, Vydyborets NV, Kovalenko OF. Analiz poshyrenosti tyreoidnoi patolohii ta zakhvoriuvanosti na nei sered naselennia Kyivskoi oblasti ta Ukrainy za 2007–2017 rr. [Analysis of the prevalence of thyroid pathology and morbidity among the population of the Kyiv region and Ukraine for 2007–2017]. International Endocrine Journal. 2018;14(3):272-277. doi: 0.22141/2224-0721.14.3.2018.136426. (Ukrainian) DOI
2. Asa SL, Mete O. Endocrine pathology: past, present and future. Pathology. 2018;50(1):111–118. doi: 10.1016/j.pathol.2017.09.003. DOI
3. Taylor P.N., Albrecht D., Scholz A. et al. Global epidemiology of hyperthyroidism and hypothyroidism. Nature reviews. Endocrinology. 2018;14(5):301–316. doi: 10.1038/nrendo.2018.18. DOI
4. Ferrari SM, Fallahi P, Elia G et al. Thyroid autoimmune disorders and cancer. Seminars in cancer biology. 2020;64:135–146. doi: 10.1016/j.semcancer.2019.05.019. DOI
5. Marakis G, Katsioulis A, Kontopoulou L et al. Knowledge, attitude and behaviour of university students regarding salt and iodine: a multicentre cross-sectional study in six countries in Europe and Asia. Archives of Public Health. 2021;79(1):1–14. doi: 10.1186/s13690-021-00593-5. DOI
6. Mamenko MYe. Profilaktyka yododefitsytnykh zakhvoriuvan': scho maie znaty ta mozhe zrobyty pediatr i likar zahal'noi praktyky? (Klinichni rekomendatsii) [Prevention of iodine deficiency disorders: what should know and can do a pediatrician and general practitioner? (Clinical practice guideline)]. Sovremennaya pediatriya. 2017;2:8-16. doi: 10.15574/SP.2017.82.8. (Russian) DOI
7. Skrypnyk NV, Marusyn OV. Dynamika zakhvoriuvanosti y poshyrenosti vuzlovykh utvoren' schytopodibnoi zalozy za desiatyrichnyi period (2006-2016 rr.) v Ukraini ta na Prykarpatti [Dynamics of incidence and prevalence of thyroid gland nodules in ukraine and in the carpathian region for ten years (2006-2016)]. Praktykuiuchyi likar. 2017;6(2):26-29. (Ukrainian)
8. Podolskyi VV, Kozar VYa, Podolskyi VV. Reproduktyvne zdorov' ia ta zakhvoriuvannia shchytopodibnoi zalozy v zhinok fertylnoho viku [Reproductive health and thyroid gland diseases in women of childbearing age]. Akusherstvo. Hinekolohiya. Henetyka. 2019;5(3):61-65. doi: 10.11603/24116-4944.2009.1.9654. (Ukrainian) DOI
9. Kjaergaard AD, Marouli E, Papadopoulou A et al. Thyroid function, sex hormones and sexual function: a Mendelian randomization study. Eur J Epidemiol. 2021;36,(3):335–344. doi: 10.1007/s10654-021-00721-z. DOI
10. Tarashchenko YuM, Kovalenko AYu, Kravchenko VI ta in. Yodo- ta selenodefitsyt u patohenezi zobnoi transformatsii shchytopodibnoi zalozy ta avtoimunnykh tyreopatii (ohliad literatury ta rezultaty vlasnykh doslidzhen) [Iodine and selenium deficiency in the pathogenesis of goitre transformation of the thyroid gland and autoimmune thyroid diseases (literature review and results of our own research)]. Endokrynolohiya. 2020;25(4):297–304.). doi:10.31793/1680-1466.2020.25-4.297. (Ukrainian) DOI
11. WHO. Assessment of iodine deficiency disorders and monitoring their elimination. Clinical Nutrition. 2001. https://iris.who.int/server/api/core/bitstreams/7872bac7-fd37-4744-863c-a794cc7807c1/content [Accessed 14 April 2025]
12. Ferrari SM, Fallahi P, Antonelli A. Environmental issues in thyroid diseases. Front Endocrinol. 2017;8:1–8. doi:10.3389/fendo.2017.00050. DOI
13. Takedani K, Notsu M, Yamauchi M. Graves' disease and vertebral fracture: Possible pathogenic link in postmenopausal women. Clin Endocrinol. 2020;93(2):204–211. doi: 10.1111/cen.14208. DOI
14. Hu S, Rayman MP. Multiple Nutritional Factors and the Risk of Hashimoto's Thyroiditis. Thyroid. 2017;27(5):597–610. doi: 10.1089/thy.2016.0635. DOI
15. Krela-Kaźmierczak I, Czarnywojtek A, Skoracka K. Is there an ideal diet to protect against iodine deficiency? Nutrients. 2021;13(2):1–15. doi: 10.3390/nu13020513. DOI

16. Esche J, Thamm M, Remer T. Contribution of iodized salt to total iodine and total salt intake in Germany. Eur J Nutrition. 2020;59(7):3163–3169. doi: 10.1007/s00394-019-02154-7. DOI
17. Tronko MD, Pasteur IP, Zamotayeva GA, Masyk SV. Ukrayins'ko-amerykans'kyy proekt z pytan' shchytovydnoyi zalozy: ohlyad naukovykh publikatsiy z epidemiolohiyi, dozymetriyi ta yodnoho zakhystu. [Ukrainian-American thyroid project: review of scientific publication on epidemiology, dosimetry and iodine protection]. Endokrynolohiya. 2018;23(2):154–166. (Ukrainian)
18. Tronko MD, Kravchenko VI. Znachennia yodu dlia orhanizmu, naibilsh vahomi yoho doslidzhennia ta perspektyvy zaprovadzhennia yodnoi profilaktyky v Ukraini [The importance of iodine for the body, the most significant studies on it, and the prospects for the introduction of iodine prophylaxis in Ukraine]. Endokrynolohiya. 2021;26(1):59–74. (Ukrainian)
19. Knobel M. Etiopathology, clinical features, and treatment of diffuse and multinodular nontoxic goiters. J Endocrinol Invest. 2016;39(4):357–373. doi: 10.1007/s40618-015-0391-7. DOI
20. Ventura M, Melo M, Carrilho F. Selenium and Thyroid Disease: From Pathophysiology to Treatment. Int J Endocrinol. 2017;17:1297658. doi: 10.1155/2017/1297658. DOI
21. Tronko MD, Kravchenko VI. Profilaktyka yodozalezhnykh zakhvoriuvan v ukraini (ohliad literatury) [Prevention of iodine-dependent diseases in Ukraine (literature review)]. Environment & Health. 2019;2:65–69. doi:10.32402/dovkil2019.02.065. (Ukrainian) DOI
22. Pankiv VI. Vplyv dysfunktsii shchytopodibnoi zalozy na reproduktyvnu systemu zhinky. Suchasni mozhlyvosti fitoterapii [The effect of thyroid dysfunction on the female reproductive system. Modern possibilities of phytotherapy]. Reproduktyvna endokrynolohiya. 2016;1(27):52–57. (Ukrainian)
23. Redford C, Vaidya B. Subclinical hypothyroidism: Should we treat? Post Reproductive Health. 2017;23(2):55–62. doi: 10.1177/2053369117705058. DOI
24. Chiovato L, Magri F, Carlé A. Hypothyroidism in Context: Where We've Been and Where We're Going. Adv Ther. 2019;36:47–58. doi: 10.1007/s12325-019-01080-8. DOI
25. Kalra AK, Das S, Bajaj R et al. Diagnosis and Management of Hypothyroidism: Addressing the Knowledge–Action Gaps. Adv Ther. 2018;35(10):1519–1534. doi: 10.1007/s12325-018-0744-7. DOI
26. Goyal G, Goyal L, Singla H. Subclinical Hypothyroidism and Associated Cardiovascular Risk Factor in Perimenopausal Females. J Midlife Health. 2020;11(1):6–11. doi: 10.4103/jmh.JMH_38_19. DOI
27. Slopien R, Owecki M, Slopien A et al. Climacteric symptoms are related to thyroid status in euthyroid menopausal women. Journal of Endocrinological Investigation. 2020;43(1):75–80. doi: 10.1007/s40618-019-01078-7. DOI
28. Ghianda SD, Tonacchera M, Vitti P. Thyroid and menopause. Climacteric. 2014;17(3):225–234. doi: 10.3109/13697137.2013.838554. DOI
29. Ragusa F, Fallahi P, Elia G et al. Hashimotos' thyroiditis: Epidemiology, pathogenesis, clinic and therapy. Best Pract Res Clin Endocrinol Metab. 2019;33)6):101367. doi: 10.1016/j.beem.2019.101367. DOI
30. Allelein S, Feldkamp J, Schott M. Autoimmune Schilddrüsenerkrankungen. [Autoimmune thyroid diseases.]. Der Internist. 2022;58(1):47–58. doi:10.17749/2313-7347/ob.gyn.rep.2022.251. (German) DOI
31. Guo Q, Wu Y, Hou Y et al. Cytokine secretion and pyroptosis of thyroid follicular cells mediated by enhanced NLRP3, NLRP1, NLRC4, and AIM2 inflammasomes are associated with autoimmune thyroiditis. Front Immunol. 2018;9:17-22. doi: 10.3389/fimmu.2018.01197. DOI
32. Pasiechko NV, Petryk KhV. Profilaktyka yododefitsytnykh zakhvoriuvan v Ternopilskomu rehioni [Prevention of iodine deficiency disorders in the Ternopil region]. Medsestrynstvo. 2013;4:11-13. (Ukrainian).
33. Taylor PN, Albrecht D, Scholz A et al. Global epidemiology of hyperthyroidism and hypothyroidism. Nat Rev Endocrinol. 2018;14(5):301-316. doi: 10.1038/nrendo.2018.18. DOI
34. Kravchenko VI, Medvedev BK. The biological role of iodine and iodine deficiency as pathogenetic factor of thyroid pathology in pregnant women and its prevention. Int J Endocrinol. 2018;14(2):111–118. doi:10.22141/2224-0721.14.2.2018.130552. DOI

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Larysa Ya. Fedoniuk
I. Horbachevsky Ternopil National Medical University
9 Valova st, 46000 Ternopil, Ukraine
e-mail: fedonyuk22larisa@gmail.com

ORCID AND CONTRIBUTIONSHIP
Larysa Ya. Fedoniuk: 0000-0003-4910-6888 A B E F
Yaroslav O. Bilyk: 0000-0001-8971-1420 B D
Nataliia V. Porokhovska: 0000-0001-5221-8544 D F
Oksana M. Matolinets: 0009-0008-6521-8867 A B E
Mariia I. Kulitska: 0000-0002-4251-5411 B E

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

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REVIEW ARTICLE

CONTENTS

New diagnostic tools for obstructive sleep apnea

Jakub Piotr Bundyra, Agata Sochocka, Alicja Sikorska, Julia Parda, Emil Mian, Zuzanna Zapart, Martyna Szepietowska, Nina Nowicka, Oliwia Zuzanna Gańska, Dominika Janik
MEDICAL STUDENT, MEDICAL UNIVERSITY OF WARSAW, WARSAW, POLAND

ABSTRACT

Aim: To evaluate how multi-night home sleep apnea testing and other ambulatory monitoring strategies can complement or, in selected patients, replace single-night in-laboratory polysomnography when diagnosing obstructive sleep apnea, with emphasis on clinical decision-making, diagnostic accuracy, feasibility, and patient experience.

Materials and Methods: PubMed and Scopus were searched for English-language publications from 2015-2025. Selected landmark studies published before 2015, one American Academy of Sleep Medicine guideline and conference abstract were also included.

Current evidence shows substantial night-to-night variability in the apnea-hypopnea index (AHI), driven by sleep position, alcohol use, and sleep-stage distribution. Single-night testing may misclassify a large proportion of mild-to-moderate cases. Modern home testing devices (e.g., peripheral arterial tonometry-based systems, accelerometry, radar, and under-mattress sensors) enable extended monitoring, reduce the first-night effect, and may improve diagnostic precision. Most patients prefer to perform tests at home due to convenience. However, it is worth emphasizing that clear instructions and easily accessible technical support are very important factors for them.

Conclusions: For uncomplicated adults with a high pre-test probability of obstructive sleep apnea, multi-night home testing can be a pragmatic first-line option, especially around diagnostic thresholds. In-laboratory polysomnography remains preferred in patients with significant comorbidities, suspected coexisting sleep disorders, or when home testing is negative, inconclusive, or technically inadequate.

KEY WORDS: obstructive sleep apnea, polysomnography, home sleep apnea testing, telemedicine, apnea-hypopnea index

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INTRODUCTION

Obstructive sleep apnea (OSA) is a common chronic disorder with intermittent hypoxia and sleep fragmentation. It is linked with cardiovascular, metabolic and neurocognitive complications [1, 2]. Despite its high prevalence, many patients remain undiagnosed or start treatment late, partly because in-laboratory testing is limited and costly [3-6].

In-laboratory polysomnography (PSG) is the reference diagnostic test [7, 8]. PSG usually records electroencephalography (EEG), electrooculography (EOG) and electromyography (EMG) plus airflow, respiratory effort, electrocardiography (ECG), oximetry and body position, so it allows sleep staging and standardized event scoring [7, 8]. However, apnea severity can change substantially between nights, so one night may not reflect typical disease burden [9-12]. This matters near diagnostic cut-offs, where an atypical night can lead to underestimation or a false-negative result [9, 10, 12]. In real-world pathways, the time from diagnosis to starting positive airway pressure (PAP) has been reported to be about 2-10 months, which supports the need for scalable alternatives [3]. This is especially relevant in high-risk groups such as patients assessed for bariatric surgery, where OSA prevalence has been estimated at 70-80%. [7] Because OSA is associated with higher postoperative cardiopulmonary complications, timely identification before major surgery is important [13].

AIM

The aim of our literature review is to provide evidence for multiday home monitoring as an alternative to single-day PSG for OSA and its complications. We focus on diagnostic accuracy, feasibility of modern home technologies, and patient experiences.

MATERIALS AND METHODS

A narrative literature review was conducted in PubMed and Scopus for publications from 2015-2025. Search terms combined concepts related to OSA,

night-to-night variability, home diagnostic testing and ambulatory monitoring technologies. Earlier landmark studies were included when they provided foundational evidence. Studies were prioritized if they compared multi-night approaches with laboratory PSG, quantified diagnostic misclassification, or reported patient experience and pathway-level outcomes.

REVIEW AND DISCUSSION

NIGHT-TO-NIGHT VARIABILITY AND RISK OF MISCLASSIFICATION

As shown by Punjabi et al. [9] multi-night recordings show that apnea severity is not a fixed trait. Systematic reviews and large cohorts demonstrate frequent crossing of conventional severity categories across nights, particularly in mild-to-moderate disease [9, 10, 12]. In modelling and real-world datasets, the probability of misclassification based on a single night has been reported in the range of roughly 20-50%, depending on the diagnostic threshold and patient phenotype [9, 12]. Pragmatically, three to four nights often provide a reasonable balance between improved precision and feasibility, with diminishing returns beyond longer monitoring in many scenarios [9, 12, 14].

DRIVERS OF NIGHT-TO-NIGHT VARIABILITY AND EVERYDAY SLEEP CONDITIONS

Laboratory studies may be affected by the first-night effect, as highlighted in the meta-analysis by Roeder et al. [10]. This refers to changes in sleep when a person sleeps in an unfamiliar environment (for example, a sleep laboratory), often with increased arousal and lighter sleep during the first night [10, 11]. It can alter sleep architecture and reduce rapid eye movement sleep, which may lower the measured event rate [10, 11]. Home recordings are usually closer to a patient's typical sleep. They capture usual body position patterns and everyday factors such as supine sleep, alcohol intake, and day-to-day changes in sleep timing and fatigue [9, 11, 15]. For this reason, averaging results from multiple home nights provides a more representative estimate of usual sleep and reduces reliance on a single potentially atypical night [9, 12, 15].

TECHNOLOGIES ENABLING MULTI-NIGHT HOME MONITORING

Conventional portable monitors can provide accurate respiratory signals but may be burdensome for repeated use [4-6, 8]. Newer devices prioritise simplified application and comfort, making multi-night protocols more practical. Validated approaches include peripheral arterial tonometry systems, [16] photoplethysmography-based fingertip or ring devices, [17, 18] mandibular movement sensors, [19] acoustic patches, [20, 21] bio-impedance patches, [22] and contactless under-mattress or radar-based sensors [12, 23]. In appropriately selected patients, these platforms show clinically acceptable agreement with laboratory PSG for obstructive events, while enabling repeated measurements in the home environment [17-25]. Several newer home sleep apnea testing (HSAT) systems use automated (including machine-learning) analysis to detect respiratory events and generate indices that facilitate multi-night monitoring [17, 20, 21]. Examples are summarized in (Table 1).

