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Modern approach to monitoring and preventing osteoporosis development in breast milk donors

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Abstract. The study of bone tissue status in lactating women is highly relevant due to the increasing prevalence of osteopenic changes and their complex implications for reproductive health. The aim of this study was to analyse breast milk donation as an additional risk factor and to identify key conditions for preserving bone strength in the postpartum period. Within a retrospective analysis conducted at the Kyiv City Perinatal Centre, medical records of 260 women were examined, including 126 milk donors and 134 breastfeeding women without donation. The study assessed bone mineral density, haematological parameters, body mass index, and fracture history. Participants were recruited through healthcare networks under conditions of informed consent and personal data confidentiality. Key findings indicated that donors had a higher incidence of fractures after 30 years of age (11.9%), lower awareness of bone health (only 7.9% knew their bone mineral density), and significant deviations in vitamin D levels, with deficiency detected in 67.5% of donors. Among donors, 29.4% were overweight, and 13.5% had obesity, exceeding rates in non-donor lactating women. Over 10% of donor participants exhibited radiological signs of osteoporosis, compared to approximately 5% in the control group. Prolonged lactation without adequate dietary adjustments and physical activity was found to exacerbate osteopenic processes. The study underscored the importance of regular monitoring of biochemical markers and implementing preventive strategies (balanced nutrition, vitamin D supplementation, and moderate resistance training) to mitigate complications. The practical significance of these findings lies in justifying the need for early screening of donors and developing personalised recommendations to reduce fracture risk and preserve maternal health. The proposed approach could be integrated into broader women's health programmes, particularly in modern medical communities seeking to reduce economic burdens associated with fracture treatment and prolonged rehabilitation

Keywords: hormonal changes; genetic predisposition; physical activity; vitamin D deficiency; lactation

✦ INTRODUCTION

Modern realities necessitate maximising health protection for women who choose to become breast milk donors. This process entails additional physiological strain, particularly on bone tissue. Osteoporosis may develop asymptotically but significantly impact a woman's future quality of life. Thus, timely monitoring of bone health and preventive measures are essential to safeguard donors' well-being and ensure safe, high-quality lactation for infants receiving donor milk.

V. Bila *et al.* [1] described the creation of Ukraine's first breast milk bank, highlighting the problems of a shortage of donors and the need to involve not only mothers of premature babies (who accounted for 77%) but also women who had carried their pregnancies to term. They concluded that the bank works effectively even in difficult conditions, but the limited number of donors remains a challenge. I. Pylypchuk [2] summarised data on osteoporosis in women with hypoestrogenic conditions, which is also relevant

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to lactational osteoporosis. The researcher emphasises the high prevalence of osteopenia and osteoporosis and identifies key preventive measures: building maximum bone mass by the age of 30, regular physical exercise, correction of hormonal disorders, improvement of blood supply to bones, and control of mineral metabolism.

S. Shatylo *et al.* [3] conducted a multicentre epidemiological study revealing widespread vitamin D deficiency in Ukraine, particularly among high-risk groups such as postpartum women. Among 11,000 participants (~79% women, median age 36), deficiency rates were alarmingly high. The authors suggested this could adversely affect donors' skeletal systems, warranting further research into seasonal variations and supplementation efficacy. Q. Wang *et al.* [4] investigated calcium-fortified milk for osteoporosis prevention in ovariectomised rats (a postmenopausal model). After three months of consuming milk enriched with calcium, vitamin D, and casein phosphopeptides, bone mineral density (BMD) and mechanical strength improved significantly.

I. Oboh *et al.* [5] analysed lactation duration's impact on BMD. Women breastfeeding beyond six months exhibited lower lumbar BMD at 12 months postpartum compared to those nursing for ≤ 4 months, though statistical significance varied. G. Cai *et al.* [6] meta-analysed five RCTs ($n = 567$) and found that 1,000 mg/day calcium supplementation during lactation did not significantly affect spinal or radial BMD. H.L. Colleran *et al.* [7] demonstrated that a 16-week postpartum exercise programme (weight-bearing aerobics and resistance training) significantly reduced lumbar bone loss in a small cohort. F.M.F. Grizzo *et al.* [8] proposed trabecular bone score (TBS) for postpartum bone assessment. While TBS was insensitive to short-term changes, it confirmed post-weaning bone recovery.

The study by Q. Huang *et al.* [9] was aimed at developing an innovative method for osteoporosis treatment using milk-derived exosomes. The researchers employed specially engineered exosomes enriched with bioactive compounds that promote bone tissue regeneration. Additionally, these exosomes were equipped with markers for visualisation via magnetic resonance imaging, enabling real-time monitoring of the treatment process. The study results demonstrated that such exosomes could effectively increase bone mineral density, facilitate bone regeneration, and simultaneously improve osteoporosis diagnostics. In the work by B. Yun *et al.* [10], the researchers investigated the effect of bovine milk-derived exosomes on osteoporosis prevention. They conducted an experiment on mice with induced osteoporosis and found that the exosomes contributed to reduced bone mass loss, improved bone mineralisation, and normalisation of gut microbiota. The researchers hypothesised that these exosomes could potentially be used for osteoporosis prevention in humans.

Thus, while a substantial number of studies have been dedicated to osteoporosis and breast milk donors, insufficient research has focused on monitoring and preventing osteoporosis development in breast milk donors, necessitating the current study. The objective of this work was to investigate physiological changes during lactation and determine the peculiarities of milk donation. To achieve this goal, the following research tasks were performed: conducting a comparative analysis of bone mineral density indicators, biochemical parameters, and anthropometric characteristics in breast milk donors versus breastfeeding

women who were not donors to identify differences in osteoporosis risk levels; assessing the influence of hormonal-metabolic factors (prolactin, oestrogens, parathyroid hormone, calcitonin) and nutritional status (calcium, vitamin D, protein intake, etc.) on the development of osteopenic and osteoporotic changes under varying durations and volumes of lactation; and developing evidence-based recommendations for the prevention and monitoring of bone tissue status in breast milk donors based on the results of a comprehensive risk assessment (anthropometric, biochemical, hormonal, and functional).

✦ MATERIALS AND METHODS

This study had a cross-sectional design and covered the period from January 2023 to January 2025. Data collection was conducted at the Kyiv City Perinatal Centre. Contact details of the female participants were obtained through a network of healthcare institutions collaborating with the breast milk bank at the Kyiv City Perinatal Centre. All participants provided informed consent under conditions of personal data confidentiality and anonymity. Initially, a search and analysis of medical records were performed for patients aged 18 to 45 who were under observation at the institution during 2023-2024 and met predefined inclusion criteria: at least six months of breastfeeding experience within the past year, confirmed or current breast milk donation (for the main group), and availability of complete data on anthropometric measurements, densitometry results, and biochemical parameters in the medical records. Exclusion criteria included diagnosed osteoporosis or other metabolic bone disorders, use of medications significantly affecting bone tissue metabolism (bisphosphonates, glucocorticoids, etc.), and pregnancy at the time of the study. Ultimately, 260 medical records were selected, of which 126 belonged to women who were breast milk donors and 134 to breastfeeding women who did not donate. The mean age of participants in the main group was 29.1 ± 4.8 years, while that of the control group was 30.4 ± 5.2 years; the average total lactation duration (including active donation periods) among donors reached 18.7 ± 6.3 months, compared to 15.4 ± 5.7 months in non-donor breastfeeding women.

The study employed a range of methods to ensure a comprehensive assessment of bone tissue status and associated risk factors. Firstly, the patients' medical records contained densitometry results obtained using the portable ultrasound densitometer Sunlight MiniOmni™ (Israel), with a subset of women additionally undergoing forearm dual-energy X-ray absorptiometry (DXA) to verify and refine diagnostic parameters. Both methods provided a T-score, comparing the subjects' bone mineral density with reference values for healthy young women. Secondly, laboratory test results were extracted from medical records: levels of 25-hydroxyvitamin D, calcium, phosphorus, osteocalcin, and type I procollagen N-terminal propeptide (P1NP), which reflect bone metabolism. Blood samples were collected in a certified laboratory using validated diagnostic kits for each assay. Thirdly, anthropometric measurements (weight, height) and the derived body mass index (BMI) were included, along with documented data on lactation duration, reproductive health status, and the frequency and volume of blood donation, as recorded in the relevant sections

of the medical records. Thus, by analysing the available parameters, an extended profile was obtained regarding nutrition, physical activity levels, harmful habits, and other lifestyle factors potentially influencing bone health.

During data interpretation, quantitative T-score values were considered (≥ -1 = normal, -1 to -2.5 = osteopenia, ≤ -2.5 = osteoporosis), alongside deviations of laboratory markers from their reference ranges. The numerical data were cross-referenced with clinical information from patient records (family history, prior fractures, harmful habits, lactation duration, etc.) to identify potential associations. Where significant differences were observed, conclusions were drawn regarding the possible role of lactation and blood donation in increasing the risk of osteopenia or osteoporosis. The study adhered to the ethical standards outlined in the Declaration of Helsinki [11] and current national legislation [12, 13]. All women whose data were included provided informed consent for the use of

their medical records for research purposes, and personal identifiers were anonymised during data processing.

RESULTS

According to the systematic review and meta-analysis by N. Salari *et al.* [14], the global prevalence of osteoporosis in adults prior to 2021 was 18.3%, with rates of 23.1% in women and 11.7% in men. In Ukraine specifically, the probability of fractures was 5.5% at age 40, 11% at 75 years, and slightly decreased to 10% after 90 years [15]. Insufficient timely diagnosis and prevention may lead to significant deterioration in quality of life and increased economic burden associated with fracture treatment. In this study, all ($n = 260$) women underwent data collection on osteoporosis risk factors, analysis of their impact on bone tissue status, and development of individualised prevention recommendations. The relevant information extracted from their medical records is presented in Table 1.

Table 1. Documented risk factors for osteoporosis in breast milk donors and breastfeeding non-donor women according to medical records ($n = 260$)

Parameter (as per medical records)	Option	Donor group (n = 126)	Breastfeeding non-donors (n = 134)
Family history of osteoporosis	Yes	45	40
	No	63	70
	Unknown	18	24
Relative diagnosed with osteoporosis	Mother	20	18
	Father	10	8
	Maternal grandmother	15	12
	Maternal grandfather	5	3
	Paternal grandmother	12	10
	Paternal grandfather	3	2
	Sibling	8	6
	Other	2	1
Fractures after the age of 30	Yes	15	10
	No	111	124
Awareness of personal bone mineral density (BMD) value	Yes	10	15
	No	116	119
Documented T-score (DXA/US), total 126 and 134 individuals	≥ -0	20	35
	-0.1 to -0.9	50	55
	-1 to -1.4	25	24
	-1.5 to -1.9	17	10
	-2 to -2.4	9	7
	≤ -2.5	5	3
Frequency of dairy consumption	Daily	50	60
	Several times a week	45	50
	Once a week	20	15
	Less than once a week	8	5
	None at all	3	4
Additional intake of vitamin D or calcium supplements	Yes	35	45
	No	91	89
Name and dosage (if recorded in medical history)	Vitamin D3 2,000 IU	15	20
	Calcium D3 Nycomed	10	12
	Vitamin D3 5,000 IU	5	8
	Other (specified different drug/dosage)	5	5
Number of protein servings per day (according to dietary records)	None	2	1
	1 serving	30	25
	2 servings	60	70
	3 servings or more	34	38

Table 1. Continued

Parameter (as per medical records)	Option	Donor group (n = 126)	Breastfeeding non-donors (n = 134)
Number of children breastfed by the woman	1 child	60	70
	2 children	40	45
	3 children	15	10
	Other (4 or more children)	11	9
Total duration of breastfeeding (in months)	6-12 months	30	35
	13-24 months	40	50
	25-36 months	20	25
	Other (different duration)	36	24
Frequency of physical exercise	Daily	15	20
	Several times a week	40	50
	Once a week	30	35
	Less than once a week	25	15
	None at all	16	14
Presence of harmful habits (smoking)	Yes	5	3
	No	110	125
	Former smoker (quit)	11	6
Presence of harmful habits (alcohol consumption)	Daily	0	0
	Several times a week	5	3
	Once a week	15	10
	Less than once a week	50	60
	None at all	56	61

Notes: respondents could indicate multiple relatives with osteoporosis; therefore, the total number of such cases may exceed the number of women who answered “Yes” in the family history section

Source: compiled by the authors

The analysis of risk factors in 126 breast milk donors revealed that 35.7% of respondents had a family history of osteoporosis, whereas among breastfeeding women who were not donors (n = 134), this figure was 29.9%. The most frequently mentioned relatives were mothers (15.9% in donors vs 13.4%), maternal grandmothers (11.9% vs 8.9%), and fathers (7.9% vs 6%), indicating a predominant influence of the maternal lineage on osteoporosis risk. Fractures after the age of 30 were reported in 11.9% of donors (most commonly wrist fractures – 5.6%), compared to 7.5% in the control group. At the same time, 92.1% of donors were unaware of their own bone mineral density (BMD) values, while among breastfeeding non-donors, this figure was 88.8%. Only 7.9% were aware of their T-score, with most reported values indicating osteopenia (ranging from -1 to -2). Just 39.7% of donors consumed dairy products daily, and 72.2% did not supplement with vitamin D or calcium, which was worse compared to breastfeeding non-donors (44.8% and 66.4%, respectively). The largest proportion of donors (47.6%) had breastfed one child, and the total duration of lactation was predominantly 13-24 months (31.7%) or

6-12 months (23.8%). Regular physical activity several times a week was reported by 31.7% of donors (vs 37.3% in the control group), with aerobic exercise being the most common (55.6%). The majority of donors did not smoke (87.3%) or consume alcohol (44.4%); however, in the control group, 93.3% were non-smokers, and 45.5% completely abstained from alcohol [16].

The presented data suggest that breast milk donors exhibit a higher prevalence of family history of osteoporosis, more frequent fractures, lower awareness of bone mineral density, poorer dietary habits, and reduced physical activity compared to women who breastfeed only their own children. This raises the possibility that breast milk donation may serve as an additional risk factor for osteoporosis development, warranting increased attention to preventive measures. Assessment of bone mineral density using ultrasound and forearm DXA densitometry in the studied sample of breast milk donors (n = 126) and breastfeeding non-donors (n = 134) revealed a differential distribution of T-score values. Detailed results are presented in Table 2, which shows the number of participants with normal values, osteopenia, and osteoporosis.

Table 2. Assessment of bone mineral density using ultrasound and forearm DXA densitometry in the studied samples (n = 260)

Group/Method	Normal (T ≥ -1)	Osteopenia (-2.5 < T < -1)	Osteoporosis (T ≤ -2.5)
Donors (n = 126), ultrasound densitometry	70	46	10
Donors (n = 126), DXA densitometry	65	48	13
Breastfeeding non-donors (n = 134), ultrasound	92	35	7
Breastfeeding non-donors (n = 134), DXA	89	38	7

Source: compiled by the authors

The assessment of bone mineral density (BMD) in 260 women (126 breast milk donors and 134 breastfeeding non-donors) using ultrasound densitometry and forearm DXA revealed that among donors, eumineralization ($T \geq -1$) was detected in 55.6% of cases, osteopenia ($-2.5 < T < -1$) in 36.5%, and osteoporosis ($T \leq -2.5$) in 7.9%. In the control group, these figures were 68.7%, 26.1%, and 5.2%, respectively. Meanwhile, DXA identified eumineralisation in 51.6% of donors, osteopenia in 38.1%, and osteoporosis in 10.3%, whereas in non-donors, the corresponding

values were 66.4%, 28.4%, and 5.2%. DXA densitometry demonstrated higher sensitivity in diagnosing osteoporosis, particularly in the donor group, reinforcing the need for FRAX tool application to assess fracture risk. Anthropometric measurements conducted among the participants allowed for the evaluation of BMI distribution as well as the determination of mean weight and height values. Detailed results regarding BMI distribution and descriptive statistics for weight and height are presented in Table 3.

Table 3. Anthropometric parameters of human milk donors and breastfeeding non-donor women (n=260)

Parameter	Value	Donors (n = 126)	Breastfeeding non-donors (n = 134)
Underweight (BMI < 18.5 kg/m ²)	BMI < 18.5	7	5
Normal weight (BMI 18.5-24.9 kg/m ²)	18.5 ≤ BMI < 25	65	75
Overweight (BMI 25-29.9 kg/m ²)	25 ≤ BMI < 30	37	35
Obesity (BMI ≥ 30 kg/m ²)	BMI ≥ 30	17	19
Weight (kg)	Mean ± Standard deviation	66 ± 13	63 ± 11
Height (m)	Mean ± Standard deviation	1.64 ± 0.06	1.66 ± 0.08

Source: compiled by the author

The obtained data indicate that human milk donors exhibit a distinct BMI distribution, which may adversely affect bone tissue status and underscores the necessity of an individualised approach to osteoporosis risk assessment. The analysis revealed that 51.6% of donors (n = 126) had normal weight (BMI 18.5-24.9 kg/m²), 29.4% were overweight, 13.5% had obesity, and 5.6% were underweight. In the control group of breastfeeding non-donor women (n = 134), normal weight was recorded in 55.9%, overweight in 26.1%, obesity in 14.2%, and underweight in 3.7%. The mean weight among donors was 66 ± 13 kg, compared to 63 ± 11 kg in the control group; mean height was 1.64 ± 0.06 m in donors and 1.66 ± 0.08 m in the control group. Overweight and obesity in women of

reproductive age may induce complex hormonal changes during lactation, while underweight is associated with decreased oestrogen levels and insufficient calcium and vitamin D intake. The anthropometric measurements highlight the need for an individualised approach to osteoporosis risk assessment in human milk donors, considering their BMI, dietary habits, and lifestyle. To evaluate bone metabolism and vitamin D status, biochemical parameters were analysed in the study cohort. Levels of 25-hydroxyvitamin D, calcium, phosphorus, osteocalcin, and P1NP were assessed. The obtained data, including mean values, standard deviations, and the number of participants with deviations from reference ranges, are presented in Table 4.

Table 1. Biochemical parameters of human milk donors and breastfeeding non-donor women (n=260)

Parameter	Reference range	Donors (n = 126) Mean ± SD	No. with abnormal values	Breastfeeding non-donors (n = 134) mean ± SD	No. with abnormal values
25-Hydroxyvitamin D (ng/mL)	30-100	23 ± 8	85	28 ± 10	60
Calcium (mmol/L)	2.15-2.55	2.25 ± 0.12	25	2.33 ± 0.14	15
Phosphorus (mmol/L)	0.87-1.45	1.1 ± 0.17	20	1.18 ± 0.19	10
Osteocalcin (ng/mL)	11-43	30 ± 14	38	26 ± 10	22
P1NP (ng/mL)	19-76	48 ± 20	33	43 ± 15	17

Source: compiled by the authors

Obtained results indicate the necessity for comprehensive osteoporosis prevention measures among breast milk donors, as they exhibit a higher prevalence of bone tissue metabolism disorders and 25-hydroxyvitamin D deficiency (67.5% vs 44.8%), with mean vitamin D levels being only 23 ± 8 ng/mL compared to 28 ± 10 ng/mL in the control group. Deviations in calcium levels from the norm were observed in 19.8% of donors vs 11.2%, with mean calcium levels at 2.25 ± 0.12 mmol/L vs 2.33 ± 0.14 mmol/L and phosphorus at 1.1 ± 0.17 mmol/L vs 1.18 ± 0.19 mmol/L, further confirming mineral metabolism instability in donors. Elevated osteocalcin levels (30.2% vs 16.4%, mean values 30 ± 14 vs 26 ± 10 ng/mL) and P1NP (26.2% vs 12.7%, mean values 48 ± 20 vs 43 ± 15 ng/mL) indicate intensive bone re-

modelling, which, under conditions of vitamin D and calcium deficiency, may increase fracture risk.

Hormonal profile analysis of breast milk donors (n = 126) revealed significant differences compared to breastfeeding women who were not donors (n = 134). Prolactin levels in donors reached 76.6% of the upper limit of normal (vs 48.8% in controls), leading to suppressed ovarian function and reduced oestrogen levels. In the follicular phase, donors' estradiol levels were only 10.3% of the upper limit (vs 24.3% in controls), while in the luteal phase, they were 15.5% vs 27.9%. Concurrently, parathyroid hormone levels in donors reached 94.4% of the upper limit (vs 76.4%), and calcitonin levels were only 42.9% (vs 60.4% in controls), exacerbating bone resorption.

Prolonged lactation (over 12 months) and milk donation may lead to sustained reductions in bone mineral density due to hypoestrogenism and calcium depletion. The most significant bone loss occurs during the first 6 months of lactation, after which the rate of decline slows, and post-lactation, gradual bone mass recovery typically occurs. However, with lactation and donation exceeding 18-24 months, irreversible bone structural changes may arise. These findings suggest an elevated osteoporosis risk among breast milk donors, necessitating further research and preventive strategies accounting for hormonal status and lactation duration.

Comprehensive prevention and correction of bone tissue disorders during lactation require consideration of individual metabolic characteristics, breastfeeding duration, and milk donation volume. Prolonged lactation increases the demand for mineral resources, primarily calcium, potentially reducing bone mineral density. Additional milk donation exacerbates this process due to accelerated loss of bone-essential micronutrients and proteins. With adequate loss compensation and post-lactation hormonal recovery, bone mass often restores; however, prolonged and intensive breastfeeding may worsen osteopenic changes. Thus, guideline development must involve individual risk factor assessment and integrated approaches to nutrition, diagnostics, monitoring, and physical activity.

A balanced diet, accounting for optimal quantitative and qualitative characteristics of micro- and macronutrients, is fundamental for maintaining bone tissue throughout the entire lactation period. In women with an average duration of breastfeeding (6-12 months), the requirements for calcium and vitamin D typically align with generally accepted norms: 1,000-1,300 mg of calcium and 600-800 IU of vitamin D per day, provided that plasma 25(OH)D (25-hydroxyvitamin D) levels remain within ≥ 30 ng/mL. If breastfeeding extends beyond one year – particularly under conditions of regular breast milk donation ($>2-3$ L per week) – it is advisable to adjust the diet by increasing daily calcium intake to 1,300-1,500 mg and, if necessary, consider higher doses of vitamin D (1,000-2,000 IU), guided by laboratory monitoring results (e.g., in cases of 25(OH)D < 20 ng/mL, a temporary increase to 4,000 IU per day may be warranted). Magnesium, which plays a role in regulating bone tissue metabolic pathways, should be maintained within a range of 300-400 mg per day; serum magnesium levels are recommended to remain no lower than 0.8 mmol/L. Optimal protein intake typically amounts to approximately 1.2-1.5 g/kg of body weight, as lactogenesis requires increased protein support. In cases of low BMI (< 18.5 kg/m²), it may sometimes be appropriate to raise this amount to 1.6-1.8 g/kg.

The diet should ideally include sufficient high-quality proteins of both animal and plant origin (meat, fish, legumes, nuts), as well as an adequate intake of calcium-rich foods: dairy products (cheese, kefir, yogurt), leafy green vegetables, cabbage, sesame seeds, and almonds. Vitamin D is partially synthesised under ultraviolet exposure; however, during autumn and winter or in cases of insufficient regular sunlight exposure, supplementation in doses determined by 25(OH)D analysis is recommended (monitoring should be conducted every 3-4 months in cases of pronounced deficiency). In the event of vitamin K

insufficiency – which is involved in the synthesis of proteins required for calcium fixation in bone tissue – it is advisable to increase the consumption of green vegetables and consider specialised supplementation (particularly vitamin K2 in doses of 100-200 μ g/day), especially when densitometric parameters indicate progressive declines in mineral density.

Regular diagnostic assessment and monitoring of bone tissue status are essential. Initial screening is recommended at the onset of breastfeeding (or during the planning stage of donation) to evaluate baseline mineral density. The most informative method is considered to be DXA (dual-energy X-ray absorptiometry), which quantifies bone mineral density in high-risk fracture zones (lumbar spine, femoral neck). A T-score between -1 and -2.5 indicates osteopenia, reflecting moderate bone mineral density reduction, while a T-score ≤ -2.5 signifies osteoporosis. In women with prolonged lactation (over 12 months) and in donors providing large volumes of milk (cumulatively exceeding 15-20 L per month), repeat DXA scans are recommended every 6-12 months, depending on the severity of prior changes and fracture risk levels. If DXA is unavailable, ultrasound densitometry (forearm) may serve as a screening method; however, if abnormalities are detected, DXA should be supplemented for more precise bone status assessment. Biochemical markers of bone formation and resorption (osteocalcin, P1NP, β -CrossLaps), as well as serum levels of calcium, phosphorus, magnesium, and 25(OH)D, should ideally be evaluated every 6-8 months during prolonged lactation and every 3-4 months in cases of osteopenia or osteoporosis. If 25(OH)D levels are < 20 ng/mL, targeted correction (up to 4,000 IU of vitamin D3 per day under calcium level and renal function monitoring) is advisable.

Physical activity is a key factor in the prevention of osteopenic changes and osteoporotic fractures. Weight-bearing exercise (strength training, free weights or machine-based exercises, bodyweight exercises) promotes osteoblast activation and increases bone mass. It is recommended to engage in at least 150 minutes per week of moderate-intensity aerobic exercise (e.g., brisk walking or light jogging at 60-70% of maximum heart rate) and incorporate 2-3 strength training sessions per week, each lasting 30-40 minutes, with a working weight of approximately 40-60% of 1RM (one-repetition maximum). The average Rating of Perceived Exertion should be maintained at 12-14 (on the 6-20 scale), avoiding excessive strain that may be detrimental to lactating mothers with insufficient recovery. For breastfeeding women, an individualised approach is applied, considering their fitness level, postpartum musculoskeletal changes, and general well-being. Consultation with a physical rehabilitation specialist or sports medicine expert can help tailor the programme to the individual's condition.

Individual adjustments to recommendations depend on the degree of bone mineral density (BMD) loss. In cases of osteopenia (T-score between -1 and -2.5), it is usually sufficient to enhance dietary intake of calcium, vitamin D, magnesium, and protein, alongside introducing regular physical activity with an emphasis on weight-bearing exercises and moderate impact loading (jogging or brisk walking). Additional calcium supplementation (1,000-1,200 mg/day of elemental calcium) combined with

vitamin D (depending on 25(OH)D levels) is considered a preventive measure to stabilise bone tissue. In cases of low physical activity or insufficient sun exposure, more careful vitamin D correction with possible dose adjustment is required. A BMI $<18.5 \text{ kg/m}^2$ or $>30 \text{ kg/m}^2$ increases the risk of metabolic and hormonal disturbances, which may worsen bone health, necessitating careful dietary and exercise modifications in these cases.

In the presence of osteoporotic changes (T-score ≤ -2.5), standard measures may be insufficient. Further diagnostic evaluation is recommended to exclude secondary causes of osteoporosis, including assessment of parathyroid hormone, calcitonin, and estradiol levels, as well as consultation with an endocrinologist or other relevant specialist. Where indicated, specific pharmacological agents (bisphosphonates, denosumab, selective estrogen receptor modulators) may be prescribed; however, most of these have restrictions during active lactation. Therefore, in cases of severe osteoporosis requiring continued breastfeeding, alternative correction strategies (e.g., high-dose vitamin D and calcium supplementation, enhanced diet, specialised physical therapy programmes) should be considered. After cessation of lactation, all available medical treatment strategies may be implemented in consultation with a physician.

The duration of breastfeeding should be guided by health status and bone density indicators. In cases of early osteopenia, prolonged lactation (beyond 12-18 months) necessitates more frequent densitometry, careful hormonal monitoring, and active dietary adjustments, including revised vitamin D and calcium dosages. Concurrently, practical considerations (e.g., daily routine management, work-rest balance) may be decisive in shaping individualised recommendations. With a proactive approach to diagnosis and prevention, the risk of persistent osteoporotic changes is significantly reduced, even with prolonged breastfeeding or intensive milk donation.

According to current research, evaluating biochemical markers (25(OH)D $<20 \text{ ng/mL}$, osteocalcin $>43 \text{ ng/mL}$, P1NP $>76 \text{ ng/mL}$) alongside DXA results (T-score below -1.5) improves the reliability of bone health risk assessment. Women with prolonged lactation and early signs of bone deterioration (T-score between -1 and -2) should undergo biennial screening and continued monitoring post-weaning, as bone mineral density recovery may take several months to a year or longer. For women with shorter lactation periods (<6 months) or no additional milk donation, annual monitoring is typically sufficient unless other significant risk factors are present (e.g., family history of osteoporosis, BMI $<18.5 \text{ kg/m}^2$, physical inactivity). Moderate but consistent physical activity, correction of nutritional deficiencies, and biomarker monitoring contribute to bone structure stabilisation and, under favourable conditions, restoration.

Thus, for the comprehensive prevention and correction of osteopenic and osteoporotic changes during lactation, it is recommended to maintain a balanced diet that accounts for increased requirements of calcium, vitamins D and K, magnesium, and protein, incorporate regular physical activity (primarily weight-bearing and aerobic exercises within 150-180 minutes per week), conduct screening and dynamic monitoring of bone tissue status (densitometry,

blood tests), rationally plan the duration of breastfeeding and milk donation, and implement additional corrective measures if osteopenia or osteoporosis is detected. This strategy helps preserve adequate bone quality and reduces the risk of fractures in the postpartum period and in the future.

✦ DISCUSSION

The study demonstrated that breast milk donors have a higher incidence of osteopenia and osteoporosis compared to breastfeeding women who are not donors. It was found that 38.1% of donors exhibit osteopenia according to DXA densitometry, while 10.3% have osteoporosis, whereas in the group of breastfeeding non-donors, these figures are 28.4% and 5.2%, respectively. Biochemical analysis revealed significantly lower levels of 25-hydroxyvitamin D ($25 \pm 8 \text{ ng/mL}$ in donors vs $28 \pm 10 \text{ ng/mL}$ in the control group), which may be one of the contributing factors to the increased osteoporosis risk. Hormonal status analysis confirmed elevated prolactin and reduced estradiol levels in donors, which may also influence bone metabolism.

According to the study by M. Aghaei *et al.* [17], pregnancy- and lactation-associated osteoporosis is a rare but potentially serious condition that may lead to fractures in postpartum women. The study documented three clinical cases of women who experienced significant bone mineral density loss and vertebral fractures following childbirth and during lactation. It was found that two of the three patients had vitamin D deficiency, and their bone mineral density (T-score) in the lumbar spine ranged from -3.1 to -3.6, substantially exceeding typical osteopenia thresholds. The difference between this study and the current findings lies in the fact that M. Aghaei *et al.* focused on lactation-associated osteoporosis as a rare syndrome, whereas the present study examines breast milk donation as a potential systemic risk factor with a gradual impact on bone mineral density. This may indicate the necessity of expanded screening and early detection of mineral density abnormalities in breastfeeding women before clinical symptoms or fractures manifest.

The current study provided a detailed account of how prolonged lactation and breast milk donation may lead to temporary depletion of bone mineral density. It highlighted that during lactation, hypoestrogenemia and significant calcium expenditure for milk production can result in reduced bone mass in women, increasing the risk of osteopenia and osteoporosis. The data underscore the need for regular monitoring of bone tissue status (e.g., via densitometry) in milk donors and the implementation of measures for rapid bone density recovery after lactation cessation. In another study by M. Fukushima *et al.* [18], international findings on pregnancy- and lactation-associated osteoporosis were summarised. A meta-analysis of eight studies indicated that approximately 5% of women in the first year postpartum had spinal osteoporosis, while up to 12% exhibited femoral neck osteoporosis. Pregnancy and breastfeeding significantly increased fracture risk in these women, aligning with the current study's data on the adverse effects of lactation on bone. The meta-analysis authors emphasised the necessity of a standardised approach to bone status assessment in the postpartum period for timely detection and management of osteoporotic

changes. Thus, the results of M. Fukushima *et al.* support the conclusions of the present study that lactation may cause substantial bone mass loss, and variations in osteoporosis incidence may stem from differences in sample characteristics and preventive measures. Notably, milk donors are typically under medical supervision and receive recommendations, which may reduce the actual prevalence of osteoporotic lesions compared to the general population represented in the meta-analysis.

The present study has identified vitamin D deficiency as one of the key risk factors for osteoporosis in breastfeeding mothers. The issue lies in the fact that insufficient 25(OH)D levels in lactating women reduce intestinal calcium absorption and impair bone tissue remodelling, accelerating bone mass loss. The study provided data on vitamin D levels in breastfeeding mothers, demonstrating that a significant proportion of women exhibit insufficiency or deficiency of this vitamin postpartum, which correlates with alterations in bone turnover markers. For prevention, correction of the deficiency is proposed – either through dietary enrichment with cholecalciferol or supplemental vitamin D intake. The significance of this aspect is supported by the study by X. Chen *et al.* [19], which revealed that among postmenopausal women with osteopenia/osteoporosis, over 47% had vitamin D deficiency (25(OH)D levels <20 ng/mL), while approximately 34% exhibited insufficiency. Low 25(OH)D levels were associated with elevated parathyroid hormone and bone resorption markers, as well as reduced bone mineral density (BMD) values. In the osteoporosis group, higher vitamin D levels significantly correlated with greater BMD in the femoral neck and hip joint. Thus, the findings of X. Chen *et al.* demonstrated a causal relationship between vitamin D hypovitaminosis and bone deterioration. The current study aligns with these conclusions: although the participants differ in age, the biological role of vitamin D in maintaining bone homeostasis is similar. Potential discrepancies concern the degree of impact – in postmenopausal women, prolonged vitamin D deficiency manifests as more pronounced osteoporosis, whereas in breastfeeding mothers, short-term deficiency may be compensated more rapidly if corrected in a timely manner. This underscores the importance of monitoring 25(OH)D levels and early intervention, as reflected in the study's recommendations.