From a physiological perspective, HSAT platforms differ in how they infer obstructive events. Peripheral arterial tonometry systems (e.g., WatchPAT) analyse changes in the PAT signal (a marker of peripheral vasoconstriction) together with oximetry to capture autonomic arousals and desaturation patterns linked to apneas and hypopneas [15, 16, 24]. Photoplethysmography fingertip or ring wearables extract pulse-wave and saturation features (often combined with actigraphy) and use automated algorithms, including deep learning, to estimate AHI-equivalent indices across multiple nights [17, 18, 25]. Mandibular movement sensors quantify characteristic jaw motion patterns associated with obstructive events, illustrating a mechanosignal-based HSAT approach [19]. Acoustic neck patches record tracheal breathing sounds and vibrations to classify respiratory events and snoring in the home environment [20, 21]. Bio-impedance patches track cyclical thoracic impedance changes as a surrogate of respiratory effort and incorporate body position, enabling repeated unattended recordings [22]. Finally, contactless under-mattress or radar-based sensors capture respiratory-related motion signals without attaching sensors to the patient, which may facilitate longer monitoring when signal quality is adequate [12, 23]. Notably, some newer fingertip- and patch-based systems integrate cloud connectivity with automated scoring, including deep-learning approaches for respiratory event detection and AHI estimation [17, 20, 21]. In high-volume settings, this may support faster triage while preserving clinician oversight for complex or discordant cases [17].

Table 1. Examples of home monitoring technologies suitable for multi-night protocols

Technology	Examples	Typical strengths / caveats
Peripheral arterial tonometry	WatchPAT ONE/300 [16] (PAT signal from a finger probe + oximetry; events inferred from autonomic responses and desaturation patterns)	Comfortable; derives events from autonomic signals; may be less suitable for complex comorbidities.
Photoplethysmography wearables	NightOwl (fingertip PPG) [17] (Pulse-wave/SpO_2 features with automated/AI analysis to estimate an AHI-equivalent index) SleepImage ring (ring PPG) [18] (Pulse-wave/SpO_2 features with algorithm-based analysis and actigraphy-derived sleep surrogates)	Very low burden; scalable to multi-night; relies on automated algorithms and sleep-wake surrogates.
Acoustic neck patch	AcuPebble SA100 [20, 21] (Adhesive neck patch; records tracheal breathing sounds/vibrations with automated scoring)	High usability; automated analysis; validation vs PSG in home settings.
Bio-impedance patches	Wesper system [22] (Bio-impedance patch monitoring thoracic effort-related impedance changes + body position)	Wireless effort signals and position; requires correct patch placement.
Contactless sensors	Under-mattress / radar-based systems [12, 23] (Contactless sensing of respiratory-related motion via under-mattress pressure/ballistocardiography signals or radar-based motion tracking)	Zero-touch, ideal for long-term monitoring; performance depends on signal quality and phenotype

Table 2. Practical triage for selecting HSAT versus PSG in suspected obstructive sleep apnea

Clinical scenario	Preferred test / next step	Rationale and practical notes
Uncomplicated adult; high pre-test probability of moderate-to-severe OSA	HSAT, preferably multi-night (≥2 nights); initiate treatment pathway if diagnostic	Guidelines support home testing for uncomplicated high-risk adults. Multi-night sampling reduces misclassification near decision thresholds [7, 9-13, 31, 32]
Uncomplicated adult; suspected mild disease or borderline severity; symptoms fluctuate	Multi-night home testing; consider PSG if results are discordant with clinical picture	Mild disease is most vulnerable to night-to-night variability. Repeated nights can improve sensitivity and ecological validity [9-12, 15, 31, 32]
Significant cardiopulmonary disease, suspected hypoventilation, neuromuscular weakness, or need for oxygen/ventilation assessment	In-laboratory PSG (add carbon dioxide monitoring when available)	Higher risk of hypoventilation/complex breathing disorders; home testing may underestimate severity.[7]
Suspected coexisting sleep disorders (central sleep apnea, parasomnia, periodic limb movements, narcolepsy) or differential diagnosis needed	In-laboratory PSG	Requires neurophysiology and broader signal set; home testing cannot reliably evaluate many comorbid sleep disorders [7, 8, 13]
HSAT negative, inconclusive, or technically inadequate but clinical suspicion remains high	Proceed to in-laboratory PSG	Guidelines recommend PSG after a negative or inadequate home study in symptomatic patients [7]
Need to accelerate access to therapy (severe symptoms, long waiting lists) after appropriate safety screening	Multi-night home testing with tele-support; fast-track treatment when diagnostic	Home pathways can shorten time to diagnosis and treatment; reserve laboratory capacity for complex cases [5, 6, 30]

PATIENT EXPERIENCE AND SUPPORT NEEDS

Most patients report higher comfort and a more natural sleep environment when testing at home. [26, 27] However, a minority prefer laboratory testing due to reassurance from professional supervision and concerns about device failure or incorrect setup [26-28]. Highly simplified devices and telemedicine-supported pathways can reduce "technostress" and improve acceptability, especially when multi-night protocols allow at least one usable night even if a recording fails [4, 16, 22, 28, 29].

According to Pendharkar et al., [27] stakeholders involved in primary-care OSA pathways emphasized

that clear education, communication and easy access to support are critical when diagnostic and therapeutic steps move beyond specialist sleep laboratories [27].

Similarly, Moffa et al. [16] reported that a telemedicine-based diagnostic service using WatchPAT® ONE was generally well accepted by patients, but highlighted the practical importance of straightforward instructions and accessible technical support for successful home recordings [16].

SYSTEM-LEVEL IMPLICATIONS

Home-based pathways can shorten time to diagnosis and treatment initiation compared with a laboratory-first approach and may reduce initial diagnostic costs [3-6, 30]. Even if multi-night testing collects more data, better classification near decision thresholds may reduce repeat testing and the downstream costs of delayed or missed diagnosis [3-6, 30]. At the health-system level, using validated HSAT for uncomplicated patients can reserve laboratory PSG capacity for more complex cases and help reduce waiting times [3, 7, 27].

In addition, Pendharkar et al., [30] evaluated an alternative care provider clinic model for severe sleep-disordered breathing, supporting the feasibility of non-traditional, pathway-based approaches to improve access while maintaining clinical oversight [30].

HEALTH-SYSTEM IMPACT AND FUTURE DIRECTIONS (AI-ENABLED HSAT)

Multi-night HSAT can help reduce diagnostic bottlenecks by moving uncomplicated assessments from sleep laboratories to home, which may shorten the time from suspected OSA to starting treatment [3, 27, 30]. In real-world pathways, delays from diagnosis to PAP therapy have been reported to be around 2-10 months [3]. In a large retrospective multi-night dataset using wearable PPG, Nygate et al. [31] found that relying on the first night would have missed a proportion of OSA cases and that many patients increased by at least one severity category on later nights [31]. Faster confirmation of clinically relevant OSA may be important in time-sensitive preoperative pathways. OSA is common among candidates for bariatric surgery (reported estimates 70-80%) and is associated with increased postoperative cardiopulmonary complications [7, 13]. Some newer devices combine simplified sensors with automated, cloud-based analysis. For example, Chen et al. [17] validated a system using deep-learning methods to detect respiratory events in the home environment [17]. More automation may reduce manual scoring workload and support scalable telemedicine models, alongside patient education and accessible technical support [16, 27, 30]. AI tools may also help with signal-quality checks, artifact detection and triage to PSG when home results are inconclusive or clinical complexity is high [7]. Future studies should evaluate AI-assisted, multi-night HSAT pathways against single-night strategies for time-to-diagnosis, patient-centred outcomes and health-system efficiency [3, 27, 30].

A multi-night approach treats the diagnostic "gold standard" as getting a representative picture of sleep over time, not just one laboratory night. Laboratory PSG is still essential in complex phenotypes, discordant cases, suspected non-obstructive sleep disorders or when home testing is non-diagnostic [7, 8, 13]. For uncomplicated adults with high pre-test probability, multi-night home monitoring may offer a practical balance between accuracy, access and patient preference [3, 4, 9, 12, 26].

IMPORTANT LIMITATIONS REMAIN

Many home monitoring technologies do not record EEG and instead use sleep-wake surrogates, which can be less accurate in patients with fragmented sleep [8, 18, 24]. In addition, reimbursement and accreditation structures may lag behind technological capability, which can slow the adoption of multi-night protocols despite evidence of efficiency benefits [3-6].

CLINICAL DECISION-MAKING: WHEN TO USE HOME TESTING AND WHEN TO REFER FOR POLYSOMNOGRAPHY

Clinical practice guidelines recommend either PSG or HSAT for diagnosing OSA in uncomplicated adults with an increased risk of moderate-to-severe disease, provided that the home study is technically adequate and interpreted within a clinical evaluation [7]. In this setting, multi-night HSAT (e.g., 2-4 nights) can better reflect everyday sleep, reduce first-night effects and limit night-to-night variability that may shift patients across severity categories [9-12, 14] (Table 2).

PSG remains preferable when the probability of measurement error or alternative diagnoses is higher, including significant cardiopulmonary disease, suspected hypoventilation, neuromuscular weakness, chronic opioid use, prior stroke, severe insomnia, or suspected non-obstructive sleep disorders (e.g., central sleep apnea, parasomnias, periodic limb movement disorder) [7,

8, 13]. Because many home platforms rely on surrogate sleep-wake measures and may underestimate event rates in fragmented sleep, careful phenotyping and follow-up are essential when home results and symptoms do not align [8, 18, 24].

This preference reflects the broader PSG signal set (including EEG-based sleep staging and, when indicated, additional monitoring such as carbon dioxide), which enables differential diagnosis and more robust phenotyping than most HSAT devices. [7, 8, 13] A practical triage approach is summarized in Table 2.

LIMITATIONS OF CURRENT EVIDENCE

Most available data on multi-night strategies are observational, device-validation studies, or pragmatic pathway evaluations. Comparative randomized studies are limited, and protocols vary in the number of nights, sensor types, and scoring algorithms. Future work should standardize multi-night thresholds, clarify which phenotypes benefit most, and evaluate implementation outcomes in routine care.

CONCLUSIONS

For uncomplicated adults at increased risk of moderate-to-severe OSA, HSAT is an appropriate diagnostic option alongside in-laboratory PSG, and multi-night protocols can reduce misclassification near diagnostic thresholds. PSG should remain the preferred test in patients with significant comorbidities or suspected coexisting sleep disorders, and it is indicated when HSAT is negative, inconclusive, or technically inadequate despite persisting clinical suspicion. Implementation should prioritise patient instructions, rapid technical support and telemedicine-enabled pathways to minimize failed studies and reduce delays in treatment initiation.

REFERENCES

1. Benjafield AV, Ayas NT, Eastwood PR, Heinzer R, Ip MSM, Morrell MJ, et al. Estimation of the global prevalence and burden of obstructive sleep apnoea: a literature-based analysis. Lancet Respir Med. 2019;7(8):687-698. doi:10.1016/S2213-2600(19)30198-5. DOI
2. Lal C, Strange C, Bachman D. Neurocognitive impairment in obstructive sleep apnea. Chest. 2012;141(6):1601-1610. doi:10.1378/chest.11-2214. DOI
3. Wickwire EM, Zhang X, Munson SH, Benjafield AV, Sullivan SS, Payombar M, et al. The OSA patient journey: pathways for diagnosis and treatment among commercially insured individuals in the United States. J Clin Sleep Med. 2024;20(4):505–514. doi:10.5664/jcsm.10908. DOI
4. Phua CQ, Jang IJ, Tan KB, Hao Y, Senin SRB, Song PR, et al. Reducing cost and time to diagnosis and treatment of obstructive sleep apnea using ambulatory sleep study: a Singapore sleep centre experience. Sleep Breath. 2021;25(1):281–288. doi:10.1007/s11325-020-02115-z DOI
5. Deutsch PA, Simmons MS, Wallace JM. Cost-effectiveness of split-night polysomnography and home studies in the evaluation of obstructive sleep apnea syndrome. J Clin Sleep Med. 2006;2(2):145–153. doi:10.5664/jcsm.26508 DOI
6. Chai-Coetzer CL, Antic NA, Rowland LS, Reed RL, et al. Primary care vs specialist sleep center management of obstructive sleep apnea and daytime sleepiness and quality of life: a randomized trial. JAMA. 2013;309(10):997–1004. doi:10.1001/jama.2013.1823. DOI
7. Kapur VK, Auckley DH, Chowdhuri S, Kuhlmann DC, Mehra R, Ramar K, Harrod CG. Clinical practice guideline for diagnostic testing for adult obstructive sleep apnea: an American Academy of Sleep Medicine clinical practice guideline. J Clin Sleep Med. 2017;13(3):479–504. doi:10.5664/jcsm.6506. DOI
8. Berry RB, Brooks R, Gamaldo CE, Harding SM, Lloyd RM, Marcus CL, Vaughn BV. The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications. Version 2.4. Darien IL: American Academy of Sleep Medicine; 2017. J Clin Sleep Med. 2017 May 15;13(5):665-666. doi: 10.5664/jcsm.657.
9. Punjabi NM, Patil S, Crainiceanu C, Aurora RN. Variability and misclassification of sleep apnea severity based on multi-night testing. Chest. 2020;158(1):365-373. doi:10.1016/j.chest.2020.01.039. DOI
10. Roeder M, Bradicich M, Schwarz EI, Thiel S, et al. Night-to-night variability of respiratory events in obstructive sleep apnoea: a systematic review and meta-analysis. Thorax. 2020;75(12):1095–1102. doi:10.1136/thoraxjnl-2020-214544. DOI
11. Stepnowsky CJ Jr, Orr WC, Davidson TM. Nightly variability of sleep-disordered breathing measured over 3 nights. Otolaryngol Head Neck Surg. 2004;131(6):837-843. doi:10.1016/j.otohns.2004.07.011. DOI
12. Lechat B, Naik G, Reynolds A, Aishah A, et al. Multinight prevalence, variability, and diagnostic misclassification of obstructive sleep apnea. Am J Respir Crit Care Med. 2022;205(5):563-569. doi:10.1164/rccm.202107-17610C. DOI
13. Laratta CR, Ayas NT, Povitz M, Pendharkar SR. Diagnosis and treatment of obstructive sleep apnea in adults. CMAJ. 2017;189(48):E1481-E1488. doi:10.1503/cmaj.170296. DOI
14. Lechat B, Scott H, Manners J, Adams R, Proctor S, Mukherjee S, et al. Multi-night measurement for diagnosis and simplified monitoring of obstructive sleep apnoea. Sleep Med Rev. 2023;72:101843. doi:10.1016/j.smrv.2023.101843. DOI

15. Tschopp S, Wimmer W, Caversaccio M, Borner U, Tschopp K. Night-to-night variability in obstructive sleep apnea using peripheral arterial tonometry: a case for multiple night testing. J Clin Sleep Med. 2021;17(9):1751-1758. doi:10.5664/jcsm.9300. DOI
16. Moffa A, Giorgi L, Nardelli D, Ferro A, et al. A new telemedicine-based sleep service using WatchPAT® ONE for patients with suspected OSA: what does the patient experience?. Sleep Breath 29, 47 (2025). doi:10.1007/s11325-024-03218-7 DOI
17. Chen KW, Tseng CH, Lee HC, Liu WT, Chou KT, Wu HT. Validation of a fingertip home sleep apnea testing system using deep learning AI and a temporal event localization analysis. Sleep. 2025;48(5):zsae317. doi:10.1093/sleep/zsae317. DOI
18. Lu M, Brenzinger L, Rosenblum L, Salanitro M, Fietze I, Glos M, et al. Comparative study of the SleepImage ring device and polysomnography for diagnosing obstructive sleep apnea. Biomed Eng Lett. 2023;13(3):343-352. https://doi.org/10.1007/s13534-023-00304-9.
19. Martinot JB, Cuthbert V, Le-Dong NN, Coumans N, et al. Clinical validation of a mandibular movement signal-based system for the diagnosis of pediatric sleep apnea. Pediatr Pulmonol. 2022;57(8):1904-1913. doi:10.1002/ppul.25320. DOI
20. Devani N, Pramono RXA, Imtiaz SA, Bowyer S, Rodriguez-Villegas E, Mandal S. Accuracy and usability of AcuPebble SA100 for automated diagnosis of obstructive sleep apnoea in the home environment setting: an evaluation study. BMJ Open. 2021;11(12):e046803. doi:10.1136/bmjopen-2020-046803. DOI
21. Sanchez Gomez J, Pramono RXA, Imtiaz SA, Rodriguez-Villegas E, Valido Morales A. Validation of a wearable medical device for automatic diagnosis of OSA against standard PSG. J Clin Med. 2024;13(2):571. https://doi.org/10.3390/jcm13020571
22. Raphelson JR, Ahmed IM, Ancoli-Israel S, Ojile J, Pearson S, Bennett N, et al. Evaluation of a novel device to assess obstructive sleep apnea and body position. J Clin Sleep Med. 2023;19(9):1643-1649. doi:10.5664/jcsm.10644. DOI
23. Edouard P, Campo D, Bartet P, Yang RY, Bruyneel M, Roisman G, Escourrou P. Validation of the Withings Sleep Analyzer, an under-the-mattress device for the detection of moderate–severe sleep apnea syndrome. J Clin Sleep Med. 2021;17(6):1217-1227. doi:10.5664/jcsm.9168. DOI
24. Lyne CJ, Hamilton GS, Turton ARE, Stupar D, Mansfield DR. Validation of a single-use and reusable home sleep apnea test based on peripheral arterial tonometry. J Clin Sleep Med. 2023;19(8):1429-1435. doi:10.5664/jcsm.10568. DOI
25. Walter J, Lee JY, Blake S, Kalluri L, et al. A new wearable diagnostic home sleep testing platform: comparison with available systems and benefits of multinight assessments. J Clin Sleep Med. 2023;19(5):865–872. doi:10.5664/jcsm.10432. DOI
26. Chua AP, Soh ZY, Abd Rahman S, Luo N, Shorey S. Lived experience of patients with sleep apnea: a systematic synthesis of qualitative evidence. Qual Life Res. 2023;32(5):1447-1467. doi:10.1007/s11136-022-03332-7. DOI
27. Pendharkar SR, Blades K, Kelly JE, Tsai WH, et al. Perspectives on primary care management of obstructive sleep apnea: a qualitative study of patients and health care providers. J Clin Sleep Med. 2021;17(1):89-98. doi:10.5664/jcsm.8814. DOI
28. Abma IL, van der Wees PJ, Veer V, Westert GP, Rovers M. Measurement properties of patient-reported outcome measures (PROMs) in adults with obstructive sleep apnea (OSA): a systematic review. Sleep Med Rev. 2016;28:18-31. doi:10.1016/j.smrv.2015.07.006. DOI
29. Taweesedt P, Najeeb H, Surani S. Patient-centered therapy for obstructive sleep apnea: a review. Medicina (Kaunas). 2022;58(10):1338. doi:10.3390/medicina58101338. DOI
30. Pendharkar SR, Tsai WH, Penz ED, Santana MJ, Ip-Buting A, Kelly J, et al. A randomized controlled trial of an alternative care provider clinic for severe sleep-disordered breathing. Ann Am Thorac Soc. 2019;16(12):1558-1566. doi:10.1513/AnnalsATS.201901-087OC. DOI
31. Nygate Y, Wodnicki J, Rusk S, Fernandez C, Hwang D, Watson NF. Evaluating the impact of multi-night home sleep apnea testing for obstructive sleep apnea diagnosis. Sleep. 2025;48(Suppl 1):A297-A298. doi:10.1093/sleep/zsaf090.0683. DOI
32. Lechat B, Loffler KA, Reynolds AC, Naik G, Vakulin A, Jennings G, et al. High night-to-night variability in sleep apnea severity is associated with uncontrolled hypertension. NPJ Digit Med. 2023;6(1):57. doi:10.1038/s41746-023-00801-2. DOI

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Jakub Piotr Bundyra

Medical student, Medical University of Warsaw,
Warsaw, Poland
e-mail: kubabundyra4@gmail.com

ORCID AND CONTRIBUTIONSHIP
Jakub Piotr Bundyra: 0009-0008-7042-1990 A D E F
Agata Sochocka: 0009-0004-5384-2014 B E
Alicja Sikorska: 0009-0009-6274-6944 D
Julia Parda: 0009-0006-9079-5936 F
Emil Mian: 0009-0006-1538-7190 F
Zuzanna Zapart: 0009-0007-0466-4508 E
Martyna Szepietowska: 0009-0000-2423-4563 E
Nina Nowicka: 0009-0004-1942-0045 B
Oliwia Zuzanna Gańska: 0009-0007-9024-0209 B
Dominika Janik: 0009-0000-8527-7428 B

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

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REVIEW ARTICLE

CONTENTS

Challenges of ensuring the protection of personal and other medical data in the field of transplantation and reproductive technologies in the digital environment

Viktor Zaborovskyy, Vasyl Manzyuk, Vasyl Fennych, Stanislav Shchoka
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

Aim: To study the legal, technical, and organisational issues of ensuring the confidentiality of personal and other medical data, in particular, in the context of the use of mobile medical applications (mHealth), various sets (bases) of medical data, in transplantology and the use of ART.

Materials and Methods: The research materials comprised scholarly publications, legal acts, analytical reports from international organizations, and judicial practice relating to the problems of ensuring confidentiality and protection of personal and medical data in healthcare, particularly in transplantology and ART within the digital environment. The search for sources was conducted using international and national scientometric and legal databases, including PubMed, Scopus, and Web of Science, as well as official repositories of regulatory and legal acts of the European Union, the United States, and Ukraine. The chronological scope of the search covered the period from 2015 to 2025, which is justified by the rapid development of digital medicine, mobile health applications (mHealth), biobanks, and the growing incidence of cyber threats in the healthcare sector during this time frame. The search was conducted using the following keywords and their combinations in Ukrainian and English: confidentiality of medical data, personal data, digital medicine, mHealth, cybersecurity, protection of medical information, transplantology, and ART.

Conclusions: Confidentiality is a fundamental component of the relationship between a doctor and a patient, which should be based on mutual trust, especially in the field of transplantology and the use of ART, which require medical professionals not only to clearly apply the norms of legislation in this area, but also to adhere to high moral and ethical standards of professional activity, taking into account, the need for permanent exchange of information between many participants in such special legal relations.

KEY WORDS: confidentiality, medical data, personal data, digitalization of medicine, mHealth applications, transplantology, ART

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INTRODUCTION

In the context of the rapid development of the use of digital technologies, especially in the field of health care (and particularly in the field of transplantology and reproductive technologies), the issue of ensuring the confidentiality of personal and other medical data is gaining particular importance. The introduction of mHealth applications, biometric and genetic technologies, as well as the expansion of the scope of application of additional reproductive technologies and transplantology, significantly expands the volume and sensitivity of medical information that is processed. This, in turn, leads to the emergence of new challenges related to cybersecurity, data deanonymization, commercial use of personal information without the consent of the subject, as well as the insufficient effectiveness of existing legal control mechanisms.

The relevance of this work also lies in the need to study the problem of ensuring trust between participants in medical legal relations, which is the basis for the effective functioning of the health care system, in particular, in the field of transplantology and reproductive technologies. In view of the above, the study of legal, organisational, and technical aspects of protecting medical information is extremely relevant both at the national and international levels.

Among the scientists who have studied individual aspects of this issue, it is appropriate to single out the works of S. Alder, P. Burcher, K. Cato, D. Chirra, D. Chornenka, R. Gupta, N. Hammond-Browning, C. Horner, S. Hosseini, P. Huyvan, R. Iyengar, S. Ikeda, S. Iribarren, H. Luu, M. Mello, A. Musienko, O. Omelchenko, V. Pishta, M. Sharma, A. Schwab, K. Spector-Bagdady, P. Stone, N. Williams and others. At the same time, a comprehensive

study of this issue, as well as the identification of ways to solve it, remained virtually unnoticed by scientists.

AIM

To study the legal, technical, and organisational issues of ensuring the confidentiality of personal and other medical data, in particular, in the context of the use of mobile medical applications (mHealth), various sets (bases) of medical data, in transplantology and the use of ART.

MATERIALS AND METHODS

Various methods of scientific knowledge form the methodological basis of an interdisciplinary approach, which includes a set of methods that allow us to study the social and legal aspects of ensuring the confidentiality of medical information in the context of the digital transfor mation of health care, especially in the field of transplantology and reproductive technologies.

The research materials comprised scholarly publications, legal acts, analytical reports from international organizations, and judicial practice relating to the problems of ensuring confidentiality and protection of personal and medical data in healthcare, particularly in transplantology and ART within the digital environment. The search for sources was conducted using international and national scientometric and legal databases, including PubMed, Scopus, and Web of Science, as well as official repositories of regulatory and legal acts of the European Union, the United States, and Ukraine.

The chronological scope of the search covered the period from 2015 to 2025, which is justified by the rapid development of digital medicine, mobile health applications (mHealth), biobanks, and the growing incidence of cyber threats in the healthcare sector during this time frame. The search was conducted using the following keywords and their combinations in Ukrainian and English: confidentiality of medical data, personal data, digital medicine, mHealth, cybersecurity, protection of medical information, transplantology, and ART. In total, more than 50 sources were analyzed in this study, including peer-reviewed scholarly articles, international guidelines, regulatory instruments (GDPR, HIPAA), court decisions, and analytical reports from specialized medical organizations.

The inclusion criteria for sources comprised: relevance to the protection of medical data in the digital environment, with emphasis on healthcare, particularly transplantology and/or ART; the presence of a clearly articulated legal, ethical, or cybersecurity-related analysis; publication in peer-reviewed academic journals or official sources. The limitations of the study consisted in the exclusion of sources that did not correspond to the research subject or were purely descriptive in nature, as well as those lacking substantiated conclusions or failing to account for the specificity of the digital environment and contemporary cybersecurity challenges.

ETHICS

All sources used in this literature review are publicly available.

REVIEW AND DISCUSSION

MODERN CHALLENGES AND THREATS TO THE CONFIDENTIALITY OF MEDICAL DATA IN THE DIGITAL AGE

The impact of information technology, in particular, on the health care sector, as well as the problems of ensuring the confidentiality of personal and other medical data of an individual, have repeatedly been the subject of our scientific research [1-4]. A significant problem in ensuring the confidentiality of personal and other medical data of an individual lies in their actual separation from other related data about the individual, as well as taking into account the diversity of sources of obtaining such information. Based on the fact that the clinical use of personal genetic and other personal data of an individual is rapidly growing, unprecedented volumes of personal data are generated and distributed in many other contexts, such as mobile application technologies, intelligent personal and home devices, online activity and the use of biometric technologies, a number of scientists question the feasibility of entering a new era of digitalization while abandoning confidentiality when using new technologies [5]. In the work «Consumer Perspectives on Privacy Protection and Sharing of Personal Digital Health Information», scientists have drawn attention to the fact that, on the one hand, interaction with the health care system and the use of mobile devices, social networks, phone applications, and retail create a huge amount of digital data reflecting a person's private health, and on the other hand, the growing collection of digital health information and the blurred boundaries between medical data and data that do not directly relate to a person's health raise concerns about the possibility of ensuring their confidentiality and security, which in some ways even contradict the benefits that such data can provide [6]. A striking example of this is the high-profile case Dobbs v Jackson Women's Health [7], in which the US Supreme Court focused, among other things, on the justified

concern that information from women's applications (for example, for tracking menstrual cycles) and for purchases on websites may contain confidential medical data, in particular, on reproductive health.

The issue of ensuring the confidentiality of personal and other medical data of an individual processed by mobile applications that are in some way related to the field of health care (mHealth applications) has been repeatedly the subject of scientific research. Without questioning the advantages that mHealth applications provide in terms of improving access to health care resources and monitoring the health status of an individual in real time [8], there are quite justified concerns about ensuring the confidentiality of the information they access, its commercial use (sale of subscriptions and sharing of user data), and inadequate compliance with privacy standards in general [9].

Although the EU, the USA and many other countries around the world have developed standards (regulations) for the protection of personal data and other confidential information of individuals, including in the health care sector (for example, in the European Union this is the General Data Protection Regulation (GDPR) [10], in the USA – the US Health Insurance Portability and Accountability Act (HIPAA) [11] and the Post-Market Management of Medical Device Cybersecurity [12]), research in this area indicates the difficulty in implementing these standards in practice and the presence of cases of unauthorized collection and distribution of data obtained from applications, in particular in the health care sector.

A study conducted by the Norwegian Consumer Council found that a number of popular applications, including those in the health and fitness sector, shared data with advertising companies without the user's informed consent [13]. A fundamental study into the privacy disclosures of mHealth applications was carried out by Australian researchers who conducted a large-scale analysis of over 20,000 applications available on Google Play (the largest mobile application marketplace). Their study found that the vast majority of applications (88%) could access and potentially share personal data, but compared to basic non-health applications, mHealth applications included fewer data collection operations in their code, transferred less user data, and demonstrated lower levels of third-party intrusion. At the same time, an analysis of the privacy policies of such applications, namely the actual transfer of user information, raised concerns, as 28.1% of mHealth applications did not offer any privacy policy text, and at least 25% of user data transfers violated what was stated in their privacy policies. Based on this study, Australian researchers conclude that the collection of users' personal information is a common practice in mHealth applications and is not always transparent and secure, and users (patients) are often not properly informed about the privacy practices of these applications and the associated privacy risks before installing and using them. Many applications in the field of medicine, health and fitness are opaque in their work and collect user data (including on behalf of hundreds of third parties) and have the potential to share data with third parties, including advertising and tracking services [14]. Many other researchers, including K. Spector-Bagdady and M.M. Mello, point out that there are many cases not only when Internet providers transfer mobile device user data to law enforcement agencies without sufficient legal grounds, but also cases of selling commercially collected user data to third parties [15].

CYBER THREATS IN DIGITAL MEDICINE: THE PROBLEM OF PERSONAL DATA LEAKAGE

Information technologies not only create new opportunities for people, but also generate new threats. The rapid development of telecommunication and computer technologies contributes to the fact that the exchange and processing of information has become more voluminous and easy, respectively, but the problem of protecting personal data from such violations of the rights of their carrier, as information leaks, unauthorized or accidental access to it, illegal copying, destruction, modification, blocking, and distribution is becoming increasingly relevant [16]. O. Omelchenko rightly notes that today, not only is the physical safety of a person relevant, but also their information security, which is becoming even more vulnerable due to the capabilities of the latest technologies [17]. In recent years, not only has the number of Internet users increased, but the form of their connection has also changed (from personal computers and mobile phones to everyday objects, such as «smart» household appliances, vehicles, and other devices connected to the network), which has complicated the procedures for cybersecurity and protecting the privacy of individuals in the digital age [18]. A.V. Musienko and V.V. Musienko hold an almost similar position, since the deepening of the digital transformation of society (for example, «a country in a smartphone»), in their opinion, along with the advantages, also increases the vulnerability of society to cyber threats, which requires strengthening cybersecurity not only in a particular country, but also in a global dimension, since network systems spread throughout the world [19].

The rapid development of information and innovative technologies, in particular in the field of healthcare,

unfortunately leads to the emergence of new illegal ways of obtaining personal and other medical data of an individual, new manifestations of criminal acts, and therefore to the need for permanent updating of methods of countering them. Leakage of medical data is not a new phenomenon, and over the past ten years, the following statistical data can be cited. Thus, one of the world leaders in the field of cybersecurity, Trend Micro Incorporated, conducted an analysis, as a result of which it was confirmed that in 2015 alone, 113.2 million medical records were stolen, which were used by attackers to illegally purchase medicines, commit tax fraud and other illegal actions [20]. In 2017, a ransomware attack (WannaCry) affected more than 200,000 devices in 150 countries, which led to mass chaos and suspension of medical services in many parts of the world [21]. The media also describes Google's attempt to enter the lucrative US healthcare market, namely through Project Nightingale, to obtain and process personal medical data of up to 50 million non-anonymised customers of Ascension, one of America's largest healthcare providers, without notification or consent from patients or their doctors [22].

While the use of digital technologies during the COVID-19 pandemic has undoubtedly had a significant positive impact on healthcare, education, pensions and other social security, this period has also been characterised by an unprecedented surge in cyberattacks, following the increase in the amount of personal data processed as a result of the pandemic. For example, in 2020 alone, more than 400 organisations and 20 million people in the US healthcare system were affected by cyberattacks [23].

In February 2024, Change Healthcare was attacked by ransomware, which resulted in the encryption of files and the theft of protected health information of approximately 190 million people. This leak was the largest leak of medical data (names, contact information, dates of birth, Social Security numbers, and other medical information) in US history and led to a disruption that lasted several weeks and seriously hampered the work of many healthcare institutions [24].

Overall, ransomware attacks have become one of the most serious and widespread threats to the healthcare sector in recent years. Critical patient data, confidential information, and even potentially catastrophic service disruptions are attractive targets for cybercriminals, as noted by R. Chirra [25].