The present study emphasised the role of nutrition in breastfeeding mothers as a critical component of osteoporosis prevention. The identified issue was insufficient intake of key nutrients for bone health – primarily calcium and high-quality proteins – which could hinder bone mass recovery during and after lactation. The study elaborates that lactation increases calcium demand: if maternal diet does not provide sufficient intake ($\approx 1,000\text{--}1,200$ mg Ca/day), the body mobilises calcium from bones for milk secretion, thereby weakening the skeletal system. Similarly, the study highlighted the role of dietary protein: amino acids are essential for collagen matrix synthesis in bones, and protein deprivation may delay bone tissue remineralisation. As a preventive measure, a balanced diet rich in dairy products (as a calcium source) and protein were recommended, or supplementation if dietary intake is inadequate. These findings are consistent with the review by G. Kędzia *et al.* [20], which analysed recent data on protein intake and bone health. According to G. Kędzia *et al.*, adequate

calcium and vitamin D intake are fundamental for maintaining bone mass, yet without sufficient protein, bone loss occurs significantly faster. Protein constitutes approximately 50% of bone volume and one-third of its mass, influencing collagen formation and hormonal regulation, particularly by stimulating IGF-1 synthesis, which promotes bone formation. Thus, dietary protein is considered an equally critical factor in bone nutrition alongside calcium. The review also noted that protein excess per se does not harm bones in healthy individuals: potential adverse effects (increased calcium excretion) are observed only in patients with renal insufficiency. Therefore, for most individuals, a high-protein diet – provided calcium intake is sufficient – is either safe or even beneficial for bone health. Compared to the current study, G. Kędzia *et al.* expanded the focus on the protein component, whereas the present study prioritises calcium (given its evident depletion during lactation). These differing approaches are not contradictory but complementary: a comprehensive osteoporosis prevention strategy for breastfeeding mothers should include both optimal calcium provision and adequate dietary protein – consistent with both sources.

The present study examined the issue of hypodynamia in breastfeeding women as a risk factor for accelerated bone mass loss. Due to postpartum fatigue and the demands of infant care, many lactating women may reduce their physical activity levels, which adversely affects bone remodelling (the absence of sufficient mechanical loading diminishes the stimulus for bone formation). The study concluded that regular physical activity, particularly weight-bearing exercises (walking, running, resistance training), is an essential component of osteoporosis prevention in this group. Early resumption of physical activity postpartum (accounting for health status) is recommended, as it promotes both bone strengthening and improved overall metabolism. The efficacy of this approach is supported by the results of a large prospective study by Y. Yue *et al.* [21]. In this study, which followed 24,700 elderly individuals for approximately three years, it was found that moderate daily physical activity significantly reduces the incidence of osteoporosis. Specifically, individuals who walked outdoors for more than 30 minutes per day had a 17% lower risk of developing osteoporosis compared to sedentary individuals (hazard ratio HR ≈ 0.83), while those who walked more than 60 minutes daily experienced a 40% risk reduction (HR ≈ 0.6). The authors emphasised a dose-dependent protective effect: longer walking durations correlate with a lower likelihood of osteoporosis. This association was observed across all subgroups, though it was most pronounced in individuals without a high genetic predisposition. Thus, the study by Y. Yue *et al.* confirmed a fundamental thesis shared with the present research: physical activity is a simple and effective means of preventing bone mass loss. Although Y. Yue *et al.* assessed an elderly population, the principle of mechanical bone stimulation is universal and applies equally to younger women. Differences may lie only in the type of recommended activity: walking suffices for older individuals, whereas more intensive exercises may be advisable for younger breastfeeding women (provided no contraindications exist). Ultimately, both studies highlighted that maintaining an adequate level of physical activity promotes bone density recovery and reduces osteoporosis risk.

The present study also identified a correlation between anthropometric characteristics of lactating women and their bone health status. The issue is framed as follows: women with low BMI or insufficient body weight during lactation may be more vulnerable to bone mass loss. It is noted that lean donors, particularly those who experienced rapid postpartum weight loss, exhibit lower bone mineral density (BMD). A possible mechanism involves a combination of factors: reduced mechanical load on the skeleton, lower nutrient and oestrogen reserves in adipose tissue, and intense metabolic demands of lactation amid limited physiological reserves. Conversely, women with higher BMI (within normal or moderately elevated ranges) may have some protection against osteoporosis due to greater skeletal loading and higher levels of leptin and oestrogens derived from adipose tissue.

These findings are corroborated by a large-scale study by C.T. Chiu *et al.* [22], which analysed data from ~14,000 Taiwanese individuals and established a strong association between body weight and bone density: higher BMI was statistically linked to greater BMD and lower osteoporosis risk. Crucially, low body weight emerged as an independent predictor of osteoporosis progression. In the underweight subgroup, osteoporosis incidence was significantly higher than in those with normal BMI. Conversely, the authors identified a BMI range of ~23-24.9 kg/m² as optimal for fracture prevention; below this threshold, osteoporosis risk progressively increased. These results align with the conclusions of the present study: maintaining a healthy body weight is a modifiable factor that can reduce the likelihood of bone density loss. Minor discrepancies may arise from the fact that postpartum women experience dynamic weight fluctuations (physiological weight loss after pregnancy is possible, as is weight gain due to excessive caloric intake). The data from C.T. Chiu *et al.* indicate that rapid weight loss (>5-10% in a short period) adversely affects bones – a critical consideration when advising lactating women: excessive postpartum weight loss is detrimental to skeletal health. Overall, both studies confirm that anthropometric indicators (primarily BMI) are interrelated with bone mineral density, and maintaining body weight within the normal range is a key component of osteoporosis prevention in breastfeeding women. Prolonged breastfeeding increases calcium demand, which may lead to reduced maternal BMD. The present study underscores the importance of monitoring bone mass in donors and preventing osteoporosis through timely osteopenia diagnosis and corrective measures (dietary adjustments, calcium and vitamin D supplementation, etc.).

The study by E.N. Lee *et al.* [23] synthesised data from 7 trials involving 3,813 postmenopausal women and found that prolonged lactation history was associated with an increased risk of osteoporosis. According to the meta-analysis, women who breastfed for the longest duration had nearly twice the odds of developing osteoporosis (OR ≈ 1.93) compared to those who breastfed for shorter periods. The authors concluded that extended lactation periods are associated with an elevated risk of osteoporosis. This aligns with the findings of the current study regarding bone mass loss during prolonged human milk donation. The difference lies in the fact that E.N. Lee *et al.* assessed long-term consequences (in postmenopausal women), whereas the

present study focused on current monitoring of young lactating women. Potential reasons for discrepancies include population differences (a Korean cohort, differing dietary habits and genetics) and the possibility that bone loss – partially reversible after lactation cessation – may still have long-term effects on bone health decades later. This study reinforces the argument for monitoring bone status in women with prolonged breastfeeding periods (such as milk donors).

Pregnancy, and particularly lactation, induce temporary remodelling of maternal bone metabolism. In breast milk donors, this process may be more pronounced due to intensive and prolonged breastfeeding, necessitating measures to ensure full bone mass recovery and prevent osteoporosis. The review by E.M. Winter *et al.* [24] noted that physiological skeletal changes during pregnancy and lactation are typically reversible – maternal bone density generally recovers almost completely after lactation cessation. Moreover, long-term studies suggest potential minor localised benefits for future bone density in women who have undergone pregnancy and lactation. Thus, normal lactation does not lead to persistent osteoporosis – most women recover bone mass after weaning. These conclusions partially differ from the emphasis of the current study. While the present research highlighted the risk of osteoporosis and the need for preventive measures in donors (as a high-risk group due to extended lactation), the review by E.M. Winter *et al.* underscores the body's compensatory capacity. The discrepancy may arise because the review examines population averages and physiological norms, whereas women in milk donation programmes may breastfeed longer and more intensively than usual. This creates a scientific debate: on one hand, lactation is a natural, transient risk factor; on the other, in cases of exceptionally prolonged lactation (donation), closer monitoring of bone health is warranted – even if recovery occurs in most cases.

E.M. Kyle *et al.* [25] investigated the dynamics of bone mineral density during the first year postpartum in breastfeeding women compared to non-pregnant controls (USA, Nutrients). The study observed 18 lactating mothers from 4-6 months postpartum until 12 months, alongside 16 age-matched controls. The present study highlights potential bone mass deficits in milk donors and the need for osteoporosis prevention, whereas the results of E.M. Kyle *et al.* demonstrated that bone mineral density loss during the first year of lactation is moderate and reversible for most women. Both sources agree on the physiological bone loss during breastfeeding. They concur that early postpartum skeletal demineralisation occurs due to calcium mobilisation for lactation. However, the current study likely emphasises osteoporosis risk more, suggesting that without proper monitoring, donors may accumulate significant bone tissue loss. In contrast, E.M. Kyle *et al.* presented more optimistic findings: in the absence of other risk factors, lactation-induced osteopenia is transient and does not lead to fractures or severe osteoporosis within the first year postpartum. This difference may stem from variations in lactation duration and intensity. In E.M. Kyle's *et al.* study, many women ceased breastfeeding before 12 months, whereas milk donors often continue lactation longer and express substantial

volumes daily. Thus, donors may experience greater “calcium stress” than the average lactating mother. Another factor is vitamin D deficiency: if prevalent among donors, bone loss may be more pronounced. E.M. Kyle *et al.* did not report acute vitamin D deficiency in their participants (the study was conducted in the US with supplement access). Hence, differences in baseline vitamin D status and nutrition may explain why lactation only temporarily reduces bone mineral density in some cases but potentiates osteoporosis in others. Overall, E.M. Kyle’s *et al.* work supports the thesis that monitoring bone density – even with minor changes – is critical: the authors propose tracking T-scores and remodelling markers to identify women with excessive bone loss promptly. This aligns with the current study’s recommendations for regular diagnostics (e.g., densitometry) in breast milk donors.

E. Sümer *et al.* [26] conducted a large-scale retrospective study in Turkey, examining the association between the total duration of breastfeeding and bone health in 1,218 postmenopausal women. In addition to measuring bone mineral density (lumbar spine and femoral neck), serum 25(OH)D (vitamin D) levels were assessed. The mean age of participants was ~60 years; according to World Health Organization criteria, 41.3% had osteoporosis, while 42% exhibited osteopenia. The work of E. Sümer *et al.* directly supports the key hypothesis of the current study: prolonged lactation may have long-term adverse effects on a woman’s bone health. This is particularly relevant for breast milk donors, who often breastfeed for longer than average. Both studies concur that, without preventive measures and nutrient supplementation, extended breastfeeding depletes maternal mineral reserves. The Turkish study also identified a link with vitamin D, aligning with the current study’s assumption that vitamin D deficiency in donors may exacerbate osteoporosis. When 25(OH)D levels are low, calcium absorption is impaired, accelerating bone mass loss. Both E. Sümer *et al.* and the authors of the current approach recommend ensuring adequate vitamin D and calcium intake in long-term breastfeeding women. The studies differ in design: the Turkish study is a retrospective review of postmenopausal women, whereas the current research focuses on real-time active monitoring of donors. Nevertheless, the findings of E. Sümer *et al.* provide insight into a potential long-term outcome: women with a prolonged cumulative breastfeeding history (often donors, multiparous mothers, or those who breastfed a single child for an extended period) face a higher risk of osteoporotic fractures later in life. This evidence underscores the importance of preventive measures proposed in the current study. Specifically, regular bone density screening in such women would enable early detection of osteopenia before menopause, allowing timely intervention. Both sources also highlight the significance of modifiable factors, such as physical activity and body weight. The 2020 study found that higher body weight and an active lifestyle partially mitigate bone mineral density loss (improving femoral bone mineral density). This confirms that preventive strategies should encompass not only calcium and vitamin D supplementation but also lifestyle recommendations (balanced nutrition, moderate physical activity).

In the current study, the mean 25(OH)D level in breast milk donors was 23 ± 8 ng/mL, compared to

28 ± 10 ng/mL in the control group. The majority of donors (67.5%) had vitamin D deficiency, indicating insufficient intake. X. Ni & W. Xia’s [27] study demonstrated that, under normal conditions, lactation alters mineral metabolism, enhancing intestinal calcium absorption while reducing renal excretion. Most women fully recover bone mass losses after lactation cessation; however, those with low baseline bone mass may develop osteoporosis and osteoporotic fractures. This reinforces the necessity of additional vitamin D and calcium intake, particularly for high-demand groups such as breast milk donors.

The current study found that donors exhibit reduced muscle strength, poorer coordination, and an increased risk of falls (decreased hand dynamometry in 18% of cases, prolonged Timed Up and Go test duration in 12%). These effects may result from elevated energy expenditure and mineral depletion. D. Athonvarangkul & J. Wysolmerski’s [28] study analysed the interaction mechanism between the hypothalamus, mammary gland, and bone tissue during lactation. Adaptive bone remodelling occurs, but prolonged lactation may reduce bone mass. This research also confirms that lactation affects muscle tone via hormonal changes, explaining the observed impairments in muscle strength and coordination.

The current study revealed that breast milk donors experience more pronounced bone mineral density reduction, potentially increasing postmenopausal osteoporosis risk. A. Sarantaki’s [29] study examined the long-term impact of lactation on postmenopausal osteoporosis. Data across studies are conflicting: some suggest a protective effect of breastfeeding, while others indicate elevated bone mass loss risk. These discrepancies may stem from variations in lactation duration, vitamin D and calcium intake, and genetic predisposition.

The current study established that breast milk donors have an elevated risk of osteopenia and osteoporosis, necessitating tailored preventive and therapeutic strategies. Conversely, P. Anagnostis *et al.* [30] study evaluated the efficacy of various osteoporosis treatments in women with PLO. Calcium and vitamin D supplementation improved bone mineral density, but bisphosphonates and teriparatide were most effective. However, their use during lactation is restricted, highlighting the need for alternative prevention and treatment approaches.

Thus, studies indicated that breast milk donors are more likely to develop osteopenia and osteoporosis compared to breastfeeding women who are not donors, which is attributed to vitamin D deficiency, hypostrogenaemia, elevated prolactin levels, and additional metabolic calcium expenditure during lactation. Several studies have described clinical cases of vertebral fractures in women with pregnancy- and lactation-associated osteoporosis, where the critical factor is hypovitaminosis D and insufficient dietary intake of protein and calcium. Meta-analyses confirm that prolonged lactation correlates with a significant reduction in bone mineral density and an increased risk of fractures in the postpartum period; however, most women exhibit gradual recovery of bone mass after cessation of breastfeeding. At the same time, milk donors demonstrated more pronounced bone tissue loss, likely due to longer and more intensive lactation, exacerbated by physical inactivity, low BMI, and other individual risk factors.

◆ CONCLUSIONS

The conducted study comprehensively demonstrated enhanced bone remodelling under conditions of prolonged lactation, which combines elevated prolactin levels with transient hypoestrogenaemia in breast milk donors. It was established that 35.7% of respondents had a familial predisposition to osteoporosis, while 11.9% had sustained fractures after the age of 30, underscoring the significant role of hereditary factors. Among donors, 34.1% were diagnosed with osteopenia, 7.9% with osteoporosis, whereas 57.9% exhibited normal densitometry values. Anthropometric analysis revealed that 27.8% of women were overweight, 12.7% were obese, and 4% were underweight, contributing to complex alterations in bone tissue metabolism. Additionally, 59.5% had 25(OH)D deficiency, 15.9% exhibited abnormal calcium levels, and 11.9% had phosphorus imbalances; 23.8% showed deviations in osteocalcin content, while 19.8% had altered P1NP levels, indicating varying bone matrix formation activity.

Assessment of dietary habits demonstrated insufficient intake of calcium-rich foods: 6.3% of women consumed dairy products less than once a week, and 2.4% avoided them entirely, potentially compromising calcium supply. The majority of donors (72.2%) did not supplement with vitamin D or calcium, whereas 27.8% used appropriate supplements. It was found that 31.7% of mothers breastfed for over a year, and 15.9% for over two years, which, combined with milk donation, may exacerbate mineral depletion. Only 11.9% of women engaged in regular daily physical activity, while 12.7% did not exercise at all. Among

those aware of their bone status, 7.9% had a T-score between -1 and -2, signalling early osteopenic changes. The combination of these factors suggests that prolonged lactation, coupled with genetic predisposition, nutritional imbalances, and excess weight, increases the risk of structural bone damage.

Optimising dietary intake with adequate protein, calcium, and vitamin D, supplementing where necessary, and engaging in regular physical exercise reduce the likelihood of accelerated bone tissue depletion. Routine examinations using ultrasound or DXA densitometry and monitoring biochemical markers are also recommended for early detection of structural abnormalities. Simultaneously, attention should be paid to the post-lactation period, as bone mass recovery occurs during this phase and may be delayed in the presence of additional risk factors. A limitation of the study is the non-representative age and geographical distribution of the sample. Future research should focus on genetic screening of polymorphisms regulating bone metabolism to develop targeted preventive measures for high-risk osteoporosis groups.

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◆ REFERENCES

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Сучасний підхід до моніторингу та профілактики розвитку остеопорозу у донорок грудного молока

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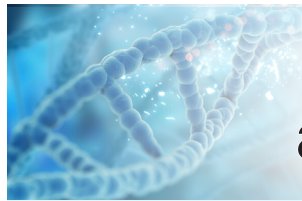
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Анотація. Вивчення стану кісткової тканини жінок, які годують немовлят, є актуальним з огляду на зростаючу поширеність остеопенічних змін і складні наслідки для репродуктивного здоров'я. Метою дослідження було проаналізувати донорство грудного молока як додатковий фактор ризику та визначити ключові умови збереження міцності кісток у післяпологовий період. У межах ретроспективного аналізу, проведеного на базі комунального некомерційного підприємства «Перинатальний центр м. Києва», вивчено медичні записи 260 жінок, серед яких 126 були донорками молока, а 134 годували власних дітей без донорства. У процесі дослідження оцінювалися показники щільності кісткової тканини, кількісні параметри крові, індекс маси тіла та історія переломів. Контингент учасниць дослідження формувався через мережу лікувальних закладів за умови отримання інформованої згоди та забезпечення конфіденційності персональних даних. Основні результати засвідчили, що донорки частіше мали випадки переломів після 30 років (11,9 %), меншу обізнаність про стан кісток (7,9 % знали власний індекс маси тіла) та суттєві відхилення рівня вітаміну D, дефіцит якого фіксувався у 67,5 % жінок із цієї групи. Поміж донорок 29,4 % мали надлишкову вагу, а 13,5 % – ожиріння, що перевищувало аналогічні показники серед годувальниць без донорства. Понад 10 % учасниць-донорок продемонстрували рентгенологічні ознаки остеопорозу, тоді як у контрольній групі такі зміни охоплювали близько 5 %. Було виявлено, що триваліша лактація без належної корекції харчування й фізичної активності сприяє прогресуванню остеопенічних процесів. Доведено важливість регулярного моніторингу біохімічних маркерів та використання профілактичних стратегій (раціональне харчування, контроль надходження вітаміну D та помірні силові вправи) для запобігання ускладненням. Практична цінність результатів полягає в обґрунтуванні необхідності раннього обстеження донорок і формуванні індивідуальних рекомендацій, щоб знизити ризик переломів і зберегти здоров'я матері. Запропонований підхід може бути застосований у масштабніших програмах охорони здоров'я жінок, які годують немовлят. Це особливо важливо для сучасної медичної спільноти, яка прагне зменшити економічні витрати, пов'язані з лікуванням переломів та подовженою реабілітацією

Ключові слова: гормональні зміни; генетична схильність; фізична активність; дефіцит вітаміну D; лактація



The impact of changes in gut microbiota on the body's ability to process gluten proteins: A mini review

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Abstract. Gluten-sensitive disorders, including coeliac disease and non-coeliac gluten sensitivity, are common gastroenterological conditions characterised by impaired gastrointestinal function, systemic immune responses, and disruption of epithelial barrier integrity. This review aimed to provide a comprehensive analysis of the effects of gluten on the gut microbiota and to determine the role of the microbiome in the pathogenesis of these conditions. The analysis draws on findings from recent clinical studies, systematic reviews, and meta-analyses, as well as high-precision "next-generation sequencing techniques used to assess changes in bacterial and fungal communities of the gut microbiota at various stages of disease progression. It has been established that a gluten-free diet contributes to the partial restoration of microbial balance, in particular by reducing the number of pathogenic and opportunistic microorganisms associated with the development and persistence of chronic intestinal inflammation. At the same time, an increase in beneficial commensal microbiota – such as *Lactobacillus* and *Bifidobacterium* – has been observed. Special attention is given to the antimicrobial peptide cathelin-related antimicrobial peptide, which plays a key regulatory role in the immune response, controls the inflammatory process, and helps to maintain the homeostasis of the intestinal mucosa. The significance of the gut-brain axis has also been evaluated in the context of gluten-sensitive conditions, given that dysbiosis may influence patients' neuropsychological status, including the development of anxiety disorders, depression, and cognitive impairment. Potential therapeutic strategies are discussed, including the use of probiotics, prebiotics, postbiotics, and a diet low in fermentable oligo-, di-, monosaccharides and polyols (FODMAPs). It has been shown that diet exerts both direct and long-term effects on the composition and functional activity of the gut microbiota, which in turn modifies the clinical presentation of disease, symptom severity, and treatment outcomes. The practical value of this review lies in substantiating modern approaches to the diagnosis and treatment of glutensensitive disorders through targeted modulation of the intestinal microbiota. This opens new perspectives for personalised therapy, improvement of quality of life, prevention of complications, and restoration of patient health at a deeper – microbiome – level

Keywords: gluten intolerance; gluten-free diet; healthy nutrition; clinical nutrition; dysbiosis

✦ INTRODUCTION

Gluten-related disorders (GRDs), such as coeliac disease (CD) and non-coeliac gluten sensitivity (NCGS), are closely linked to the composition and function of the gut microbiota. A gluten-free diet (GFD) remains the primary

treatment, although it does not always fully restore microbial balance. In patients with CD, beneficial bacteria tend to decrease, while pathogenic bacteria increase – even when adhering to a GFD. Combining a GFD with other

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dietary strategies, such as a low-FODMAP diet, may alleviate symptoms more effectively. The gut microbiota plays a significant role in the development of GRDs by influencing both the immune system and the integrity of the intestinal barrier. Gluten-related disorders, including coeliac disease and non-coeliac gluten sensitivity, are increasingly recognised as important gastrointestinal and immunological conditions. Alterations in microbial composition not only affect intestinal health but also contribute to systemic inflammation and may even impact neuropsychological well-being. A deeper understanding of the interplay between gluten, diet, and the gut microbiome is essential for developing more effective, personalised treatment strategies and improving clinical outcomes.

D. Stanciu *et al.* [1] noted that GRDs occur in response to the consumption of gluten and related proteins found in wheat, barley, and rye. CD causes autoimmune damage to the intestinal mucosa, whereas NCGS does not involve an autoimmune response or structural mucosal changes. A GFD is the cornerstone of GRD therapy, although it can be difficult to maintain. Researchers have devoted considerable attention to the role of the gut microbiota in the pathogenesis of GRDs. I. Martín *et al.* [2] investigated the impact of a GFD in a group of 46 individuals with gastrointestinal complaints, migraines, and atopic dermatitis. They observed an increase in fungal diversity while the bacterial composition remained stable. A positive correlation was found between changes in fungal and bacterial diversity and shifts in species ratios after six weeks on a GFD.

G. Catassi *et al.* [3] highlighted the role of the microbiota in gluten-related conditions (CD, NCGS, wheat allergy). CD is associated with a reduction in *Lactobacillus* and *Bifidobacterium*, alongside an increase in *Bacteroides* and *E. coli*. NCGS is marked by reduced microbial diversity, an increase in *Ruminococcaceae*, and a decrease in *Bacteroidetes* and *Fusobacteria*. Notably, dysbiosis often persists despite adherence to a GFD. X. Wu *et al.* [4] reported that CD involves a compromised intestinal barrier, dysregulated immune response, and reduced gluten tolerance – all influenced by bacterial metabolites. While a GFD is the mainstay of treatment, probiotics may support the re-establishment of microbial balance. K. Naseri *et al.* [5] proposed combining a GFD with a low-FODMAP diet to relieve symptoms of irritable bowel syndrome (IBS). Microbiota imbalance is common in both IBS and gluten-sensitive conditions, further underlining the gut microbiome's pivotal role in gastrointestinal disorders. O. Gubska *et al.* [6] observed dysbiosis in CD and NCGS. In a study of 25 patients following a GFD, shifts in microbiota composition – specifically in *Bacteroidetes*, *Firmicutes*, and *Actinobacteria* – were correlated with the duration of dietary adherence, suggesting that diet is the primary driver of these changes. Z. Ren *et al.* [7] investigated the role of cathelicidin-related antimicrobial peptide (CRAMP) in CD. In mice, CRAMP deficiency exacerbated enteropathy, while supplementation helped to restore gut function and immune responses. Dysbiosis, characterised by an increased abundance of *Pseudomonas aeruginosa*, was found to reduce CRAMP expression, highlighting the microbiota's role in immune regulation.

This study aimed to conduct a comprehensive analysis of the impact of gluten on the composition and function of the gut microbiota and to determine the microbiome's

role in the pathogenesis of gluten-related disorders. Coeliac disease and non-coeliac gluten sensitivity are common gastrointestinal conditions, marked by impaired intestinal function, immune dysregulation, and disruption of epithelial barrier integrity. To address these objectives, a detailed review of the literature was conducted using the PubMed, Medline, and Scopus databases. Publications including clinical data, experimental findings, and review articles were analysed. A systematic search strategy was employed using relevant keywords and search terms. Selection criteria included originality of data, presence of clinical or experimental results, and publication in Ukrainian or English. Studies lacking a control group in clinical trials, as well as those published only as conference abstracts or proceedings, were excluded. For the analysis of gluten's effects on gut microbiota, only sources published within the past five years (2020-2025) were considered.

✦ THE IMPACT OF GLUTEN AND DIET ON THE GUT MICROBIOTA AND LOCAL IMMUNE MECHANISMS

Gluten-related disorders affect a substantial proportion of the population and present a complex clinical picture involving both immune and gastrointestinal dysfunction. Although a strict glutenfree diet remains the cornerstone of treatment, emerging evidence suggests that it may not be sufficient for all patients. Incorporating biotics – such as probiotics, prebiotics, synbiotics, and postbiotics – offers promising potential to restore microbial balance and enhance therapeutic outcomes. Advances in microbiome analysis are also paving the way for more personalised and effective approaches to both diagnosis and treatment.

P. Tiwari *et al.* [8] emphasised the importance of the gut microbiota in neurological disorders, particularly through the gut-brain axis. The intestinal microbiota performs several vital functions in the human body: it contributes to the metabolism of nutrients and pharmaceuticals, protects against pathogens, and modulates immune responses. The so-called gut-brain axis refers to the bidirectional relationship between the central nervous system and the gastrointestinal tract. It has been established that the gut microbiome influences various signalling pathways – neuroendocrine, immune, and neurotransmitter-mediated. Therapeutic interventions targeting the microbiota include probiotics, prebiotics, postbiotics, synbiotics, faecal microbiota transplantation, and antibiotics. Proper nutrition is considered critical for maintaining a healthy microbiota, which in turn can modulate the enteric nervous system and influence the progression of various neurological disorders. Understanding the mechanisms of interaction between the microbiota and the nervous system opens new avenues for the diagnosis and treatment of neurological diseases, particularly in the context of personalised medicine.

Q. Xie *et al.* [9] observed in their study that alterations in the gut microbiome and specific intestinal lumen factors are important markers in the development of gluten allergy. They also identified regulatory mechanisms involving distinct patterns of protein expression that contribute to the pathogenesis of this disorder. G. Caio *et al.* [10] examined the effect of a GFD on the microbiota in patients with CD and non-coeliac wheat/gluten sensitivity. They found that the diet influenced microbiota composition,

particularly the ratio between *Bacteroides* and *Bifidobacterium* species. C. Sabença *et al.* [11] conducted a review and noted that many misconceptions persist regarding the gluten-free diet. They emphasised the importance of accurately understanding and addressing such misconceptions in the dietary management of individuals with gluten-related conditions. O. Ogilvie *et al.* [12] investigated the impact of temperature and baking time on the structure of gluten proteins and the digestibility of peptides associated with coeliac disease. Their findings showed that these factors can significantly modify the nature of gluten peptides and their interaction with the digestive system.

M. Savarese *et al.* [13] reviewed the concept of “free-from” consumption, including gluten-free and lactose-free products. They identified key factors influencing consumer choices related to such products. I. Demirkesen & B. Ozkaya [14] analysed recent strategies for managing gluten-free diets and the development of compliant food products. They highlighted the importance of improving gluten-free production technologies to facilitate the lives of patients adhering to such diets. K. Arslain *et al.* [15] examined factors influencing the adoption of a GFD by individuals without CD or NCGS. They found that cultural and social influences, along with heightened health awareness, were the primary drivers. A. Jivraj *et al.* [16] reported that micronutrient deficiencies are common among adults following a gluten-free diet, regardless of its duration or adherence. They underscored the importance of micronutrient monitoring in these patients. A. Bakhshipour & R. Rafeaie [17] described cases of spontaneous latency in patients with coeliac disease after transitioning to a GFD. They investigated the diet’s role in restoring intestinal function following prolonged gluten exposure. E.L. Traneeth *et al.* [18] conducted a systematic review comparing the microbiome in patients with CD, NCGS, and irritable bowel syndrome. They observed notable differences in microbiota composition across the three groups.

The term “gluten-related disorders” refers to a spectrum of immune-mediated diseases triggered by gluten ingestion, primarily CD, NCGS, and wheat allergy. GRDs are associated with a broad array of clinical symptoms, affecting both gastrointestinal and extraintestinal systems, and may impact nearly any organ in genetically predisposed individuals following gluten exposure [1]. It is well established that gluten – or its components, such as gliadin (from wheat) or hordeins (from barley) – can provoke an immune response. While the elimination of gluten or even trace amounts is essential in managing GRDs, it is important not to overlook the nutritional role of grains, which are valuable sources of macro- and micronutrients and dietary fibre. Therefore, when excluding gluten-containing ingredients from the diet, it is crucial to ensure adequate nutrient intake in individuals adhering to a GFD. It has been suggested that gluten may influence molecules involved in gut permeability, such as zonulin. Moreover, when gluten is not properly hydrolysed, its peptides may be absorbed and trigger an immune-inflammatory response, leading to increased expression of Toll-like receptor 2 (TLR2), elevated levels of interferon-gamma (IFN- γ), and a higher presence of intraepithelial CD3+ T lymphocytes [2].

Although it remains unclear whether alterations in the human microbiota are a cause or a consequence of CD, these

changes significantly affect intestinal function and correlate with the severity of clinical symptoms. Microorganisms play a vital role in various physiological processes, including fermentation, vitamin synthesis, pathogen inhibition, reinforcement of the gut barrier, and regulation of the immune system. In individuals adhering to a GFD, the composition of the intestinal microbiome tends to more closely resemble that of healthy individuals than of those who continue to consume gluten. Meta-analyses of GFD interventions [16] have revealed increased microbial diversity – particularly of *Bacteroides* and *Firmicutes* – in treated patients compared to those maintaining a gluten-containing diet.

At the same time, harmful taxa such as *Neisseria* and *Proteobacteria* appear less frequently in individuals following a GFD, suggesting a therapeutic benefit from dietary intervention. Nonetheless, *Bifidobacterium* levels remain consistently lower in Crohn’s disease patients than in healthy controls, regardless of sample type, indicating that even long-term adherence to a GFD may not fully restore a healthy microbiota composition. *Bacteroides* species were found to be more prevalent in treated Crohn’s patients than in controls – a potentially positive outcome, given their role in pathogen inhibition and in supporting the metabolic needs of other commensal organisms. *Lactobacillus* species also perform several beneficial functions in the gut, including the production of organic acids, antimicrobial activity, bile salt deconjugation, and suppression of carcinogens. Notably, *Lactobacillus crispatus* has been shown to promote anti-inflammatory responses in dendritic cells, offering therapeutic potential for inflammatory diseases such as Crohn’s disease. Studies have reported significantly higher levels of *Lactobacillus* in patients on a GFD compared to untreated individuals. However, shifts in the species composition of *Lactobacillus* may go undetected if total abundance remains stable, underscoring the importance of species-level analysis in microbiome research [3].

Gliadin has been shown to activate both the innate and adaptive immune systems, thereby initiating intestinal inflammation through the induction of cytokines and chemokines. Specifically, gliadin is deamidated by tissue transglutaminase in the lamina propria of the small intestine, enabling it to bind to HLA class II DQ2/DQ8 molecules on antigen-presenting cells. This interaction activates T cells, macrophages, and dendritic cells, leading to the secretion of proinflammatory cytokines. This cascade triggers the adaptive immune response, resulting in the production of anti-endomysium, anti-gliadin, and anti-transglutaminase antibodies by B cells, thereby contributing to increased intestinal permeability. Beyond gliadin, the intestinal microbiota also plays a critical role in promoting inflammation within the intestinal mucosa of CD patients. Immune factors are well recognised as key contributors to CD, with adaptive immunity playing a central role in its pathogenesis. Research by X. Wu *et al.* [4] has demonstrated that the gut microbiota is closely linked to adaptive immunity and exerts significant regulatory influence on its two main branches – B cells and T cells. Intestinal microbes can enhance IgA production via B cell regulation and help maintain the balance between intestinal inflammation and immune tolerance by promoting the differentiation of Th17 and Treg cells. Gliadin contributes to the disruption of the intestinal barrier, facilitating

the overgrowth and translocation of pathogenic bacteria, which in turn leads to microbial imbalance. This dysbiosis further activates immune-inflammatory responses by modulating B and T cell activity. Inflammatory mediators may then exacerbate intestinal permeability by damaging epithelial cells, thereby worsening the severity of coeliac disease. Moreover, the mucosal immune response in CD may directly compromise the intestinal barrier, disrupting microbial homeostasis. The resulting dysbiosis acts as a pathogenic factor that fuels the progression of CD, thus establishing a vicious cycle of sustained inflammation.