According to Microsoft Threat Intelligence, healthcare was one of the most affected industries last year, and ransomware attacks have increased by 300% in recent years (one of the factors is Russia providing a safe haven for ransomware groups). In 2024, nearly 400 US healthcare facilities were affected by ransomware, causing network outages, offline systems, delays in critical medical procedures, and rescheduled appointments, costing facilities up to $900,000 per day in downtime alone [26].

PECULIARITIES OF ENSURING CONFIDENTIALITY IN THE FIELD OF TRANSPLANTOLOGY AND APPLICATION OF REPRODUCTIVE TECHNOLOGIES

Despite all the positive aspects of the development of the field of transplantology and additional reproductive technologies, the issue of ensuring the confidentiality of personal and other medical data of all participants in medical-legal relations remains important. This issue has repeatedly been the subject of scientific research, and in many cases, scientists focus their attention on the problem of ensuring a balance between the need to form large databases containing primarily medical data of patients and the need to ensure their confidentiality [2].

Protection of private, in particular, personal and medical data of a person is an element of a comprehensive information protection system that must ensure not only the personal security of a person (protect the inviolability of private life), but also maintain a balance of interests of the individual, society and the state in the field of information processing [27]. Society, the state, and each individual, on the one hand, are interested in increasing the amount of medical information, and on the other hand, it is necessary to create appropriate conditions for their collection, storage and protection, primarily in cyberspace. As V.I. Pishta rightly notes, the Ukrainian legislator must develop special legal norms to regulate relations in the field of healthcare, in connection with the use of digital technologies (regarding the encryption of medical data, control of access to information and proper authentication of users, conducting security audits of information systems to identify potential risks), which is necessary to eliminate potential threats to the security of medical information and violation of its confidentiality [28].

Ensuring the confidentiality of medical information, as noted by P.S. Krakhmalov and H.V. Mulyar, is one of the basic principles that contributes to the protection of patients' rights and increases the level of trust in both medical professionals and the health care system as a whole. Without a doubt, confidentiality is a fundamental component of the relationship between a doctor and a patient, and their relationship is based on mutual trust (the patient trusts the doctor if he acts in the best interests of the patient, and the doctor - when the pa-

tient is honest and provides him with all the necessary information to provide proper medical care) [29].

K. Horner and P. Burcher draw attention to the specifics of the trust relationship between the patient and the doctor in the case of the use of additional reproductive technologies, taking into account, first of all, that the surrogate mother must provide prior consent to the disclosure of confidential information about her (contractual waiver of confidentiality), and therefore her ability to be completely open and honest with her doctor in all cases (even regarding accidents or errors) is questioned, based on the fact that the doctor may indicate this in the medical documentation, and as a result, this may be the basis for legal liability for breach of the surrogacy contract. The physician should be aware that this may negatively impact his or her relationship with the patient (even before any misconduct by the patient occurs or without the physician disclosing confidential information) and the quality of care, as the surrogate mother may not provide important information necessary for her care during pregnancy [30].

The issue of the peculiarity of the relationship between the patient and the doctor was also investigated by O.S. Bilanov [31], noting that a person is most vulnerable at the moment when they seek medical help. In his opinion, one of the most vulnerable are patients who seek surrogacy services, since they share all personal information with the doctor regarding the health status of both the man and the woman, and therefore such relationships are based not only on trust, but also require medical professionals to have high moral and ethical standards and clear legal norms aimed at protecting information, based primarily on the need for constant exchange of information between all participants in the surrogacy relationship (biological parents, medical institution and surrogate mother of the child).

The individual's confidence in the confidentiality of one's personal and other medical data, as well as the existence of a trusting relationship, for example, between subjects of medical legal relations, are the determining conditions that encourage an individual to provide permission (informed consent) to receive and further process such data. Many owners of medical data, as scientists note, support the use of their data for research to improve the quality of medical services, at the same time, they are concerned about the possible violation of the confidentiality of their information or improper use or processing of data [32]. Providing an individual's medical data to support scientific research, medical innovations and other initiatives in the field of health care is a voluntary act. However, the provision of such data and their further processing in many cases faces significant difficulties, primarily due to issues of ensuring their confidentiality and security (the most critical concern is related to the potential risk that an individual can be further identified from their data [33]), so reliable guarantees for the protection of the rights of individuals who provide (grant) their medical data are extremely important [34].

The need for a balanced legislative mechanism for providing information and access to it, as well as ensuring the confidentiality of information about a person's health and other information that is a type of information about a person, O.A. Chaban considers as an important element of the implementation of online services and electronic health care, which are components of the development of cooperation and bringing our national legislation into line with European standards in the areas of information society development (Chapter 14) and public health (Chapter 22) [35] of the Association Agreement between Ukraine and the European Union. Cooperation in the field of public health primarily involves increasing the level of security in the field of health care as a prerequisite for sustainable development and economic growth of our state.

Cooperation in the exchange of health data, as well as ensuring their confidentiality and cybersecurity, are crucial conditions for ensuring respect for human rights (primarily in the area of privacy) and providing them with quality medical care. Such cooperation is important in any area of health care, but, as scientists note, in organ transplantation, effective data harmonisation is crucial due to the complex interaction of factors and the need for large, high-quality data sets for accurate analysis of results, which are constantly not only increasing but also improving [36]. It should be noted that the practice of data sharing in medical research is widely supported by governments around the world (including the European Commission, which considers «open access to publications and research data» as a cornerstone of its «Open Science» policy), as well as by numerous international organizations (e.g., the World Medical Association (WMA), the World Health Organization) and national institutions (in particular, the National Institute for Health and Care Research of the United Kingdom and the National Institute of Health of the United States), which proceed from the need for continuous improvement in the field of openness and transparency of research, as well as effective management and exchange of medical data [37].

Despite the huge potential of using various sets (bases) of medical data, primarily in the field of transplantology and the application of reproductive technologies, the following problematic aspects of their formation and application can be identified, namely: the complexity of developing and ensuring a reliable cybersecurity

infrastructure [25]; fragmentation and the presence of other shortcomings in the processes of data generation [36]; the need to ensure confidentiality and protection of personal data, for example, of donors and recipients [38]; the presence of various jurisdictional difficulties regarding the cross-border exchange of medical data [37], the insufficient level of digital literacy of all participants in medical legal relations and a number of other problematic aspects of their formation and application, which were investigated in more detail in one of our scientific works [39].

CONCLUSIONS

Given the continuous process of increasing the use of information and innovative technologies in the field of healthcare, as well as the increase in the volume of personal and other medical data of an individual (and the diversification of their types), it is becoming increasingly difficult to ensure confidentiality and proper protection of such information, primarily from unauthorized disclosure and possible further misuse.

Although the EU, the USA, and many other countries of the world have developed standards (regulations) for the protection of personal data and other confidential information of individuals, including in the field of healthcare, research in this area indicates the difficulty in implementing these standards in practice and the presence of cases of unauthorized collection and dissemination of such data about an individual. This is due, in particular, to the fact that the rapid development of the use of information technologies, especially in the field of healthcare, unfortunately leads to the emergence of new illegal ways of obtaining personal and other medical data of an individual, new manifestations of criminal acts, and based on the scale of the use of medical information, this problem is becoming global.

Despite positive trends in the field of transplantology and reproductive technologies, the problem of maintaining the confidentiality of personal and other medical data of all participants in medical-legal relations remains relevant. This issue is regularly studied by scientists, who usually emphasise the difficulty of achieving a compromise between the need to create large-scale databases with patients' medical information and the need to guarantee their confidentiality. Without a doubt, confidentiality is a fundamental component of the relationship between a doctor and a patient, and their relationship is based on mutual trust, especially in the field of transplantology and the use of additional reproductive technologies, which require medical professionals not only to clearly apply the norms of legislation in this area, but also to adhere to high moral and ethical standards of professional activity, taking into account, first of all, the need for permanent exchange of information between many participants in such special legal relations.

It is necessary to realize that cooperation in the field of exchange of medical data (especially in the field of transplantology and the use of additional reproductive technologies), as well as ensuring their confidentiality and cybersecurity are determining conditions in terms of ensuring compliance with human rights (primarily in the field of privacy) and providing them with high-quality medical care. In addition, a person's confidence in ensuring the confidentiality of their personal and other medical data, as well as the presence of trusting relationships, for example, between subjects of medical legal relations, are determining conditions that encourage a person to provide permission (informed consent) to receive and further process such data. Therefore, the issues of disclosing the essence of legal, organisational and technical methods of ensuring confidentiality and proper protection of medical information remain relevant.

REFERENCES

1. Zaborovskyy V, Ustych O, Popovych T, Manzyuk V. Certain ethical issues that arise when using 3D bioprinting technology. Wiad Lek. 2025;78(4):915-920. doi: 10.36740/WLek/203904. DOI
2. Zaborovskyy VV. Vyklyky ta perspektyvy zastosuvanni innovatsiynykh tekhnolohiy v sferi okhorony zdorov''ya (transplantolohiya i DRT) ta problemy zabezpechennya konfidentsiynosti medychnoyi informatsiyi u voyennyy chas [Challenges and prospects for the application of innovative technologies in the field of health care (transplantation and ART) and the problems of ensuring the confidentiality of medical information in wartime]. Analitychno-porivnyal'ne pravoznavstvo. 2025;2:545-552. doi: 10.24144/2788-6018.2025.02.81. (Ukrainian) DOI
3. Zaborovskyy V, Stupnyk Ya, Hetsko M, Chervko P. Legal conflicts in medical practice and methods of their resolution. Wiad Lek. 2023;76(11):2517-2524. doi: 10.36740/WLek202311128. DOI
4. Zaborovskyy VV, Stoyka AV. Deyaki yurydychni aspekty zastosuvannya tekhnolohiy virtual'noyi real'nosti v psykhoterapiyi [Some legal aspects of the use of virtual reality technologies in psychotherapy]. Zakarpat·s'ki pravovi chytannya: materialy KHII Mizhnarodnoyi naukovo-praktychnoyi konferentsiyi (m. Uzhhorod, 29-30 kvitnya 2020 roku). Uzhhorod: RIK-U. 2020, p.347-354. https://dspace.uzhnu.edu.ua/server/api/core/bitstreams/84e17aba-fc13-44c3-8a06-24e105dd3c75/content [Accessed 30 October 2025] (Ukrainian)
5. Schwab AP, Luu HS, Wang J, Park JY. Genomic privacy. Clin Chem. 2018;64:1696-1703. doi: 10.1373/clinchem.2018.289512. DOI

6. Gupta R, Iyengar R, Sharma M et al. Consumer Views on Privacy Protections and Sharing of Personal Digital Health Information. JAMA Netw Open. 2023. doi: 10.1001/jamanetworkopen.2023.1305. DOI
7. Dobbs v Jackson Women's Health Organization. US. 2022. https://www.supremecourt.gov/opinions/21pdf/19-1392_6j37.pdf [Accessed 30 October 2025]
8. Tighe J, Shand F, Ridani R et al. Ibobbly mobile health intervention for suicide prevention in Australian Indigenous youth: a pilot randomised controlled trial. BMJ Open. 2017. doi: 10.1136/bmjopen-2016-013518 pmid:28132007. DOI
9. Iribarren SJ, Cato K, Falzon L, Stone PW. What is the economic evidence for mhealth? a systematic review of economic evaluations of mhealth solutions. PLoS One. 2017. doi: 10.1371/journal.pone.0170581. DOI
10. EU General Data Protection Regulation. https://gdpr-info.eu/ [Accessed 30 October 2025]
11. Health Insurance Portability and Accountability. Act of 1996. https://aspe.hhs.gov/reports/health-insurance-portability-accountability-act-1996 [Accessed 30 October 2025]
12. US Food and Drug Administration. Guidance on the Postmarket Management of Cybersecurity in Medical Devices. 2016. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/ postmarket-management-cybersecurity-medical-devices/ [Accessed 30 October 2025]
13. Consumer Council of Norway. Out of Control. 2020. https://fil.forbrukerradet.no/wp-content/uploads/2020/01/2020-01-14-out-of-control-final-version.pdf [Accessed 30 October 2025]
14. Tangari G, Ikram M, Ijaz K et al. Mobile Health and Privacy: Cross Sectional Study. BMJ. 2021. doi: 10.1136/bmj.n1248. DOI
15. Spector-Bagdady K, Mello MM. Protecting the Privacy of Reproductive Health Information After the Fall of Roe v Wade. JAMA Health Forum. 2022;3(6). doi: 10.1001/jamahealthforum.2022.2656. DOI
16. Huyvan PD. Pravovi zasady zakhystu personal'nykh danykh [Legal principles of personal data protection]. Nauk. visn. Uzhhor. nats. un-tu. Seriya: Pravo. 2018;51(1):87-91. https://dspace.uzhnu.edu.ua/bitstreams/48e4ff4d-97cd-4c7e-b8fe-005262c48a12/download [Accessed 30 October 2025] (Ukrainian)
17. Omelchenko O. Zakhyst biobankamy personal'nykh danykh donoriv u konteksti pryntsypu vidkrytosti nauky [Omelchenko O. Protection of donors' personal data by biobanks in the context of the principle of open science]. Pidpryyemnytstvo, hospodarstvo i pravo. 2017;12:54-57. http://pgp-journal.kiev.ua/archive/2017/12/13.pdf [Accessed 30 October 2025] (Ukrainian)
18. Mulligan SP, Linebaugh CD. Data Protection Law: An Overview, Congressional Research Service. 2019. https://sgp.fas.org/crs/misc/R45631.pdf [Accessed 30 October 2025]
19. Musienko AV, Musienko VV. Aktual'ni aspekty normatyvno-pravovykh mekhanizmiv zakhystu personal'nykh danykh v elektronnykh medychnykh reyestrakh v Ukrayini [Current aspects of regulatory and legal mechanisms for protecting personal data in electronic medical registers in Ukraine]. Dictum Factum. 2022;1(11):17-22. https://df.duit.in.ua/index.php/dictum/article/view/213 [Accessed 30 October 2025] (Ukrainian)
20. Elektronnyy retsept v Ukrayini – pohlyad yurystiv [Electronic prescription in Ukraine – the view of lawyers]. 2019. https://www.legalalliance.com.ua/novini/elektronnij-recept-v-ukraini-poglad-uristiv/ [Accessed 30 October 2025] (Ukrainian)
21. Tully J, Selzer J, Phillips JP et al. Healthcare challenges in the era of cybersecurity. Health Secur. 2020;18(3): 228-231. doi: 10.1089/hs.2019.0123. DOI
22. Schneble ChO, Elger BS, Shaw DM. Google's Project Nightingale highlights the necessity of data science ethics review. EMBO Mol Med. 2020;12(3). doi: 10.15252/emmm.202012053. DOI
23. Ikeda S. Wave of cyber attacks hits US healthcare system as FBI warns of coordinated criminal campaign. Singapore: CPO Magazine. 2020. https://www.cpomagazine.com/cyber-security/wave-of-cyber-attacks-hits-us-healthcare-system-as-fbi-warns-of-coordinated-criminal-campaign/ [Accessed 30 October 2025]
24. Alder S. Judge Sets Deadline for Motions to Dismiss Claims in Change. Healthcare Data Breach Lawsuits. 2025. https://www.hipaajournal.com/change-healthcare-responding-to-cyberattack/ [Accessed 30 October 2025]
25. Chirra DR. Mitigating Ransomware in Healthcare: A Cybersecurity Framework for Critical Data Protection. Revista de Inteligencia Artificial en Medicina. 2021;12(1):495-513.
26. US Healthcare at risk: Strengthening resiliency against ransomware attacks. 2024. https://www.microsoft.com/en-au/security/security-insider/emerging-threats/US-healthcare-at-risk-strengthening-resiliency-against-ransomware-attacks#footnoteref4 [Accessed 30 October 2025]
27. Yesimov SS. Zakhyst personal'nykh danykh u konteksti rozvytku dynamichnykh system [Personal data protection in the context of the development of dynamic systems]. Naukovyy visnyk derzhavnoho universytetu vnutrishnikh sprav. 2013;3:198-207. http://nbuv.gov.ua/UJRN/Nvlduvs_2013_3_25 [Accessed 30 October 2025] (Ukrainian)
28. Pishta VI. Zakonodavchyy analiz zakhystu medychnoyi informatsiyi u zv"yazku z vykorystannyam tsyfrovykh tekhnolohiy [Legislative analysis of the protection of medical information in connection with the use of digital technologies. Scientific Research and Innovation]. Scientific Research and Innovation: proceedings of the 2nd International Scientific and Practical Internet Conference, April 3-4, 2023. FOP Marenichenko V.V. Dnipro, Ukraine. https://dspace.uzhnu.edu.ua/items/7ef1335d-f859-441c-a499-19490908c58c [Accessed 30 October 2025] (Ukrainian)

29. Krakhmalov PS, Mulyar HV. Pravovi aspekty zabezpechennya konfidentsiynosti medychnoyi informatsiyi v Ukrayini [Legal aspects of ensuring the confidentiality of medical information in Ukraine]. Yurydychnyy naukovyy elektronnyy zhurnal. 2024;6:84-88. doi: 10.32782/2524-0374/2024-6/19 (Ukrainian) DOI
30. Horner C, Burcher P. A surrogate's secrets are(n't) safe with me: patient confidentiality in the care of a gestational surrogate. J Med Ethics. 2021;47(4):213-217. doi: 10.1136/medethics-2017-104518. DOI
31. Bilanov OS. Pravove rehulyuvannya medychnoyi tayemnytsi u dohovirnykh vidnosynakh surohatnoho meterynstva [Legal regulation of medical secrecy in contractual relations of surrogacy]. Aktual'ni problemy vitchyznyanoyi yurysprudentsiyi. 2022;4:18-23. https://repository.pdmu.edu.ua/500 [Accessed 30 October 2025] (Ukrainian)
32. Liu Y, Yang C, Liu Q et al. PDPHE: Personal Data Protection for Trans-Border Transmission Based on Homomorphic Encryption. Electronics. 2024;73(10):1-23. doi: 10.3390/electronics13101959. DOI
33. Voigt TH, Holtz V, Niemiec E et al. Willingness to donate genomic and other medical data: results from Germany. Eur J Hum Genet. 2020;28(8):1000-1009. doi: 10.1038/s41431-020-0611-2. DOI
34. Gaballah SA, Abdullah L, Alishahi M et al. Anonify: Decentralized Dual-level Anonymity for Medical Data Donation. Proceedings on Privacy Enhancing Technologies (PETS). 2024;3:94-108. doi: 10.56553/popets-2024-0069. DOI
35. Chaban OA. Pravo fizychnoyi osoby na tayemnytsyu pro stan zdorov"ya v Ukrayini [The right of an individual to confidentiality about health status in Ukraine]: dys. … kand. yuryd. nauk. Kyyiv, 2018:222 https://www.researchgate.net/publication/371206051_Pravo_fizicnoi_osobi_na_taemnicu_pro_stan_zdorova_v_Ukraini [Accessed 30 October 2025] (Ukrainian)
36. Hosseini SA, Kazemzadeh R, Foster BJ et al. New Tools for Data Harmonization and Their Potential Applications in Organ Transplantation. Transplantation. 2024;108(12):2306-2317. doi: 10.1097/TP.0000000000005048. DOI
37. Hammond-Browning N, Williams NJ. Ethical data management and sharing in uterus transplantation – reflections and recommendations. International Legal and Ethical Perspectives on Uterus Transplantation. Elgar Studies in Health and the Law Cheltenham. 2024. doi: 10.4337/9781803920498.00021. DOI
38. Chornenka DS. Biomedychni ta etychni pryntsypy transplantatsiyi v konteksti zakhystu prav lyudyny ta zastosuvannya Konventsiyi OV"YEDO [Biomedical and ethical principles of transplantation in the context of human rights protection and application of the Convention on the Elimination of All Forms of Discrimination against Women]. Naukovyy visnyk Uzhhorods'koho Natsional'noho Universytetu. Seriya PRAVO. 2023;80(1):141-150. doi: 10.24144/2307-3322.2023.80.1.20. (Ukrainian) DOI
39. Zaborovsky VV. Problemni aspekty formuvannya ta zastosuvannya medychnykh baz danykh v umovakh tsyfrovoyi transformatsiyi sfery okhorony zdorov"ya [Problematic aspects of the formation and application of medical databases in the context of digital transformation of the healthcare sector]. Innovative Research in Science and Economy: Collection of Scientific Papers with Proceedings of the 1st International Scientific and Practical Conference (Brussels (Belgium), July 30 – August 1, 2025). Brussels: International Science Unity. 2025, pp.108-110. https://isu-conference.com/wp-content/uploads/2025/07/Brussels_Belgium_30.07.25.pdf [Accessed 30 October 2025] (Ukrainian)

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR:
Viktor Zaborovskyy
Uzhhorod National University
14 Universytetska St, 88000 Uzhhorod, Ukraine
e-mail: zaborovskyviktor@gmail.com

ORCID AND CONTRIBUTIONSHIP
Viktor Zaborovskyy: 0000-0002-5845-7535 A B D
Vasyl V. Manzyuk: 0000-0003-2133-1573 B D
Vasyl P. Fennych: 0000-0003-2649-1322 F
Stanislav Shchoka: 0000-0002-7165-2191 B D

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

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CASE STUDY

CONTENTS

Spontaneous coronary artery dissection following induced vaginal delivery

Agata Stawska, Szymon Kaczyński, Małgorzata Głogiewicz, Rafał Adamczak
DEPARTMENT AND CLINIC OF OBSTETRICS, GYNECOLOGICAL DISEASES AND ONCOLOGIC GINECOLOGY, JAN BIZIEL UNIVERSITY HOSPITAL No. 2 IN BYDGOSZCZ, BYDGOSZCZ, POLAND

ABSTRACT

Cardiovascular diseases represent a significant threat to the health and life of women during pregnancy and the postpartum period, accounting for 13% of all maternal deaths and constituting the third most common cause of perinatal mortality. A particular cause of myocardial infarction in young women, including pregnant patients, is spontaneous coronary artery dissection (SCAD).The occurrence of spontaneous coronary artery dissection may be preceded by triggering factors such as intense physical exertion, intense Valsalva manoeuvres, emotional or physical stress, labour, as well as exposure to exogenous hormones and β hCG. Despite the increasing number of reported cases, the true incidence of pregnancy related SCAD remains unknown. The presented clinical case describes an acute coronary syndrome in a 32-year-old woman in the early postpartum period, secondary to spontaneous coronary artery dissection. The patient was qualified for urgent coronary angiography, which revealed critical, ninety-percent stenosis of the left anterior descending coronary artery (LAD). Following percutaneous transluminal coronary angioplasty, effective myocardial reperfusion was achieved. Young women with low cardiovascular risk and without clinically significant risk factors may develop acute myocardial ischaemia.

KEY WORDS: acute myocardial ischaemia, spontaneous coronary artery dissection, postpartum period

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INTRODUCTION

Cardiovascular diseases represent a significant threat to the health and life of women during pregnancy and the postpartum period, accounting for 13% of all maternal deaths and constituting the third most common cause of perinatal mortality [1]. One of the most serious cardiovascular complications is myocardial infarction. According to the European Society of Cardiology, and in line with the "Fourth Universal Definition of Myocardial Infarction" (2018), myocardial infarction is defined as the presence of acute myocardial injury confirmed by abnormal biochemical markers in a clinical setting consistent with acute myocardial ischaemia [2]. A particular and diagnostically challenging cause of myocardial infarction in young women, including pregnant patients, is spontaneous coronary artery dissection (SCAD). By definition, spontaneous dissection of an epicardial coronary artery is not associated with atherosclerosis or injury [3]. The incidence of SCAD still remains underestimated; however, it is estimated to account for 1-4% of acute coronary syndrome cases [3]. The most frequently identified risk factor is fibromuscular dysplasia (FMD) - a nonatherosclerotic, noninflammatory vascular disease characterised by abnormal arterial wall growth. FMD is diagnosed in up to 72% of SCAD cases [4]. The occurrence of spontaneous coronary artery dissection may be preceded by triggering factors such as intense physical exertion, intense Valsalva manoeuvres, emotional or physical stress, labour, as well as exposure to exogenous hormones and βhCG. Despite the increasing number of reported cases, the true incidence of pregnancyrelated SCAD remains unknown. A review of the existing literature indicates 510 described cases of this complication up to 2023 [5]. Given the serious clinical consequences and diagnostictherapeutic challenges, SCAD in the peripartum period deserves particular attention.

CASE REPORT

We present the case of a 32-year-old patient admitted electively to the Pregnancy Pathology Unit due to gestational diabetes treated with diet at 39+0 weeks of

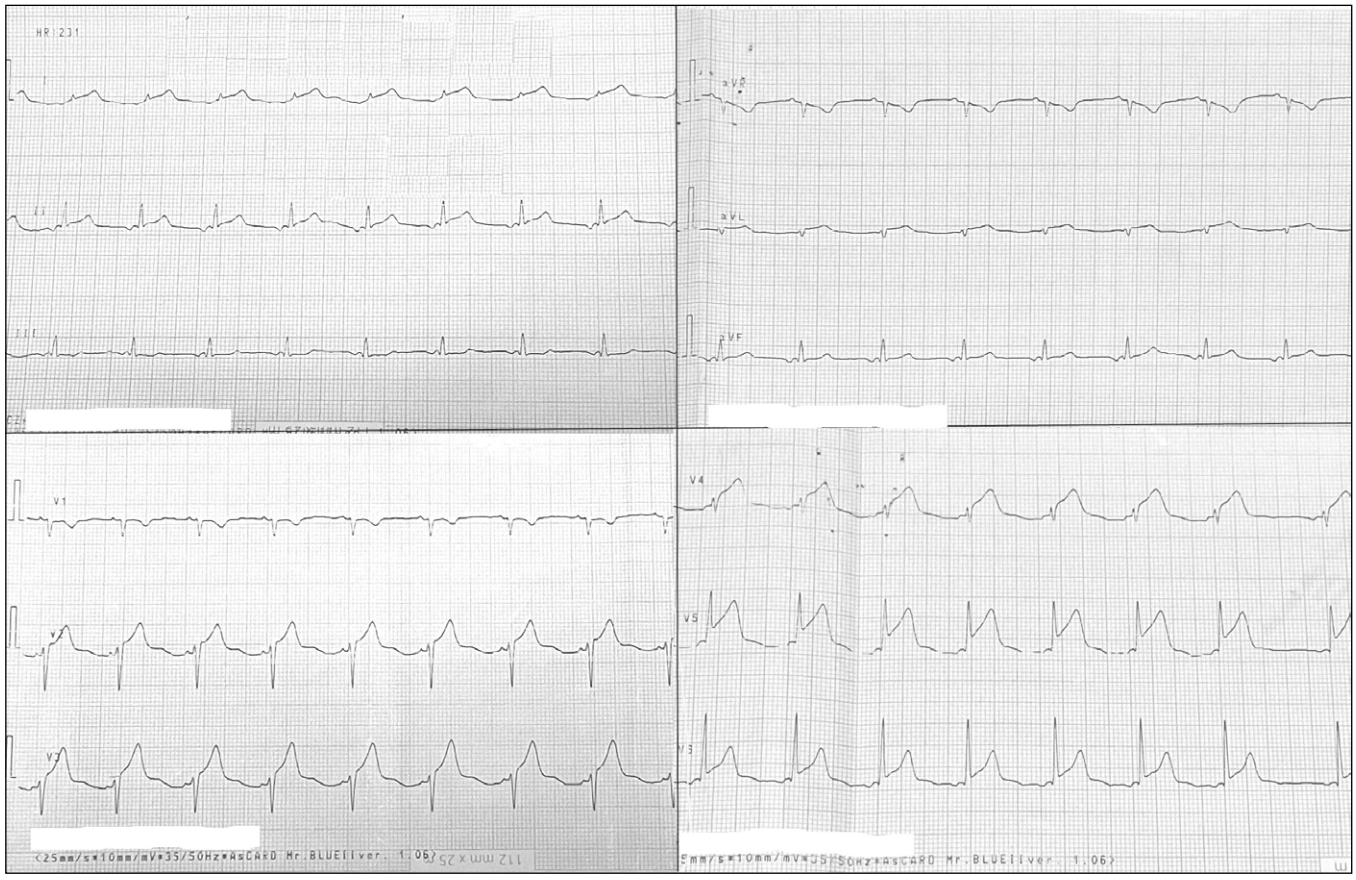

Fig. 1. Follow up electrocardiogram showing Pardee wave in precordial leads
Source: Own materials

gestation. The pregnancy was additionally complicated by well-controlled hypothyroidism. Her obstetric history included one uncomplicated vaginal delivery three years earlier. The patient was qualified for induction of labour with 5 units of oxytocin. Labour analgesia was provided with remifentanil at a rate of 0.1 mg/hour. The first stage of labour lasted 1 hour and 45 minutes, and the second stage lasted 6 minutes. A live term male infant was delivered, with a birth weight of 3200 g and an Apgar score of 10. Due to incomplete delivery of the placenta, uterine curettage was performed. A vaginal mucosal tear was sutured. Peripartum blood loss was estimated at 300 ml.

Twelve hours after delivery, the patient began reporting chest pain, nausea, and vomiting. Additional diagnostics revealed ST-segment elevation in leads V5 - V6 on electrocardiography (ECG) and elevated high-sensitivity troponin T (hsTnT) levels of 18 ng/l, forming the basis for diagnosing acute myocardial injury. A followup ECG demonstrated evolution of changes into Pardee waves over the anterior wall leads and elevated N-terminal pro-B-type natriuretic peptide (NT pro-BNP) levels of 208 pg/ml (Fig. 1).

Computed tomography angiography of the chest excluded pulmonary embolism. The patient was transferred to the Cardiology Clinic for further management, where she was qualified for urgent coronary angiography. This revealed critical, ninety-percent stenosis of the left anterior descending coronary artery (LAD) in segment s7 with a contrast defect suggesting thrombus. The vessel lumen was significantly constricted, with possible stenosis in segment s8 **(**Fig. 2, 3). Percutaneous transluminal coronary angioplasty (PTCA) was performed without complications, with implantation of five drug-eluting stents (DES), covering the dissection proximal to the stents and achieving reperfusion with optimal residual stenosis (Fig. 4).

Myocardial infarction secondary to spontaneous coronary artery dissection was suspected. After PTCA, persistent distal vasospasm was observed without visible dissection at the distal stent edges. Dual antiplatelet therapy (DAPT) after percutaneous coronary intervention (PCI) was initiated in accordance with current guidelines, using ticagrelor and acetylsalicylic acid (ASA). Effective myocardial reperfusion was achieved three hours after symptom onset. Pharmacological suppression of lactation with cabergoline was necessary to initiate optimal treatment. On the first day after PCI, transthoracic echocardiography (TEE) revealed akinesia of the apex of the heart and adjacent segments of the

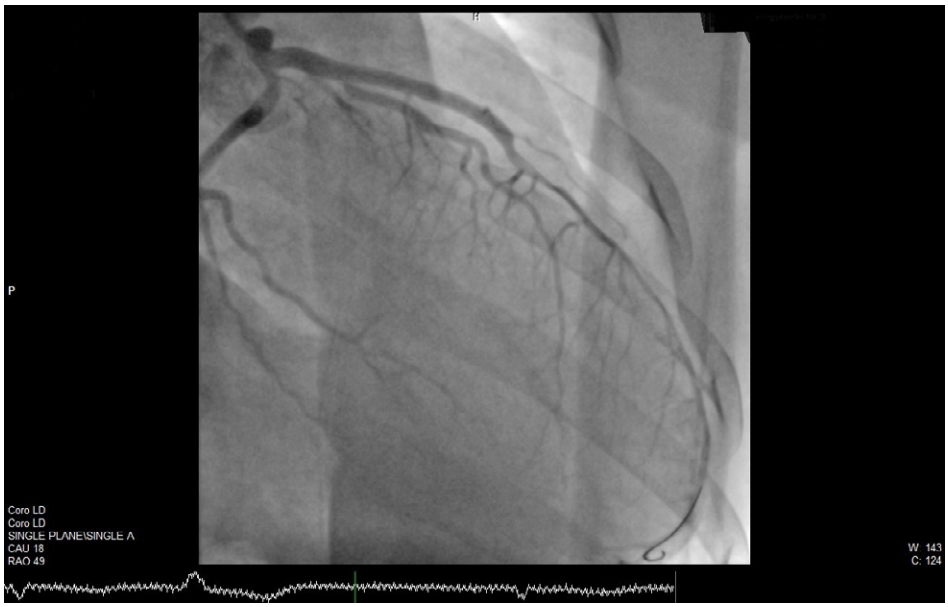

Fig. 2. Coronary angiography – LAD dissection in s7 with critical stenosis
Source: Own materials