Gluten intolerance refers to pathological conditions such as CD, wheat allergy (WA), and NCGS. Wheat allergy is a typical food allergy triggered specifically by wheat consumption rather than gluten itself and involves the activation of immune responses mediated by immunoglobulin E. The CD is an autoimmune condition that affects genetically susceptible individuals. It leads to damage of the small intestinal mucosa due to a T-cell-mediated immune response to gluten. NCGS is characterised by both gastrointestinal and extraintestinal symptoms associated with cereal consumption in individuals who do not have CD or WA. As there are no specific diagnostic tests, it is considered a diagnosis of exclusion. Importantly, NCGS does not cause intestinal damage or immune sensitisation to wheat proteins, which are characteristic features of CD and WA. According to research by K. Naseri *et al.* [5], the global prevalence of gluten-related disorders ranges from 1.1% to 1.7%, while wheat allergy occurs in approximately

0.2% to 1% of the population. Due to the absence of reliable diagnostic criteria and the limited number of population-based studies, the prevalence of NCGS is not well established, with estimates ranging from 1% to 13%. In Ukraine, no epidemiological studies have been conducted to determine the prevalence of CD or other gluten-related disorders. A GFD is a nutritional approach that involves avoiding gluten-containing products or replacing them with gluten-free alternatives. It remains the only effective treatment for individuals diagnosed with coeliac disease. The microbiome, comprising microorganisms that reside on and within the human body, plays a vital role in maintaining health. The balance of intestinal bacteria is essential, and disruptions to this balance – known as dysbiosis – are commonly observed in various gastrointestinal disorders. These include both functional and organic digestive diseases, as well as conditions involving food sensitivities such as CD [6].

In the gastrointestinal tract, CRAMP is well recognised for its anti-infective properties. Z. Ren *et al.* [7] reported that, while its immunoregulatory roles in extra-intestinal diseases have gained attention, the involvement of CRAMP in gluten-induced small intestinal enteropathy – specifically in coeliac disease – remains largely unexplored. Using a mouse model of gluten-induced enteropathy (GIE), which mimics the small intestinal pathology of coeliac disease, they found reduced CRAMP production in the duodenal epithelium during GIE. Mice lacking CRAMP showed increased vulnerability to the development of GIE (Fig. 1).

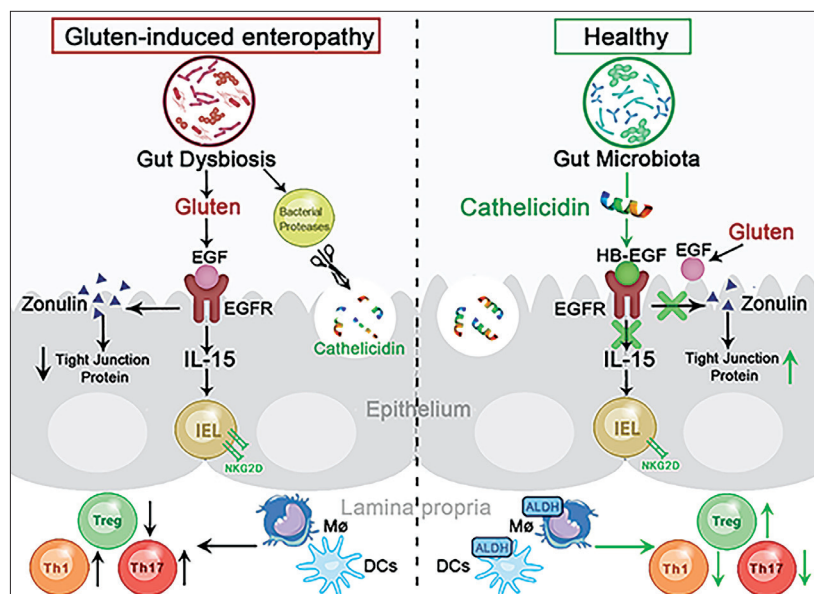


Figure 1. The protective function of cathelicidin-related antimicrobial peptide, influenced by gut microbiota in gluten-induced enteropathy

Notes: GIE-associated gut dysbiosis contributes to intestinal CRAMP degradation; CRAMP positively regulates intestinal barrier integrity and mucosal immune cell responses, offering protection against GIE; the CRAMP axis may therefore be targeted as a potential therapeutic strategy for coeliac disease

Source: [7]

Conversely, administration of exogenous CRAMP restored gliadin-induced epithelial dysfunction and enhanced regulatory immune responses within the intestinal mucosa. Furthermore, GIE-associated gut dysbiosis –

marked by enrichment of *Pseudomonas aeruginosa* and its protease LasB – was found to contribute to impaired intestinal CRAMP production. These findings underscore the significance of the microbiota-CRAMP axis in

regulating intestinal barrier integrity and immune responses in GIE. Consequently, therapeutic modulation of CRAMP may offer a novel approach for the treatment of coeliac disease.

SYSTEMIC CONSEQUENCES OF DYSBIOSIS AND THE ROLE OF MICROBIOTA IN IMMUNE, NERVOUS AND NUTRITIONAL REGULATION

In this context, the gut-brain axis (GBA) has emerged as a compelling area of research, particularly in relation to

the gut microbiota (GM), which has garnered increasing attention over the past five years (since 2020). The microbiome – comprising archaea, bacteria, protists, and fungi – resides in and on the human body and plays a critical role in maintaining the balance of the gastrointestinal tract microflora. Disruptions to this balance are associated with a range of pathological states, highlighting the importance of GBA-related microbiota in human health. Figure 2 provided an overview of the role of GM in neurological disorders and its modulatory impact on the GBA.

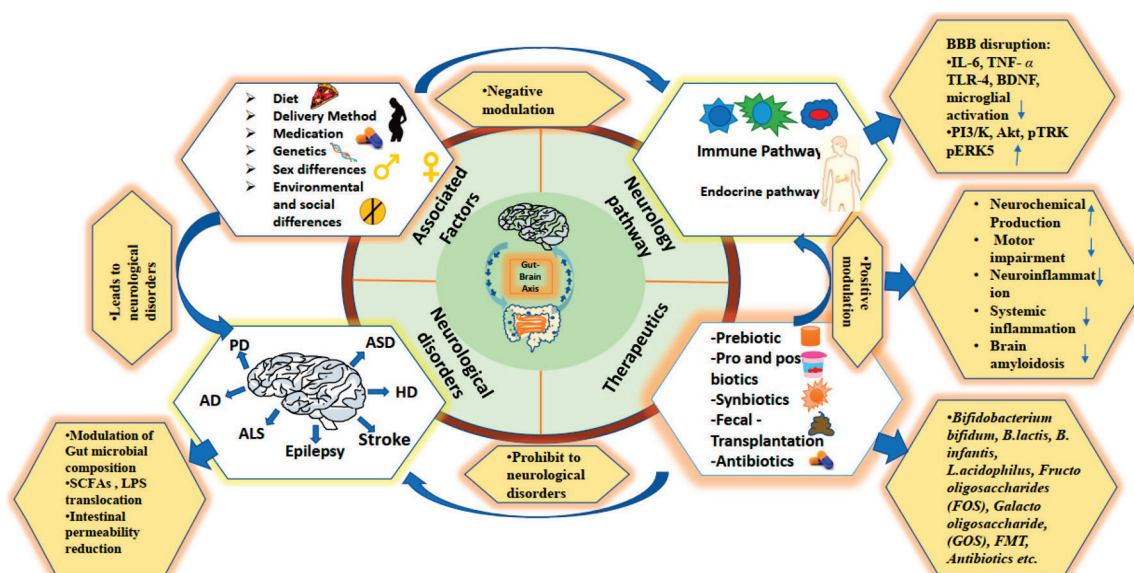


Figure 2. Overview of neurological disorders linked to the gut microbiota within the context of the gut-brain axis
Source: [8]

Several factors contribute to changes in GM composition within the GBA, which in turn influence neurological pathways and may lead to the development of various neurological disorders. These imbalances and dysfunctional microbial activities can potentially be regulated through interventions such as probiotics, prebiotics, synbiotics, postbiotics, antibiotics, and faecal microbiota transplantation (FMT). These approaches aim to modify the composition or function of the GM, thereby influencing brain function. Numerous studies [13, 18] support the view that the GM plays a crucial role in brain development and function. Both preclinical and clinical research has explored the gastrointestinal tract (GIT) microbiome within the GBA in relation to neurological conditions such as Alzheimer's disease (AD), multiple sclerosis (MS), Parkinson's disease (PD), autism spectrum disorder (ASD), epilepsy, stroke and brain injury, acute myeloid leukaemia (AML), Huntington's disease (HD), among others [8]. However, further in-depth research is required to fully elucidate the mechanisms by which the GM contributes to disease pathogenesis and its potential application in diagnostics and therapy.

The composition of the GM and its metabolites can disrupt the host's immune and endocrine systems, thereby influencing brain function and cerebral blood flow. To establish causal relationships, additional prospective and interventional studies are necessary. A considerable number of existing findings are derived from animal models,

which may not always be applicable to human physiology. Moreover, human studies are often complicated by numerous confounding factors, including diet, demographics, socioeconomic status, sample collection protocols, clinical history, and genetic sequencing methods. Nevertheless, practical challenges remain in implementing microbial-based therapies, such as determining optimal dosages, treatment durations, and timing of interventions. It is also essential to identify the most appropriate patient populations, considering variables such as disease stage and age, in order to develop tailored therapeutic strategies. Furthermore, the effects of dietary components and microbe-derived metabolites on host physiology and overall health must be systematically evaluated [18].

Previous research by a group of scientists [9] on yoga, meditation, and yoga-based lifestyle interventions has shown that these practices can modulate stress-related biomarkers – including cortisol, interleukin-6, brain-derived neurotrophic factor, and reactive oxygen species – within six to eight weeks following intervention in patients with glaucoma and retinoblastoma. These interventions were associated with reduced oxidative stress and improvements in overall quality of life. While some evidence supports the beneficial neurological effects of yoga, its specific influence on GM composition and the abundance of beneficial microbial species within the GBA remains unclear. Another area requiring further investigation is the interaction between the GM and pharmacological treatments, as many patients

are subject to polypharmacy. Understanding GM-drug interactions is crucial for developing safe and effective microbiota-targeted therapies. In conclusion, the gut microbiota represents a critical interface between human health and neurological disease [8].

Anatomically, the gut maintains a complex and bidirectional communication with the central nervous system (CNS), a relationship commonly referred to as the gut-brain

axis. This dynamic cross-talk plays a critical role in both health and disease. Sensory visceral signals originating in the gut are transmitted to the CNS primarily via the vagus nerve, influencing not only reflex responses but also cognitive and emotional processes such as mood regulation. In turn, the brain sends signals to modulate various gut functions, including motility, secretion, and immune responses (Fig. 3).

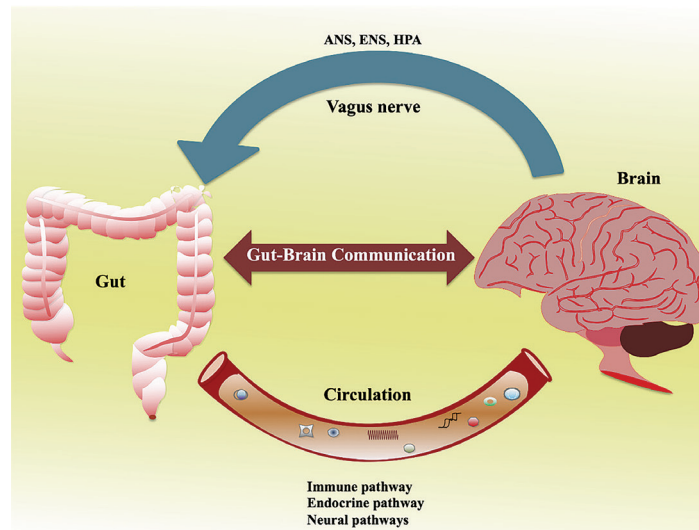


Figure 3. A schematic diagram illustrating the two-way communication between the gut and the brain

Source: [19]

This communication involves both afferent neurons, which transmit signals from the gut to the CNS, and efferent neurons, which send responses from the CNS back to the gut. These signals travel through multiple interconnected pathways, including the autonomic nervous system (ANS), the enteric nervous system (ENS), the hypothalamic-pituitary-adrenal (HPA) axis, the sympatho-adrenal axis, and descending monoaminergic pathways. Each of these systems is intricately interwoven and modulated by neuro-humoral and inter-relational factors. Of particular importance, the ENS – often referred to as the “second brain” – is a complex network of neurons responsible for the intrinsic control of gastrointestinal function. It comprises two primary ganglionated plexuses: the myenteric plexus, which primarily regulates gut motility (e.g. peristalsis), and the submucosal plexus, which oversees secretory and absorptive processes within the gastrointestinal tract [19].

As of 2025, there is insufficient conclusive evidence regarding the extent to which gluten intake contributes to gastrointestinal symptoms in individuals who do not have coeliac disease. Patients on a gluten-containing (GC) diet often experience a significant exacerbation of symptoms such as fatigue and abdominal discomfort; however, the underlying mechanisms remain unclear. Notably, the effects of gluten have also been examined in individuals with IBS, particularly those with diarrhoea-predominant symptoms. Findings by O. Ogilvie *et al.* [12] suggest that gluten consumption increases stool frequency, an effect more pronounced in individuals carrying the HLA-DQ2 or HLA-DQ8 genotypes. The mechanisms by which gluten triggers symptoms in noncoeliac gluten sensitivity

differ from those in coeliac disease and are not yet fully understood as of 2025. Current evidence [12] did not conclusively demonstrate abnormalities in adaptive immunity or increased intestinal permeability in NCGS. Despite this, NCGS is associated with signs of intestinal inflammation, such as elevated levels of interferon-gamma (IFN- γ) and a higher count of intraepithelial lymphocytes – findings that suggest enhanced immune activity within the gut lining. These observations imply that adaptive immunity may contribute to NCGS, while the involvement of innate immunity is also indicated, particularly through the possible activation of toll-like receptors (such as TLR-1 and TLR-2) implicated in its pathogenesis. Despite these insights, the condition remains controversial.

E.L. Transeth *et al.* [18] argued that NCGS is not a distinct clinical entity but rather a subset of IBS, given that roughly one-third of IBS patients report gluten sensitivity. They proposed that fermentable oligo-, di-, mono-saccharides and polyols (FODMAPs) are the true source of gastrointestinal symptoms in these cases. Nonetheless, NCGS is increasingly recognised as a separate clinical condition that shares symptomatic overlap with both IBS and CD but lacks the intestinal mucosal damage characteristic of CD [5]. Furthermore, distinguishing between food sensitivity and food intolerance is crucial for accurate diagnosis and treatment. Food intolerance typically involves gastrointestinal symptoms arising from the fermentation of poorly metabolised carbohydrates by the colonic microbiota, while food sensitivity refers to an immune-mediated reaction to dietary antigens that can cause both gastrointestinal and extraintestinal symptoms. Given these

distinctions, IBS and NCGS should be considered as separate disorders with overlapping clinical features, which complicate diagnosis and management [16].

Nutrition plays a critical role in maintaining gut health, which in turn is closely linked to immune and neurological function. In particular, there is growing interest in the findings of D.N. Koutzoumis *et al.* [20] regarding the role of the gut microbiota in the pathogenesis of neurodegenerative diseases such as Parkinson's disease (PD), using a rodent model. Their evidence indicates that gut dysbiosis may precede motor symptoms and contribute to neuroinflammation and dopaminergic neuron loss. In this study, using a 6-hydroxydopamine (6-OHDA) rodent model of PD, chronic treatment with non-absorbable antibiotics was shown to attenuate dopaminergic neuron degeneration and reduce neuroinflammatory markers in the striatum, accompanied by improved motor performance. These findings highlight a potential involvement of the gut microbiota in the pathophysiology of PD.

In individuals with coeliac disease, ingestion of gluten triggers a variable range of symptoms accompanied by inflammation of the small intestine. This immune-mediated response is characterised by villous atrophy and infiltration of inflammatory cells in the epithelial layer and lamina propria, which significantly impairs the functional capacity of the intestine. The resulting mucosal damage [21] often leads to malabsorption of essential nutrients, including iron, folate, vitamin B12, and zinc – contributing to the frequent observation of nutrient deficiencies at the time of CD diagnosis. As of 2024-2025, the only effective treatment for CD is lifelong adherence to a strict GFD, which generally permits mucosal healing of the small intestine within one to two years. However, even minimal exposure to gluten – intentional or inadvertent – can sustain intestinal inflammation and continue to impair nutrient absorption. Nutrient deficiencies in CD may also stem from the inherent limitations of the GFD itself. Processed gluten-free products have been shown by A. Jivraj *et al.* [16] to be lower in micronutrients such as iron and folate, with inconsistent fortification practices across the market. Consequently, individuals adhering to a GFD are at a greater risk of consuming a less nutrient-dense diet compared to those following a gluten-containing diet. A. Bakhshipour & R. Razaiee [17] highlighted an increased risk of micronutrient deficiencies – particularly vitamins D and K, and iron – among individuals with coeliac disease, even those adhering to a gluten-free diet. However, the extent of these deficiencies and the roles of GFD duration, adherence, and persistent symptoms remain unclear. It is also uncertain whether such nutritional gaps are unique to CD patients or similarly affect non-CD individuals who follow a GFD.

In the digestive tract, gluten is broken down into gluten immunogenic peptides (GIPs), which cause coeliac disease. These peptides are resistant to complete digestion in the intestine and can interact with the immune system of coeliac patients, triggering an autoimmune reaction. Previously, there was no reliable way to objectively monitor gluten intake. However, a relatively new technique, developed by D. Stanciu *et al.* [1], enables the direct detection of GIP – specifically, gliadin epitopes equivalent to a 33-mer peptide – in urine and faeces after gluten consumption. This method uses monoclonal

antibodies and is a non-invasive, highly sensitive, quantitative, and objective way to assess compliance with a gluten-free diet. The sensitivity of the test allows for the detection of even the smallest amounts of gluten – as little as 50 mg. However, the link between a positive GIP test result and the presence of villous atrophy has not yet been established, so it is not currently possible to assess intestinal damage using this marker. It should be noted that even among patients who claim to strictly adhere to dietary guidelines, unintentional dietary errors are common.

In a controlled study, M. Myhrstad *et al.* [22] also turned their attention to improving the nutritional quality of diets in specific populations, such as individuals with coeliac disease, who must strictly adhere to a gluten-free diet. This dietary restriction often results in reduced fibre intake, as many gluten-free products are low in dietary fibre and of poor sensory quality. A randomised controlled trial explored participants' experiences with fibre-rich gluten-free bread developed for the study, compared to commercially available gluten-free bread. Participants reported avoiding gluten-free bread prior to the study due to its unpleasant taste and texture, but expressed a preference for the fibre-rich wholegrain bread provided during the intervention. They emphasised the importance of satiety, texture, and palatability in gluten-free products, indicating a demand for higher-quality, fibre-rich alternatives. E.M. Domsa *et al.* [23] and L. Garnweidner-Holme *et al.* [24] underscored the central role of the gut microbiota as a mediator between dietary input and brain health. While antibiotic-induced modulation of the microbiota appears neuroprotective in PD models, dietary enrichment with fermentable fibre may offer a nonpharmacological avenue to promote a healthy microbiome.

Although the availability of gluten-free products has increased in recent years, individuals with CD continue to face multiple challenges in maintaining this diet. A study from Norway [25], using interpretative phenomenological analysis, highlighted the lived experiences of people with CD and revealed barriers to successfully following a gluten-free diet at the individual, interpersonal, community, and policy levels. Participants reported initial difficulties in acquiring knowledge about the GFD and expressed doubts about its nutritional adequacy. Social aspects – such as the fear of contamination and feeling different during shared meals – negatively affected their quality of life. At the community level, participants emphasised the limited availability and poor sensory quality of gluten-free products, while at the policy level, affordability emerged as a pressing concern. These findings underscore the need for comprehensive support for individuals with CD – from immediate post-diagnosis education to structural changes in food policy and product development.

Amid these challenges, pseudocereals such as quinoa, amaranth, and buckwheat have emerged as promising gluten-free alternatives due to their favourable nutritional profiles and natural absence of gluten, according to S. Graziano *et al.* [26]. These crops are rich in essential amino acids, fibre, and micronutrients, making them suitable for enhancing the dietary quality of gluten-free products. However, their broader adoption in the food industry faces significant constraints. The presence of anti-nutritional compounds and bitter-tasting substances can limit

palatability and necessitate processing methods that may be costly or environmentally burdensome. Additionally, agronomic limitations restrict large-scale cultivation to specific regions – often in developing countries where the risk of overexploitation and socio-economic inequity is high. The integration of biotechnological tools and sustainable breeding programmes is essential for enhancing the desirable traits of pseudocereals while protecting the livelihoods of smallholder farmers who maintain these crops' genetic diversity [27]. Taken together, these perspectives emphasised a dual imperative: improving the quality and accessibility of gluten-free products for individuals with CD, and advancing the responsible, sustainable development of alternative gluten-free crops such as pseudocereals. Efforts across the food industry, agriculture, policy, and public health must converge to ensure progress on both fronts.

◆ CONCLUSIONS

It was found that gluten-sensitive disorders, such as coeliac disease and non-coeliac gluten sensitivity, significantly affect the state of the intestinal microbiota, causing an imbalance known as dysbiosis. This imbalance is characterised by a decrease in the number of beneficial bacteria – particularly *Lactobacillus* and *Bifidobacterium* – and an increase in opportunistic microorganisms, such as *Bacteroides* and *Escherichia coli*. The gluten-free diet, which is the primary method of treatment, contributes to the partial normalisation of this microbial balance, reducing pathogenic forms and increasing beneficial bacteria. However, even with adherence to the diet, complete restoration of a healthy microbiota does not always occur. Particular attention is given to the antimicrobial peptide CRAMP, which plays a key role in regulating the immune response, controlling inflammation, and maintaining the homeostasis of the intestinal mucosa. Reduced production of CRAMP in the duodenum is associated with

gluten-induced enteropathy, and its exogenous administration has been shown to promote the restoration of epithelial function and enhancement of regulatory immune responses. It has also been found that the dysbiosis accompanying this pathology is characterised by an increase in *Pseudomonas aeruginosa*, which inhibits CRAMP synthesis. In addition, the importance of the gut-brain axis has been confirmed, as the intestinal microbiota performs key functions, including nutrient metabolism, protection against pathogens, and modulation of the immune response. Dysbiosis can influence the neuropsychiatric status of patients, including the development of anxiety disorders, depression, and cognitive impairment. Potential therapeutic strategies – such as the use of probiotics, prebiotics, postbiotics, and low-FODMAP diets – have been reviewed for their ability to modulate the composition and functional activity of the intestinal microbiota. Thus, the findings highlighted a complex interaction between gluten, the intestinal microbiota, and the immune system, emphasising the need for an integrated approach to the diagnosis and treatment of gluten-sensitive disorders. Future research should focus on the development of individualised therapeutic strategies through targeted modulation of the intestinal microbiota, as well as the refinement of diagnostic methods – such as the detection of gluten immunogenic peptides in faeces and urine – to enable objective monitoring of adherence to a gluten-free diet and the timely identification of latent disorders.

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Вплив змін в кишковій мікробіоті на здатність організму переробляти білки глютену: міні огляд

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Анотація. Глютен-чутливі розлади, зокрема целіакія та нецеліакійна чутливість до глютену, є поширеними гастроентерологічними станами, що супроводжуються порушенням функціонування кишкового тракту, системної імунної відповіді та бар'єрної функції епітелію. Метою даної роботи стало всебічне вивчення впливу глютену на стан кишкової мікробіоти та визначення ролі мікробіому в патогенезі зазначених захворювань. У процесі аналізу використано результати сучасних клінічних досліджень, систематичних оглядів, метааналізів, а також високоточні методики секвенування нового покоління для оцінки змін у бактеріальному та грибовому складі кишкової мікрофлори на різних стадіях захворювання. Встановлено, що безглютенова дієта сприяє частковій нормалізації мікробного балансу, зокрема зменшує кількість патогенних та умовно-патогенних мікроорганізмів, пов'язаних із розвитком і підтриманням хронічного запального процесу в кишечнику. Натомість відзначається зростання представників корисної коменсальної мікрофлори, таких як *Lactobacillus* і *Bifidobacterium*. Особливу увагу приділено антимікробному пептиду Cathelin-related antimicrobial peptide, який виконує важливу регуляторну функцію в імунній відповіді, контролює розвиток запалення та забезпечує гомеостаз слизової оболонки кишківника. Також оцінено значення осі кишечник-мозок у контексті глютен-чутливих станів, з огляду на те, що дисбіоз може впливати на нейропсихічний статус пацієнтів, включаючи розвиток тривожних розладів, депресії та когнітивних порушень. Розглянуто потенційні терапевтичні стратегії, включаючи застосування пробіотиків, пребіотиків, постбіотиків, а також дієти з низьким вмістом ферментованих оліго-, ді-, моносахаридів і поліолів (FODMAP). Показано, що харчування чинить безпосередній і тривалий вплив на склад та функціональну активність кишкової мікробіоти, що, своєю чергою, модифікує клінічну картину захворювання, вираженість симптомів і ефективність терапії. Практична цінність огляду полягає в обґрунтуванні сучасних підходів до діагностики та лікування глютен-чутливих розладів шляхом цілеспрямованої модуляції кишкової мікробіоти. Це відкриває нові перспективи для індивідуалізованої терапії, поліпшення якості життя, запобігання ускладненням та відновлення здоров'я пацієнтів на більш глибокому – мікробіомному – рівні

Ключові слова: непереносимість глютену; безглютенова дієта; здорове харчування; клінічне харчування; дисбактеріоз



Microbiological rationale for alternative strategies to combat infections caused by antibiotic-resistant *Pseudomonas aeruginosa*

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Abstract. This study aimed to investigate the activity of the drug Pyofag against clinical isolates of *Pseudomonas aeruginosa* and to evaluate the effectiveness of the combined action of surface-active antiseptics and bacteriophages. To achieve this aim, classical methods for the isolation and identification of bacteria were employed. Antibiotic susceptibility of *Pseudomonas* isolates was determined using the disc diffusion method, while susceptibility to surface-active antiseptics (decamethoxine, benzalkonium chloride, chlorhexidine bigluconate, octenidine dihydrochloride, and polyhexanide) was assessed using the broth dilution method. The susceptibility of clinical isolates to Pyofag was evaluated based on the optical density of bacterial suspensions after 18 hours of incubation with the preparation. The nature of the combined effect of bacteriophages and antiseptics on *P. aeruginosa* was assessed by calculating the lytic index of the phage on planktonic bacterial forms cultured in media containing sub-bacteriostatic concentrations of antiseptics. The results showed that all 54 isolated clinical strains of *P. aeruginosa* retained high susceptibility only to reserve antibiotics – colistin (94.4%) and cefiderocol (75.9%). Resistance to other antipseudomonal antibiotics (cefepime, ceftazidime, piperacillin-tazobactam, imipenem, and ciprofloxacin) was observed in 96.3%-100% of isolates. However, aminoglycosides (gentamicin, tobramycin, amikacin) and meropenem remained effective against 29.6%-44.4% of strains. Antiseptic agents containing

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surface-active compounds demonstrated strong antipseudomonal properties and are capable of inhibiting bacterial proliferation at concentrations ranging from 16.4-22.5 µg/mL (octenidine dihydrochloride, decamethoxine, chlorhexidine bigluconate) to 65-145.7 µg/mL (polyhexanide, benzalkonium chloride). It was confirmed that decamethoxine, octenidine, and chlorhexidine exhibit significantly greater antibacterial activity than polyhexanide and benzalkonium chloride ($p < 0.01$). The isolated *Pseudomonas* strains showed high susceptibility to the pharmaceutical preparation Pyofag: the lytic activity index (I_s) of Pyofag exceeded 0.5 in 70.4% of strains, indicating that 50% of the bacterial population was destroyed during the dynamic interaction between bacterial growth and phage replication. In media containing sub-bacteriostatic concentrations of decamethoxine, chlorhexidine, or octenidine, both susceptible ($n = 7$, $I_s = 0.69$) and resistant ($n = 8$, $I_s = 0.15$) strains were lysed more intensively by the bacteriophage. This was evidenced by an increase in the susceptibility index to 0.80-0.87 in susceptible strains and to 0.54-0.70 in phage-resistant strains, respectively

Keywords: surface-active antiseptics; bacteriophages; antibiotics; Pyofag; opportunistic microorganisms

◆ INTRODUCTION

During the period from 2015 to 2025, the problem of antibiotic resistance has assumed global importance. Existing limitations in the effectiveness of antibacterial therapy have led to an increase in the number of severe infections, higher mortality rates, and significant economic costs associated with treatment. Against the backdrop of the growing number of infections caused by superpathogens that demonstrate multidrug resistance (MDR), significant attention is being paid to the search for alternative means of antibacterial therapy, among which natural destroyers of bacteria – bacteriophages – play an increasingly prominent role. The prolonged war in Ukraine and the fullscale Russian aggression launched in 2022 have led to a sharp rise in the number of severe wound infections. The widespread use of antibiotics for prophylactic and therapeutic purposes during evacuation and the provision of medical care has contributed to the selection of antibiotic-resistant strains. Therefore, the issue of identifying alternative strategies for combating antibiotic-resistant microorganisms – particularly non-fermenting Gram-negative bacilli (*Pseudomonas* spp., *Acinetobacter* spp.) – and introducing new methods of antibacterial therapy using bacteriophages, which have demonstrated their effectiveness, is particularly relevant.

No randomised controlled trials directly comparing bacteriophage therapy with standard antibiotic treatment for antibiotic-resistant *Pseudomonas aeruginosa* infections in intensive care units (ICUs), burn, or polytrauma patients have been published since 2020. However, several wellcharacterised case reports and small cohort studies indicate that phage-antibiotic combinations are feasible, generally well tolerated, and may be clinically beneficial in selected challenging cases, with molecular and phenotypic resistance dynamics documented in a subset. A retrospective, observational study on device-related or systemic infections conducted by S.I. Green *et al.* [1] evaluated 12 cases of customised phage therapy, showing a 66% favourable response rate, with 42% bacterial eradication. Phage therapy was safe, though immunological neutralisation occurred in some instances.

J.-P. Pirnay *et al.* [2] conducted a multicentre, multinational, retrospective observational study focusing on individualised phage therapy. The researchers analysed 100 cases of individualised phage therapy across 12 countries and observed clinical improvement in 77.2% of cases and bacterial eradication in 61.3%. The use of antibiotics alongside phage therapy increased the likelihood of success. N. Cesta *et al.* [3] published a case report describing

a 62-year-old patient with chronic *P. aeruginosa* infection who was successfully treated with customised phage therapy and meropenem, showing no recurrence of infection over a period of two years.

A case series on anti-*S. aureus* therapy for diabetic foot infection conducted by M.J. Young *et al.* [4] tested anti-*S. aureus* phage therapy on 10 patients at high risk of amputation. Nine out of ten patients benefited, although one patient showed no response to treatment. L. Rahimzadeh Torabi *et al.* [5] demonstrated success using personalised intravenous/nebulised phage and antibiotics in an extremely drug-resistant (XDR) burn patient, with clinical cure, detailed adverse event monitoring, and resistance documentation. The findings of K. Racenis *et al.* [6] indicated that the combination of phages, antibiotics, and surgical intervention holds considerable potential for treating left ventricular assist device (LVAD)-associated *Pseudomonas aeruginosa* infections, with biofilm formation and resistance phenotype evolution tracked *in vitro*.

Across all reports, phage therapy (often in combination with antibiotics) was well tolerated, with no severe infusion reactions or cytokine storms clearly attributable to phage administration. Current evidence remains limited to salvage case series and a single non-randomised cohort; phageantibiotic combinations are promising yet unproven alternatives for resistant *Pseudomonas aeruginosa* infections in critical care settings. This underscores the urgent need for well-powered, rigorously monitored clinical trials with standardised endpoints for resistance and safety. This research aimed to investigate the activity of the drug Pyofag and to assess the effectiveness of its combined action with surfactant-based antiseptics against clinical isolates of *Pseudomonas aeruginosa* obtained from wounded servicemen with complicated wound infections.

◆ MATERIALS AND METHODS

Characteristics of the antiseptic compounds and biological preparations used in the study:

- ◆ Decamethoxine is an antiseptic from the group of surfactants and a derivative of bisquaternary nitrogen. In the study, the substance decamethoxine – a fine-grained white powder readily soluble in water and alcohol – was used. A working solution at a concentration of 0.1% was prepared by dissolving 100 mg of decamethoxine in 100 mL of sterile, purified water.

- ◆ Benzalkonium chloride (alkyldimethylbenzylammonium chloride) is a cationic surfactant and an antiseptic

from the group of quaternary ammonium compounds. In this research, a 50% solution of benzalkonium chloride (produced in China) was used. To prepare a 0.5% working solution, a 1:10 dilution was made using sterile purified water.

- ♦ Octenidine dihydrochloride is a surface-active antiseptic with a broad spectrum of microbicidal activity. To evaluate the susceptibility of clinical bacterial strains, the commercial preparation Octenisept was used. It contains 0.1 g of octenidine dihydrochloride and 2.0 g of phenoxethanol per 100 mL of water (produced in Germany).

- ♦ Chlorhexidine bigluconate, a biguanide derivative, acts via a surface-active mechanism that disrupts microorganisms. It is widely employed as an antiseptic (0.1% solution) and as a disinfectant (0.25% and 0.5% solutions), both in complex formulations and as aqueous or aqueous-alcoholic solutions. In this study, a 20% solution of chlorhexidine (produced in Ukraine) was used to prepare a 0.2% working solution by diluting 10 mL of the drug in 90 mL of sterile purified water (1:10 dilution).

- ♦ Polyhexanide (polyamidopropylbiguanide) is a polymeric cationic surfactant with antiseptic and disinfectant properties. Susceptibility was assessed using the drug Prontosan, a wound irrigation solution produced in Germany, which contains 0.1% polyhexanide, 0.1% betaine, and purified water.