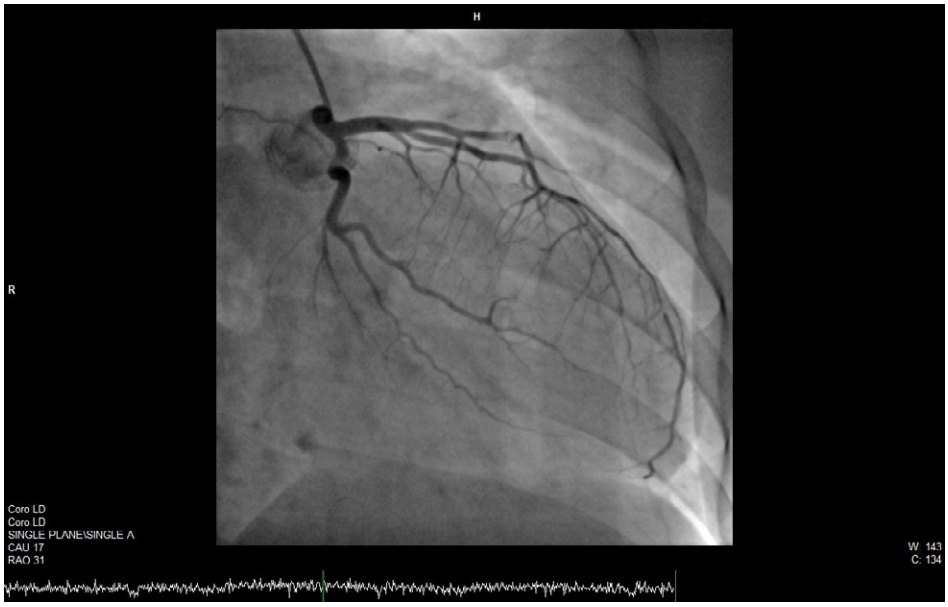

Fig. 3. Coronary angiography – LAD dissection in s7 with critical stenosis
Source: Own materials

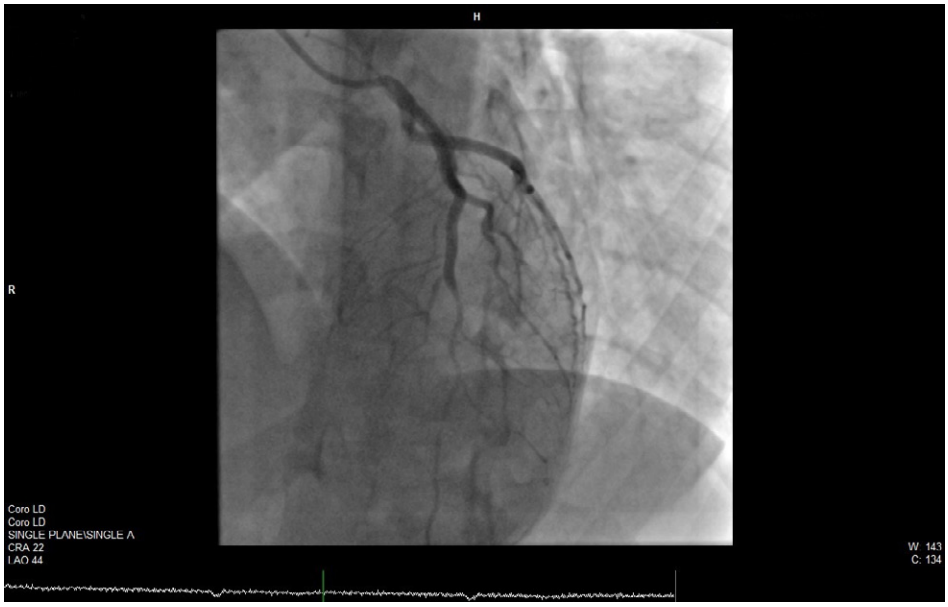

Fig. 4. Coronary angiography after percutaneous coronary intervention
Source: Own materials

anterior, inferior, and interventricular septal walls, as well as the midsegments of the anterior wall and septum, with hyperkinesia of the remaining left ventricular segments. Left ventricular ejection fraction (LVEF) was estimated at 48%.

Additionally, separation of pericardial layers was noted (in parasternal long-axis view, PLAX) up to 2 mm behind the posterior wall of the left ventricle, up to 5 mm at the apex, and in the subcostal view anterior to the right ventricle. Cardiac chamber dimensions and wall thickness remained unchanged. Follow-up hsTnT levels increased to 5583 ng/l. Mixed hyperlipidaemia was diagnosed, with elevated lowdensity lipoprotein cholesterol (LDL-C) of 152 mg/dl and triglycerides (TG) of 190 mg/dl. In subsequent days of hospitalization, significant progression of pericardial effusion was observed, up to 6 mm in PLAX behind the posterior wall of the left ventricle and 10 mm at the apex, without signs of cardiac chamber compression.

Treatment for pericarditis was initiated with non-steroidal anti-inflammatory drugs and colchicine, in accordance with current guidelines [7], resulting in reduction of effusion in followup TEE. Due to suspicion of myocardial infarction secondary to spontaneous coronary artery dissection and further propagation of dissection during the procedure, follow-up coronary angiography was performed, confirming optimal PTCA outcome, excluding DES thrombosis, and demonstrating proper healing of dissection at distal stent edges without impaired flow and with vessel lumen relaxation. The patient was qualified for further conservative management. She has remained under cardiology outpatient followup for one year, with no further cardiac events.

DISCUSSION

Among pregnant and postpartum women, myocardial infarction (PAMI) is a significant cause of morbidity and mortality. Its incidence is difficult to estimate. Based on a 2017 metaanalysis by Gibson et al., PAMI occurs at a rate of 3.34 per 100,000 pregnancies, with a maternal mortality rate of 0.20 per 100,000 pregnancies.[8] Acute myocardial infarction (AMI) occurs more frequently in pregnant women than in non-pregnant women of reproductive age, with hormonal and haemodynamic changes typical of pregnant patients considered the primary contributors. Additional risk factors include maternal age, hypertension, gestational diabetes, smoking, and thrombophilia [9, 10]. Spontaneous coronary artery dissection is recognised as the most common cause of myocardial infarction in pregnant and postpartum women, despite its rarity in the general population [3, 5, 11, 12]. This may be related to hormonal changes occurring during pregnancy, particularly in the peripartum period, which affect oestrogen and progesterone receptors in coronary vessels. These changes may weaken the arterial wall and lead to vessel injury, resulting in clinical symptoms [3, 11].

Two types of SCAD are distinguished. Spontaneous coronary artery dissection occurring during pregnancy or within 12 weeks postpartum is defined as pregnancy-associated SCAD (P-SCAD). In contrast, spontaneous coronary artery dissection unrelated to pregnancy (NP- SCAD) may occur at any time in a woman's life [5]. In the presented case, apart from gestational diabetes, no identifiable risk factors for acute myocardial ischaemia were present. The patient was young and had no pre-existing comorbidities. It is possible that the cumulative hormonal changes of a second pregnancy contributed to weakening of the coronary arterial wall and the onset of myocardial ischaemia. The occurrence of P-SCAD may be associated with very high progesterone levels and rapid hormonal fluctuations in the peripartum period [5, 13]. Additionally, both physical and emotional stress associated with labour may further contribute to AMI. The timing of symptom onset in this case confirms that the early postpartum period is associated with increased risk of complications, particularly when pregnancy is completed by caesarean section or when labour is complicated by postpartum haemorrhage. Most acute cardiac events associated with P-SCAD described in the literature occurred in the third trimester or within 30 days postpartum [5, 14]. According to Saw et al., as many as 64% of patients with coronary artery dissection developed symptoms immediately before delivery or within one week postpartum [15]. Due to a nonspecific chest or back pain and to the young age and absence of typical risk factors, acute myocardial ischaemia may not be initially suspected in patients with SCAD. Symptoms such as chest or back pain in pregnant or postpartum women should prompt clinical vigilance, and ECG should be performed. In this case, ECG demonstrated ST-segment elevation in leads V5 - V6, and troponin T levels were elevated. In an analysis of 120 P-SCAD cases by Havakuk et al., 75.5% of patients presented STsegment elevation myocardial infarction, and 72% of SCAD cases were located in the left anterior descending artery [16]. A more common ST-segment elevation in ECG is caused by more frequent changes in left coronary artery, which may result in more extensive myocardial injury [17]. In the presented case, critical occlusion of the left anterior descending artery was diagnosed during urgent coronary angiography, and five stents were implanted. Coronary angiography remains the gold standard for diagnosing coronary artery disease, with the additional advantage of enabling therapeutic interventions such

as angioplasty or stenting. Intravascular ultrasound or optical coherence tomography may be used during angiography for more precise diagnosis, particularly when SCAD is suspected [18–20]. Appropriate diagnostic planning and timely treatment in this case enabled rapid reperfusion of the occluded vessel. SCAD management includes conservative (watch-fulwaiting) strategies, pharmacotherapy as an adjunct to conservative management, revascularisation, or coronary stenting. Stenting is recommended in cases of significantly occluded coronary arteries, haemodynamic instability, persistent chest pain, or persistent ST-segment elevation [5]. Unfortunately, in SCAD, stenting may extend the dissection, impair healing, or contribute to vessel occlusion [3, 21]. It should also be noted that patients with P-SCAD are at higher risk of treatment failure and complications such as coronary artery bypass grafting (CABG), cardiogenic shock, mechanical circulatory support, or maternal death [5, 16]. Therefore, appropriate treatment selection and long-term follow- up are crucial. In the presented case, early pericarditis developed after acute myocardial ischaemia, as pericardium responded to standard therapy. No longterm complications occurred, and echocardiography demonstrated a left ventricular ejection fraction above 48%. The patient remains under long-term follow-up.

CONCLUSIONS

Young women with low cardiovascular risk and without clinically significant risk factors may, under the influence of multiple factors inherent to the peripartum period, develop acute myocardial ischaemia. Women in the postpartum period are particularly exposed to the risk of spontaneous coronary artery dissection due to intense physical exertion, emotional stress, and Valsalva manoeuvres during labour. The presented case highlights the need for close monitoring of maternal wellbeing in the early postpartum period and prompt response to any concerning symptoms. It also indicates the importance of close interdisciplinary collaboration to ensure the highest standard of perinatal care.

REFERENCES

1. Knight M, Kenyon S, Brocklehurst P, et al. Saving lives, improving mothers' care: lessons learned to inform future maternity care from the UK and Ireland confidential enquiries into maternal deaths and morbidity 2009-2012. MBRRACE-UK: Saving Lives, Improving Mothers' Care, 2023.
2. Thygesen K, Alpert JS, Jaffe AS, et al. ESC Scientific Document Group , Fourth universal definition of myocardial infarction. Eur Heart J. 2019;40,(3):237-269, doi: 10.1093/eurheartj/ehy462. DOI
3. Hayes SN, Kim ESH, Saw J, et al. American Heart Association Council on Peripheral Vascular Disease; Council on Clinical Cardiology; Council on Cardiovascular and Stroke Nursing; Council on Genomic and Precision Medicine; and Stroke Council. Spontaneous Coronary Artery Dissection: Current State of the Science: A Scientific Statement From the American Heart Association. Circulation. 2018;8;137(19):e523-e557. doi: 10.1161/CIR.0000000000000564. DOI
4. Saw J, Aymong E, Sedlak T, et al. Spontaneous coronary artery dissection: association with predisposing arteriopathies and precipitating stressors and cardiovascular outcomes. Circ Cardiovasc Interv. 2014;7(5):645-55. doi: 10.1161/CIRCINTERVENTIONS.114.001760. DOI
5. Zeven K. Pregnancy-Associated Spontaneous Coronary Artery Dissection in Women: A Literature Review. Curr Ther Res Clin Exp. 2023; 1; 98: 100697. doi: 10.1016/j.curtheres.2023.100697. DOI
6. Valgimigli M, Bueno H, Byrne RA, et al. 2017 ESC focused update on dual antiplatelet therapy in coronary artery disease developed in collaboration with EACTS: The Task Force for dual antiplatelet therapy in coronary artery disease of the European Society of Cardiology (ESC) and of the European Association for Cardio-Thoracic Surgery (EACTS). Eur Heart J. 2018; 39(3), 213-260. doi: 10.1093/eurheartj/ehx419. DOI
7. Adler Y, Charron P, Imazio M, et al. ESC Scientific Document Group, 2015 ESC Guidelines for the diagnosis and management of pericardial diseases: The Task Force for the Diagnosis and Management of Pericardial Diseases of the European Society of Cardiology (ESC) Endorsed by: The European Association for Cardio-Thoracic Surgery (EACTS). Eur Heart J. 2015; 36(42), 2921–2964. doi: 10.1093/eurheartj/ehv318. DOI
8. Gibson P, Narous M, Firoz T, et al. WHO Maternal Morbidity Working Group. Incidence of myocardial infarction in pregnancy: a systematic review and meta-analysis of population-based studies. Eur Heart J Qual Care Clin Outcomes. 2017; 3(3), 198-207. doi: 10.1093/ehjqcco/qcw060. DOI
9. Smilowitz NR, Gupta N, Guo Y, et al. Acute myocardial infarction during pregnancy and the puerperium in the United States. Mayo Clin Proc. 2018; 93(10), 1404-1414. doi: 10.1016/j.mayocp.2018.04.019. DOI
10. Ladner HE, Danielsen B, Gilbert WM. Acute myocardial infarction in pregnancy and the puerperium: a population-based study. Obstet Gynecol. 2005; 105(3): 480–484. doi: 10.1097/01.AOG.0000151998.50852.31. DOI
11. Elkayam U, Jalnapurkar S, Barakkat MN, et al. Pregnancy-associated acute myocardial infarction: a review of contemporary experience in 150 cases between 2006 and 2011. Circulation. 2014;129(16):1695-1702. doi: 10.1161/CIRCULATIONAHA.113.002054. DOI

12. Edupuganti MM, Ganga V. Acute myocardial infarction in pregnancy: Current diagnosis and management approaches. Indian Heart J. 2019;71(5):367-374. doi: 10.1016/j.ihj.2019.12.003. DOI
13. Maas A, Bouatia-Naji N, Persu A, et al. Spontaneous coronary artery dissections and fibromuscular dysplasia: Current insights on pathophysiology, sex and gender. Int J Cardiol. 2019; 286: 220-225. doi: 10. 1016/j.ijcard.2018.11.023. DOI
14. Vijayaraghavan R, Verma S, Gupta N, et al. Pregnancy-related spontaneous coronary artery dissection. Circulation. 2014;130:1915-1920. doi: 10.1161/CIRCULATIONAHA.114.011422. DOI
15. Saw J, Starovoytov A, Humphries K, et al. Canadian spontaneous coronary artery dissection cohort study: In-hospital and 30-day outcomes. Eur Heart J. 2019; 40(15): 1188-1197. doi: 10.1093/eurheartj/ehz007. DOI
16. Havakuk O, Goland S, Mehra A, et al. Pregnancy and the risk of spontaneous coronary artery dissection: an analysis of 120 contemporary cases. Circ Cardiovasc Interv. 2017;10:e004941. doi: 10.1161/CIRCINTERVENTIONS.117.004941. DOI
17. Tweet MS, Hayes SN, Codsi E, et al. Spontaneous coronary artery dissection associated with pregnancy. J Am Coll Cardiol. 2017;25;70(4):426-435. doi: 10.1016/j.jacc.2017.05.055. DOI
18. Ismail S, Wong C, Rajan P, et al. ST-elevation acute myocardial infarction in pregnancy: 2016 update. Clin Cardiol. 2017;40(6):399-406. doi: 10.1002/clc.22655. DOI
19. Amsterdam EA, Wenger NK, Brindis RG, et al. 2014 AHA/ACC guide line for the management of patients with non-ST-elevation acute cor onary syndromes: a report of the American College of Cardiology/ American Heart Association Task Force on Practice Guidelines. Circulation. 2014;130:e344-e426. doi: 10.1016/j.jacc.2014.09.017. DOI
20. O'Gara PT, Kushner FG, Ascheim Deborah D, et al. 2013 ACCF/AHA guideline for the management of ST-elevation myocardial infarction: executive summary: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Circulation. 2013;127:529-555. doi: 10.1016/j.jacc.2012.11.018. DOI
21. Cade JR, Szarf G, de Siqueira ME, et al. Pregnancy-associated spontaneous coronary artery dissection: Insights from a case series of 13 patients. Eur Heart J Cardiovasc Imaging. 2017;18(1):5461. doi: 10.1093/ehjci/jew021. DOI

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Agata Stawska
Department and Clinic of Obstetrics,
Gynecological Diseases and Oncologic Ginecology,
Jan Biziel University Hospital No. 2 in Bydgoszcz,
Bydgoszcz, Poland
e-mail: akowalewska89@gmail.com

ORCID AND CONTRIBUTIONSHIP

Agata Stawska: 0000-0002-5265-4415 A B C D
Szymon Kaczyński: 0000-0003-3227-3292 B C D
Małgorzata Głogiewicz: 0000-0001-6770-2064 B C D
Rafał Adamczak: 0000-0001-7479-5940 E F

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

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CASE STUDY

CONTENTS

Reconstruction of the hip stump

Yurii O. Bezsmertnyi, Viktor I. Shevchuk, Dmytro V. Bondarenko, Olexandr Y. Branitsky
NATIONAL PIROGOV MEMORIAL MEDICAL UNIVERSITY, VINNYTSIA, UKRAINE

ABSTRACT

The formation of a functional limb stump after combat injuries with extensive soft tissue damage is challenging. After amputations or revision surgeries, long femoral stumps may remain. Although functionally inferior to tibial stumps, they can still provide satisfactory movement and performance. However, prolonged prosthesis use or poor-quality muscle grafting can lead to complications, such as skin breakdown, trophic issues, and inability to continue prosthetic use. These problems are partly due to the mismatch between the area of the bone stump end and the applied body weight.

Ilizarov compression-distraction osteosynthesis, radiographic assessment, and gait analysis before and after treatment using the GaitRite system.

We describe a case of femoral stump reconstruction following a mine-blast injury. Prosthetic fitting was complicated by massive scars covering the bone fragments. A method was developed to expand the distal end of the femoral stump via distraction osteogenesis using the Ilizarov apparatus. This approach created a stable platform with a significantly larger bearing surface. For the first time, the bearing surface area was increased twofold, reducing soft tissue trauma and enhancing the load-bearing capacity of the prosthesis.

The proposed reconstruction method significantly enlarges the distal femoral stump area, creates a mushroom-shaped end, decreases load per unit area, ensures even pressure distribution within the prosthetic socket, and improves the patient's functional outcome.

KEY WORDS: prosthetics, Ilizarov technique, bearing surface, distraction method, hip amputation, increase in bearing surface area

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INTRODUCTION

The main requirements for good prosthetics are maximum lever strength and pressure on the end of the cushion, which brings walking in the process closer to natural walking by transmitting information through the sensory nervous system. In addition, stump support is the most effective counteraction to the development of osteoporosis [1].

Transfemoral amputation is one of the most extensive types of amputation during war and peace. In the immediate and long term, it dramatically affects the quality of life, including phantom and local pain, leg and joint pain [2-5]. Existing studies on amputation surgery are more concerned with transtibial amputations [6,7]. They pay special attention to bone synostosis to reduce the pressure in the receiving sleeve, eliminate pain syndrome, and maximize the load of the distal part for the purpose of force closure between the stump and the receiving sleeve [7,8]. By increasing the bearing surface, a more even pressure distribution in the contact surface of the prosthesis can be achieved.

According to [9,10], prosthetic comfort depends on the pressure distribution at the interface between the prosthetic socket and the residual limb end. Uneven pressure distribution on the residual limb causes residual limb end pain, damage to the dermofibrous lining, and decreased motivation to use the prosthesis. The rejection rate due to high residual limb pressure is 60 [11]. These data indicate the need to find ways to increase the support surface of the femoral stump end.

There is no information in the literature about the prospects of improving the functional qualities of the residual limb in transfemoral diaphyseal amputation.

CASE REPORT

PATIENT AND STUDY DESIGN

A 27-year-old male combatant, previously in good health, with no associated diseases, received multiple wounds to the right shin, foot and knee joint as a result of a mine explosion. During the evacuation stages,

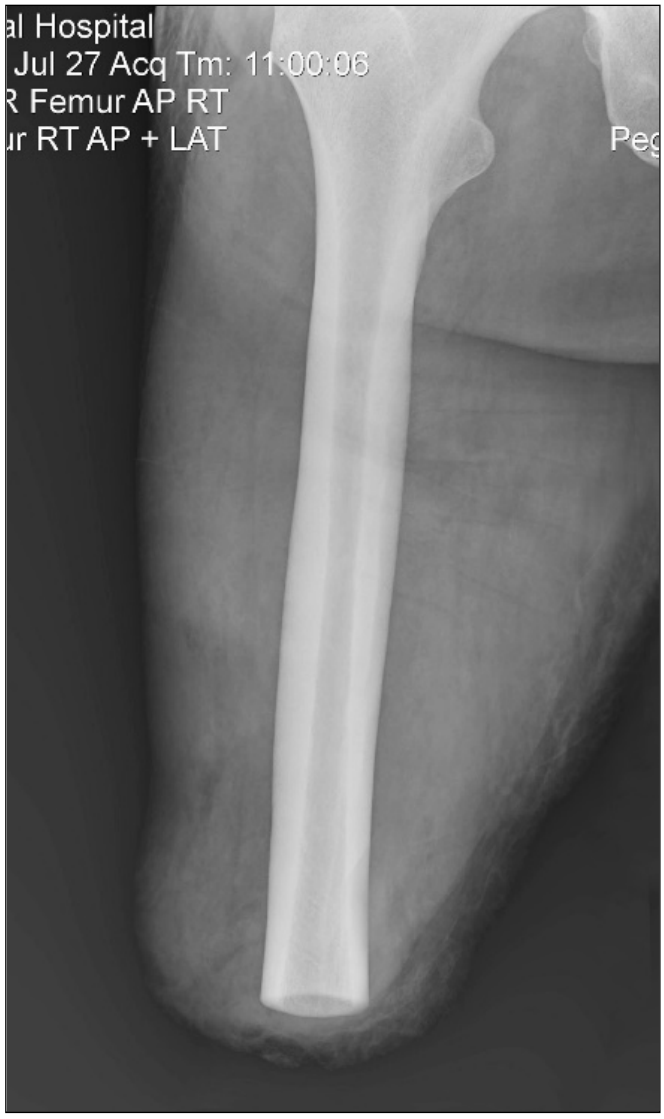

Fig. 1. Radiograph of the hip stump before surgery
Picture taken by the authors

the lower third of the thigh was amputated. Due to extensive suppuration a month later, reamputation was performed at the border of the lower and middle thirds of the thigh. (Fig. 1). The wound healed with secondary healing. After 4.5 months, primary prosthetics was performed. At first he used crutches, and after 34 days he started walking with a cane. Periodically the scar surface of the residual limb became inflamed due to penetration by the end of the residual limb into the bone. Walking in the prosthesis was accompanied by pain and inflammation.

Written informed consent was obtained from the patient for the case details and images to be published.

METHODS

Complete blood count, urine test, calcium, albumin, phosphorus, magnesium, parathyroid hormone, vitamin D, X-ray of the stump upon admission, during reconstruction and at specific intervals, ultrasound to determine the area of the support surface, assessment of gait parameters in the prosthesis on the Gaitrite biomechanical track (UK) before surgery and in the long term. Osteotomy of the stump bone, application of the Ilizarov apparatus (compression, distraction, fixation), anaesthetic for 2 days after surgery and for 8-9 days after the start of distraction, calcium, vitamin D.

CASE PRESENTATION

After preparing the surgical field, two cross pins were inserted into the proximal femoral metaepiphysis perpendicular to the limb axis, taking into account the anatomy of the sciatic nerve. 4 cm below the pins in the sagittal plane, a 3.5 mm diameter self-tapping rod was inserted perpendicular to the femur. The pins and rod are fixed in two rings of the device. The rings are connected to each other by threaded rods. The femur was isolated by a flap incision of the skin, subcutaneous tissue and fascia. Scar tissue was removed. The pointed bone was rounded and shortened by 1 cm. After perineural injection of 1% novocaine solution, the sciatic, saphenous and posterior femoral nerves were shortened. In the cortical layer, 4 linear periosteum incisions were made proximally from the end of the bone stump in accordance with the directions of the planned corticotomies. Three bone and periosteum plates measuring 2.5 x 0.5 cm and 1.5 mm thick were formed. Two oblique corticotomies of the femur were made from the end of the stump in the proximal direction at an angle of 45 ° to the outer and inner cortical layer with an oscillating saw. The latter were broken with a bit. 2 bone and periosteum fragments in the form of triangles 2.5 cm long and 0.9 cm wide were formed. The resulting bone and periosteum plates were placed on the mother bed and temporarily fixed with a bone holder.Under the control of an electron-optical transducer, 2 parallel pins with stop pads 10 mm apart were passed through the proximal parts of the formed grafts and the mother bone in the frontal plane, without reaching the bone with 4 mm of stop pads. A cannulated drill with a diameter of 3 mm was used to make channels in both grafts and the mother bone along the pins. The pins with stop pads were pulled to the bone and fixed in threaded brackets. In this way, the proximal parts of the grafts are fixed, which will prevent their axial displacement during distraction. Two pins with stop pads were passed through the distal parts of the formed grafts at an angle of 30° to the bone axis. Proximally, the pins are fixed in the tension rods and to the overlying apparatus with the help of attachments and brackets. Distally, the pins are

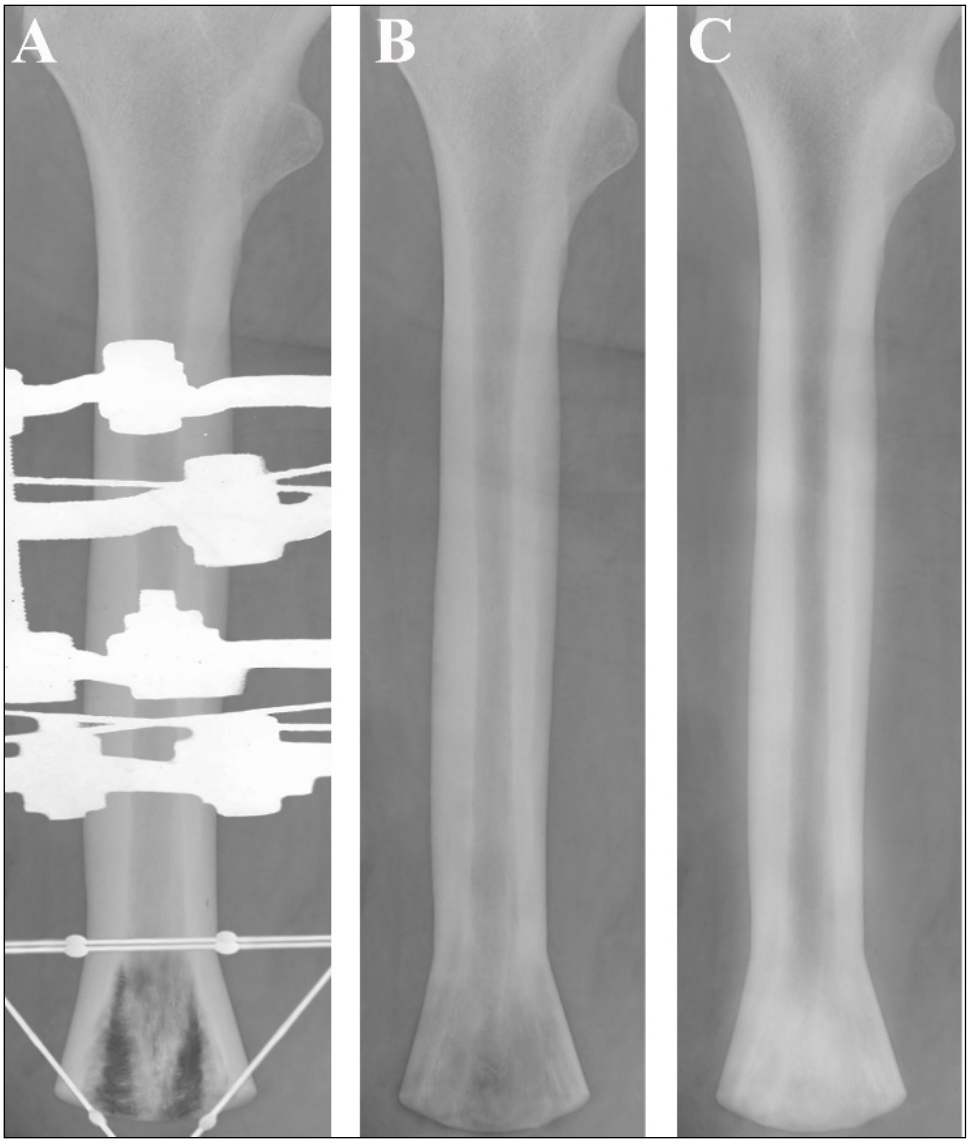

Fig. 2. Radiographs of the hip stump: A) during distraction, B) 1 year after surgery, C) 4 years after surgery
Picture taken by the authors

fixed to the anterior half-ring of the apparatus with the help of pins clamps (not quite rigidly, so that the pins can easily slip into the clamp during future distraction). The half-ring is rigidly connected to the above rings using threaded brackets (Fig. 2A).

The postoperative wound is closed. Classically, according to Ilizarov, the distraction rate should correspond to 1 mm per day, which in most cases allows to obtain a regenerate. In this case, since 2 oblique osteotomies were performed, distraction was performed in fractions of 0.25 mm 4 times a day for 30 days. As a result, endosteal-periosteal regenerates are formed due to abundant vascularization. Vascularization is carried out due to the vascular network of soft tissues, periosteum and medullary vessels, including a nutricia.

After 7 days the one-hour dosovana distraction of both grafts was started on the string-pulls for 30 days. Then the apparatus was set for fixation until full mineralization of the regenerates. After 65 days the apparatus was removed.

Radiography was performed once every 12 days during the distraction period and once a month during the fixation period. The magnitude of diastasis and the nature of the course of osteogenesis were determined.

At X-ray examination after 12 days, signs of periosteal callus with cloud-like shadows were detected at the point of contact along the edges of the grafts. After 30 days of distraction (day 37 after surgery), cloud-like shadows of medium intensity were detected over the entire area of the distraction regenerate. After one month of fixation (2 months after surgery), the regenerate was filled with a homogeneous shadow of high intensity. By the end of fixation (3 months after surgery), fusion of bone trabeculae was observed. A cortical-diaphyseal layer was formed along the edges of the regenerates, which indicated the maturity of the distraction regenerates. The bone stump acquired the appearance of a fungus. The area of the supporting surface of the femur before the operation was 4.0 cm2, and after the operation – 10.9 cm^2.

Table 1. Patient's walking parameters before and after treatment according to the GaitRite system.

	Before treatment			After 1 year			After 4 year		
	healthy	**prosthetics**	**Dif**	**healthy**	**prosthetics**	**Dif**	**healthy**	**prosthetics**	**Dif**
Step Time (sec)	1.34	1.19	0.15	0.66	0.71	0.05	0.61	0.59	0.02
Cycle Time (sec)	1.32	1.93	0.61	1.19	1.36	0.17	1.12	1.09	0.03
Step Length (cm)	34.71	14.36	20.35	51.91	45.74	6.17	55.78	50.9	4.88
Stride Length (cm)	82.23	74.95		86.47	80.18		104.31	103.65	
Single Support (%GC)	73.1	25.8		39.6	33.2		41.4	39.7	
Double Support (%GC)	44.2	97.8		36.8	40.3		24.3	26.1	
Mean Normalized Velocity	0.57			0.83			1.06		
FAP Scope	53			88			91		

Source: compiled by the authors of this study

BEFORE SURGERY

On admission to the clinic, the presence of an amputation stump at the border of the middle and lower thirds was noted. On the end surface of the residual limb there is an extensive scar with an area of 14.2 cm2 fused to the bone fillet penetrating the tissue. The muscles on the lateral and anteroposterior surfaces are fused to the scar, tightened proximally so that the residual limb has acquired a cone-shape. During active and passive movements in the hip joint, pain syndrome occurs in the stump of the sciatic nerve and the scar tissue on the end of the stump. On radiographs, the femoral stump shows moderate osteoporosis.

AFTER SURGERY

An educational and training prosthesis and then a permanent prosthesis were made.

The patient was examined one year (Fig. 2B) and 4 years (Fig. 2C) after surgery. After 4 years, the femoral stump is moderately conical in shape. The skin is normal in color. Uses a prosthesis with a rigid receiving cavity with a contact bottom. The entire surface of the residual limb, including its end, is in direct contact with the rigid walls and the bottom of the receiving sleeve. When standing on "both legs", he loads the prosthesis like a healthy leg, feeling stability and no pain. Gait is rhythmic and stable. The stride size of the healthy and prosthetic limbs is almost identical. Walking on level and uneven surfaces, on inclines, climbing stairs is performed without restrictions. The patient can withstand direct loading while standing on the residual limb without the prosthetic socket. The turn of the prosthetic foot corresponds to the position of the foot of the healthy limb. Radiologically, the shape of the bone stump end is preserved. The remodeling of the bone tissue of the residual limb is complete. The area of the supporting surface of the residual limb is 10.9 cm^2.