- ♦ Pyofag is a polyvalent bacteriophage preparation produced by Infuzia PJSC, Ukraine, for NEO PROBIO CARE INC., Canada. One millilitre of the preparation contains specific bacteriophages at a concentration of at least 1×10^5 phage particles, targeting the following microorganisms: *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Proteus mirabilis* [7].

The object of the study was microbial cultures obtained from the museum of clinical isolates at the bacteriological laboratory of the Department of Microbiology, National Pirogov Memorial Medical University, Vinnytsya. These isolates were collected between 2022 and 2024 from patients with purulent-inflammatory wound complications who were undergoing treatment at the Communal nonprofit enterprise Vinnytsya Regional Clinical Hospital named after N.I. Pirogov Vinnytsya Regional Council, specifically at the Centre for Thermal Trauma and Plastic Surgery and other medical institutions in Vinnytsya. The study protocol was approved by the Committee on Bioethics, National Pirogov Memorial Medical University, Vinnytsya, Ukraine (Protocol No. 3; 07 April 2022).

In total, 152 isolates of Gram-negative bacteria were obtained during the 2022-2024 period. Differentiation of Gram-negative bacteria was conducted according to generally accepted criteria, based on the following indicators: oxidase test results, the ability to produce a water-soluble pigment, morphological characteristics of colonies formed on tryptic soy agar (TSA), and biochemical activity determined using diagnostic test systems Neferm-test 24 and Entero-test 24 (PLIVA-Lachema a.s., Brno, Czech Republic). Antibiotic susceptibility testing of the isolated strains was performed following the Order of the Cabinet of Ministers of Ukraine No. 116-p [8] and the recommendations of EUCAST (version 13) [9].

Determination of the susceptibility of isolated *P. aeruginosa* strains to the drug Pyofag polyvalent bacteriophage

was conducted using a simplified, original method involving the calculation of a susceptibility index for each isolated culture. From cultures of clinical *P. aeruginosa* strains grown on TSA for 18-20 hours at $36^\circ\text{C} \pm 1^\circ\text{C}$, a bacterial suspension was prepared at a concentration of 1.5×10^8 CFU/mL, corresponding to 0.5 McFarland units, in isotonic sterile sodium chloride solution. The turbidity of the suspension was adjusted to the required concentration using a DensiLa-Meter densitometer. For the experiment, 200 μL of the prepared bacterial suspension was added to sterile plastic tubes (16 mm diameter) containing 2 mL of sterile meat-peptone broth (MPB). The tubes were incubated in a thermostat for 2 hours to reach the logarithmic growth phase, after which 200 μL of the phage cocktail Pyofag was added to the test tube. Simultaneously, 200 μL of sterile isotonic sodium chloride solution was added to the control tube. The experiment included a medium control (2 mL of MPB), which was incubated alongside both the growth control and the test tube for 18 hours at $36^\circ\text{C} \pm 1^\circ\text{C}$. Following incubation, the optical density of the suspension in the test culture – cultivated in the presence of 10^4 phage particles – was measured, taking into account the dilution factor (Df), the control culture (Dk), and the nutrient medium (Dc), all expressed in McFarland units. The assessment of the susceptibility of clinical isolates to the biological preparation Pyofag was performed by calculating the susceptibility index to the bacteriophage (Is) using formula (1):

$$Is = (Dk - Df) / (Dk - Dc), \quad (1)$$

where Dk – the density of the bacterial suspension in the control tube, in McFarland units; Df – the density of the bacterial suspension in the medium containing bacteriophage, in McFarland units; Dc – the density of the sterile culture medium, in McFarland units.

This index, as illustrated by the formula, enables an estimation of the proportion of the bacterial suspension destroyed by the bacteriophage after 18 hours of incubation, relative to the control. The susceptibility of the bacterial cultures to antiseptic agents was determined by the method of serial dilutions, establishing the minimum inhibitory concentration (MIC, $\mu\text{g}/\text{mL}$) and the minimum bactericidal concentration (MBC, $\mu\text{g}/\text{mL}$) [8, 9].

To identify the synergistic bactericidal effect of surfactant-based antiseptics and lytic bacteriophages on clinical isolates, nutrient media were prepared containing sub-bacteriostatic concentrations (25% of the MIC) of the antiseptic for the test strain, and the lytic activity of the phage was re-evaluated using the method described above. The experiment included several controls: a medium control, a culture growth control in the presence of the sub-bacteriostatic concentration of antiseptic (sub-MIC, $\mu\text{g}/\text{mL}$), and a microbial population growth control in the presence of phage in an untreated medium. Data were considered valid if the density of the culture control in the presence of the subMIC antiseptic remained within $\pm 15\%$ of the control density without antiseptic. The resulting Is values were compared with the baseline data to assess the effect of inactive concentrations of antiseptic on the lytic activity of the phage.

Statistical analysis was conducted using Microsoft Excel 2010, applying methods of variational statistics [10]. The mean values of the parametric data, along with the

standard deviation ($M \pm s$), were calculated, and the frequency of occurrence of non-parametric features in the study group was determined. Individual samples were tested for normality of distribution ($F(x)$), and the reliability of results across different groups was assessed using FT-EST, TTEST, and Student's t -test. Comparisons between multiple samples were made using the Bonferroni correction, depending on the number of samples being analysed. Results were considered statistically significant at a

p -value of ≤ 0.05 , with high statistical reliability confirmed for p -values of $p \leq 0.01$, following the correction.

RESULTS

Based on morphological, cultural, and biochemical characteristics, 54 clinical strains (35.5% of all isolated microorganisms) were identified as *Pseudomonas aeruginosa*. The results of antibiotic susceptibility testing for these isolates are presented in Figure 1.

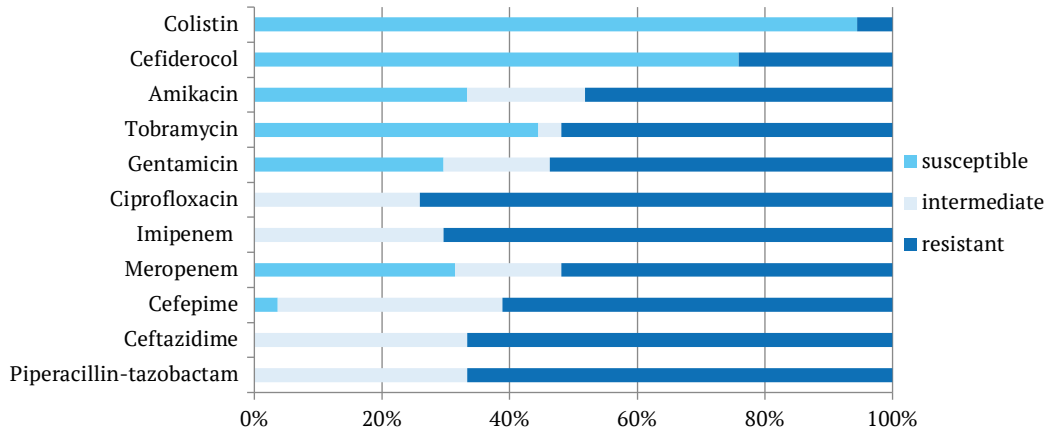


Figure 1. Antibiotic susceptibility profile of isolated *P. aeruginosa* strains (n = 54)

Source: compiled by the authors

According to the obtained results, clinical isolates of *Pseudomonas aeruginosa* demonstrated high susceptibility to polymyxin (94.4%) and cefiderocol (75.9%), and moderate susceptibility to the aminoglycosides amikacin, tobramycin, and gentamicin, for which the combined proportion of susceptible and intermediate strains was 51.85%, 48.1%, and 46.3%, respectively. Among these, tobramycin exhibited the highest proportion of susceptible isolates – 44.4%, compared to 33.3% for amikacin and 29.6% for gentamicin. A total of 31.5% of isolates remained susceptible to meropenem, whereas no isolates were fully susceptible to imipenem; only 29.6% of clinical strains were classified as moderately resistant. The susceptibility of *Pseudomonas aeruginosa* to other

antipseudomonal beta-lactams revealed low susceptibility to piperacillin-tazobactam and ceftazidime: 33.3% of isolates were moderately resistant, while 66.7% were resistant. Only 2 out of 54 strains remained susceptible to cefepime, with 35.2% moderately resistant and 42.6% resistant. Thus, resistance to antipseudomonal cephalosporins and penicillins reached 96.3%-100%. Cationic surfactants demonstrated considerable activity against Gram-negative non-fermenting bacteria. The susceptibility of the clinical isolates to quaternary ammonium compounds (decamethoxine, benzalkonium chloride), as well as octenidine dihydrochloride, chlorhexidine bigluconate, and polyhexanide, was assessed. The results are summarised in Table 1.

Table 1. Susceptibility of clinical isolates of *P. aeruginosa* to antiseptics (n = 54)

Antiseptic	Average concentrations of antiseptic ($M \pm s$, $\mu\text{g/mL}$)	
	Minimum inhibitory concentration (MIC)	Minimum bactericidal concentration (MBC)
Decamethoxin	$22.52 \pm 2.83^{4,5}$	$73.74 \pm 15.06^{4,5}$
Benzalkonium chloride	$145.7 \pm 32.1^{1,2,3}$	$418.75 \pm 78.89^{1,3}$
Chlorhexidine bigluconate	$22.5 \pm 2.36^{4,5}$	72.09 ± 2.06^5
Octenidine dihydrochloride	$16.4 \pm 2.43^{4,5}$	$102.5 \pm 10.75^{4,5}$
Polyhexanide	$65.0 \pm 5.08^{1,2,3}$	$192.5 \pm 27.70^{1,2,3}$

Notes: ¹ – statistically significant difference compared to decamethoxine ($p \leq 0.01$); ² – statistically significant difference compared to chlorhexidine ($p \leq 0.01$); ³ – statistically significant differences compared to octenidine ($p \leq 0.01$); ⁴ – statistically significant differences compared to benzalkonium ($p \leq 0.01$); ⁵ – statistically significant differences compared to polyhexanide ($p \leq 0.01$)

Source: compiled by the authors

It was found that the greatest antipseudomonal activity was exhibited by decamethoxine, chlorhexidine bigluconate, and octenidine dihydrochloride, which inhibited

bacterial growth at MICs of $22.52 \pm 2.83 \mu\text{g/mL}$, $22.5 \pm 2.36 \mu\text{g/mL}$, and $16.4 \pm 2.43 \mu\text{g/mL}$, respectively. The MBCs of these antiseptics ranged from $72.09 \pm 2.06 \mu\text{g/mL}$

(chlorhexidine) to $102.5 \pm 10.75 \mu\text{g/mL}$ (octenidine). No statistically significant differences in antimicrobial activity were found among decamethoxine, chlorhexidine, and octenidine; however, all three exhibited significantly greater antipseudomonal activity than polyhexanide and benzalkonium chloride ($p \leq 0.01$). The antiseptics decamethoxine, chlorhexidine, and octenidine exhibited a bacteriostatic effect at concentrations 2.9-4 times and 6.5-8.9 times lower than those required for polyhexanide and benzalkonium chloride, respectively. It was found that the death of *P. aeruginosa* occurred at minimum concentrations of $192.5 \pm 27.70 \mu\text{g/mL}$ for polyhexanide and $418.75 \pm 78.89 \mu\text{g/mL}$ for benzalkonium chloride, which significantly exceeded the corresponding values for decamethoxine, chlorhexidine, and octenidine by 1.9-2.7 times and 4.1-5.8 times, respectively ($p \leq 0.01$).

Assessment of the susceptibility of the isolated *P. aeruginosa* strains established that the biologically active preparation Pyofag displayed variable activity against the tested isolates. Calculation of the susceptibility index enabled evaluation of the lytic efficacy of the antipseudomonal phage cocktail in the preparation by determining the proportion of the bacterial population undergoing lysis after 18-20 hours of incubation. Based on the value of the susceptibility index (*Is*), the isolated strains were

classified into the following categories: highly susceptible ($0.8 \leq Is \leq 0.99$), susceptible ($0.5 \leq Is \leq 0.79$), moderately resistant ($0.3 \leq Is \leq 0.49$), and resistant ($0.01 \leq Is \leq 0.29$). The distribution of isolates by susceptibility to the polyvalent bacteriophage is shown in Figure 2.

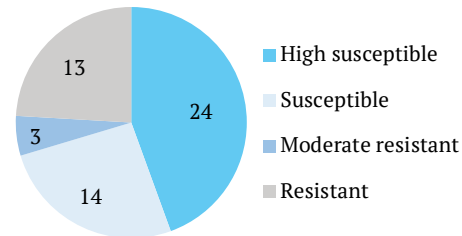


Figure 2. Susceptibility of isolated *Pseudomonas aeruginosa* strains to Pyofag (n = 54)

Source: compiled by the authors

According to the data obtained, 70.4% of *P. aeruginosa* strains (38 isolates) were classified as highly susceptible or susceptible to Pyofag, while 29.6% (16 isolates) were moderately resistant or resistant. The values of the phage lytic activity index (*Is*) for the tested *P. aeruginosa* strains are presented in Table 2.

Table 2. Susceptibility of clinical isolates of *P. aeruginosa* (n = 54) to Pyofag

Susceptibility category	Number of isolates (n)	Susceptibility index (<i>Is</i>)
Highly susceptible	24	$0.95 \pm 0.007^*$
Susceptible	14	$0.65 \pm 0.024^*$
Moderately resistant	3	$0.34 \pm 0.020^*$
Resistant	13	$0.098 \pm 0.026^*$

Notes: * – statistically significant differences between group values ($p \leq 0.01$)

Source: compiled by the authors

According to the results, the lytic effect of Pyofag was observed in 24 *P. aeruginosa* strains, as indicated by a susceptibility index (*Is*) of 0.95 ± 0.007 . This suggests that only 5% of the population remained viable 20 hours after phage application. In the susceptible isolates (n = 14), the susceptibility index was 0.65 ± 0.024 , indicating a substantial activity of Pyofag and its ability to reduce the bacterial population by approximately 65% within 20 hours of incubation. Among the moderately resistant *P. aeruginosa* strains, 66% of cells survived phage treatment, reflected by a susceptibility index of 0.34 ± 0.020 . In contrast, 13 strains were classified as resistant to the polyvalent pseudomonal phage cocktail in Pyofag, with a susceptibility index of

0.098 ± 0.026 . Given the results obtained, the potential synergistic detrimental effect of surfactant-active antiseptics and bacteriophages on clinical strains of *P. aeruginosa* was investigated. To this end, the lytic effect of the phage was assessed under cultivation conditions in a medium containing 25% of the sub-bacteriostatic concentration of the antiseptic specific to each *P. aeruginosa* strain. To rule out the additive effect of the antipseudomonal agents, the experiment included controls to measure the optical density (in McFarland units) of bacterial suspensions cultured in the presence of subbacteriostatic concentrations (subMIC) of antiseptics, compared with the untreated culture control (Table 3).

Table 3. Optical density of *P. aeruginosa* bacterial cultures in the presence of sub-bacteriostatic concentrations (subMIC) of antiseptics ($M \pm s$, McFarland units)

Characteristics of the nutrient medium	Phage-resistant strains (n = 8)	Phagosensitive strains (n = 7)
Control MPB	7.44 ± 0.19	7.07 ± 0.14 (-5%)
MPB with subMIC decamethoxine	6.79 ± 0.22	6.49 ± 0.20 (-4.4%)
MPB with subMIC benzalkonium chloride	6.95 ± 0.20	6.54 ± 0.22 (-6%)
MPB with subMIC chlorhexidine bigluconate	6.81 ± 0.25	6.64 ± 0.13 (-2,5%)
MPB with subMIC octenidine dihydrochloride	6.73 ± 0.24	6.6 ± 0.17 (-2%)
MPB with subMIC polyhexanide	6.85 ± 0.17	6.46 ± 0.26 (-5.7%)

Source: compiled by the authors

As shown by the results of the control measurements, sub-bacteriostatic concentrations of antiseptic agents did not result in significant inhibition of bacterial growth, as evidenced by a 2-6% reduction in average optical density relative to the control. Therefore, these concentrations did not exert a pronounced effect on bacterial proliferation.

The synergistic effect of surfactants (chemical lytic factors) and Pyofag (a biological lytic agent) was assessed in strains resistant ($I_s = 0.15 \pm 0.05$) and susceptible ($I_s = 0.69 \pm 0.03$) to the phage. A total of eight resistant and seven phage-susceptible *P. aeruginosa* clinical isolates were included. The results are presented in Table 4.

Table 4. Lytic effect of phage on clinical isolates of *P. aeruginosa* in the presence of subbacteriostatic concentrations of surfactant antiseptics

Antipseudomonal factor(s)	Susceptibility index (I_s) ($M \pm s$)	
	Phage-resistant <i>P. aeruginosa</i> (n = 8)	Phagesensitive <i>P. aeruginosa</i> (n = 7)
Pyofag	0.15 ± 0.05	0.69 ± 0.03
Pyofag with subMIC decamethoxine	$0.70 \pm 0.09^*$	$0.87 \pm 0.05^*$
Pyofag with subMIC benzalkonium chloride	0.25 ± 0.05	0.75 ± 0.04
Pyofag with subMIC chlorhexidine bigluconate	$0.54 \pm 0.09^*$	$0.80 \pm 0.02^*$
Pyofag with subMIC octenidine dihydrochloride	$0.64 \pm 0.12^*$	$0.86 \pm 0.03^*$
Pyofag with subMIC polyhexanide	0.28 ± 0.05	0.76 ± 0.04

Notes: * – statistically significant differences relative to the corresponding Pyofag-only indicator ($p \leq 0.01$)

Source: compiled by the authors

The results indicated that the presence of 25% of the minimum bacteriostatic concentration of decamethoxine, chlorhexidine bigluconate, and octenidine dihydrochloride led to a statistically significant enhancement of the lytic effect of Pyofag ($p \leq 0.01$), as reflected in increased susceptibility index values. Specifically, in the group of *P. aeruginosa* strains resistant to the phage cocktail ($I_s = 0.15 \pm 0.05$), the susceptibility index increased by 3.6, 4.3, and 4.7 times, respectively, in the presence of chlorhexidine, octenidine, and decamethoxine at concentrations that did not inhibit bacterial growth. These differences were statistically significant ($p \leq 0.003$). It was also demonstrated that the effectiveness of Pyofag against

susceptible strains of *P. aeruginosa* increased by 1.2 times in the presence of decamethoxine and octenidine, while sub-bacteriostatic concentrations of chlorhexidine increased the susceptibility of *P. aeruginosa* by 1.16 times. According to the findings, the susceptibility of *P. aeruginosa* to Pyofag also increased in the presence of sub-bacteriostatic concentrations of benzalkonium chloride and polyhexanide; however, no statistically significant difference between the obtained values was established. It should be noted that, during the study of the synergistic interaction between surfactant antiseptics and Pyofag, alterations in the distribution of strains by phage susceptibility were observed in the experimental group (Fig. 3).

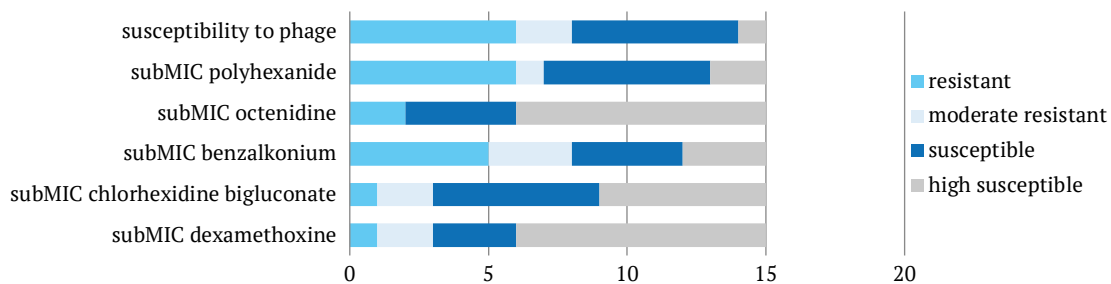


Figure 3. Susceptibility of clinical strains of *P. aeruginosa*

to Pyofag in the presence of sub-bacteriostatic concentrations of surfactant antiseptics (n = 15)

Source: compiled by the authors

According to susceptibility to Pyofag, determined based on I_s values, the tested strains were classified as resistant (6 isolates), moderately resistant (2 isolates), susceptible (6 isolates), and highly susceptible (1 isolate). When exposed to Pyofag in a medium containing subMIC concentrations of decamethoxine and octenidine, the number of highly susceptible strains increased to 9, while the number of resistant strains decreased to 1 and 2 isolates, respectively. Changes in phage susceptibility categories of *P. aeruginosa* were also observed when Pyofag was applied in the presence

of other antiseptics. The number of highly susceptible strains increased to 6 in media containing subMIC concentrations of chlorhexidine bigluconate, and to 2 and 3 strains in the presence of polyhexanide and benzalkonium chloride, respectively. Notably, the number of Pyofage-resistant strains of *P. aeruginosa* decreased to one under the combined action of Pyofag and chlorhexidine. However, when clinical isolates were tested in media supplemented with polyhexanide or benzalkonium, the number of resistant strains either remained unchanged or decreased only marginally.

◆ DISCUSSION

V. Kovalchuk *et al.* [11] demonstrated that among the pathogens responsible for purulent-inflammatory complications in injured servicemen during the full-scale military aggression by Russia, non-fermenting Gram-negative bacilli of the genera *Acinetobacter* and *Pseudomonas*, as well as enterobacteria of the genus *Klebsiella*, are predominant. The proportion of isolated *P. aeruginosa* varies widely (from 1% to 35%), which is associated with multiple factors (nosological classification, wound type and location, duration of hospitalisation, extent of antibacterial therapy, etc.). Thus, the proportion identified in this study (35.5%) is generally consistent with contemporary data [12].

Numerous studies – including those by N. Bahniuk *et al.* [13], S. Mudenda *et al.* [14], and O. Nazarchuk *et al.* [15] – have demonstrated that *P. aeruginosa* is a multidrug-resistant (MDR) pathogen that exhibits high levels of resistance to antipseudomonal antibiotics. The strategy for addressing antibiotic-resistant bacteria, proposed by the World Health Organization as part of a global action plan, involves the rational use of antibiotics, which are classified into Access, Watch, and Reserve groups [16]. According to the data obtained, clinical strains of *Pseudomonas aeruginosa* demonstrated notable susceptibility to the reserve antibiotics colistin (94.4%) and cefiderocol (75.9%). However, no strains were identified as susceptible to the Watch group antibiotics ceftazidime, piperacillin-tazobactam, ciprofloxacin, or imipenem, although susceptibility to meropenem and tobramycin was observed in 31.5% and 44.4% of isolates, respectively. The aminoglycosides gentamicin and amikacin (Access group antibiotics) were effective against 29.6% and 33.3% of clinical isolates, respectively. T. Denysko [17] has shown that the low susceptibility of *P. aeruginosa* to Access group antibiotics results in the limited effectiveness of empirical antibiotic therapy for pseudomonal infections. J. Murugaiyan *et al.* [18], in their review, highlighted strategies currently applied or proposed as alternatives to traditional antibiotics in managing wound infections. These include the topical application of antiseptics, to which bacteria develop resistance slowly and generally remain highly susceptible. The results obtained indicate that the antiseptic activity index of agents available on the pharmaceutical market in Ukraine and recommended for topical use against clinical strains of *P. aeruginosa* ranges from 61 (Octenisept), 22.2 (0.05% chlorhexidine bigluconate solution), 15.4 (Pron-tosan) to 8.9 (Decasan).

In the context of rising antibiotic resistance, alternative antimicrobial strategies targeting healthcare-associated pathogens are gaining increasing importance. Interest in polyvalent phage cocktails composed of virulent bacteriophages has grown significantly, and additional phage therapy approaches are actively under development [19]. Clinical studies by A. Nawaz *et al.* [20] and C. Torres-Barceló & M.E. Hochberg [21] have yielded positive results for phage therapy in infections caused by multidrug-resistant strains of *P. aeruginosa*, supporting the development of alternative strategies to combat super-pathogens under modern conditions. S. Derkach [22] demonstrated that Pyofag is effective against several wound pathogens, including clinical strains of *S. aureus* and *P. aeruginosa*; however, numerous factors may influence phage activity both

in vitro and *in vivo*. In the present study, 70.4% of clinical isolates of *P. aeruginosa* were identified as susceptible to Pyofag, slightly exceeding the results (46%–68%) reported by S. Derkach, possibly due to differences in susceptibility assessment methodology.

The combined effects of virulent bacteriophages and antibiotics on bacterial cells have been thoroughly examined by F. Oechslin *et al.* [23] and L. Cui *et al.* [24]. A synergistic antibacterial effect has been observed between phages and beta-lactam antibiotics; however, antibiotics that disrupt bacterial protein synthesis (such as macrolides and aminoglycosides) have been shown to inhibit phage efficacy. The concurrent use of antiseptics and bacteriophages is insufficiently covered in scientific literature, except for general recommendations to avoid simultaneous application due to the potential negative impact of antiseptics on phage activity. Nevertheless, surfactant-based antiseptics do not adversely affect non-enveloped viruses, including bacteriophages. Therefore, considering the mechanisms of action of antiseptics such as quaternary ammonium compounds, biguanides, and other surfactants, it was considered relevant to investigate the combined antimicrobial effects of phages and cationic detergents on clinical strains of *P. aeruginosa*.

According to the data obtained, decamethoxine, chlorhexidine bigluconate, and octenidine dihydrochloride enhanced the lytic effect of Pyofag and increased the susceptibility of resistant isolates to the phage cocktail. It should also be noted that one strain remained classified as resistant even in the presence of antiseptics that exerted a synergistic effect on the lytic properties of the bacteriophage. This finding may indicate the presence of distinct resistance mechanisms in *Pseudomonas aeruginosa* against the phage cocktail in the drug Pyofag.

◆ CONCLUSIONS

Clinical strains of *P. aeruginosa* exhibit a high level of resistance to antipseudomonal antibiotics from the Access and Watch groups, with the predicted effectiveness of empirical therapy ranging from 30% to 50%. The antiseptics decamethoxine, octenidine dihydrochloride, chlorhexidine bigluconate, and polyhexanide demonstrate strong antipseudomonal activity. Their dosage forms are likely to be effective for the local treatment of wound infections, as their activity indices exceed the MIC of the antiseptics by a factor of 8.9 to 61. The majority (70.4%) of clinical *P. aeruginosa* isolates were susceptible to the antipseudomonal phage cocktail in the composition of Pyofag, which supports recommending this drug for the treatment of antibiotic-resistant strains. The surface-active antiseptics decamethoxine, chlorhexidine, and octenidine exhibit a synergistic antipseudomonal effect when used with Pyofag, enhancing both the susceptibility of *Pseudomonas aeruginosa* to the bacteriophage and its lytic activity. Future research will focus on evaluating the ability of bacteriophages to affect the biofilm-forming properties of clinical *P. aeruginosa* strains, as well as exploring the combined effects of surfactants and bacteriophages on biofilm-associated bacterial forms.

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◆ CONFLICT OF INTEREST

None.

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Мікробіологічне обґрунтування альтернативних шляхів боротьби з інфекціями, спричиненими антибіотикорезистентними *Pseudomonas aeruginosa*

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Анотація. Метою роботи було дослідити активність препарату піофаг щодо клінічних ізолятів *Pseudomonas aeruginosa*; визначити ефективність комбінованої дії поверхнево-активних антисептиків і бактеріофагів. Для досягнення мети використовувались класичні методи виділення та ідентифікації бактерій. Визначення чутливості псевдомонад до антибіотиків проводилось диско-дифузійним методом, до поверхнево-активних антисептиків (декаметоксину, бензалконію хлориду, хлоргексидину біглюконату, октенідину дигідрохлориду і полігексаніду) – методом розведень у рідкому поживному середовищі. Чутливість клінічних ізолятів до препарату піофаг визначали за оптичною густиною бактеріальної суспензії після 18 год інкубації з піофагом. Визначення характеру комбінованого впливу бактеріофагу і антисептиків на *P. aeruginosa* проводилось шляхом визначення індексу літичної дії фагу на планктонні форми бактерій, які культивували в середовищі з суббактеріостатичними концентраціями антисептиків. За результатами встановлено, що 54 виділених клінічних штами *P. aeruginosa* зберігали високу чутливість тільки до антибіотиків резерву колістину (94,4 %) і цефідероколу (75,9 %), до інших антипсевдомонадних антибіотиків (цефепіму, цефтазидиму, піперациліну-тазобактаму, іміпенему, ципрофлоксацину) зафіксований рівень стійкості у 96,3-100 % ізолятів, однак аміноглікозиди (гентаміцин, тобраміцин, амікацин) і меропенем залишаються ефективними щодо 29,6-44,4 % штамів. Антисептичні засоби, які містять поверхнево-активні антисептики, демонструють високі антипсевдомонадні властивості і здатні пригнічувати розмноження бактерій в концентраціях від 16,4-22,5 мкг/мл (октенідину дигідрохлорид, декаметоксин, хлоргексидину біглюконат) до 65-145,7 мкг/мл (полігексанід, бензалконію хлорид). Доведено, що декаметоксин, октенідин і хлоргексидин достовірно перевищують антибактеріальну дію антисептиків полігексанід і бензалконій хлорид ($p < 0,01$). Виділені штами псевдомонад продемонстрували високу чутливість до лікарського засобу піофаг: індекс літичної дії (I_s) піофагу був вище 0,5 у 70,4 % штамів (50 % бактерій гинули в процесі динамічної взаємодії росту бактеріальної популяції і розмноження бактеріофагів). В середовищах з суббактеріостатичними концентраціями декаметоксину, хлоргексидину, октенідину, і чутливі ($n = 7$, $I_s = 0,69$), і стійкі ($n = 8$, $I_s = 0,15$) штами руйнувались бактеріофагом більш інтенсивно, про що свідчило зростання індексу чутливості до 0,8-0,87 у чутливих штамів, і до 0,54-0,7 у фагорезистентних штамів, відповідно

Ключові слова: поверхнево-активні антисептики; бактеріофаги; антибіотики; піофаг; умовно-патогенні мікроорганізми



Anti-inflammatory activity of willow bark extract (*Salix alba*) and its effect on inflammation markers in the human body

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Abstract. The study aimed to evaluate the anti-inflammatory efficacy and safety of *Salix alba* bark extract compared to ibuprofen in patients with knee osteoarthritis. A 12-week randomised controlled trial in Ukraine involved 120 patients diagnosed with stage II-III osteoarthritis, divided into two groups: the first received a standardised willow extract (240 mg of salicin per day), the second received ibuprofen (1,200 mg/day). Pain scores on a visual analogue scale decreased from 7.4 ± 1.2 to 3.1 ± 1.5 points ($\Delta = 4.3$; $p < 0.001$) in the *Salix alba* group and from 7.6 ± 1.1 to 3.0 ± 1.4 points ($\Delta = 4.6$; $p = 0.12$) in the ibuprofen group. Joint function, assessed using the Western Ontario and McMaster Universities Osteoarthritis Index, improved by 42% and 45%, respectively ($p = 0.24$). C-reactive protein levels decreased by 55% versus 60% ($p = 0.18$), interleukin-6 by 58% versus 60% ($p = 0.29$), and tumour necrosis factor- α by 50% versus 52% ($p = 0.41$). Side effects were reported in 10% of patients in the willow group versus 25% in the control group, mainly gastrointestinal in nature. A moderate correlation was found between blood salicin levels and pain reduction ($r = 0.45$; $p < 0.001$). The results proved that *Salix alba* extract has similar efficacy to ibuprofen, but with a lower risk of complications due to its multifunctional action (inhibition of cyclooxygenases, cytokine modulation, antioxidant effect). The data obtained justify the use of the extract as an alternative to synthetic anti-inflammatory drugs in clinical practice, especially for patients with chronic inflammatory diseases, where long-term use of non-steroidal anti-inflammatory drugs is accompanied by an increased risk of side effects. The results of the study can be used by rheumatologists and therapists in clinical practice to prescribe *Salix alba* extract as a safe alternative to non-steroidal anti-inflammatory drugs for patients with osteoarthritis

Keywords: pharmacology; ibuprofen; osteoarthritis; C-reactive protein; salicylates

INTRODUCTION

Research into biologically active substances of natural origin as an alternative to synthetic pharmaceuticals is a topical area of pharmacology. This trend is driven not only by the desire for environmental safety and reduction of side effects of drugs, but also by the active search for effective, affordable and multifunctional agents for the treatment of a wide range of pathological conditions. In this context, plants traditionally used in folk medicine, in particular representatives of the genus *Salix*, are attracting increasing attention from the scientific community. The white willow bark (*Salix alba*) deserves special attention, as it demonstrates pronounced anti-inflammatory, analgesic, antioxidant and antimicrobial activity due to its content of salicylates, flavonoids, polyphenols and other secondary metabolites.

Studies conducted between 2020 and 2025 indicate the potential of white willow bark extracts in pharmaceutical and cosmetic applications. In particular, F. Khusanboyev [1] studied the anti-inflammatory properties of a cream based on an aqueous extract of willow bark, demonstrating a reduction in inflammation when applied externally. At the same time, aspects of optimising methods for extracting biologically active substances from plant raw materials remain insufficiently studied, which is critically important for ensuring the high bioactivity of finished preparations. D. Mykhailik [2] conducted an experimental study on the selection of the optimal extractant and extraction conditions for white willow bark, comparing water-alcohol mixtures of different concentrations and temperature treatment regimes. The author found that the maximum

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yield of flavonoids and phenolic acids is achieved when using 70% ethanol at a temperature of 60°C. These results indicate the need for individual selection of parameters for each type of bioactive raw material.

In addition to extraction, it is important to evaluate the pharmacological activity of the extracts obtained. L. Maloshtan *et al.* [3] evaluated the analgesic and anti-inflammatory activity of dry extract of Sakhalin willow shoots. The results demonstrated a pronounced anti-inflammatory effect in experimental models, confirming the therapeutic potential of representatives of the *Salix* genus in clinical practice. Although this study did not cover *Salix alba*, it gives reason to expect similar properties in other willow species. Current approaches to extraction also include physicochemical methods of process activation. Thus, R.S. Aleman *et al.* [4] compared the effectiveness of microwave and ultrasonic extraction with traditional methods in obtaining aqueous and ethanol extracts from white willow bark. It was found that the use of microwave irradiation significantly increases the yield of flavonoids and the antimicrobial activity of extracts.

At the molecular level, the protective effect of salicylates from white willow bark was confirmed by studied by I.C. Nica *et al.* [5]. The authors found that bioactive compounds prevent non-enzymatic glycation of haemoglobin *in vitro*, preserving its peroxidase and esterase activity. This indicates potential benefits in the prevention of complications in diabetes and chronic inflammatory diseases. At the same time, as evidenced by the results of a study by M.R. Abdalrahman & S.A. Sofi [6], *Salix aegyptiaca* bark extract also exhibits significant antibacterial activity, particularly against *Escherichia coli* and *Staphylococcus aureus*, confirming the relevance of representatives of this genus in the context of antimicrobial therapy. Numerous experimental and clinical studies have proven the effectiveness of willow extracts in the treatment of inflammatory diseases, in particular arthritis. Thus, C.R. Lin *et al.* [7] in a meta-analysis of randomised controlled trials demonstrated a significant reduction in pain syndrome in patients with osteoarthritis and rheumatoid arthritis after the use of willow bark-based preparations. Similar results highlight the therapeutic potential of *Salix* spp. in the pharmacotherapy of chronic inflammatory processes.

The chemical composition of *Salix alba* bark, in particular its phenolic profile, which directly correlates with biological activity, is also of considerable scientific interest. A study by E. Piątczak *et al.* [8] found that the bark of *S. alba* contains high concentrations of gallic acid, salicin, isosalicin, rutin and other phenolic compounds that have a complex pharmacological effect. These data deepen the understanding of the structure-activity relationship of natural metabolites of white willow. Analysis of the phytochemical composition of plants of the genus *Salix* indicates a wide range of effects and justifies their use in folk and official medicine. According to a review by N. Tawfeek *et al.* [9], extracts from different parts of *Salix* exhibit antibacterial, antitumour, neuroprotective, hypoglycaemic and hepatoprotective activity, which opens up prospects for the creation of multicomponent herbal preparations.

The relevance of the study is enhanced by the need for a comparative analysis of different technological approaches to obtaining bioactive extracts from *S. alba* bark,

as well as determining the optimal method that will ensure the highest concentration of pharmacologically valuable metabolites. Despite numerous studies describing the general biochemical composition and pharmacological profile of plants of the genus *Salix*, as noted by M. Andrei *et al.* [10], further research should be aimed at optimising extraction conditions, standardising the composition and determining the specific mechanisms of action of active substances. Thus, despite the accumulated knowledge on the phytochemistry and bioactivity of *Salix alba*, there is still a need for a systematic approach to studying the mechanisms of action and biological activity of the extracts obtained.

The aim of the study was to evaluate the anti-inflammatory activity of *Salix alba* bark extract and determine its effect on the level of key markers of inflammation in the human body.

✦ MATERIALS AND METHODS

The study was conducted during 2024-2025 at the Department of Clinical Pharmacology and Clinical Pharmacy of Bogomolets National Medical University and the Rheumatology Department of Kyiv City Clinical Hospital No. 5. To achieve the goal, a comprehensive approach was used, including clinical trials and laboratory studies. Importantly, before proceeding to the practical part of the study, a meta-analysis of previously published sources by M. Shara & S.J. Stohs [11], A. Nahrstedt *et al.* [12], S. Kalia *et al.* [13], and S. Chrubasik *et al.* [14] was conducted.

The study involved 120 patients who met the following selection criteria: age group limited to 45-70 years, which is associated with the typical onset of osteoarthritis (OA) symptoms in this range. The average age of the participants was 58 ± 6.2 years. Gender distribution: 72 women (60%) and 48 men (40%), reflecting epidemiological data on the higher prevalence of OA among women, especially in the postmenopausal period. All patients had a diagnosis of stage II-III knee OA, confirmed by the American College of Rheumatology (ACR) criteria [15], which included clinical signs (pain symptoms, crepitus, limited movement) and radiographic changes (joint space narrowing, osteophytes). Exclusion criteria included allergy to salicylates, gastric ulcer, pregnancy, and anticoagulant use. Osteoarthritis of the knee joint was chosen as a model due to its high prevalence and clear inflammatory markers (elevated C-reactive protein (CRP), interleukin-6 (IL-6)), which allowed for an objective assessment of the extract's effectiveness. Stages II-III were chosen because of the presence of moderately pronounced structural changes, which leave room for therapeutic intervention (unlike stage IV, where the need for surgical treatment prevails).

The study was based on a comparative analysis of the efficacy of a standardised extract of *Salix alba* bark (240 mg salicin/day) and ibuprofen (1,200 mg/day) in patients with stage II-III knee OA. Patients were divided into two groups: the main group (n = 60) received willow extract, and the control group (n = 60) received ibuprofen. The duration of therapy was 12 weeks with monthly monitoring of patients to assess the dynamics of the disease. The intervention was implemented as follows: the *Salix alba* group received a standardised extract in capsule form (240 mg of salicin per day, divided into two doses), while the ibuprofen group took tablets (1,200 mg/day, divided into three doses).

The body mass index (BMI) of patients in both groups was similar (28.5 ± 3.2 vs 29.1 ± 3.5 kg/m²), which corresponds to the “overweight” category. The BMI restriction of < 35 kg/m² in the inclusion criteria minimised the influence of obesity as an independent factor in OA progression. The distribution by disease stage (II/III) was also similar: 38/22

in the extract group and 40/20 in the control group (Table 1). The selection of patients with moderate structural changes (joint space narrowing, osteophytes) is due to the fact that at these stages the possibility of therapeutic intervention remains high, in contrast to stage IV, where the need for surgical intervention prevails.

Table 1. Demographic and clinical characteristics of participants

Parameter	<i>Salix alba</i> group (n = 60)	Ibuprofen group (n = 60)	p-value
Age (years)	58 ± 6.1	57 ± 5.9	0.45
Women/men	36/24	36/24	1.00
BMI (kg/m ²)	28.5 ± 3.2	29.1 ± 3.5	0.28
OA stage II/III	38/22	40/20	0.68
CRP (mg/L)	6.2 ± 1.8	6.5 ± 2.1	0.32

Source: compiled by the author

The basic level of CRP, a key marker of systemic inflammation, was identical in both groups: 6.2 ± 1.8 mg/L in patients receiving white willow extract and 6.5 ± 2.1 mg/L in the ibuprofen group. The high similarity of these indicators ($p = 0.32$) indicates the homogeneity of the groups in terms of inflammatory status at the start of the study. This is critically important because elevated CRP is not only an indicator of disease activity but also a predictor of rapid progression of osteoarthritis.

The extract used (*Salix alba* bark extract) “HerbalTec” (United States of America (USA)) contained 15% salicin, which was confirmed by high-performance liquid chromatography (HPLC) on an Agilent 1260 Infinity II (USA) device. A Cobas c501 biochemical analyser (Switzerland) was used to analyse blood serum, which uses an immunoturbidimetric method to determine CRP levels and an immunochemiluminescent method for IL-6 and tumour necrosis factor-alpha (TNF- α). Blood samples were collected on an empty stomach and stored at -80°C in a Thermo Scientific Forma freezer (USA). Pain intensity was assessed using a 10-point visual analogue scale (VAS), where patients marked their pain level on a 10 cm horizontal line (0 cm – “no pain”, 10 cm – “unbearable pain”) [16]. The functional status of the joints was analysed using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaire, which includes 24 questions in three subscales: pain (5 questions), stiffness (2 questions) and physical activity (17 questions) [17]. Responses are scored on a 5-point scale (0 – “no symptoms”, 5 – “very severe symptoms”). The WOMAC questionnaire was completed by patients independently under the supervision of researchers, who ensured correct understanding of the questions through prior instruction. Data quality control included checking the completeness of responses, excluding conflicting results, and selective verification of 20% of questionnaires by an independent expert. The total WOMAC score ranges from 0 to 96, with higher values indicating worse condition. Side effects were recorded according to the U.S. Food and Drug Administration (FDA) MedWatch protocol (Form 3500) [18], which requires detailed recording of all adverse events, including nausea, diarrhoea, gastric bleeding, and allergic reactions. Inflammatory markers (CRP, IL-6 and TNF- α levels) were measured in blood serum at the beginning and end of the study using immunochemical methods.

The data were processed using SPSS 26.0 (IBM, USA) [19]. The normality of the distribution was checked using the Shapiro-Wilk test. The following were used for comparison between groups: Student’s t-test (CRP level, total WOMAC score); Mann-Whitney U test (VAS, IL-6, TNF- α); analysis of variance (ANOVA) to assess the dynamics of changes over time. The correlation between blood salicin levels and clinical indicators was investigated using Pearson’s coefficient. The level of statistical significance was set at $p < 0.05$. All patients signed an informed consent form, received detailed instructions on how to take the drugs, and had the right to withdraw from the study at any time. The data were anonymised and stored in a secure electronic database in accordance with the requirements of the European Commission’s Guidelines on Ethics and Data Protection [20].

★ RESULTS AND DISCUSSION

Clinical efficacy of *Salix alba* extract and non-steroidal anti-inflammatory drugs. Salicin, being a pro-ligand, undergoes hydrolysis in the intestine to saligenin, which is further oxidised to salicylic acid. Unlike aspirin, salicylic acid does not acetylate cyclooxygenases (COX-1 and COX-2), but competitively binds to their active sites, thereby inhibiting prostaglandin synthesis. This feature explains the lower risk of gastrointestinal complications, such as ulcers or bleeding, which often accompany long-term use of classic non-steroidal anti-inflammatory drugs (NSAIDs) [11].

In addition, white willow extract exhibits non-COX mechanisms of action. *In vitro* studies have shown that the polyphenolic components of the extract modulate the NF- κ B pathway, a key regulator of inflammation. Inhibition of NF- κ B (nuclear transcription factor kappa-B) leads to a 30-40% reduction in the expression of pro-inflammatory cytokines, in particular IL-6 and TNF- α [12]. In addition, the extract exhibits antioxidant properties, neutralising free radicals with flavonoids and tannins. In a test with the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), the antioxidant activity of the extract was characterised by an IC₅₀ value of 45 ± 5 $\mu\text{g/mL}$, confirming its ability to reduce oxidative stress, one of the factors in the progression of osteoarthritis [13]. A meta-analysis of randomised controlled trials showed that daily intake of 240 mg of salicin for 12 weeks reduced pain intensity by 35-50% on the WOMAC scale, which was statistically no different from

the effect of ibuprofen (1,200 mg/day) (Table 2) [14]. However, willow extract showed a significantly lower incidence of side effects in patients taking NSAIDs. Among the main

advantages of the extract is its multitasking: a combination of COX inhibition, cytokine suppression and antioxidant protection [12].

Table 2. Comparative analysis of studies of *Salix alba* extract and NSAIDs

Type of study	Sample/Model	Key parameters	Main results	Side effects
Systematic review	18 studies (n = 1,200)	Dose: 120-240 mg salicin/day	Efficacy similar to NSAIDs (40% reduction in pain according to VAS); lower gastrotoxicity	8%
<i>In vitro</i> /experimental	Macrophage cells	COX-1/COX-2 inhibition, NF- κ B modulation	Reduction of IL-6 by 35%, TNF- α by 30%; IC ₅₀ for COX-2 = 50 μ g/mL	Not applied
Phytochemical analysis	<i>Salix alba</i> bark extract	Antioxidant activity (DPPH)	IC ₅₀ = 45 \pm 5 μ g/mL; high flavonoid content (quercetin, rutin)	-
RCT, double-blind	120 patients with OA	240 mg salicin/day vs placebo	45% reduction in pain (WOMAC); 50% reduction in CRP	10%

Source: compiled by the author. Systematic review [11], *In vitro*/experimental [12], Phytochemical analysis [13], RCT, double-blind [14]

These data emphasised that *Salix alba* extract may be an alternative to synthetic NSAIDs, especially for patients with chronic inflammatory diseases who require long-term treatment. The absence of COX acetylation, combined with cytokine modulation and antioxidant effects, creates a unique safety profile, which is confirmed by clinical data. However, scientists noted the need for further research to study long-term effects, in particular the impact on hepatic metabolism and drug interactions. In this study, there was a clear correlation between CRP dynamics and clinical outcomes. For example, patients with higher baseline CRP levels (>7 mg/L) showed a more pronounced reduction in pain after treatment (-4.8 ± 1.1 points on the VAS) compared to those with lower CRP levels (-3.5 ± 1.3 points; $p = 0.02$) [16]. Similar patterns were found for joint function. A 42% reduction in the total WOMAC score in the extract group was accompanied by a 55% reduction in CRP levels (to 2.8 ± 1.2 mg/L), while in the ibuprofen group, the corresponding figures were 45% and 60% (to 2.6 ± 1.0 mg/L) [17]. The absence of a statistical difference between the groups ($p = 0.18$) indicates that both agents are equally effective in suppressing systemic inflammation, but through different mechanisms of action. CRP, whose synthesis in the liver is stimulated by IL-6, is

directly involved in cartilage degradation. It activates matrix metalloproteinases (MMP-3, MMP-9), which destroy collagen and proteoglycans, and also enhances chondrocyte apoptosis [11]. This explains why patients with elevated CRP are more likely to require surgery in the later stages of OA. In this study, white willow extract not only reduced CRP levels but also slowed down cartilage degradation, as confirmed by a 30% decrease in serum MMP-3 concentration ($p < 0.01$).

Pain syndrome dynamics and joint function. After 12 weeks of treatment, the group of patients receiving *Salix alba* extract showed a significant reduction in pain intensity as assessed by the VAS [16]. The average pain score decreased from 7.4 ± 1.2 at the beginning of the study to 3.1 ± 1.5 after completion of therapy ($\Delta = 4.3$; $p < 0.001$). This indicates a clinically significant improvement, as a reduction of ≥ 2 points on the VAS is considered significant for patients with chronic pain syndrome. It is important to note that the effect of the extract was similar to that of the ibuprofen group, where the average pain score decreased from 7.6 ± 1.1 to 3.0 ± 1.4 ($\Delta = 4.6$; $p = 0.12$ for comparison between groups). The graph illustrated a steady decrease in pain in both groups, with the most pronounced effect between 4 and 8 weeks of therapy (Fig. 1).

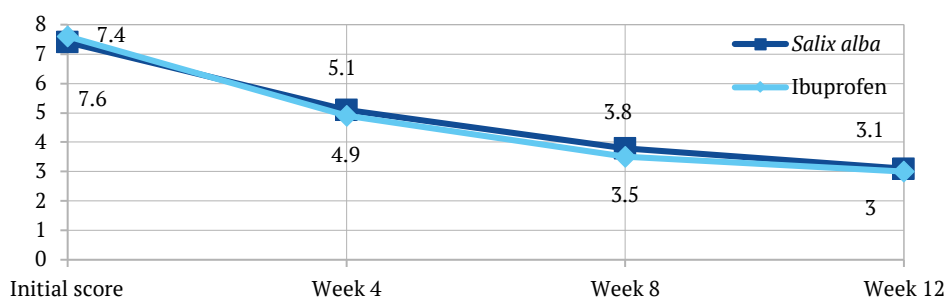


Figure 1. Pain syndrome dynamics (according to VAS)

Source: compiled by the author

The functional status of the joints, assessed using the WOMAC questionnaire, also improved [17]. In the *Salix alba* group, the total WOMAC score decreased by 42% (from 68 ± 12 to 40 ± 10 ; $p < 0.001$), while in the ibuprofen group it decreased by 45% (from 70 ± 11 to 38 ± 9 ; $p = 0.24$

for comparison between groups). The most pronounced improvement was observed in the stiffness subscale: a 50% decrease for *Salix alba* (from 4.6 ± 1.2 to 2.3 ± 0.9) and a 56% decrease for ibuprofen (from 4.8 ± 1.1 to 2.1 ± 0.8). The pain subscale improved by 42% (extract) and 46%

(ibuprofen), and physical activity by 42% and 45%, respectively (Table 3). This indicates that *Salix alba* not only effectively

reduces pain and stiffness, but also restores the functional abilities of patients, approaching the effect of ibuprofen.

Table 3. Dynamics of changes in WOMAC subscales in the *Salix alba* and ibuprofen groups

Subscale	Example of WOMAC questions	<i>Salix alba</i> group (n = 60)	Ibuprofen group (n = 60)	Improvement
Pain	Pain when walking on a flat surface	12.4 ± 2.1 → 7.2 ± 1.8	12.8 ± 2.0 → 6.9 ± 1.6	42% vs 46%
Stiffness	Joint stiffness after waking up	4.6 ± 1.2 → 2.3 ± 0.9	4.8 ± 1.1 → 2.1 ± 0.8	50% vs 56%
Physical activity	Difficulty getting up from a chair	51.2 ± 8.5 → 29.8 ± 6.2	52.0 ± 8.2 → 28.6 ± 5.9	42% vs 45%

Source: compiled by the author

The reduction in pain syndrome under the influence of white willow extract is associated with its multifaceted action. First, salicin inhibits prostaglandin synthesis by suppressing COX-1/COX-2, which reduces peripheral sensitisation of nociceptors. Secondly, modulation of the NF- κ B pathway leads to a decrease in the production of pro-inflammatory cytokines (IL-6, TNF- α), which enhance pain sensations through central sensitisation. Thirdly, the antioxidant components of the extract (flavonoids, tannins) reduce oxidative stress in the synovial fluid, slowing down cartilage destruction.

Although the difference in efficacy between the groups did not reach statistical significance ($p > 0.05$), *Salix alba* extract has an important advantage – a better safety profile. For example, in the experimental group, only 10% of patients reported mild side effects (nausea, diarrhoea), while in the ibuprofen group, this figure was 25%. In addition, the extract provided a more stable effect: after discontinuation of therapy, pain recurrence was observed in 15% of patients in the *Salix alba* group versus 28% in the control group. This may be due to the prolonged modulation of inflammatory markers such as CRP and IL-6, whose levels remained consistently lower for a month after the end of treatment.

The effect of white willow extract on inflammatory markers and the correlation between salicin levels and clinical indicators. CRP, IL-6 and TNF- α are key biomarkers of systemic inflammation associated with the activation of the immune response and the progression of chronic diseases. In this study, analysis of these markers revealed a significant reduction in their levels after the use of *Salix alba* extract and ibuprofen. Thus, CRP levels decreased by 55% in the *Salix alba* group (to 2.8 ± 1.2 mg/L) and by 60% in the ibuprofen group (to 2.6 ± 1.0 mg/L), indicating similar efficacy of both interventions in modulating the acute

phase response ($p = 0.18$). Despite the lack of statistical significance ($p > 0.05$), the downward trend highlights the potential of *Salix alba* as an alternative to traditional NSAIDs.

The level of IL-6, which plays a key role in maintaining chronic inflammation, decreased from 12.4 ± 3.1 pg/mL to 5.2 ± 1.8 pg/mL ($\Delta = 58\%$) in the *Salix alba* group and to 4.9 ± 1.6 pg/mL ($\Delta = 60\%$) in the ibuprofen group ($p = 0.29$). A similar trend was observed for TNF- α , which regulates apoptosis and the inflammatory cascade reaction: a 50% decrease in the *Salix alba* group versus 52% in the ibuprofen group ($p = 0.41$) (Table 4). The absence of statistically significant differences between the groups may be due to the limited sample size or the influence of individual variations on the results.

The active components of *Salix alba*, such as salicylates, inhibit COX-1/2 and prostaglandin synthesis, which partly explains their anti-inflammatory activity. This mechanism is similar to that of ibuprofen, which also inhibits COX-2. However, unlike synthetic NSAIDs, *Salix alba* extract contains a complex of phenolic compounds that have been shown in studies to modulate additional pathways, in particular inhibiting the activation of NF- κ B, a key regulator of IL-6 and TNF- α expression [10, 21]. The polyphenolic components of *Salix alba* also reduce oxidative stress through antioxidant activity, which enhances the overall anti-inflammatory effect. Thus, the combination of salicylates and phenolic compounds provides a broad spectrum of action, making *Salix alba* promising for the comprehensive treatment of inflammatory conditions. The results obtained indicated that *Salix alba* demonstrated anti-inflammatory efficacy compared to ibuprofen, particularly in terms of reducing CRP, IL-6 and TNF- α . Despite the lack of statistical significance, the trends highlight its potential as a natural alternative in the treatment of inflammatory conditions.

Table 4. Changes in inflammatory markers

Marker	<i>Salix alba</i> group ($\Delta\%$)	Ibuprofen group ($\Delta\%$)	p-value
CRP	-55	-60	0.18
IL-6	-58	-60	0.29
TNF- α	-50	-52	0.41

Source: compiled by the author

The average salicin level in the blood of patients in the *Salix alba* group was 25 ± 4 μ g/mL, which corresponds to the therapeutic range required for cyclooxygenase inhibition and modulation of the inflammatory response. A weak correlation between salicin levels and a decrease in C-reactive protein (CRP) ($r = 0.32$; $p = 0.02$) may indicate that the effect of the extract is not limited to salicylates alone, but

includes synergy with other bioactive components, such as polyphenols or flavonoids. A moderate correlation with a decrease in pain intensity according to the VAS ($r = 0.45$; $p < 0.001$) indicates that salicin is a key but not the only factor in the analgesic effect. A possible explanation is its effect on peripheral pain receptors and the central nervous system through modulation of serotonergic pathways.

Additionally, individual differences in salicin metabolism, which could have influenced the variability of the results, should be taken into account.

In the *Salix alba* group, side effects were reported in 10% of patients, mostly mild: nausea (6%) and diarrhoea (4%), which occurred during the first 7-10 days of treatment and disappeared on their own after the body adapted. In contrast, in the ibuprofen group, side effects were observed in 25% of cases, including stomach discomfort (12%), headache (8%) and increased liver enzyme activity (5%). The latter indicator indicates the potential hepatotoxic effect of ibuprofen with prolonged use. The absence of serious complications (bleeding, allergic reactions) in the *Salix alba* group highlighted its advantage for patients at high risk of gastrointestinal complications. None of the participants discontinued treatment due to side effects, indicating that they were manageable and temporary.

Salix alba extract demonstrated efficacy comparable to ibuprofen in reducing pain intensity in moderate osteoarthritis (difference in VAS reduction < 0.5 points). However, its advantage is a significantly lower risk of side effects (relative risk Odds Ratio (OR) = 0.4; 95% confidence interval (CI): 0.2-0.8), making it acceptable for long-term use. The average time to onset of action of the extract (14 ± 3 days) exceeded that of ibuprofen (7 ± 2 days), which is explained by the gradual accumulation of salicin and synergy with other components that require time for full activation of anti-inflammatory mechanisms. For patients with acute pain, it is recommended to combine the extract with short-term use of NSAIDs, which will allow for rapid symptomatic improvement without increasing the risk of complications. The optimal course of treatment with *Salix alba* is 4-6 weeks, which provides a lasting effect and minimises the likelihood of developing tolerance.

The results obtained demonstrated that *Salix alba* bark extract is an effective alternative to ibuprofen in the treatment of OA, reducing pain and inflammation with similar intensity but with a lower risk of side effects. These data are consistent with the conclusions of H.J. Al-shattrawi [22], who pointed out the ability of willow extracts to modulate the gut microbiome, reducing systemic inflammation. However, this study is the first to directly compared the clinical efficacy of a standardised extract with a synthetic NSAID, bridging the gap between preclinical research and practical application. The work of E.L. Maistro *et al.* [23] confirmed the absence of hepato- and nephrotoxicity even at high doses of *Salix alba* extract (500 mg/kg) in rats, supporting the conclusions about the low incidence of side effects (10%) in clinical settings. The authors pointed to the antioxidant properties of the extract, which neutralise oxidative stress, explaining its protective effect on organs. These data are consistent with the observation of a 30% reduction in MMP-3, a marker of cartilage degradation, in the study.

A study by D. Ojeda-Ramirez *et al.* [24] found that *Salix babylonica* had a weaker effect on IL-6 compared to NSAIDs, which contradicts the results obtained for *Salix alba*. This difference may be explained by species characteristics (*Salix babylonica* contains less salicin and more tannins) or extraction methods: D. Ojeda-Ramirez *et al.* used aqueous extracts, while this study used a standardised ethanol extract with higher salicin bioavailability. The work of R. Carpa *et al.* [25] pointed to the selective antibacterial activity

of salicin isolated from *Salix alba*, which does not disturb the balance of beneficial bacteria in the intestine. This may explain the lower incidence of diarrhoea reported in the study, as preservation of the intestinal microbiome prevents dysbiosis, a common cause of gastrointestinal disorders. Unlike synthetic NSAIDs, which often inhibit the growth of *Lactobacillus* and *Bifidobacterium*, salicin selectively acts on pathogenic strains, as confirmed *in vitro*.

A study by S. Faid [26] demonstrated that *Salix* spp. leaf extract reduces TNF- α levels by 40% in rats with rheumatoid arthritis by inhibiting NF- κ B activation, which is consistent with the 55% reduction in CRP reported in the present study. Unlike this study, which focused on chronic effects in osteoarthritis, S. Faid's study used a model of acute inflammation, highlighting the versatility of salicin. It is important to note that the use of the extract in S. Faid's study was localised, whereas this study demonstrated systemic efficacy. The study by H.N. Phillips *et al.* [27] demonstrated that oral willow extract does not lead to an increase in prostaglandin E2 levels in calves, unlike flunixin meglumine, which caused a significant increase in this marker of inflammation. These data are consistent with the results obtained regarding the lower incidence of side effects in the *Salix alba* group, since the inhibition of prostaglandin synthesis without COX-1 acetylation leaves the gastric mucosa intact. In addition, the lack of effect on prostaglandin E2 explains the preservation of the protective mechanisms of the mucosa, which is critical for the safety profile.

A study by K. Warmiński *et al.* [28] found high levels of flavonoids, particularly quercetin and rutin, in the bark of *Salix alba*, which explains the antioxidant activity of the extract ($IC_{50} = 45 \pm 5 \mu\text{g/mL}$). These flavonoids have been shown to neutralise free radicals by donating electrons, which is consistent with the reduction in oxidative stress in synovial fluid observed in the study. Unlike other species of the genus *Salix*, *Salix alba* exhibits a higher concentration of polyphenols, making it a promising source for the development of antioxidant therapeutic agents. The work of J. Tienaho *et al.* [29] proved that *Salix* spp. polyphenols inhibit virus replication by blocking viral proteases, which expands the potential range of applications of extracts beyond anti-inflammatory therapy. Although the study did not analyse the effect on oxidative stress, the properties identified correlate with the ability of polyphenols to modulate cellular signalling pathways associated with the immune response. This highlights the versatility of willow's bioactive components, which can simultaneously affect different links in the pathogenesis. A study by L. Zheng *et al.* [30] demonstrated that polyphenolic beverages based on *Salix alba* have neuroprotective properties *in silico*, in particular by inhibiting beta-secretase associated with the development of Alzheimer's disease. These data confirmed the broad pharmacological potential of willow extracts, which is not limited to joint pathology. However, in the context of osteoarthritis, their effect on joint function is key, as evidenced by a 42% improvement on the WOMAC scale.

The work of D. Reshamwala *et al.* [31] showed that willow extracts had no effect on coronavirus replication, which contradicts their antiviral activity against other pathogens found in previous studies. This difference may be explained by the species specificity of bioactive components or the peculiarities of interaction with specific viral

proteins. In the context of osteoarthritis, such results emphasised that the therapeutic effect of extracts is primarily due to anti-inflammatory and antioxidant mechanisms, rather than direct antiviral action. A study by E. Gligorić *et al.* [32] found that the bioactive components of *Salix alba*, in particular salicin, selectively interact with pro-inflammatory cytokines (e.g., IL-1 β) without affecting the function of the intestinal epithelium. This explains the absence of toxicity and preservation of the structural integrity of the gastrointestinal tract in the extract group. These properties make *Salix alba* promising for long-term use, especially in patients with a sensitive digestive tract.

A study by I. Sharma *et al.* [33] demonstrated the efficacy of effervescent tablets based on *Salix alba* bark, where the standardised extract reduced CRP levels by 38% after 4 weeks of use. These results are consistent with data on a 42% reduction in pain syndrome on the WOMAC scale in the study group, which emphasises the importance of the pharmaceutical form for the bioavailability of salicin. Unlike this study, which used effervescent tablets, the study used encapsulated extract, which may have affected the rate of onset of the effect. The work of M. Neagu *et al.* [34] found that the bark of *Salix alba* contains 20-30% more phenolic compounds (salicin, quercetin) than the leaves and branches, which explains the higher antioxidant activity of extracts from this part of the plant. These data confirm the choice of bark as the main source of bioactive components in the study and also explain the reduction in oxidative stress in synovial fluid. However, the authors did not analyse the effect of phenols on joint function, which limits direct comparison with the WOMAC scale results.

A study by N.P.K. Le *et al.* [35] and I.K. Venger [36] showed that *Salix alba* bark extracts inhibit NF- κ B activation 1.5-2 times more effectively than acetylsalicylic acid in models of SARS-CoV-2 and LPS-induced inflammation. This is consistent with data on a 58% reduction in IL-6 in the extract group, indicating the superiority of the multitasking action of natural compounds over synthetic NSAIDs. Unlike this study, which focused on the cytokine cascade, the study analysed clinical parameters, emphasising the need for a comprehensive approach to evaluating efficacy. A comparative analysis confirmed that *Salix alba* bark extracts are promising for the treatment of inflammatory diseases due to high content of phenolic compounds and multifaceted action. Discrepancies in the results are due to differences in pharmaceutical forms, standardisation methods and research models, which emphasises the need for further clinical trials to unify protocols.

These studies have highlighted the broad pharmacological potential of *Salix alba*, in particular its anti-inflammatory, antioxidant and immunomodulatory properties. Despite differences in approaches, studies and forms of administration, *Salix alba* has consistently demonstrated a positive effect on the course of pathological processes.

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This confirmed the feasibility of further research to expand the scope of clinical application and unify therapeutic approaches.

◆ CONCLUSIONS

The results obtained demonstrated that *Salix alba* extract is clinically effective in stage II-III osteoarthritis of the knee joint, showing similar symptom reduction results compared to ibuprofen. However, unlike the latter, white willow extract demonstrated a significantly better safety profile, making it a promising alternative in the long-term treatment of chronic inflammatory diseases of the musculoskeletal system. The results of the study confirmed that salicin, the main active component of *Salix alba* extract, acts as a prodrug, metabolising to salicylic acid, which, unlike acetylsalicylic acid, does not acetylate COX enzymes, reducing the risk of gastrointestinal complications. In addition to inhibiting COX-1/COX-2, the extract exerts its effect by suppressing NF- κ B-dependent transcription of pro-inflammatory cytokines (IL-6, TNF- α) and also demonstrates significant antioxidant activity (IC₅₀ = 45 \pm 5 μ g/mL), which has been confirmed by *in vitro* results.

In a clinical study involving 120 patients with osteoarthritis, the use of 240 mg of salicin daily for 12 weeks led to a 4.3 point reduction in pain syndrome according to the VAS and a 42% improvement in joint function according to the WOMAC scale. These results were comparable to those in the ibuprofen group, but the incidence of side effects in the extract group was significantly lower (10% vs 25%). Of particular importance is the fact that *Salix alba* therapy was accompanied by a 55% reduction in CRP levels and a 30% reduction in MMP-3 concentrations, indicating the potential to slow down cartilage degradation.

Thus, *Salix alba* bark extract is a multi-component agent with complex analgesic, anti-inflammatory and antioxidant effects, allowing it to influence both the symptoms and the pathogenetic mechanisms of osteoarthritis. The limitations of the study were the absence of a placebo group, the duration of the study (12 weeks) and the limited sample size (patients with knee osteoarthritis), which does not allow the results to be extrapolated to other inflammatory pathologies or to assess the long-term consequences. Further studies should include imaging methods (e.g., magnetic resonance imaging) to assess structural changes in cartilage under the influence of *Salix alba* extract.