The patient's walking parameters were measured using the GaitRite system. The examination was performed when the patient came to the clinic (before surgery) and during control examinations after 1 and 4 years. The results of the patient's walking parameters before and after treatment are shown in Table 1.

Prior to treatment, the patient had a significant limp, which was confirmed by a significant asymmetry of the time and geometric parameters of the steps.

The difference in foot support time (Step Time) was 0.15 s with a noticeable decrease in the duration of support on the prosthesis base. The difference in the duration of steps (Cycle Time) was 0.61 s, with a clear increase in the duration of the prosthetic limb step. The largest difference of 20.35 cm was in the Step Length, with the prosthetic limb's step being only 14.36 cm.

The time of support (Single Support) on the foot of the prosthetic limb was 2.8 times shorter (25.8%) than on the opposite foot (73.1%), the same proportion differed in the index of double support (Double Support), that

Fig. 3. Patient with prosthesis
Picture taken by the authors

is, when the healthy limb was the support, the duration of support increased by 2.2 times (97.8%). According to the study, the average normalized speed was 0.57. The FAP Scope functional capacity index was 53 points.

Such a pronounced asymmetry of the patient's walking was caused by the presence of pain at the end of the stump.

In 1 year after the reconstruction of the stump, a significant improvement in the quality of walking was noted. The time of support decreased by half to 0.66 s for the healthy and 0.71 s for the prosthetic limb with a difference of 0.05 s. The step duration also normalized due to a decrease in the parameter of the prosthetic limb to 1.36 seconds and became more symmetrical. The length of steps decreased, but still remained noticeable – 6.17 cm. The length of the short step increased to 51.91 cm for the healthy and 45.74 cm for the prosthetic limb, the length of the long step also increased, although not as noticeably, by an average of 5 cm. The proportional indicators of single and double support became more symmetrical compared to the pre-treatment data. The overall FAP Scope score was 80 points.

After 4 years, the walking performance for both limbs became almost symmetrical. The length of steps and the duration of support became the same, the difference was 0.02 and 0.03, respectively. A slight difference of 4.88 cm remained in the length of the short step, but the length of the long step became the same and increased significantly to 103.65 cm. The percentages of the stride parameters - single and double support - have equalized. The speed of movement almost doubled. At the end of the rehabilitation period, the functional capacity index FAP was 91 points, which corresponds to the indicators of healthy people. The results obtained indicate the formation of a stable walking pattern and the feasibility of performing such operations.

The positive dynamics of walking recovery was obtained, due to the elimination of pain and a significant increase in the area of the bearing surface.

The patient works as a security guard. He walks 12-13 km per day (Fig. 3).

When determining the indications for lower limb prosthetics, one of the most important factors is the mental and physical condition of the patient. A healthy psyche, positive attitude and motivation contribute to a speedy prosthesis and recovery. Somatic conditions (cardiovascular disorders, paralysis, urinary and fecal incontinence) require an individualized approach and sometimes even refusal of prosthetics. In the absence of mental and physical impediments, the most important factor for successful prosthetics is the suitability of the residual limb for prosthetics.

Formerly painful scars are caused by secondary wound healing when the underlying tissues are involved in the inflammatory process. They are easily traumatized and ulcerated when using prostheses. In cases where the residual limb is of sufficient length and painful scars are combined with other pathologies (neuroma, high muscle location, osteophyte), reimputation is indicated. Retraction of muscles and their attachment to the skin scar occurs due to insufficient attention to the muscle suture. The muscles truncated during amputation or reamputation contract and fuse with the scar, thus obtaining a false point of attachment. As a result, muscle strength decreases and the residual limb loses functionality. The muscles fused with the scar do not perform their inherent functions. The energy of their contraction is spent unproductively.

Knee disarticulation stands apart. On the one hand, it has disadvantages due to the fear of complications with wound healing and problems with prosthetics due to the club-like shape [12,13]. On the other hand, the high energy efficiency of such a residual limb is very attractive. The residual limb after disarticulation allows direct load transfer, which is a physiologic method of weight bearing, and has a larger surface area than a diaphyseal residual limb [14]. In order to reduce clubbing, it has been proposed to reduce the volume of the residual limb by trimming the medial, lateral, and posterior outgrowths and removing the patella cup, which somewhat simplifies prosthetics.

Unfortunately, in military settings, localization of the wound does not always allow for knee disarticulation.

In addition, after unilateral disarticulation in the knee joint, it is not possible to position the center of rotation of the knee joint of the prosthesis in accordance with the center of rotation of the same joint of the healthy leg, so it is necessary to prescribe heavy low-functional prostheses with superimposed splints.

The suitability of the residual limb is understood as painlessness, moderately conical shape, the condition of the adjacent muscles, the ability to enter into proper interaction of the prosthesis with full contact of the residual limb over the entire surface of the socket. The most valuable qualities are leverage strength and the ability to fully load the end of the residual limb. Transfemoral diaphyseal amputations are performed in 78%.

In modern prostheses, it is the end surface of the residual limb that requires close contact and maximum support, which makes prosthetics closer to natural walking. Attempts to relieve the end surface by transferring the support to the area above the residual limb result in impaired venous and lymphatic outflow. At the same time, the bearing capacity of the end surface is the only way to counteract the development of osteoporosis and to maximize walking in the prosthesis.

To a certain extent, the amount of loading depends on the soft tissue at the residual limb end and the quality of the prosthetic socket. The surgeon's task is to perform muscle grafting for both function and soft tissue framing of the end bone surface. In addition to closing the residual limb, muscle plasty involves volume reduction, contour correction, and preservation of antagonist agonist muscle tone [14-16].

In total-contact prosthetics, preserving the ability to perceive the limb requires their tension to be at least 61% [16].

The advantage of the proposed method is to reduce the load in the prosthesis on the distal fossa and to compensate for the loss of mechanical load. Certainly, this can be achieved after disarticulation when bearing capacity is preserved or with femoral shortening osteotomy. Unfortunately, the researchers did not study the effect of prosthetics on the nature of clinical and functional problems, although they note that the patient's mobility index was significantly higher after knee disarticulation compared to transfemoral amputation.

The use of the proposed method of reconstruction of the diaphyseal femoral stump is based on the creation of compression-distraction forces in the places of contacts of the formed grafts with the parent bone and dosed displacement of bone fragments in the frontal plane.

At the stage of compression the creation of permanent immobility at the junction of the grafts with the maternal bed is a necessary condition for the formation of bone fusion due to proliferating skeletogenic tissue. Distraction begins in the period of the greatest reparative reaction before the beginning of ossification of the interlayer. Fractional dilatation of the device subsystems by 0.5 mm per day on each side leads to stretching of connective tissue bridges formed during the compression period. During the distraction period (40 days), the new formation of transversely oriented bone beams continues at the border between the bone sections of the regenerate and the connective tissue layer. This leads to the formation of large-filament spongy bone

with a lamellar structure. Periosteal bone formation due to osteoperiosteal plates plays a definite positive role in this process [6]. The maturation of fibrous structures and their replacement by newly formed bone (ossification) increases. Completion of ossification, sufficient volume and density of the regenerate allow removing the Ilizarov apparatus and starting functional rehabilitation. It continues until the reorganization processes ensure the formation of an organotypic structure of the newly formed end of the bone stump capable of providing the possibility of functional loading.

All forces of a static or dynamic nature between the patient and the prosthesis are transferred through the socket-stump contact surface [7,8]. Theoretically, the pressure can be minimized by increasing the surface area of the residual limb end - creating a maximum support surface, since pressure (P) equals force/area (P=F/A). The task of the surgeon and prosthetist is to achieve a uniform pressure distribution in the contact surface of the prosthesis [7,8]. The possibility of full contact increases the hydrostatic pressure in the receiving sleeve and improves the distribution of body weight on the prosthesis. The more intensively the distal part of the residual limb is loaded, the more reliable is the "force closure" between the residual limb and the prosthesis socket, the more painless is walking on the prosthesis. A study of walking dynamics has shown that reducing pressure at the end of the residual limb promotes symmetry of joint moments, which approximates the walking behavior of humans without amputation [18]. According to [19] the skin and soft tissues of the residual limb form an important contact with the socket of the prosthesis, and the shape of the bone residual limb end plays a leading role.Therefore, it is important to perform muscle plasty with miodesis. In order to close potentially important areas for prosthetics, it is necessary to preserve the tone of the agonist-antagonist muscles. Such tensioning is only possible with total-contact prosthetics, which was seen in this case.

A well-formed, painless, moderately conical stump with an enlarged oval bone end was an indication for the fabrication of a rigid socket. The rational shape of the receiving sleeve must meet certain clinical and biomechanical requirements: 1) not to allow concentrated pressure on some parts of the residual limb and not to leave others unloaded, 2) to provide conditions for the functioning of the residual limb muscles, not to compress vessels and nerves, 3) to prevent the formation of soft tissue roll in the area of the adductor muscles and perineum, not to allow venous stasis, chafing, naminas. 4) limit displacement of the femur in the soft tissues of the residual limb, reduce piston-like movements and phenomena of external-internal instability, 5) prevent rotation of the sleeve relative to the residual limb, especially in the rolling phase, and do not impede normal sitting.

One of the advantages of the proposed method is that it eases the load on the distal sulcus and provides a faster walk [17]. The distribution of force over a larger surface area protects against soft tissue destruction. We agree with the statement [17] that the preservation of muscle strength plays an important role after transfemoral amputation, which allows for gait with minimal deviations. An increase in the area of the bone bearing surface on the receiving sleeve compensates for the loss of mechanical load transfer by 30% [20,21].

The functional difference of the proposed method is the development of a rollback force during a fast walk.

The technique can be used in reconstructive interventions after traumatic and oncological amputations in young and middle-aged people who want to lead an active lifestyle associated with a profession that requires prolonged movement during the day. In the case of vascular diseases and in the older age group, such interventions are risky. In the postoperative period, infectious complications are possible - inflammation of the tissues near the studs. In this case, the staple is removed and another staple is performed in healthy tissues. In this observation, there were no complications.

The criteria for restrictions before surgery are: stumps after amputation due to thrombobliterative vascular diseases, stump osteomyelitis, pustular skin inflammation, insufficient mastery of the external fixation device, the need for skin treatment around the stump, purulent-inflammatory skin diseases, diabetes mellitus, taking glucocorticosteroids, bisphosphonates within the last 3 months, chronic kidney disease, hyperthyroidism and hypothyroidism, chronic heart failure, tuberculosis, systemic diseases.

Potential limitations are associated with prolonged use of external fixation devices. This is the risk of infection and inflammation of the soft tissues around the spikes, their eruption and delayed union, which depends on the nature of the bone structure, its vascularization, and the selected distraction speed.

CONCLUSIONS

Thus, the use of the developed method of reconstruction of the distal part of the femoral stump will allow to significantly increase its area, create a mushroom-like shape of the end of the stump, reduce the load per unit area, achieve a uniform distribution of pressure in the prosthesis sleeve, and increase the patient's functional capabilities.

REFERENCES

1. Shevchuk V, Bezsmertnyi Y, Branitsky O et al. Remodeling of the fibula stump after transtibial amputation. Orthop Res Rev. 2024; 16:153-62. doi:10.2147/ORR.S459927. DOI
2. Felder JM 3rd, Skladman R. Translating technique into outcomes in amputation surgeries. Mo Med. 2021;118(2):141-6.
3. Bosse MJ, Morshed S, Reider L et al. Transtibial amputation outcomes study (TAOS): Comparing transtibial amputation with and without a tibiofibular synostosis (Ertl) procedure. J Orthop Trauma. 2017;31(1):63-9. doi: 10.1097/BOT.0000000000000791. DOI
4. Hobusch GM, Döring K, Brånemark R, Windhager R. Advanced techniques in amputation surgery and prosthetic technology in the lower extremity. EFORT Open Rev. 2020;26;5(10):724-41. doi: 10.1302/2058-5241.5.190070. DOI
5. Tirrell AR, Kim KG, Rashid W et al. Patient-reported outcome measures following traumatic lower extremity amputation: A systematic review and meta-analysis. Plast Reconstr Surg Glob Open. 2021;9(11):e3920. doi: 10.1097/GOX.0000000000003920. DOI
6. Bezsmertnyi YO, Shevchuk VI, Bezsmertnyi OY et al. Bone bridge transtibial amputation by an innovative technique. JAAOS Glob Res Rev. 2024;8:e24.00063. doi: 10.5435/JAAOSGlobal-D-24-00063. DOI
7. Shevchuk V, Bezsmertnyi Y, Bezsmertnyi O, Branitsky O. Ertl surgery on the short fibula stump after war injury. Med Glas (Zenica). 2024;21(2):379-84. doi: 10.17392/1744-21-02. DOI
8. Mollaee S, Fuentes-Aguilar RQ, Huegel JC et al. A pneumatic reconfigurable socket for transtibial amputees. Int J Numer Method Biomed Eng. 2024;40(2):e3801. doi: 10.1002/cnm.3801. DOI
9. Ali S, Abu Osman NA, Eshraghi A et al. Interface pressure in transtibial socket during ascent and descent on stairs and its effect on patient satisfaction. Clin Biomech. 2013;28(9-10):994-9. doi: 10.1016/j.clinbiomech.2013.09.004. DOI
10. Rajtukova V, Hudak R, Zivcak J et al. Pressure distribution in transtibial prostheses socket and the stump Interface. Procedia Eng. 2014;96:374-81. doi:10.1016/j.proeng.2014.12.106. DOI
11. Baars EC, Schrier E, Dijkstra PU, Geertzen JHB. Prosthesis satisfaction in lower limb amputees. Medicine (Baltimore). 2018;97(39):e12296. doi: 10.1097/MD.0000000000012296. DOI
12. Murakami T, Murray K. Outcomes of knee disarticulation and the influence of surgical techniques in dysvascular patients: A systematic review. Prosthet Orthot Int. 2015;40(4):423–435. doi: 10.1177/0309364615574163. DOI
13. Albino FP, Seidel R, Brown BJ et al. Through knee amputation: technique modifications and surgical outcomes. Archives Plastic Surg. 2014;41(5):562–70. doi: 10.5999/aps.2014.41.5.562. DOI
14. Pinzur MS. Outcomes-oriented amputation surgery. Plast Reconstr Surg. 2011;127:241-7. doi: 10.1097/PRS.0b013e318200a409. DOI
15. Firth GB, Masquijo JJ, Kontio K. Transtibial Ertl amputation for children and adolescents: a case series and literature review. J Child Orthop. 2011;5(5):357-62. doi: 10.1007/s11832-011-0364-0. DOI
16. Song H, Israel EA, Gutierrez-Arango S et al. Agonist-antagonist muscle strain in the residual limb preserves motor control and perception after amputation. Commun Med (Lond). 2022;2:97. doi: 10.1038/s43856-022-00162-z. DOI
17. Kingsbury T, Thesing N, Collins JD et al. Do patients with bone bridge amputations have improved gait compared with patients with traditional amputations? Clin Orthop Relat Res. 2014;472(10):3036-43. doi:10.1007/s11999-014-3617-7. DOI
18. Alhossary A, Ang WT, Chua KSG et al. Identification of secondary biomechanical abnormalities in the lower limb joints after chronic transtibial amputation: A proof-of-concept study using SPM1D Analysis. Bioengineering (Basel). 2022;9(7):293. doi:10.3390/bioengineering9070293. DOI
19. Bramley JL, Worsley PR, Bader DL et al. Changes in tissue composition and load response after transtibial amputation indicate biomechanical adaptation. Ann Biomed Eng. 2021;49(12):3176-88. doi: 10.1007/s10439-021-02858-0. DOI
20. Russell EE, MillerRH. Maintenance of muscle strength retains a normal metabolic cost in simulated walking after transtibial limb loss. PLOS ONE. 2018;13(1):e0191310. doi: 10.1371/journal.pone.0191310. DOI
21. Jiang WB, Sun SZ, Li C et al. Anatomical basis of the support of fibula to tibial plateau and its clinical significance. J Orthop Surg Res. 2021;16(1):346. doi: 10.1186/s13018-021-02500-8. DOI

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Viktor I. Shevchuk
National Pirogov Memorial Medical University
56 Pirogova St, 21018 Vinnytsia, Ukraine
e-mail: shevchukndiri@gmail.com

ORCID AND CONTRIBUTIONSHIP
Yurii O. Bezsmertnyi: 0000-0002-1388-7910 A B D
Viktor I. Shevchuk: 0000-0003-1105-4795 A B D E F
Dmytro V. Bondarenko: 0000-0001-8305-5899 B D E
Olexandr Y. Branitsky: 0000-0003-0507-3092 B D E

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

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