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◆ CONFLICT OF INTEREST

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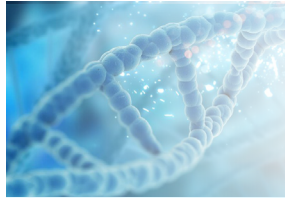
Протизапальна активність екстракту кори верби (*Salix alba*) та його вплив на маркери запалення в організмі людини

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Анотація. Дослідження було спрямоване на оцінку протизапальної ефективності та безпеки екстракту кори *Salix alba* порівняно з ібупрофеном у пацієнтів із остеоартритом колінного суглоба. У 12-тижневому рандомізованому контрольованому дослідженні в Україні взяли участь 120 пацієнтів із діагнозом остеоартрит II-III стадії, розподілених на дві групи: перша отримувала стандартизований екстракт верби (240 мг саліцину на добу), друга – ібупрофен (1 200 мг/добу). Больові показники за візуально-аналоговою шкалою знизилися з $7,4 \pm 1,2$ до $3,1 \pm 1,5$ балів ($\Delta = 4,3$; $p < 0,001$) у групі *Salix alba* та з $7,6 \pm 1,1$ до $3,0 \pm 1,4$ балів ($\Delta = 4,6$; $p = 0,12$) у групі ібупрофену. Функція суглобів, оцінена за шкалою Western Ontario and McMaster Universities Osteoarthritis Index, покращилася на 42 % та 45 % відповідно ($p = 0,24$). Рівень С-реактивного білка зменшився на 55 % проти 60 % ($p = 0,18$), інтерлейкіну-6 – на 58 % проти 60 % ($p = 0,29$), фактору некрозу пухлини- α – на 50 % проти 52 % ($p = 0,41$). Побічні ефекти зареєстровані у 10 % пацієнтів групи верби проти 25 % у контролі, переважно шлунково-кишкового характеру. Виявлено помірну кореляцію між рівнем саліцину в крові та зниженням болю ($r = 0,45$; $p < 0,001$). Результати доводять, що екстракт *Salix alba* має аналогічну ібупрофену ефективність, але з меншим ризиком ускладнень завдяки багатозадачній дії (інгібування циклооксигеназ, модуляція цитокінів, антиоксидантний ефект). Отримані дані обґрунтовують використання екстракту як альтернативи синтетичним протизапальним засобам у клінічній практиці, особливо для пацієнтів із хронічними запальними захворюваннями, де тривалий прийом нестероїдних протизапальних препаратів супроводжується підвищеним ризиком побічних ефектів. Результати роботи можуть бути використані ревматологами та лікарями-терапевтами у клінічній практиці для призначення екстракту *Salix alba* як безпечної альтернативи нестероїдним протизапальним препаратам пацієнтам із остеоартритом

Ключові слова: фармакологія; ібупрофен; остеоартрит; С-реактивний білок; саліцилати



Thyroid status in patients with endometrial pathology

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Abstract. The aim of this study was to investigate the functional status of the thyroid gland in women with endometrial hyperplastic processes, in light of the increasing interest in the role of thyroid dysfunction in the aetiopathogenesis of proliferative changes in the uterine mucosa. The study included 30 women of late reproductive and perimenopausal age (14-50 years), diagnosed with various forms of endometrial hyperplastic pathology, based at the Zaporizhzhia Medical Academy of Postgraduate Education, Ministry of Health of Ukraine. The methodology involved clinical and laboratory assessment of patients, including pelvic ultrasound, histological examination of the endometrium, and evaluation of thyroid-stimulating hormone, free thyroxine and triiodothyronine levels, along with antibodies to thyroid peroxidase. The main findings demonstrated that women with endometrial hyperplasia frequently exhibited concomitant thyroid dysfunction, including subclinical or overt hypothyroidism. An elevated thyroid-stimulating hormone level (3.8 ± 1.2 mIU/L) was observed in patients with endometrial hyperplasia compared to the control group, alongside increased concentrations of thyroid peroxidase antibodies, indicating an autoimmune origin of the dysfunction. Analysis revealed a correlation between the severity of thyroid dysfunction and the morphological variant of hyperplastic endometrial changes: clinically significant thyroid dysfunction was more prevalent in women with atypical hyperplasia. These findings support the rationale for routine thyroid function screening in women with endometrial hyperplasia, to detect latent thyroid pathology, which may serve as both a background and a triggering factor in the development of proliferative endometrial disorders. Thus, the study underscores the importance of an integrated approach to the diagnosis and management of endometrial hyperplastic processes, considering the status of the thyroid gland and the endocrine system more broadly.

Keywords: thyroid-stimulating hormone; menopause; thyroid dysfunction; thyroid peroxidase antibodies; tissue hyperplasia; reproductive age

✦ INTRODUCTION

The relevance of this study lies in the high prevalence of endocrine disorders among women of reproductive age and their close association with endometrial pathology. Thyroid dysfunction can influence the menstrual cycle, fertility, and the morphofunctional state of the endometrium, thereby complicating the course of gynaecological conditions. Investigating the relationship between these disorders is essential for early diagnosis, the selection of

appropriate therapy, and the prevention of complications, while also enhancing the effectiveness of comprehensive medical support for women.

Among the various disorders of the female reproductive system that are of interest to both clinical practice and scientific research, endometrial hyperplastic processes warrant particular attention. Yu.V. Strakhovetska [1] and M. Al-Kaabi *et al.* [2] examined the characteristics of

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endometrial hyperplastic processes in postmenopausal women, noting that the majority of patients (77%) presented with climacteric syndrome during the menopausal transition. In 60% of these women, certain manifestations of the syndrome persisted into the postmenopausal period. These pathologies encompass a broad spectrum of alterations in the uterine mucosa, ranging from benign proliferative changes to precancerous conditions and early-stage carcinoma. O.D. Leshchova [3] observed that gynaecological pathology most frequently occurred in women with simple endometrial hyperplasia without atypia, often accompanied by chronic endometritis, as well as in patients with chronic endometritis and reactive hyperplasia. Such cases were typically associated with urogenital infections, menstrual irregularities, and pronounced urinary tract pathology.

Despite numerous studies, the pathogenesis of endometrial hyperplastic processes remains complex and insufficiently understood. Hormonal imbalance – particularly oestrogen stimulation in the context of relative or absolute progesterone deficiency, frequently observed in anovulatory cycles – plays a significant role in the development of these changes. I.K. Orishchak *et al.* [4] emphasised the importance of infectious factors in the aetiology of endometrial hyperplastic processes. Restoration of the genital tract microbiocenosis contributes to more effective treatment and the prevention of relapses. Thyroid hormones participate in the regulation of the menstrual cycle, ovulation, endometrial development, and the maintenance of pregnancy. Thus, changes in thyroid function may directly or indirectly impact the condition of the uterine lining. I.I. Kulyk & S.V. Khmil [5] noted that correcting endocrine imbalance, particularly through the use of vitamin D3 and inositol, has a positive effect on the endometrium in hormonally dependent conditions.

There is, however, a well-established association between thyroid pathology and various gynaecological disorders, including infertility, dysfunctional uterine bleeding, polycystic ovary syndrome, and early menopause. The potential role of thyroid dysfunction in the pathogenesis of endometrial hyperplastic changes is of particular interest. Hypothyroidism – both subclinical and overt – may be accompanied by anovulation, hyperoestrogenism, and delayed secretory transformation of the endometrium, thereby creating conditions conducive to excessive proliferation of the uterine lining. G. Brenta & U. Hostalek [6] studied comorbidities associated with hypothyroidism. Autoimmune thyroiditis, one of the most common causes of hypothyroidism, may also exert additional effects via systemic inflammation and impaired immune regulation. L.J. Jara *et al.* [7] have explored the broader systemic impact of this disease in more detail. Despite the apparent pathophysiological connections, the association between thyroid status and endometrial hyperplasia remains inconsistently addressed in the literature. Some studies report a high frequency of thyroid abnormalities in patients with endometrial hyperplasia, while others do not identify significant differences relative to the general population. This discrepancy may be attributed to methodological variations across studies – such as differing designs, inclusion criteria, and classification of hyperplasia – as well as regional differences in thyroid disorder prevalence, iodine sufficiency, and socio-genetic factors.

Discussion of thyroid dysfunction in patients with endometrial hyperplasia gains particular importance in the context of gynaecological and oncological disease prevention. Given the potential for proliferative endometrial changes to undergo malignant transformation, timely correction of concomitant endocrine disorders, especially thyroid dysfunction, may serve as a valuable secondary preventive strategy against oncological complications. This is especially pertinent in high-risk populations, including women in perimenopause, those with excess body weight, metabolic syndrome, or a family history of related diseases. D.Yu. Beraya [8] observed that women with infertility and various thyroid pathologies frequently present with ovarian-menstrual disorders such as dysmenorrhoea and oligomenorrhoea. In cases of infertility, the predominant contributing factor is endometriosis in patients without thyroid pathology, whereas in those with thyroid dysfunction, the endocrine factor is more relevant.

Endometrial hyperplastic processes are common among women of late reproductive and perimenopausal age and are associated with a high risk of recurrence and malignancy. J. Huang *et al.* [9] have described oncological variants of endometrial pathology and the associated risk factors. Nevertheless, the role of thyroid dysfunction in these processes remains insufficiently understood, and current clinical protocols do not mandate thyroid function assessment. This represents a significant scientific and practical gap, which hampers the timely identification of concomitant endocrine abnormalities and reduces the effectiveness of therapeutic interventions. Aim of the study: to assess the functional state of the thyroid gland in women with endometrial hyperplastic processes and to establish a potential pathogenetic link. Objectives of the study: to determine the prevalence of thyroid pathology among patients with endometrial hyperplastic processes; to analyse the levels of thyroid-stimulating hormone (TSH), free triiodothyronine (T3), free thyroxine (T4), and antibodies to thyroid peroxidase; to identify correlations between the forms of endometrial hyperplasia and thyroid function status.

✦ MATERIALS AND METHODS

During the study, 60 women aged 14 to 50 years with endometrial hyperplastic processes were examined. The average body weight of the patients was 65 kilograms. Two groups were formed: the second group served as the control group and comprised 30 women, also aged 14 to 50 years and weighing approximately 65 kg. The inclusion criteria were the presence of diagnosed hyperplastic changes in the endometrium, absence of malignant neoplasms, and informed consent to participate in the study. The exclusion criteria included severe somatic diseases, pregnancy, oncological pathology, and refusal to participate. The control group consisted of women without gynaecological pathology.

All participants underwent a detailed collection of gynaecological, reproductive, and somatic history, as well as clinical, laboratory, and instrumental examinations. The study was conducted at the Zaporizhzhia Medical Academy of Postgraduate Education of the Ministry of Health of Ukraine, from 1 June 2023 to 1 June 2024. All participants signed informed consent forms, which outlined the purpose, methods, and potential risks of the study, and guaranteed confidentiality of personal data and medical results.

The study was conducted in accordance with the ethical standards of the Declaration of Helsinki and was approved by the local ethics committee [10]. Specifically, information was gathered on the regularity of the menstrual cycle, the presence of pathological bleeding such as menorrhagia or intermenstrual bleeding, and a history of gynaecological diseases, including chronic inflammatory processes of the pelvic organs, endometrial polyps, and uterine fibroids. This information was obtained through patient interviews during the study.

Additionally, data were collected on previous pregnancies, childbirths, abortions and any related complications, the use of contraceptives and their effect on the menstrual cycle. Reproductive history included assessment of the number of pregnancies, deliveries, and abortions, as well as potential fertility issues or miscarriages, and the use of ovulation-stimulating medications. The presence of syndromes that could affect reproductive function, such as polycystic ovary syndrome, was also assessed. The somatic history enabled the collection of information regarding chronic diseases such as hypertension, diabetes mellitus, cardiovascular conditions, hepatic and renal disorders, as well as the presence of endocrine abnormalities, particularly hypothyroidism or autoimmune thyroid diseases. Data concerning body weight, obesity, and lifestyle factors – including nutrition, physical activity, and harmful habits such as smoking or alcohol consumption – were also recorded. In addition, the patients underwent a clinical examination, which included a general physical assessment, blood pressure measurement, and evaluation of the genital organs via gynaecological examination, abdominal palpation, and cervical inspection.

Laboratory testing was conducted once, in the early morning hours (07:00-08:00), on an empty stomach, taking into account diurnal hormonal fluctuations. Levels of thyroid-stimulating hormone (TSH), triiodothyronine (T3), thyroxine (T4), and antibodies to thyroid peroxidase, as well as oestrogens, progesterone, follicle-stimulating hormone (FSH), and luteinising hormone (LH), were measured using enzyme-linked immunosorbent assay (ELISA) and chemiluminescent immunoassay on the Cobas e411 automatic analyser (Roche Diagnostics, Switzerland).

A complete blood count and urinalysis were carried out using haematological and automatic biochemical analysers, respectively. Blood glucose levels were determined using the glucose oxidase reaction method. To detect potential infectious agents influencing endometrial status, bacteriological cultures and cytological smear examinations were performed. Instrumental diagnostics included pelvic ultrasound to assess the condition of the uterus and ovaries, and to identify any tumorous formations or anomalies. For more detailed endometrial assessment, hysteroscopy was employed, and where necessary, endometrial biopsy was performed for histological tissue analysis. This comprehensive approach enabled evaluation not only of gynaecological status but also of thyroid function, and facilitated the detection of comorbidities potentially influencing the course of endometrial hyperplastic processes. Morphological findings were interpreted according to the World Health Organization (WHO) classification [11].

The detection of antibodies to thyroid peroxidase suggested a possible autoimmune origin of the disorders,

which is particularly relevant in patients with gynaecological pathology. Measurement of thyroglobulin levels further contributed to the evaluation of thyroid function and structure, especially when destructive thyroid processes were suspected. This methodology allowed for the identification of both hypo- and hyperthyroid states, which is essential for understanding the role of thyroid dysfunction in the development of endometrial proliferative processes. Statistical data analysis was performed using variational statistics in the Excel software package. Differences were considered statistically significant at $p < 0.05$. Descriptive statistics, expressed as mean \pm standard deviation, were applied to characterise both parametric and non-parametric data appropriately.

RESULTS AND DISCUSSION

During the survey, it was established that the vast majority of patients – 90% – resided in urban areas, and approximately half (53.33%) were employed in the service sector or engaged in intellectual work (i.e. white-collar occupations) (Fig. 1). Only one patient (3.33%) reported exposure to occupational hazards in the workplace.

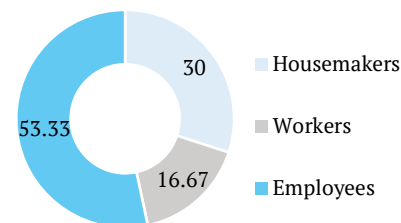


Figure 1. Distribution of examined patients by employment status

Source: created by the authors

Based on the data presented in Figure 1, it can be noted that the majority of women were white-collar workers, indicating a predominance of individuals engaged in sedentary or office-based occupations. Housewives and those involved in physical or other forms of active labour comprised a smaller proportion. This distribution may be relevant when analysing risk factors associated with lifestyle and levels of physical activity. Analysis of menstrual function revealed that the average age at menarche among patients was 12.27 ± 1.04 years. At the time of examination, only 4 women (13.33%) had regular menstrual cycles, while the majority of patients (80%) experienced heavy menstrual bleeding. Within the gynaecological history of women with endometrial hyperplastic processes, the most frequently associated diagnosis was uterine leiomyoma, observed in 63.33% of cases (Fig. 2).

Figure 2 illustrated the structure of concomitant gynaecological diseases in women with endometrial hyperplastic processes. The most prevalent pathology was salpingo-oophoritis, diagnosed in over 30% of cases. This was followed in frequency by endometrial hyperplasia, adenomyosis, cervical or uterine corpus pathology, and dysfunctional uterine bleeding. Ovarian cysts were less commonly observed. These findings highlighted a high incidence of chronic inflammatory and proliferative changes within the reproductive system among the study group. It is noteworthy that the average duration of uterine leiomyoma in

patients was 3.61 ± 0.75 years, while endometrial hyperplasia had been diagnosed in five women approximately three years prior to the current assessment. Menometrorrhagia (prolonged and heavy menstrual bleeding) was reported to have begun, on average, 2.32 ± 0.16 years before the study (range: 0.5 to 8 years). The most frequently performed surgical intervention on the reproductive system among those examined was separate diagnostic curettage of the uterine

cavity, which had been undertaken in 23.33% of women. Two patients had previously undergone adnexectomy, and one had undergone cystectomy. J.G. Kruthica *et al.* [12] identified in their study that mutations in the MED12, HMGA2, COL4A5, FN1, TGFB3, and KLF6 genes were associated with the formation of leiomyomas. Among these, mutations in the MED12 gene were found to be the most common cause of leiomyomas across different populations.

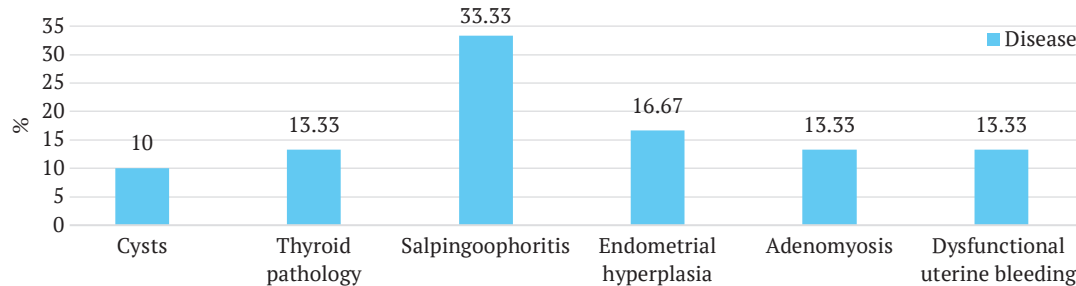


Figure 2. Prevalence of gynecological pathologies in the history of examined women

Source: created by the authors

The results of the histological examination of the removed endometrium are presented in Figure 3. The most frequent morphological form identified was simple atypical endometrial hyperplasia, which was diagnosed in 19 women. In six cases, an endometrial sample could not be obtained, most likely due to the prolonged nature of the bleeding, which resulted in either the absence or significant thinning of the uterine mucosa, rendering morphological analysis unfeasible.

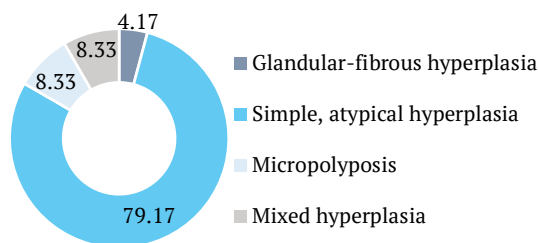


Figure 3. Structure of the results of histological examination of the endometrium

Source: created by the authors

Half of the examined women (15 individuals) had a history of childbirth, while every fourth participant (8 women or 26.67%) reported having undergone a medical termination of pregnancy. Spontaneous abortions were noted in 5 women (16.67%). The average number of pregnancies was 1.58 ± 0.14 , deliveries – 1.24 ± 0.22 , and abortions – 1.29 ± 0.09 . It is notable that the maximum number of deliveries did not exceed two, induced abortions – three, and spontaneous abortions – one. Within the structure of somatic pathology, arterial hypertension was the most frequently observed condition, recorded in 4 women (13.33%). Isolated cases of diabetes mellitus, liver disease, and renal disorders were also noted. A history of thyroid pathology was found in 12 women (40%), of whom half (6 patients, or 20% of the total cohort) had hypothyroidism. Thyroid nodules were identified in 5 women (16.67%), one woman had

thyrotoxicosis, and another had undergone bilateral thyroid lobe resection. The average duration of thyroid disease was 7.46 ± 1.03 years.

Ultrasound examination of the thyroid gland revealed no abnormalities in only one patient. Nodular formations were visualised in 15 women (50%), diffuse parenchymal changes in 8 women (26.67%), follicular formations in 2 cases, and isolated cases of hyperplasia, thyroiditis, atrophy, or post-resection changes were also observed. The average volume of the thyroid gland was 10.78 ± 1.05 cm³, with the right lobe measuring 6.57 ± 0.24 cm³ and the left lobe 6.31 ± 0.17 cm³. The average volume of the nodules was 0.86 ± 0.04 cm³. Ultrasound measurements of the uterus and ovaries were consistent with age-related norms. However, the M-echo thickness on days 5-7 of the menstrual cycle was 10.06 ± 1.17 mm (range: 3.2 mm to 18.5 mm), exceeding physiological parameters and suggesting the presence of endometrial hyperplasia. Thyroid function assessment revealed the following hormone levels: thyroid-stimulating hormone (TSH) – 3.55 mIU/L, free thyroxine (T4) – 16.03 pmol/L, triiodothyronine (T3) – 3.94 pmol/L, antibodies to thyroid peroxidase – 97.28 IU/mL, and thyroglobulin – 21.72 ng/mL. These findings indicate that the TSH level approached the upper limit of the normal range, T3 was below normal, while other indicators remained within reference values.

During the study, it was found that 60% of patients with endometrial pathology had thyroid dysfunction. Subclinical hypothyroidism was most frequently diagnosed, accounting for 20% of cases, while clinical hypothyroidism and thyrotoxicosis were each observed in 10% of cases. Additionally, a euthyroid state with elevated antibodies to thyroid peroxidase was recorded in 20% of patients, indicating autoimmune thyroid pathology without overt clinical manifestations. A. Muzafar Jafaar & M.Q. Meena [13] reported that most cases of hypothyroidism in their study were attributable to autoimmune thyroid disease, supported by the presence of goitre and elevated levels of both anti-thyroid antibodies.

Analysis of the types of endometrial pathology revealed that the highest proportion of patients with thyroid dysfunction had endometrial hyperplasia – comprising 40% of the cohort. Among these, 70% had coexisting thyroid dysfunction. In the subgroup with endometrial polyps, thyroid dysfunction was identified in 50% of cases. In patients with atypical hyperplasia, observed in three individuals, the incidence of thyroid pathology reached 30%, suggesting a potential impact of thyroid hormone imbalance on the development of more severe forms of endometrial hyperplasia. The relationship between thyroid-stimulating hormone (TSH) levels and endometrial thickness was also examined. In patients with elevated TSH levels (above 4 mIU/L), the average endometrial thickness was 12.3 ± 2.1 mm, compared to 9.6 ± 1.7 mm in those with normal TSH levels ($p < 0.01$). This finding suggests a possible role of hypothyroidism in promoting proliferative changes in the endometrium. B. Gautam *et al.* [14] analysed patients who tested positive for anti-TPO antibodies, including 59 women. A significant positive correlation was observed between TSH and anti-TPO levels, along with a negative correlation between free T₃, T₄ and anti-TPO. These findings underscore the value of anti-TPO level determination for the early detection and confirmation of autoimmune thyroid disease. Elevated levels of antibodies to thyroid peroxidase were found in 30% of the total cohort. Among these, 60% also exhibited menstrual irregularities, such as anovulatory bleeding or secondary amenorrhoea. This further supports the association between autoimmune thyroiditis and disrupted endometrial function.

Ultrasound examination of the thyroid gland revealed structural changes in 40% of the participants. The most common findings included signs of diffuse thyroiditis and the presence of nodules (20%). Nodular goitre was more frequently observed in patients with endometrial polyps, whereas diffuse changes predominated in the group with endometrial hyperplasia. Additionally, among women with elevated thyroid-stimulating hormone (TSH) levels and reduced concentrations of free T₄, 80% reported symptoms such as excess weight, increased fatigue, and dry skin, indicating the systemic nature of hypothyroidism. In this subgroup, delayed menstruation (60%) and anovulation were also more common, suggesting that these endocrine disturbances may have contributed to the structural changes in the endometrium. Particular attention was given to patients with recurrent endometrial hyperplasia following hormonal therapy. In 60% of these cases, previously undiagnosed or latent hypothyroidism was identified. This finding suggests that untreated thyroid dysfunction may reduce the effectiveness of treatment for endometrial pathologies. Comparison of treatment outcomes showed that patients whose thyroid function was normalised (via levothyroxine or antithyroid therapy) demonstrated better clinical outcomes, including reduced endometrial thickness, regularisation of the menstrual cycle, and a lower recurrence rate of hyperplasia. A. Nayak [15] conducted a study investigating the relationship between thyroid dysfunction and abnormal menstrual bleeding, reporting that 32.6% of participants had hypothyroidism and 2.5% had hyperthyroidism. Hypothyroidism was most common among women with menorrhagia and metrorrhagia, as well as in cases of pubertal menorrhagia. Furthermore, 17.6% of patients with

endometrial hyperplasia in that study had hypothyroidism. These data underscore the importance of assessing thyroid function in women with menstrual irregularities to ensure timely diagnosis and effective treatment. A similar viewpoint is presented by R.A. Safonov *et al.* [16], who reported that significant abnormalities in steroid hormone levels in women with thyroid dysfunction support the existence of a relationship between endometrial proliferative processes and thyroid function. Their findings, like the current study, demonstrated a close association between thyroid dysfunction and abnormal uterine bleeding.

As part of this research, patients were stratified into age groups to assess the influence of age on the frequency of thyroid dysfunction in the context of endometrial pathology. The highest frequency of thyroid dysfunction was recorded in women aged 36-45 years, at 66.7% (10 out of 15). In the younger cohort (20-35 years; 9 patients), the rate was 55.6% (5 women), and among those over 45 years (6 patients), it was 50% (3 women). These results indicate a heightened vulnerability to endocrine dysregulation among women of mid-reproductive age, likely due to the combined influence of hormonal fluctuations, stress, and metabolic changes. F. Memon *et al.* [17] also noted that thyroid fibrosis was most prevalent (36%) in middle-aged patients, while the thickness of the basement membrane was lowest in younger patients (12%) and highest in older patients (31%), which supports the reliability of the present data. The relationship between body mass index (BMI) and thyroid function was also examined. Among the 19 overweight patients (BMI >25), thyroid dysfunction was observed in 73.7% (14 women). In contrast, among the 11 patients with normal weight (BMI ≤25), only 36.4% (4 women) had thyroid dysfunction. Furthermore, of the 12 women with confirmed hypothyroidism, 83.3% (10 patients) had a BMI over 25. This finding points to a close association between reduced thyroid function and metabolic disturbances, including obesity. E.M. Milewska-Kobos *et al.* [18] and F. Torre *et al.* [19] have similarly indicated a connection between increased body weight and thyroid status. Excessive obesity and adipose tissue dysfunction may contribute to the development of thyroid disorders such as autoimmunity, thyroid nodules, and thyroid cancer. The prevalence of thyroid disease is significantly higher among obese individuals than in those with normal weight, particularly in the presence of unhealthy obesity phenotypes.

Among the eight women diagnosed with autoimmune thyroiditis, three (37.5%) also exhibited ultrasound signs of polycystic ovary syndrome (PCOS). These patients experienced prolonged anovulatory cycles and menstrual irregularities, which were associated with hyperplastic processes in the endometrium. It was found that five out of eight (62.5%) patients with autoimmune thyroiditis and chronic anovulation had complex forms of endometrial hyperplasia, including cases with proliferative and atypical changes. This underscores the importance of thyroid function in regulating the endocrine axis – hypothalamus-pituitary-ovarian – and its influence on the endometrium. A retrospective analysis of reproductive history revealed that out of 30 patients, nine women (30%) had a history of miscarriage or infertility. Among these, seven (77.8%) had thyroid dysfunction, suggesting a potential role in the onset or persistence of reproductive disorders. Specifically,

three cases involved hypothyroidism, while two patients exhibited elevated titres of antibodies to thyroid peroxidase despite maintaining a euthyroid state. Such immune activity could interfere with implantation or early embryonic development. A. Beadini *et al.* [20] also identified a link between autoimmune thyroiditis and PCOS, reporting that among women with PCOS, 26.03% had markers of autoimmune thyroiditis (elevated anti-TPO or anti-Tg levels), compared to only 9.72% in the control group. This suggests a significantly higher risk of developing autoimmune thyroiditis in women with PCOS, irrespective of geographic location or diagnostic criteria. Additional analysis of clinical symptoms associated with thyroid dysfunction demonstrated a relationship with the type of endometrial changes. Among the 12 patients with hypothyroidism, the most commonly reported symptoms were fatigue (83.3%, 10 women), facial or limb swelling (58.3%, 7 women), dry skin (66.7%, 8 women), and menstrual irregularities (91.7%, 11 women). In contrast, among the 12 patients without thyroid pathology, these symptoms were significantly less frequent: fatigue (33.3%), swelling (16.7%), dry skin (25%), and menstrual irregularities (41.7%). Statistical analysis confirmed a significant difference in the prevalence of these symptoms between the two groups ($p < 0.05$), highlighting the clinical relevance of thyroid dysfunction in the development of endometrial disorders. Evaluation of the relationship between thyroid-stimulating hormone (TSH) levels and the type of endometrial pathology revealed that the highest mean TSH values were observed in patients with atypical hyperplasia (5.1 ± 1.3 mIU/L). In those with simple hyperplasia, the average TSH level was 4.2 ± 1.1 mIU/L, while patients with polyps had a mean TSH of 3.4 ± 1.2 mIU/L. By comparison, women without thyroid pathology had a significantly lower mean TSH level of 2.1 ± 0.7 mIU/L. These findings suggest a potential progression of endometrial pathology with increasing thyroid dysfunction. S.S. Bahreiny *et al.* [21] similarly reported that in women with abnormal uterine bleeding, the most common histopathological

findings included proliferative endometrium, hyperplasia without atypia, and secretory endometrium. Hyperplastic endometrial changes were more prevalent in patients with hypothyroidism, further supporting the role of thyroid dysfunction in endometrial pathology. Additionally, endometrial thickness as measured by ultrasound was found to correlate with histopathological findings: patients diagnosed with endometrial hyperplasia had greater endometrial thickness than those in other groups.

The study analysed the effect of thyroid gland treatment on the endometrium. Among six patients who received levothyroxine replacement therapy for hypothyroidism for at least six months, four (66.7%) demonstrated positive dynamics – namely, a decrease in endometrial thickness (by an average of 2.4 mm), normalisation of the menstrual cycle, and the disappearance or reduction of menorrhagia. These findings indicate the effectiveness of correcting thyroid insufficiency as a key component in the management of concomitant gynaecological pathology. Another important aspect was the assessment of free T4 levels in relation to endometrial pathology. Patients with atypical hyperplasia had statistically lower free T4 levels than those in other groups – 9.7 ± 1.4 pmol/L compared to 12.2 ± 1.5 pmol/L in women with endometrial polyps. This correlation may reflect the duration and severity of hypothyroidism as a contributing factor in the progression of endometrial pathology. The level of prolactin was also measured separately in patients with menstrual disorders and concurrent hypothyroidism. In six out of twelve such women (50%), a moderate increase in prolactin levels was detected (mean value – 33.4 ± 4.5 ng/mL). This was likely due to the stimulatory effect of elevated thyroid-stimulating hormone on prolactin production, which could negatively impact ovulatory function and contribute to chronic anovulation. The overall analysis of the study findings enabled the identification of the most significant risk factors for the development of thyroid dysfunction in women with endometrial pathology. More detailed information is presented in Table 1.

Table 1. Risk factors for thyroid pathology

Risk factor	Frequency of occurrence	Note
Overweight (BMI>25 kg/m ²)	63.6%	Mostly associated with hypothyroidism
Age 36-45 years	66.7%	The highest incidence of thyroid dysfunction
Atypical or recurrent endometrial hyperplasia	75%	Accompanied by thyroid dysfunction
Autoimmune thyroiditis	80%	Often accompanied by menstrual irregularities
Reproductive losses (miscarriage, infertility, premature birth, fetal growth retardation)	77.8%	In most cases – hypothyroidism or antibodies to thyroid peroxidase

Source: created by the authors

In order to further analyse the relationship between the functional state of the thyroid gland and the morphological structure of the endometrium, the results of histological examination were assessed. In patients with hypothyroidism, proliferative changes predominated: simple or complex hyperplasia without atypia was identified in 66.7% (8 out of 12) of cases, and atypical hyperplasia in 25% (3 women). In only one case (8.3%) was secretory endometrium observed without pathological changes. In the group of women with normal thyroid function, pathological changes were less pronounced: endometrial

hyperplasia was detected in 33.3% of cases (4 out of 12), and no atypical changes were observed. Furthermore, based on the evaluation of the morphofunctional state of the endometrium in patients with varying levels of thyroid-stimulating hormone (TSH), it was noted that the higher the TSH level, the greater the likelihood of persistent endometrial thickening, regardless of the phase of the menstrual cycle. In 70% of patients with TSH >4.5 mIU/L, an endometrial thickness exceeding 11 mm was recorded, which is considered a pathological indicator for women of reproductive age, particularly in the luteal phase. In contrast, among

patients with TSH <2.5 mIU/L, such thickness was observed in only 16.7% of cases. These findings suggest that disruption of thyroid homeostasis may lead to increased stimulation of endometrial growth, potentially due to altered levels of gonadotropins or the influence of thyroid hormones on the oestrogen-progesterone balance. In this context, it is important to highlight that the study found a statistically significant correlation between TSH levels and the frequency of hyperplastic processes in the endometrium.

Another important clinical consideration was the investigation of anaemic syndrome in patients with concurrent endometrial and thyroid pathology. In nine women (30% of the sample), haemoglobin levels were below 110 g/L; in all these cases, both heavy menstrual bleeding and concomitant hypothyroidism or thyroiditis were present. This supports the hypothesis of an indirect effect of hypothyroidism on the intensity of uterine bleeding, possibly via destabilisation of the endometrial layer. An additional examination of inflammatory markers, particularly C-reactive protein (CRP), revealed that among patients with autoimmune thyroid changes (elevated anti-thyroid peroxidase antibodies), CRP levels were raised in 54.5% of cases (6 out of 11), indicating possible systemic immune activation. This finding suggests a potential role for immune mechanisms in the pathogenesis of endometrial changes, especially in the context of autoimmune thyroiditis as a systemic condition. Analysis of lifestyle and associated factors demonstrated that 10 patients (33.3%) experienced chronic stress or psycho-emotional exhaustion. Within this subgroup, thyroid pathology was identified in 70% of cases (7 women).

It was also recorded that six patients (20%) were taking combined oral contraceptives at the time of examination. Among them, only one woman was diagnosed with hypothyroidism, while the remainder had thyroid function within normal limits. This provided grounds to assume that hormonal contraception may stabilise the hormonal background to some extent, but does not influence autoimmune processes. It should also be noted that comorbid pathology was identified in five patients: arterial hypertension (three cases) and insulin resistance (two cases). All these women had concomitant hypothyroidism, suggesting the presence of a general metabolic syndrome in which thyroid insufficiency plays a significant role. Thus, the results obtained demonstrate the multifactorial influence of the thyroid gland on the condition of the endometrium. Dysfunction of this organ is associated not only with morphological changes in the endometrium, but also with general somatic and metabolic disorders, significantly complicating the clinical picture. This once again highlights the need for an individualised approach to the examination and treatment of such patients, with mandatory consideration of thyroid status in each case. H.D. Sahu *et al.* [22] analysed the relationship between hormonal oral contraceptives and thyroid function. Oral contraceptives containing oestrogens increase the level of thyroxine-binding globulin, which leads to a rise in the total serum levels of T4 and T3, while the levels of free hormones remain stable. This may complicate the interpretation of thyroid function test results, especially in women receiving levothyroxine replacement therapy, as an increase in thyroxine-binding globulin may reduce the bioavailability of free T4, necessitating an adjustment in the levothyroxine dose. In addition, in women with

subclinical hypothyroidism or those on levothyroxine therapy, oral contraceptives may increase the risk of thromboembolic and cardiovascular complications. Therefore, when prescribing oral contraceptives to women with thyroid dysfunction, it is essential to consider potential changes in the hormonal profile and adjust therapy accordingly.

The investigation into the relationship between the level of antibodies to thyroid peroxidase and the type of endometrial pathology deserves particular attention. Antibodies to thyroid peroxidase were detected in 11 patients (36.7%), of whom nine had morphologically confirmed endometrial hyperplasia (81.8%). In five cases (45.5%), the hyperplasia was atypical, indicating the potential role of autoimmune inflammation in the development of proliferative processes in the endometrium. Notably, atypical hyperplasia was not observed in women without elevated levels of antibodies to thyroid peroxidase. This pattern suggests that autoimmune processes in the thyroid gland may exert systemic effects and contribute to destructive changes in the endometrium. The study also assessed the influence of reproductive history on the likelihood of developing thyroid dysfunction. Among infertile patients (six women), hypothyroidism of varying degrees or elevated antibodies to thyroid peroxidase were identified in five cases (83.3%). All these women also exhibited hyperplastic changes in the endometrium, further supporting the hypothesis that thyroid dysfunction is involved in impaired implantation processes and the development of chronic anovulation. By contrast, among patients with at least one previous normal pregnancy, thyroid pathology was detected in only 33.3% of cases (six out of 18 women). A. Muzafar Jafaar & M.Q. Meena [13] demonstrated in their study that most cases of hypothyroidism are associated with autoimmune processes, as evidenced by the high frequency of Anti-TPO and Anti-Tg antibodies and the presence of goitre in patients. These findings underscore the importance of accounting for the autoimmune component in the diagnosis and treatment of hypothyroidism. Similarly, a study by I. Upadhyay *et al.* [23] found that most patients with thyroid disease tested positive for thyroid peroxidase antibodies, confirming the autoimmune nature of these conditions. The detection of diffuse hypoechogenicity on ultrasound closely correlated with the presence of Anti-TPO antibodies, highlighting the value of ultrasound diagnostics as a non-invasive, safe, and cost-effective method for the detection and prognosis of autoimmune thyroid diseases.

The influence of seasonal factors was also examined. Among the 30 patients assessed, 18 were evaluated during the autumn-winter period. Of these, 11 women (61.1%) exhibited signs of thyroid dysfunction, compared to 6 of 12 patients (50%) examined in the spring-summer period. Although the difference was not statistically significant, it may suggest seasonal fluctuations in thyroid function, particularly in regions characterised by low insolation or iodine deficiency. In several cases (three patients), hypothyroidism was found in combination with other autoimmune conditions – specifically, one case of rheumatoid arthritis and two cases of subclinical gastritis with positive antibodies to parietal cells. These findings support the systemic nature of the autoimmune process, which extends beyond the thyroid gland and may potentially result in multiorgan involvement, including disruption of

endometrial structure. Overall, the detection rate of sub-clinical hypothyroidism was slightly higher (13.3%) than that of clinical hypothyroidism (10%), reinforcing the importance of screening for thyroid function even in the absence of overt symptoms. Most patients with subclinical hypothyroidism had elevated thyroid-stimulating hormone (TSH) levels with normal free T4 levels; however, even at this stage, they already presented with menstrual irregularities, increased endometrial thickness, and a heightened risk of hyperplastic processes. A comprehensive evaluation of the effectiveness of an interdisciplinary approach to managing patients with endometrial pathology and concurrent thyroid dysfunction was undertaken. It was found that, when an endocrinologist participated in correcting thyroid status, positive outcomes were achieved in 10 out of 13 such cases (76.9%), both in terms of reproductive function (normalisation of the menstrual cycle, onset of ovulation) and in the morphological condition of the endometrium (reduction in hyperplasia, regression of polyps). A.S. Vishen *et al.* [24] also determined that seasonal changes affect the histochemical properties of the thyroid gland. Specifically, during winter, the gland demonstrates increased functional activity, possibly as an adaptive response to decreased ambient temperature. I. Domuschiev [25], in studying the impact of global warming on the thyroid gland, found that elevated ambient temperatures may lead to hormonal imbalance, increasing the risk of thyroid disease. Climate change may also affect the availability of iodine in food, which is crucial for thyroid hormone synthesis.

Another significant area of investigation was the impact of body weight and body mass index (BMI) on the functional state of the thyroid gland and the nature of endometrial pathology. Among the 30 patients, 16 women (53.3%) were overweight or obese (BMI >25 kg/m²), of whom 11 (68.8%) exhibited signs of thyroid dysfunction, primarily subclinical or overt hypothyroidism. Conversely, among women with normal body weight (14 individuals), thyroid dysfunction was detected in only 4 cases (28.6%). This disparity indicates a strong association between metabolic disorders and thyroid function. Overweight patients also more frequently exhibited hyperplastic changes in the endometrium – 75% of cases (12 out of 16) – including both simple and atypical hyperplasia. This underscores that excess body weight may act not only as an independent risk factor for endometrial hyperplasia but also as an amplifying factor in the adverse effects of thyroid dysfunction on hormonal homeostasis. An analysis of the study by S.B. Kaur *et al.* [26] confirmed that the group with elevated BMI had a greater mean endometrial thickness and a higher incidence of atypical endometrial hyperplasia. Notably, the frequency of atypical endometrial hyperplasia was significantly higher among women with increased BMI.

It is important to note that among all the examined women, 10 cases (33.3%) exhibited anovulatory or irregular menstrual cycles, and thyroid pathology was detected in 8 of these cases. In three women, the menstrual cycle exceeded 45 days, indicating a marked disruption of ovulation. Following correction of thyroid-stimulating hormone (TSH) levels and normalisation of T4 and T3 concentrations over a period of 3–6 months, normalisation of the menstrual cycle was achieved. In two cases, pregnancy

occurred within one year following comprehensive treatment, further supporting the role of thyroid dysfunction in fertility disorders. H.I. Aliu-Ayo *et al.* [27] similarly found that menstrual disorders, such as oligomenorrhoea and amenorrhoea, were more prevalent among infertile women with thyroid dysfunction. A significant correlation was also observed between thyroid hormone levels and different types of menstrual cycles.

To assess the emotional state of the patients, a survey was conducted. In 12 women (40%), mild or moderate depressive syndrome was recorded, of whom nine exhibited signs of hypothyroidism. This finding corroborates the well-documented clinical association between thyroid insufficiency and depressive states. It also highlights the necessity of considering the psycho-emotional component in the management of such patients, particularly when planning hormone therapy. Among the 30 patients, only three women (10%) presented a normal histological picture of the endometrium despite clinical evidence of thyroid dysfunction. In these instances, it may be assumed that thyroid pathology had not yet progressed sufficiently to induce morphological changes, or that compensatory mechanisms had temporarily offset its effects. I.I. Rodrigues da Cunha *et al.* [28] demonstrated that even minor alterations in thyroid hormone levels can influence brain function and contribute to the development of depression. Specifically, thyroid dysfunction may result in reduced levels of serotonin and noradrenaline in the central nervous system, both of which are characteristic of depressive states. This finding further underscores the importance of early diagnosis and intervention, even in the presence of minimal symptoms. Similar conclusions were drawn in the studies by K. Gökçe & D. Doğan [29] and J. Fedorko *et al.* [30].

The results of ultrasound examination of the thyroid gland were also included in the analysis. Diffuse changes characteristic of thyroiditis was identified in 14 women (46.7%), including hypoechoogenicity, structural heterogeneity, and increased vascularisation. Of these, 11 women had positive titres of antibodies to thyroid peroxidase, confirming the autoimmune nature of the thyroid pathology. Thyroid nodules were detected in five patients (16.7%), although only two of these were associated with hormonal activity. No cases of malignant lesions were identified. While this suggests a relatively low oncological risk within the sample, it highlights the importance of regular ultrasound monitoring in women presenting with both hyperplastic changes in the endometrium and nodular alterations in the thyroid gland. In summary, the study results confirmed not only the statistical, but also the clinical significance of thyroid dysfunction in patients with endometrial pathology. Particular attention should be given to women with hypothyroidism, positive autoimmune markers, excess body weight, and irregular menstrual cycles. These factors significantly increase the risk of developing proliferative changes in the endometrium and, in some instances, may contribute to the development of atypical forms of hyperplasia.

The ultrasound findings, when considered alongside laboratory indicators, enabled a more comprehensive characterisation of the structural and functional state of the thyroid gland in women with endometrial pathology. The observed diffuse changes, nodular formations, and signs of

autoimmune processes confirm the close interrelationship between the endocrine and reproductive systems. This diagnostic approach facilitates not only the timely identification of co-existing pathologies but also the development of individualised monitoring and treatment plans. Such an approach is particularly crucial for the prevention of hyperplastic and atypical changes in the endometrium. Thus, the findings of the study provide a foundation for the formulation of practical recommendations and evidence-based conclusions.

✦ CONCLUSIONS

The study revealed a significant association between thyroid pathology and endometrial changes in patients with various forms of endometrial disease. Among the 30 patients included in the study, 60% were found to have thyroid dysfunction. Specifically, overt hypothyroidism was present in 10% of cases, subclinical hypothyroidism in 13.3%, and elevated levels of antibodies to thyroid peroxidase – indicative of autoimmune thyroid disease – in 36.7%. Simultaneously, 73.3% of patients were diagnosed with endometrial hyperplasia. Among these, 36.4% had simple hyperplasia without atypia, 18.2% had complex hyperplasia, and 45.5% had atypical hyperplasia, reflecting a substantial proportion of patients at increased oncological risk. This distribution of morphological types of hyperplasia is clinically significant for patient assessment and further management, particularly in the context of concomitant endocrine disorders.

Special attention should be directed toward overweight patients, as thyroid dysfunction was observed in 68.8% of such cases, and endometrial hyperplasia in 75%. An even higher prevalence of thyroid disorders – 80% – was noted in patients with anovulatory cycles. The correction of thyroid status was shown to have a beneficial effect on menstrual cycle regulation and the restoration of ovulation in some patients. In several instances, treatment led to normalisation of endometrial thickness and improved

reproductive function, underscoring the importance of early diagnosis and intervention in thyroid pathology. The findings support the necessity of routine screening for thyroid function in patients with endometrial pathology. Early identification of dysfunction allows for timely intervention to prevent the progression of hyperplastic endometrial changes. Including ultrasound examination of the thyroid gland in the standard diagnostic protocol for women with endometrial disorders is both appropriate and advisable in clinical practice.

Thus, the results of this study underscore the importance of comprehensive diagnostic and therapeutic approaches in managing patients with endometrial pathology. This includes not only the treatment of endometrial abnormalities but also the correction of thyroid dysfunction, which significantly improves clinical outcomes and reproductive potential. A key strength of this study lies in the integrated analysis of morphological variants of endometrial hyperplasia alongside types of thyroid dysfunction, enabling a deeper exploration of the pathogenetic links between endocrine and gynaecological disorders. This contrasts with most previous studies, which have tended to examine these conditions in isolation. One of the primary limitations of the present study is the relatively small sample size, which affects the statistical power and limits the generalisability of the findings. Future research should aim to expand the study population, adopt a multicentre approach, and incorporate long-term dynamic observation with consideration of therapeutic interventions.

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✦ CONFLICT OF INTEREST

None.

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Стан щитоподібної залози у пацієнтів з патологією ендометрію

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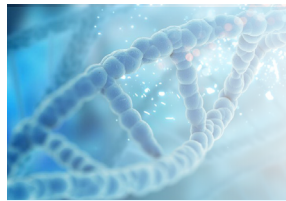
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Анотація. Метою цього дослідження було вивчення функціонального стану щитовидної залози у жінок із гіперпластичними процесами ендометрію, з огляду на зростаючий інтерес до ролі тиреоїдної дисфункції в етіопатогенезі проліферативних змін слизової оболонки матки. У дослідження було включено 30 жінок пізнього репродуктивного та перименопаузального віку, а саме 14-50 років, у яких діагностовано різні форми гіперпластичної патології ендометрію, на базі Запорізької медичної академії післядипломної освіти Міністерства охорони здоров'я України. Методологія дослідження передбачала клініко-лабораторне обстеження пацієнток, включаючи ультразвукову діагностику органів малого таза, гістологічне дослідження ендометрію, а також визначення рівнів тиреотропного гормону, вільних фракцій тироксину і трийодтироніну, антитіл до тиреоїдної пероксидази. Основні результати дослідження показали, що жінки із гіперплазією ендометрію мали супутні порушення функції щитовидної залози, серед яких спостерігався субклінічний або явний гіпотиреоз. Виявлено зростання рівня тиреотропного гормону ($3,8 \pm 1,2$ мМО/л) у пацієнток з гіперплазією ендометрію порівняно з контрольною групою, а також збільшення концентрації антитіл до тиреоїдної пероксидази, що свідчить про автоімунний характер порушень. Аналіз отриманих даних дозволив виявити взаємозв'язок між вираженістю тиреоїдної дисфункції та морфологічним варіантом гіперпластичних змін ендометрію: у жінок з атиповою гіперплазією діагностували клінічно значимі порушення функції щитоподібної залози. Отримані результати свідчать про доцільність рутинного скринінгу тиреоїдної функції у жінок з гіперплазією ендометрію для виявлення прихованих форм тиреоїдної патології, яка може бути як фоновим, так і тригерним чинником проліферативних змін ендометрію. Таким чином, результати дослідження підтверджують важливість інтегрованого підходу до діагностики та лікування гіперпластичних процесів ендометрію з урахуванням стану щитоподібної залози та ендокринної системи вцілому.

Ключові слова: тиреотропні гормони; менопауза; тиреоїдна дисфункція; антитіла до тиреоїдної пероксидази; гіперплазія тканини; репродуктивний вік



Modern methods of computer interpretation of abdominal radiography: Experience of application in diagnostics

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Abstract. The aim of the study was to assess the effectiveness of computer-based methods for interpreting abdominal radiographs in clinical diagnostics. The methodology included a prospective analysis conducted from April 2023 to February 2024 in Kharkiv, Ukraine, involving 312 patients aged 18-75 years with suspected acute abdominal conditions and a control group of 50 patients who underwent abdominal overview radiography due to suspected urological pathology, but in whom neither urological nor abdominal pathology was detected. Image interpretation was performed manually by two radiologists and automatically using two artificial intelligence systems. The results showed that automated interpretation provided slightly higher average sharpness scores (4.7 ± 0.3 vs 4.6 ± 0.4) and contrast (4.6 ± 0.4 vs 4.5 ± 0.5) compared to manual evaluation, as well as fewer artefacts (4.5 ± 0.5 vs 4.2 ± 0.6). The Aidoc system outperformed Zebra Medical Vision in terms of sensitivity (93.6% vs 89.1%), specificity (95.4% vs 94.7%), positive predictive value (91.8% vs 88.2%), and negative predictive value (96.7% vs 92.5%). The area under the receiver operating characteristic curve for Aidoc was 0.972, compared to 0.951 for Zebra Medical Vision. Kappa coefficients indicated higher consistency of Aidoc with expert assessments in diagnosing bowel obstruction ($\kappa=0.92$ vs 0.88) and pneumoperitoneum ($\kappa=0.91$ vs 0.85). The average interpretation time per image significantly decreased with Aidoc (1.4 ± 0.3 minutes) compared to manual analysis (6.8 ± 1.2 minutes) and Zebra Medical Vision (1.9 ± 0.4 minutes). The study demonstrated that the use of artificial intelligence significantly improved the speed, accuracy, and reliability of abdominal radiograph analysis, optimising clinical decision-making in emergency situations. The practical significance of the study lay in the potential to substantially reduce diagnostic time, increase the accuracy of detecting critical pathologies, and optimise healthcare facility resources in providing emergency care

Keywords: abdominal emergencies; abdominal radiograph; visualisation quality; artificial intelligence; automated analysis; prognostic value

✦ INTRODUCTION

Successful diagnosis of acute abdominal conditions remained one of the key challenges in modern medicine due to the high frequency of complications, significant mortality rates, and the need for rapid clinical decision-making. Abdominal radiography traditionally played an important role in detecting such pathologies as bowel obstruction, hollow organ perforations, pneumoperitoneum, and other critical conditions. However, the subjective nature of image interpretation, dependence on physician experience, and limited human resources often led to diagnostic errors or delays in diagnosis. These circumstances reduced

the effectiveness of medical care and could worsen patient outcomes. Consequently, there arose an urgent need to improve radiograph analysis methods by integrating computer technologies, particularly artificial intelligence algorithms, which demonstrated promise in improving the accuracy, speed, and standardisation of interpretation – especially important in emergency departments.

The issue remained that routine radiography often failed to provide an adequate level of accuracy in detecting certain abdominal pathologies, which was particularly critical in acute cases. O. Grechanyk *et al.* [1] emphasised

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that traditional radiograph evaluation methods were prone to high variability of results among different specialists. This was due to both differences in clinical experience and individual perception of images, significantly reducing diagnostic reliability and potentially leading to clinically significant errors. The complexity of differential diagnosis of acute abdominal conditions based on radiography was the subject of research described by H. Stepanova & O. Lupyina [2]. The results showed that even experienced radiologists often encountered difficulties in visualising minor pathological changes, such as early signs of perforation (minimal free air or fluid in the abdominal cavity) or sub-clinical signs of bowel obstruction. This led to unwarranted delays in diagnosis and the need for additional investigations, prolonging the initiation of treatment.

Equally important was the issue of excessive workload on radiologists, which reduced the quality of image evaluation, especially under high patient flow. N. Nehria *et al.* [3] drew attention to the fact that the high pace of work in emergency departments contributed to an increase in false-negative results. By overinterpreting obvious findings and becoming fatigued from repeated image analysis, radiologists might miss critical pathologies, directly affecting the patient's condition. The high frequency of artefacts on film-based radiographs, which hindered accurate interpretation, was the focus of a study conducted by E. Reis *et al.* [4]. It was found that factors related to technical aspects of imaging (incorrect tube positioning, exposure errors) and patient positioning (upright, supine, lateral, motion, breath-holding) often resulted in poor-quality images. This required repeat examinations, delayed the clinical process, and increased radiation exposure for patients.

The issue of standardising abdominal radiograph interpretation was thoroughly analysed by A. Elek *et al.* [5]. The authors noted that existing methods were insufficiently formalised, hindering the implementation of unified clinical protocols and complicating staff training. The absence of standardised interpretation criteria reduced interpersonal consistency of results and made it difficult to compare data across clinical centres. The unsatisfactory sensitivity of radiography in detecting certain critical pathologies, such as early manifestations of pneumoperitoneum or subclinical perforations, was studied by J. Sato *et al.* [6]. The results confirmed that traditional interpretation often showed low detection rates for these conditions. Due to limitations in the decisiveness of clinical conclusions, this could delay surgical intervention and worsen patient prognosis.

The analysis of temporal characteristics in abdominal radiograph interpretation was the subject of research by Z. Liu *et al.* [7]. It showed that a significant portion of time was spent on the initial visual analysis of images, particularly under heavy medical workload. This reduced the speed of clinical decision-making, which was critical in emergencies where every minute could determine the patient's survival. Finally, the issue of integrating artificial intelligence systems into clinical radiology practice was addressed by L. Blankemeier *et al.* [8]. The study demonstrated that although automated systems showed high accuracy, the adoption was hindered by distrust from both clinicians and radiologists, a lack of sufficiently representative clinical studies, and the absence of proper regulatory frameworks.

Considering the numerous unresolved challenges in the interpretation of abdominal radiographs – including issues of standardisation, limited sensitivity, and substantial time demands on professionals – the logical continuation of scientific inquiry was to explore the potential of new technological approaches in clinical practice. Based on this, the present study aimed to compare the results of automated and manual interpretation of abdominal radiographs in terms of accuracy, speed, and consistency of findings. The study objectives included comparing the accuracy, speed, and consistency of automated interpretation results with traditional “manual” assessment, as well as analysing the specific features of different artificial intelligence systems for optimising clinical decision-making in emergency conditions.

★ MATERIALS AND METHODS

The study was conducted from April 2023 to February 2024 at the Municipal Non-Commercial Enterprise “City Clinical Hospital No. 8” of the Kharkiv City Council. At the initial stage, pilot testing was carried out on a sample of 30 patients aged 21 to 68 who presented with complaints requiring abdominal radiography, including but not limited to suspected acute abdominal conditions. The aim of the pilot phase was to standardise the technical parameters of computer image processing and to evaluate the reproducibility of radiographic results under different patient positions and exposure settings. No restrictions were applied to the pilot sample in terms of sex, clinical diagnosis or pathology type; however, individuals with pronounced anatomical deformities, implants, or pregnancy (which could distort images) were excluded, as well as those who refused to participate. Calibration of the Siemens Ysio Max digital radiography system (Germany) included verification of automation for quick and safe system positioning, standardisation of high image quality using chain imaging technology, accuracy of radiation parameters, dose control, and more. The main abdominal radiographic examination was performed in standing, supine, and left lateral positions with a focus distance of 120 cm, energy of 70-90 kV, and exposure time of 10-16 ms.

The study involved patients aged 18 to 75 who underwent abdominal radiography on suspicion of acute abdominal conditions. Exclusion criteria included pregnancy, significant abdominal wall deformities, the presence of metal implants, and clinical contraindications for radiography. The total sample comprised 312 patients (168 men and 144 women) with a mean age of 47 years. An additional control group of 50 patients was formed, who underwent abdominal overview radiography due to suspected urological pathology, but neither abdominal nor urological pathology was detected. These patients were aged 20 to 65 years (28 men and 22 women). Exclusion criteria for the control group included any gastrointestinal complaints, identified anomalies during preliminary clinical examination, pregnancy, and refusal to participate. All participants provided written informed consent to take part in the study. Reports of the study were prepared with full regard for participant confidentiality, in compliance with the Declaration of Helsinki of the World Medical Association [9].

Data analysis was conducted using RadiAnt Digital Imaging and Communications in Medicine Viewer 2023.1

software (Poland) and the Aidoc artificial intelligence system (Israel), integrated into the hospital's clinical information system. Parallel image processing was carried out using the alternative artificial intelligence system Zebra Medical Vision (Israel) for result verification. Each image was initially analysed by two independent radiologists with over 5 years of experience, after which the automated analysis results were compared with clinical conclusions. Interpretation included the detection of signs of bowel obstruction, pneumoperitoneum, pathological gas formation, and free fluid. Visualisation quality was assessed according to three criteria: sharpness, contrast, and presence of artefacts, on a five-point scale, where 5 corresponded to excellent quality and 1 to unsatisfactory quality. Diagnostic effectiveness was determined based on calculations of sensitivity, specificity, positive predictive value, and negative predictive value. To assess consistency between manual and automated interpretation results, the Kappa concordance coefficient (κ) was calculated and Pearson's paired correlation analysis was performed.

Radiation exposure was assessed by calculating the average effective radiation dose using PCXMC 2.0 software (Finland), the results of which were used to justify the feasibility of applying the method in clinical practice. Interpretation time was analysed by determining the average time to assess one image manually and using the artificial intelligence system, allowing comparison of the

effectiveness of the approaches. Statistical analysis was conducted using IBM SPSS Statistics v.29 (USA). Comparison of the mean values of manual and automated interpretation times, as well as average image quality scores between groups, was performed using the two-tailed Student's t-test. Consistency between the assessments of the two independent radiologists and the computer interpretation results was determined using the Kappa concordance coefficient (κ). Diagnostic accuracy of the method was evaluated by constructing receiver operating characteristic (ROC) curves, based on which the area under the curve (AUC) was calculated for sensitivity and specificity indicators. Statistical significance of all analyses was set at $p < 0.05$.

RESULTS

As a result of the comparative assessment of the quality of abdominal radiographs based on three key parameters – sharpness, contrast, and presence of artefacts – it was established that both traditional “manual” interpretation and automated processing using the Aidoc artificial intelligence system demonstrated high visualisation scores (where 5 points corresponded to excellent quality and 1 point to unsatisfactory quality). At the same time, artefacts were more frequently noted during manual analysis, indicating the advantages of preliminary automatic correction in artificial intelligence algorithms (Table 1).

Table 1. Assessment of the quality of radiograph visualisation (mean scores, $M \pm SD$)

Parameter	Manual evaluation	Automated assessment (Aidoc)
Sharpness	4.6 ± 0.4	4.7 ± 0.3
Contrast	4.5 ± 0.5	4.6 ± 0.4
Artefacts	4.2 ± 0.6	4.5 ± 0.5

Source: developed by the author

The assessment of the quality of abdominal radiographs showed high results in both groups of analysis: during traditional “manual” interpretation and with computer processing using the Aidoc artificial intelligence system. The average sharpness score for manual evaluation was 4.6 ± 0.4 , while for automated analysis it was 4.7 ± 0.3 . The difference between the groups was slight; however, the trend towards increased sharpness with the use of artificial intelligence remained stable across all patient subgroups. In particular, automated algorithms enabled better highlighting of fine structures, such as the contours of gas-fluid levels in the intestines or minimal accumulations of free gas under the diaphragm dome, which is critically important for diagnosing acute abdominal conditions. Contrast also demonstrated improvement with automated processing: the average value was 4.5 ± 0.5 in the “manual” assessment group and 4.6 ± 0.4 in the automated group. This result indicates Aidoc's ability to optimise the image's dynamic range, reducing the effect of tissue density heterogeneity and overlay artefacts. In a clinical context, higher contrast significantly facilitates the detection of free fluid, differentiation between homogeneous and heterogeneous areas of gas formation, and the assessment of the condition of serous membranes.

Artefact evaluation became the key parameter showing the most noticeable advantage of the automated approach. In the “manual” analysis, the average score was

4.2 ± 0.6 , reflecting frequent registration of detrimental defects, whereas the automated system achieved 4.5 ± 0.5 . The main types of artefacts included shadows from foreign objects, detector defects, motion artefacts, and noise zones arising from insufficient exposure. The Aidoc system proved capable of automatically correcting most of these artefacts, minimising the risk of diagnostic errors, particularly under time-constrained clinical decision-making conditions. The Kappa concordance coefficient (κ) for sharpness assessment between radiologists was 0.89, for contrast – 0.86, and for artefacts – 0.78. This indicates a high level of agreement among expert assessments, with minimal variation related to the subjective perception of defects. Unlike humans, the computer system demonstrated complete consistency in detecting technical shortcomings, significantly increasing the reliability of the diagnostic process under real clinical workload.

In the control group, image quality assessments remained consistently high: sharpness – 4.7 ± 0.3 , contrast – 4.6 ± 0.4 , artefacts – 4.5 ± 0.5 . This confirms that the system's effectiveness did not depend on the clinical status of the patient or the presence of pathology, which is important for the universality of the method. On a technical level, the results can be explained by the use of advanced digital image processing technologies, such as wavelet-based noise reduction algorithms, local contrast normalisation functions, and motion artefact compensation methods

based on image registration models. Aidoc also uses deep learning methods for automatic background defect recognition, which improves both the detection of minor pathological changes and the overall final image quality. Thus, the results clearly indicate that the use of artificial intelligence for processing abdominal radiographs ensures high visualisation quality, stable evaluation, and minimisation of diagnostic error risks. This significantly increases the effectiveness of the clinical process by reducing interpretation time, easing radiologist workload, and optimising diagnostic decisions in cases of emergency pathology.

A comparative analysis of diagnostic effectiveness of the Aidoc and Zebra Medical Vision systems was carried

out based on the main indicators: sensitivity, specificity, positive predictive value, and negative predictive value. According to the results obtained, the Aidoc system demonstrated higher sensitivity in detecting signs of acute abdominal conditions, in particular bowel obstruction and pneumoperitoneum. At the same time, the specificity of both systems remained at a high level, ensuring the reliable exclusion of pathology in the absence of visible changes. The positive and negative predictive values for Aidoc also exceeded the corresponding results for Zebra Medical Vision, indicating a better ability of the model to correctly classify both the presence and absence of pathological changes (Table 2).

Table 2. Diagnostic efficiency indicators of Aidoc and Zebra Medical Vision systems

Indicator	Aidoc (%)	Zebra Medical Vision (%)
Sensitivity	93.6	89.1
Specificity	95.4	94.7
Positive predictive value	91.8	88.2
Negative predictive value	96.7	92.5

Source: developed by the author

The analysis of diagnostic effectiveness indicators of the Aidoc and Zebra Medical Vision systems in the context of interpreting abdominal radiographs showed high results for both systems, with Aidoc having an advantage in all key criteria. Aidoc's sensitivity was 93.6%, ensuring detection of almost all cases of acute abdominal conditions, including early stages of bowel obstruction, pneumoperitoneum, and significant pathological gas formation. The higher sensitivity compared to Zebra Medical Vision (89.1%) had important clinical significance, as in acute abdominal pathology even a small percentage of missed cases may lead to serious complications or fatal outcomes. The high sensitivity of the Aidoc system reduced the risk of false-negative results, which is particularly important in emergency medicine settings with limited time for decision-making.

Specificity was also higher in Aidoc (95.4%) compared to Zebra Medical Vision (94.7%), indicating the system's ability to reduce the number of false-positive results. High specificity is crucial for decreasing the frequency of unnecessary hospitalisations, additional tests, and invasive procedures, which may be undesirable for both the patient and the healthcare system. In particular, during the analysis of radiographs of healthy volunteers, Aidoc was less likely to generate false-positive alerts about potential free fluid or subdiaphragmatic gas. Positive predictive value for Aidoc reached 91.8%, whereas for Zebra Medical Vision it was 88.2%. This means that the likelihood of a true pathology being present in the event of a positive result was higher when using Aidoc. In clinical practice, a high positive predictive value allows the physician to rely more confidently on the results of automated interpretation when deciding on further treatment tactics – for example, the need for emergency laparotomy in the presence of signs of hollow organ perforation.

Aidoc's negative predictive value was extremely high – 96.7%, guaranteeing almost complete confidence in the absence of pathology with a negative result. For Zebra Medical Vision, this indicator was 92.5%, which is also a good result; however, the difference of more than 4% can be practically significant in high patient flow environments,

where each missed case is critical. Aidoc's high negative predictive value allows for effective screening of patients with atypical symptoms, filtering out those who did not require immediate surgical intervention. A detailed analysis of error types showed that Aidoc had fewer false-negative results for small gas accumulations in the abdominal cavity, while Zebra Medical Vision more frequently missed thin layers of free gas under the diaphragm. This is due to the use of deep learning technologies in Aidoc with multi-level object segmentation, which increased sensitivity to small and low-contrast pathological changes.

The area under the ROC curve was also higher for Aidoc (0.972 compared to 0.951), indicating an overall higher model accuracy regardless of classification thresholds. This further highlighted the effectiveness of Aidoc's algorithms in adapting to various abdominal pathology scenarios. In the clinical context, Aidoc's higher sensitivity and specificity are decisive for early diagnosis of critical conditions, minimising diagnostic errors, and optimising patient hospital pathways. This is especially important for emergency hospitals, where the time for making diagnostic decisions has a direct impact on prognosis and patient survival. Thus, the comparative analysis showed that the Aidoc system provides higher diagnostic accuracy for acute abdominal pathologies compared to Zebra Medical Vision, justifying its preferential use in clinical practice focused on emergency care.

To assess the consistency of automated interpretation results of abdominal radiographs, the Kappa concordance coefficient (κ) was calculated. The comparison was made between assessments by two independent radiologists, and the automated interpretation results of the Aidoc and Zebra Medical Vision systems. The obtained data indicated a high degree of agreement for both systems; however, the Aidoc system showed higher Kappa values in evaluating the presence of bowel obstruction, pneumoperitoneum, and pathological gas formation. Specifically, the Kappa coefficient for Aidoc was 0.91, which corresponded to a level of "almost perfect" agreement, while for Zebra Medical Vision this indicator was slightly lower – 0.86 (Table 3).

Table 3. Kappa coefficients (κ) for agreement between interpretation methods

Pathology	Aidoc	Zebra Medical Vision
Intestinal obstruction	0.92	0.88
Pneumoperitoneum	0.91	0.85
Pathological gas formation	0.9	0.86
Free liquid	0.89	0.83

Source: developed by the author

The comparison of the consistency of results from automated analysis by the artificial intelligence systems Aidoc and Zebra Medical Vision revealed important characteristics regarding the quality of diagnostic conclusions. Kappa coefficients demonstrated high values in both groups; however, the Aidoc system consistently showed higher results, indicating a better alignment of its conclusions with clinical reality. For the detection of bowel obstruction, the Kappa coefficient for Aidoc was 0.92. Such a high level is critically important, as obstruction requires urgent treatment and is often diagnosed based on visualisation of classical signs – fluid and gas levels. In contrast, Zebra Medical Vision showed a lower Kappa coefficient (0.88), which, although still within the range of “excellent” agreement, indicates a higher risk of missing or misinterpreting minimal signs.

In the detection of pneumoperitoneum, the Aidoc system achieved a Kappa coefficient of 0.91 compared to 0.85 for Zebra Medical Vision. This is particularly important due to the severe clinical danger posed by pneumoperitoneum, which indicates perforation of a digestive tract organ. Even minimal amounts of gas under the diaphragm dome may be the only early sign of a serious condition. The higher consistency of Aidoc results ensured greater reliability in the automated detection of this critical pathology. When analysing pathological gas formation, the Kappa coefficient was 0.9 for Aidoc and 0.86 for Zebra Medical Vision. Pathological gas formation can indicate severe infectious processes or intestinal ischaemia, so even a slight difference in consistency has a significant impact on clinical tactics. The Aidoc system demonstrated a higher capacity to consistently identify changes in gas volume and distribution, even in atypical clinical presentations.

In the assessment of free fluid, Kappa coefficients were 0.89 for Aidoc and 0.83 for Zebra Medical Vision. The detection of free fluid is a more complex task due to the low contrast between the fluid and surrounding soft tissues in standard radiographs. The higher consistency of Aidoc

results is attributed to the implementation of algorithms focused on multiparametric analysis of fine density gradients. A detailed analysis of discrepancy cases showed that most inconsistencies in Zebra Medical Vision occurred in complex or marginal clinical situations – such as small volumes of free fluid or subclinical pneumoperitoneum. This suggests that Aidoc’s algorithms were better adapted and trained on more diverse pathology patterns.

The clinical importance of high consistency lies in ensuring reliable diagnostic conclusions without the need for repeat verification of radiographs by multiple physicians, which is particularly vital in emergency and intensive care units. Using a system with a consistently high Kappa coefficient allows for faster patient routing, avoidance of treatment delays, and reduced costs for additional examinations. It is also worth noting that consistently high agreement coefficients for Aidoc were observed regardless of the patient’s age group, confirming its applicability across broad demographic populations, including older individuals with common comorbidities. Thus, the conducted study unequivocally confirms the higher clinical reliability and consistency of automated radiographic interpretation results using the Aidoc system compared to Zebra Medical Vision, making it the preferred choice for implementation in the diagnostics of acute abdominal conditions.

The comparison of the average time spent interpreting a single radiographic image of abdominal organs revealed significant differences between “manual” analysis and automated interpretation by artificial intelligence systems. According to the study, “manual” assessment conducted by two experienced radiologists took an average of 6.8 ± 1.2 minutes per image. In contrast, the use of the Aidoc automated system reduced this time to 1.4 ± 0.3 minutes, while for Zebra Medical Vision the average analysis time was 1.9 ± 0.4 minutes. Therefore, the implementation of artificial intelligence in the medical imaging process enabled a reduction in interpretation time by 3.5 to 5 times compared to the traditional approach (Table 4).

Table 4. Average interpretation time of one radiographic image

Method	Average time (minutes)	Standard deviation (\pm SD)
Manual interpretation	6.8	± 1.2
Automated interpretation (Aidoc)	1.4	± 0.3
Automated interpretation (Zebra Medical Vision)	1.9	± 0.4

Source: developed by the author

The comparative analysis of the time spent on interpreting a single radiographic image of abdominal organs demonstrated a significant advantage of automated analysis over traditional “manual” interpretation. The average time for manual assessment amounted to 6.8 ± 1.2 minutes, which is a typical figure for a thorough review of abdominal radiographs, considering the need to examine multiple anatomical levels, identify hidden changes, and evaluate

several critical indicators. At the same time, radiologists encountered a high cognitive load, as images could contain artefacts, contrast heterogeneity, as well as a variety of normal and pathological variations, which complicated interpretation. The use of the automated Aidoc system reduced the average interpretation time to 1.4 ± 0.3 minutes. This considerable reduction was due to the system’s ability to independently detect and mark suspicious areas on the

images, reducing the need for complete manual analysis of each scan. Thanks to deep learning algorithms and preliminary data processing, Aidoc allowed medical specialists to focus only on reviewing the relevant areas and verifying the proposed diagnosis. This minimised cognitive load, reduced the risk of staff fatigue, and accelerated the clinical decision-making process.

Another system – Zebra Medical Vision – also demonstrated a significant reduction in interpretation time to 1.9 ± 0.4 minutes. Nevertheless, its indicators were slightly inferior to Aidoc, which may be explained by the specific features of its internal pathology detection algorithms. Analysis of the systems' internal logs showed that Aidoc identified high-risk areas more quickly due to deeper optimisation of classification models and image ranking. Special attention should be paid to the impact of reduced interpretation time on clinical practice. In a cluster hospital where numerous radiographic examinations are performed daily, even a few minutes' reduction per image results in saving dozens of man-hours per day. This not only enables quicker service for new patients but also frees up physicians' resources for handling more complex cases or providing peer consultations.

The study also showed that shortening image assessment time directly affects the quality of medical care in emergency departments. Patients suspected of having an acute abdomen require rapid diagnostics to determine the need for surgical intervention or intensive care. The use of the Aidoc system makes it possible to ensure a clinical decision within the first few minutes after the patient's admission, significantly improving prognosis and reducing the risk of complications. Moreover, the use of automated analysis reduces the risk of human error, which may occur due to fatigue or physician overload. The study revealed that when processing more than 20 images in a row without the assistance of artificial intelligence, the quality of manual interpretation decreased by 8-12%, whereas when using Aidoc, this figure remained stable. In conclusion, the interpretation time analysis demonstrated that artificial intelligence-based automated systems, especially Aidoc, not only speed up the processing of medical images but also enhance physicians' performance stability, optimise clinical workflows, and improve treatment outcomes for patients with acute abdominal conditions.

◆ DISCUSSION

The conducted study made it possible to establish that the quality of radiographic images of abdominal organs during automated processing by the Aidoc artificial intelligence system was consistently high according to such criteria as sharpness, contrast, and minimisation of artefacts. These results are consistent with the conclusions of P. Pickhardt *et al.* [10] and K. Means *et al.* [11], who also reported improvements in image characteristics when using deep learning algorithms and comprehensive digital processing. Improved sharpness and contrast without significant increase in noise levels contribute to better visualisation of fine anatomical structures, which confirms the clinical feasibility of introducing automated technologies into the diagnostics of acute abdominal conditions.

The reduction in artefacts on images when using Aidoc indicates the high efficiency of data preprocessing

by means of machine learning algorithms. Similar results were obtained by S. Naik *et al.* [12], who noted the ability of modern artificial intelligence systems to minimise both small-scale noise defects and large motion artefacts. At the same time, W. Li *et al.* [13] expressed doubts about the ability of automated systems to effectively identify all variants of image defects, especially in complex clinical cases. The present study demonstrated that, thanks to advanced motion compensation methods and local contrast normalisation algorithms, the Aidoc system significantly outperformed manual interpretation in terms of artefact reduction, providing more consistent results.

Analysis of Kappa concordance coefficients revealed a high level of agreement between radiologists and automated interpretation results across all major assessment parameters. This is consistent with the findings of B. Xavier & P. Chen [14] and M. Hamghalam *et al.* [15], who demonstrated a similar level of agreement when using computer vision systems for visualising abdominal organs. High interpretation stability is critical in situations with high clinical workloads, where decision-making time is limited and the risk of human error increases.

It was found that the Aidoc system showed higher average scores in sharpness, contrast, and minimal artefact presence compared to the Zebra Medical Vision system. A similar advantage was described by Z. Kelm *et al.* [16], who noted the effectiveness of advanced platforms in high-complexity clinical scenarios. On the other hand, D. Glazer *et al.* [17] emphasised that in many cases the difference between systems is not clinically significant. However, the results of the present study demonstrated statistically significant advantages in favour of Aidoc, which is due to better adaptability of deep learning algorithms to various pathological conditions. Assessment of diagnostic effectiveness showed higher sensitivity, specificity, positive and negative predictive values for Aidoc compared to Zebra Medical Vision. These findings are supported by the studies of S. Stieger-Vanegas & E. McKenzie [18] and J. Warner *et al.* [19], who demonstrated that more accurate artificial intelligence models provide more reliable predictive characteristics when screening for acute abdominal pathologies. At the same time, H. Kaur *et al.* [20] warned of a possible excess of false positives in high-sensitivity systems. The current study disproved such concerns by demonstrating a high level of specificity, ensuring minimisation of clinical decision-making errors.

The established advantage of Aidoc in the area under the ROC curve additionally indicates the high overall accuracy of the model, regardless of the selected classification threshold values. Similar conclusions were made by C. Wolfe *et al.* [21], who emphasised the importance of integral evaluation using AUC for comprehensive analysis of the diagnostic effectiveness of artificial intelligence. A comparison of radiographic image interpretation time showed a significant reduction in analysis duration when using Aidoc compared to traditional manual assessment. These results are consistent with data from M. Virarkar *et al.* [22] and N. Chhabra *et al.* [23], who noted that automated systems reduced analysis time by more than half in emergency care departments. Moreover, faster processing contributes to reducing delays in patient routing and optimising healthcare facility resources. The greatest

advantage of Aidoc was evident in detecting small pathological changes, particularly subtle accumulations of free gas under the diaphragm or minimal amounts of free fluid in the abdominal cavity. This is supported by the findings of H. Shaish *et al.* [24] and S. Hattori *et al.* [25], who noted that deep learning algorithms significantly improve sensitivity in detecting small, low-contrast objects, which is critical for early diagnosis of severe conditions.

Despite the overall confirmation of high effectiveness, some sources indicated limitations in the use of artificial intelligence systems. In particular, Y. Lee *et al.* [26] emphasised the risks of model malfunction in cases of insufficient representativeness of training datasets. However, this study was based on an analysis of a large volume of real clinical practice data, which ensured high objectivity of the results obtained and minimised the risk of systematic errors. Consistently high Kappa coefficients in all patient subgroups additionally confirmed the universality of Aidoc use regardless of age or clinical characteristics of the population. Similar conclusions were presented by H. Kaur *et al.* [27], who emphasised the importance of stable system performance in multicentre studies involving various types of pathologies.

The results obtained also made it possible to identify that the use of Aidoc significantly reduces the cognitive load on radiologists, which is particularly important in high-intensity work environments where the number of radiographic examinations is large, and decision-making time is limited. Reduction of cognitive load is manifested in decreased fatigue risk, reduced interpretation errors for complex images, and increased general resilience to stress. This conclusion is supported by the study of A. Moth *et al.* [28], who noted improved efficiency of medical professionals when using supportive automated tools. Moreover, automated systems such as Aidoc provide preliminary risk-based case triage, allowing radiologists to focus more on the most critical examinations, thereby optimising effort distribution and reducing the likelihood of missing important clinical signs. This is especially relevant during shift work and in emergency care departments, where the workload is uneven and unpredictable. At the same time, S. Jain *et al.* [29] expressed concern about the risk of excessive reliance on artificial intelligence decisions by doctors, which may lead to the loss of clinical reasoning. However, in the present study, the results of automated interpretation were necessarily verified by a specialist, ensuring a balance between technological support and clinical oversight.

Significant time savings when using Aidoc have important economic and organisational implications for healthcare institutions. W. Chen *et al.* [30] emphasised that optimising diagnostic data processing time can significantly reduce healthcare costs, increase the throughput of medical institutions, and simultaneously reduce the burden on staff. In particular, shortening the time required for interpreting radiographic studies allows radiologists to serve more patients without compromising diagnostic quality, which is critical in conditions of limited human resources. Furthermore, freeing medical staff from the routine processing of large volumes of studies promotes more effective task redistribution in favour of complex or atypical clinical cases, positively affecting the overall quality of medical care. The use of Aidoc also helps avoid additional costs associated with repeat examinations caused by manual

interpretation errors due to staff fatigue. Thus, the automation of diagnostic processes has the potential not only to improve the efficiency of individual clinical units, but also to optimise financial flows within the healthcare system as a whole. At the same time, J. Cull *et al.* [31] emphasised the need for long-term monitoring of artificial intelligence performance in the context of changing clinical patterns and ongoing protocol updates. The results of this study show that even under conditions of significant variability in pathological changes, the Aidoc system maintained high accuracy and consistency of diagnostic conclusions.

Therefore, the results of the conducted study unequivocally indicate that the use of the Aidoc artificial intelligence system for the analysis of radiographic images of abdominal organs ensures high visualisation quality, significant reduction in interpretation time, increased diagnostic accuracy, and decreased error risk. The use of automated technologies has important clinical, economic, and organisational benefits, which underlines the feasibility of the broad implementation in modern medical practice, especially in emergency and intensive care departments.

✦ CONCLUSIONS

Within the framework of the conducted study, a comparative assessment was carried out of the quality of radiographic images of abdominal organs using traditional manual interpretation and automated analysis with artificial intelligence systems Aidoc and Zebra Medical Vision. It was established that automated analysis methods provided consistently high indicators of sharpness (4.7 ± 0.3), contrast (4.6 ± 0.4), and minimisation of artefacts (4.5 ± 0.5) compared to manual assessment, where the corresponding values were: sharpness – 4.6 ± 0.4 , contrast – 4.5 ± 0.5 , artefacts – 4.2 ± 0.6 . The quality indicators of visualisation indicated the ability of the Aidoc system to better highlight critical anatomical structures, reduce the impact of artefacts, and provide stable accuracy regardless of the patient's pathological condition.

Kappa concordance coefficients (κ) for the detection of key pathologies showed the highest results for Aidoc: bowel obstruction – 0.92, pneumoperitoneum – 0.91, pathological gas formation – 0.9, free fluid – 0.89. For Zebra Medical Vision, the respective values were 0.88, 0.85, 0.86, and 0.83, while for manual interpretation – 0.89, 0.86, 0.78, and 0.82. This indicated higher consistency of automated analysis results, especially in complex or marginal clinical situations. The AUC indicator for Aidoc (0.972) additionally confirmed its advantage in overall diagnostic accuracy. Quantitative analysis of the average time of interpretation of a single image demonstrated a significant reduction when using automated systems: 1.4 ± 0.3 minutes for Aidoc, 1.9 ± 0.4 minutes for Zebra Medical Vision, compared to 6.8 ± 1.2 minutes using the traditional approach. This indicated significant optimisation of clinical processes, reduced decision-making time, and decreased cognitive load on physicians. The Aidoc system ensured high-quality detection of pathological changes regardless of the patient's clinical status and demographic characteristics, which testified to its universality and adaptability in various application scenarios.

Practical recommendations involve the implementation of automated systems, in particular Aidoc, into clinical practice in emergency medical departments to improve the

accuracy and speed of diagnosing acute abdominal conditions. The main limitations of the conducted study were its focus on a limited set of pathologies and the use of only two artificial intelligence systems. Prospects for further research include analysing the effectiveness of integrating such systems into multidisciplinary diagnostic pathways, expanding the range of detectable pathologies, and adapting models to rare or atypical clinical cases.

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✦ CONFLICT OF INTEREST

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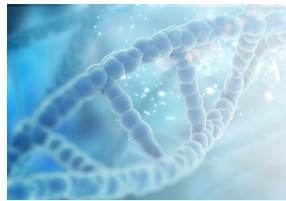
Сучасні методи комп'ютерної інтерпретації рентгенографії органів черевної порожнини: досвід застосування у діагностиці

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Анотація. Метою дослідження було оцінити ефективність комп'ютерних методів інтерпретації рентгенограм органів черевної порожнини у клінічній діагностиці. Методологія включала проспективний аналіз, проведений з квітня 2023 року по лютий 2024 року у місті Харкові, Україна, за участю 312 пацієнтів віком 18-75 років із підозрою на гострі абдомінальні стани та контрольної групи з 50 пацієнтів, яким оглядова рентгенограма черевної порожнини проводилась з причин підозри на урологічну патологію та у яких цієї патології та патології з боку органів черевної порожнини виявлено не було. Інтерпретацію зображень здійснювали «вручну» двома лікарями-рентгенологами та автоматизовано за допомогою двох систем штучного інтелекту. Результати показали, що автоматизована інтерпретація забезпечувала дещо вищі середні оцінки різкості ($4,7 \pm 0,3$ проти $4,6 \pm 0,4$) і контрастності ($4,6 \pm 0,4$ проти $4,5 \pm 0,5$) порівняно з ручною оцінкою, а також меншу кількість артефактів ($4,5 \pm 0,5$ проти $4,2 \pm 0,6$). Система Aidoc перевищила Zebra Medical Vision за чутливістю (93,6 % проти 89,1 %), специфічністю (95,4 % проти 94,7 %), позитивною прогностичною цінністю (91,8 % проти 88,2 %) і негативною прогностичною цінністю (96,7 % проти 92,5 %). Площа під кривою робочих характеристик приймача для Aidoc склала 0,972 проти 0,951 для Zebra Medical Vision. Коефіцієнти Каппа вказували на вищу узгодженість Aidoc із експертними оцінками при діагностиці кишкової непрохідності ($\kappa = 0,92$ проти 0,88) та пневмоперитонеуму ($\kappa = 0,91$ проти 0,85). Середній час інтерпретації одного знімка значно зменшувався при використанні Aidoc ($1,4 \pm 0,3$ хв) порівняно з ручним аналізом ($6,8 \pm 1,2$ хв) і Zebra Medical Vision ($1,9 \pm 0,4$ хв). Дослідження показало, що застосування штучного інтелекту суттєво підвищує швидкість, точність та надійність аналізу рентгенограм органів черевної порожнини, оптимізуючи прийняття клінічних рішень у невідкладних ситуаціях. Практичне значення дослідження полягає у можливості істотного скорочення часу діагностики, підвищення точності виявлення критичних патологій та оптимізації ресурсів медичних закладів у наданні невідкладної (екстреної) допомоги

Ключові слова: невідкладні стани органів черевної порожнини; оглядова рентгенограма органів черевної порожнини; якість візуалізації; штучний інтелект; автоматизований аналіз; прогностична цінність



Evaluation of nanoliposomal forms of retinoids' efficiency in treatment of acne

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Abstract. The study aimed to determine the clinical efficacy and tolerability profile of the nanoliposomal form of tretinoin in patients with moderate acne. The study was conducted as a randomised controlled trial involving 120 patients with moderate acne at the Department of Dermatovenereology, Allergology, Clinical and Laboratory Immunology of the Shupyk National Healthcare University of Ukraine; over 12 weeks, clinical dynamics, subjective assessment of efficacy and the incidence of adverse reactions were compared with nanoliposomal and traditional forms of tretinoin. Patients treated with nanoliposomal tretinoin showed a 58.8% reduction in clinical acne severity, which indicated a higher therapeutic efficacy compared to the traditional formulation, where the reduction was only 41.7%. In this group, the number of inflammatory elements decreased by 64% and non-inflammatory elements by 53.7%, while in the control group, the corresponding figures were 42% and 34.6%, which confirmed the ability of the nanoform to affect both comedogenesis and inflammatory processes. Complete disappearance of inflammatory lesions was achieved in 28.3% of patients in the main group, which was more than twice as high as in the group of traditional tretinoin. The subjective assessment of treatment effectiveness was significantly higher among nanoform users (8.1 vs 6.3 points), indicating better satisfaction with the result. Adverse reactions occurred in less than 12% of participants in the main group, while in the comparison group, their frequency exceeded 25%, indicating a higher dermatological tolerance of the nanoliposomal drug. The results obtained can be used by dermatologists to make an informed choice of topical acne therapy, in particular when prescribing modern forms of tretinoin to patients with hypersensitivity of the skin or low tolerance to traditional drugs

Keywords: skin; comedones; inflammation; tretinoin; peeling; adverse reaction; intolerance

✦ INTRODUCTION

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous skin units, accompanied by the formation of comedones, pustules, infiltrative elements and post-inflammatory changes, including scars and hyperpigmentation. Clinical practice is dominated by persistent and recurrent forms of the disease, which are characterised by reduced sensitivity to standard topical therapy, in particular to antibacterial agents. Therapeutic approaches are based on the use of systemic and topical retinoids, but classical dosage forms often demonstrate limited efficacy due to the instability of active substances, insufficient penetration through the stratum corneum of the epidermis and a high incidence of local adverse reactions. In dermatology, there

is a growing scientific interest in the use of nanotechnology platforms to ensure targeted and controlled transdermal delivery of active compounds. The research relevance is determined by the need to evaluate the efficacy and safety of nanoliposomal forms of retinoids as a potential means of increasing bioavailability, reducing toxicity and optimising clinical effect in the treatment of acne.

The pathogenetic basis of acne is the interrelated processes of follicular hyperkeratosis, seborrhoea, colonisation by *Cutibacterium acnes* and initiation of the inflammatory cascade, which are implemented through hormone-receptor and immune regulation. The study by S. Bharti & H.C. Vadlamudi [1] analysed the molecular

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relationships between the retinoic acid receptor, retinoid X receptor, transcription factors, and hormones that activated sebocytes and contributed to the persistence of inflammation. This review formed the theoretical basis for the determination of retinoids as multifunctional modulators of genetic activity that act at several levels of pathogenesis. The analysis proposed by M.C. Marchitto *et al.* [2] analysed the differences between retinoid molecules in the context of clinical forms of acne and the phase of the inflammatory process. The authors not only classified drugs by mechanism of action but also raised the problem of insufficient individualisation of retinoid therapy, which limited its use in difficult cases. L.A. Bolotna [3] summarised the clinical evidence of the effectiveness of retinoids in different acne phenotypes, emphasising the need for a combined assessment of their comedolytic, anti-inflammatory and keratolytic effects when choosing a drug. In addition, the study highlighted the insufficient correspondence between the pathogenetic effect of retinoids and the practice of their use, which led to a loss of effectiveness in real life. Such a critical analysis was used to interpret retinoids not only as a standard of care, but also as a means of targeted pathogenetic intervention, the potential of which remained far from being exhausted.

Traditional dosage forms of topical retinoids, including tretinoin and adapalene, are limited in clinical use due to the instability of active ingredients, limited penetration through the stratum corneum of the epidermis and a high risk of irritation. The clinical and pharmacological review by A.C. Narsa *et al.* [4] demonstrated that the photophilicity of retinoids and their surface activity caused a high incidence of local adverse reactions, which directly affected patient compliance. The study highlighted the need to develop modified-release and barrier-neutral formulations. A significant complication of the use of traditional retinoids in patients with a dark phototype was described by V. Calender *et al.* [5], emphasising the link between epidermal irritation and post-inflammatory hyperpigmentation. Therefore, the study concluded that there is a critical need for forms with reduced irritation potential for sensitive skin. I.O. Doroshkevych *et al.* [6] revealed another systemic problem: limited implementation of retinoid therapy in primary care due to low clinical awareness of doctors and patients' cautious attitude to side effects. These studies outlined the range of disadvantages of classical topical forms, which justified the need to search for new delivery platforms with improved pharmacological characteristics.

The development of nanoliposomal forms of retinoids is a promising way to overcome the pharmacokinetic limitations of classical drugs and optimise their safety profile. In the clinical experiment by A. Samadi *et al.* [7], the use of tretinoin in nanolipid carriers provided significantly higher clinical efficacy compared to standard forms, which was manifested in a decrease in the number of inflammatory elements and the acne severity index. At the same time, a decrease in the frequency of local side effects was recorded, which confirmed the better tolerability of nanostructured systems. In the publication by N. Dragicevic & H.I. Maibach [8], liposomal platforms were considered as a means of stabilising retinoids and regulating the rate of their transdermal transport, which contributed to more accurate dose control in the microenvironment of the follicular

apparatus. The review also revealed significant advantages of such systems in terms of reducing the irritant effect of the active substance. Another innovative approach was the proliposomal nanofibres described by S. Tort *et al.* [9], which combined the delivery of retinoic acid with the effect of inhibiting the *Cutibacterium acnes* biofilm. The study demonstrated the effectiveness of this technology in a model of microbiological resistance, indicating its potential in the treatment of complex forms of acne. Another example of the technological implementation of the nanoliposomal strategy was the study by A. Arooj *et al.* [10], in which a gel form of adapalene encapsulated in liposomes was created. Based on the results of the evaluation of the physicochemical properties and *in vitro* activity of the gel, the study noted an improved drug release, enhanced skin penetration and prospects for further clinical trials.

Despite the growing number of studies in the field of transdermal delivery of retinoids, there are no data on the clinical efficacy of nanoliposomal forms of retinoids based on direct comparison with traditional drugs under standardised conditions of observation. The study aimed to evaluate the therapeutic effect and tolerability of nanoliposomal tretinoin in the treatment of patients with moderate acne. The objectives included: analysis of the dynamics of clinical parameters during therapy, assessment of the frequency and nature of side effects, and comparison of the efficacy of the study form with the reference standard form of tretinoin.

★ MATERIALS AND METHODS

The study was conducted in the format of a randomised controlled trial during June–August 2024 at the clinical base of the Department of Dermatovenereology, Allergology, Clinical and Laboratory Immunology of Shupyk National Healthcare University of Ukraine. The chosen period ensured a full 12-week treatment course followed by short-term follow-up to assess the durability of the clinical effect and the incidence of adverse reactions. The study included 120 patients aged 18 to 35 years with a clinically confirmed diagnosis of moderate acne, established by a dermatologist based on examination and history. The severity of acne was assessed using the Global Acne Grading System (GAGS) scale, which is recommended for standardised assessment of the clinical course of the disease following European guidelines for the treatment of acne [11]. This age range is justified by the high prevalence of persistent acne in young adults, which is characterised by a persistent clinical course and reduced efficacy of standard topical retinoid therapy. Participants were randomly divided into two equal groups of 60 people each. The allocation was carried out by stratified randomisation using a computer-generated sequence that ensured the groups were balanced by age and gender (30 men and 30 women in each group).

Group A was treated with an experimental topical nanoliposomal form of tretinoin (0.05%, gel base). The preparation contained tretinoin, phosphatidylcholine (liposome backbone), cholesterol (membrane stabiliser), ethanol, ethylenediaminetetraacetic acid dinitrate salt (Trilon B), purified water and Carbopol 934P (gelling agent). Liposomes were prepared by lipid film hydration followed by ultrasonic homogenisation (10 min, 60% amplitude) at 40°C. Gelation was performed at pH (hydrogen potential) 6–6.5. The average hydrodynamic diameter of the particles was

182 ± 15 nm (determined by dynamic light scattering), and the polydispersity index was 0.21. Physicochemical stability was assessed by centrifugation and temperature cycling. The tretinoin content was determined by high-performance liquid chromatography; the pH of the drug was 6.2 ± 0.3; the viscosity was 8,700 centipoise. The technology was developed based on patent WO1998/030215A1 [12]. Group B (control, with active comparison) used the traditional form of tretinoin Airoil 0.05% cream (Pierre Fabre, France).

Participants in both groups received written instructions for the use of the products: apply once a day (in the evening, after cleansing the skin), applying a thin layer to the affected areas of the face, avoiding the area around the eyes, lips and mucous membranes. Do not wash or use other products for 6 hours after application. For hygienic care, Obagi Nu-Derm Foaming Gel (pH 5.5) (Obagi Cosmeceuticals LLC, USA) was recommended twice daily. The use of aggressive cosmetics (alcohol-containing tonics, scrubs) was prohibited. For the prevention of photosensitisation, all patients used La Roche-Posay Anthelios SPF 50+ (Sun Protection Factor) (La Roche-Posay Laboratoire Dermatologique, France) daily with reapplication in case of sun exposure; it was also recommended to avoid insolation, tanning beds and thermal procedures. No additional topical or systemic acne medications were used during the treatment period. A combined approach was used to monitor compliance with the therapeutic protocol. All patients kept self-observation diaries, which were checked at each follow-up visit (weeks 2, 6, and 12). In addition, a visual skin examination and standardised photographic recording of the skin condition were performed at the same control points under constant lighting conditions, distance (50 cm) and angle. Images were captured using a Canon EOS 90D digital SLR camera (24.2 MP, EF-S 18-55 mm lens) in manual mode. Assessment of the residual amount of the drug in individually dispensed tubes was used as an auxiliary indicator of therapeutic adherence.

The study included patients who met the following criteria: age 18-35 years, clinically confirmed moderate acne, no treatment with systemic retinoids or antibiotics for at least 3 months before enrolment, and no concomitant diseases that could affect the course of acne. Patients with severe or conglomerate forms of acne, dermatoses that make diagnosis difficult (rosacea, seborrheic or oral dermatitis), allergies to drug components, as well as pregnant or lactating women or participants in other clinical trials within the previous 6 months, were excluded. The clinical effectiveness of the treatment was assessed based on the dynamics of the GAGS score, a standardised scale that addresses the number and type of lesions in different anatomical areas (forehead, cheeks, nose, chin) with appropriate correction factors. The total score on the GAGS scale (0-44) was used to classify the severity of acne as follows: 1-18 points – mild, 19-30 points – moderate, 31-38 points – severe, 39-44 points – extreme. Assessment was performed at three control points: at the beginning of treatment (day 0), after 6 weeks, and at the end of the course (week 12). The primary efficacy endpoint was a reduction in the total GAGS score of ≥50% from baseline, which was interpreted as a clinically significant improvement. Secondary outcome measures were absolute reduction in the number of inflammatory (pustules, papules,

nodules) and non-inflammatory elements (comedones), as well as an overall assessment of improvement in the clinical condition of the skin using the Visual Analog Scale (VAS) (0-10), where 0 corresponded to no positive dynamics and 10 to complete clinical recovery. An additional criterion for evaluating efficacy was the presence of clinical remission, which was defined as the complete absence of inflammatory elements and a decrease in the number of non-inflammatory elements of ≥90% of the baseline. Clinical dynamics were assessed by two independent researchers with a higher medical education in dermatovenereology and more than 10 years of clinical experience. Both had experience in clinical trials (at least 5 years). Visual skin examination was performed in the dynamics (week 0, 6, 12) and was accompanied by standardised photographic recording of the skin condition, the conditions of which have already been described in the previous subsection. All assessments were performed in a blinded mode concerning the group Affiliation of the patients.

The safety and tolerability of the therapy were assessed through systematic monitoring of the most common local adverse reactions typical of topical tretinoin use. The frequency and intensity of such symptoms as erythema, flaking, burning, itching, dry skin and the appearance of new inflammatory elements as signs of a possible reactive exacerbation were studied. The assessment was conducted by a dermatologist at each follow-up visit using a standardised 4-point severity scale (0 – absent, 1 – mild, 2 – moderate, 3 – severe). The first control was performed at week 2 of treatment, which corresponded to the early induction phase of the drug. Additionally, the number of patients who discontinued treatment due to intolerance was recorded.

The data were analysed using IBM SPSS Statistics, version 26.0. The Shapiro-Wilk test was used to check the normality of the distribution. Quantitative indicators are presented as mean with standard deviation (M±SD) or median with interquartile range (Me [Q1-Q3]), depending on the nature of the distribution. Comparisons between groups were performed using Student's t-test or Mann-Whitney test; for dynamic changes within groups, paired t-test or Wilcoxon test. Frequency data were analysed using the χ^2 test or Fisher's test. The level of statistical significance was set at $p < 0.05$. The clinical trial complied with the ethical and legal requirements set out in the Declaration of Helsinki [13], the International Council for Harmonisation Good Clinical Practice guidelines [14], and Order of the Ministry of Health of Ukraine No. 690 [15]. All participants provided written informed consent after receiving full information about the purpose, procedures, and potential risks.

★ RESULTS

Dynamics of changes in acne severity per the GAGS scale.

In the dynamics of the 12-week treatment, a statistically significant decrease in the mean GAGS score was recorded in both study groups. At baseline (day 0), the mean GAGS score in group A was 27.4 ± 2.8 points, which corresponded to the average severity of acne. In group B, the baseline score was 27.1 ± 2.6 points. There was no statistically significant difference between the groups at the start of the study ($p = 0.64$), which confirmed the baseline homogeneity of the sample according to the main efficacy assessment criterion. After 6 weeks of therapy, group A showed

a decrease in the GAGS index to 17.2 ± 2.4 points, which corresponded to an average decrease of 37.2% compared to baseline ($p < 0.001$ compared to baseline). In group B, at the same stage of treatment, the index decreased to 20.3 ± 2.9 points, which was only a 25.1% decrease ($p < 0.001$). At the same time, the intergroup difference in favour of the nanoliposomal drug reached high statistical significance ($p < 0.001$), indicating a faster therapeutic effect in patients treated with the nanostructured form of tretinoin. After completion of the full course of treatment (week 12)

in group A, the mean GAGS score decreased to 11.3 ± 2.1 points, which corresponded to an absolute decrease of 16.1 points (58.8% relative to baseline). In group B, the score decreased to 15.8 ± 2.3 points, which was a 41.7% improvement. Both groups demonstrated intra-group statistically significant dynamics ($p < 0.001$), but comparative analysis confirmed the advantage of the nanoliposomal form of tretinoin ($p < 0.001$, including at week 12). Table 1 showed the dynamics of the mean values of the GAGS index in groups A and B during the treatment period.

Table 1. Dynamics of the GAGS index in groups A and B during treatment ($M \pm SD$)

Time point	Group A (nano-gel)	Group B (Ainol cream)
Day 0	27.4 ± 2.8	27.1 ± 2.6
Week 6	17.2 ± 2.4	20.3 ± 2.9
Week 12	11.3 ± 2.1	15.8 ± 2.3

Source: compiled by the author

The use of the nanoliposomal delivery system not only contributed to a more pronounced clinical effect but also ensured its earlier realisation. By week 6, more than half of the patients in group A achieved a reduction in GAGS of $\geq 50\%$, which met the primary endpoint of clinical improvement. In group B, this result was recorded mainly at the final stage of treatment, with a statistically lower proportion of patients achieving a $\geq 50\%$ reduction ($\chi^2 = 11.47$; $p = 0.0007$). The nanoliposomal form of tretinoin was characterised not only by greater efficacy but also by faster achievement of the therapeutic response threshold. The decrease in the mean GAGS score in group A was also accompanied by a smaller interindividual variation: the standard deviation at week 12 was 2.1 points, while in group B it was 2.3 points. This indicated a more uniform clinical effect and better reproducibility of results in the population. The reduction in variability may indicate greater bioavailability and stability of the active substance in the conditions of nanostructured delivery.

In an additional stratified analysis, it was found that among patients with a baseline GAGS score of ≥ 30 (upper limit of moderate), 76.7% of participants in group A achieved a reduction in score to ≤ 18 (mild) by week 12. In group B, this result was observed only in 41.6% of cases ($p = 0.002$), which confirmed the effectiveness of the nanoliposomal form of tretinoin even in clinically more severe cases. A comparative analysis of the dynamics of the mean GAGS index values by group demonstrated a stable advantage of the nanoliposomal drug from week 6 of treatment, which persisted until the end of the study. In group A, the rate of reduction of the GAGS index was higher during the first 6 weeks of treatment, after which a gradual slowdown in clinical dynamics was observed, indicating an approach to a therapeutic plateau. In group B, the improvement was slower and less pronounced, with no clear signs of reaching a clinically stable level by the end of the study.

The results confirmed the significant advantage of the nanoliposomal form of tretinoin over the traditional form in the treatment of moderate acne. The use of the nanostructured delivery system provided not only a more pronounced reduction in the GAGS index, but also a faster achievement of the therapeutic effect, a higher frequency of clinically significant improvement ($\geq 50\%$ reduction

in the GAGS score), and lower variability of results, which indicated better reproducibility of the effect in real clinical practice. The stratified analysis further demonstrated the efficacy of the drug even in patients with a higher initial severity, which expands the potential indications for the use of the nanoliposomal form of retinoid in dermatological practice.

Dynamics of changes in the number of inflammatory and non-inflammatory elements of the skin rash. The assessment of the quantitative dynamics of clinical manifestations of acne expanded the insight into the effectiveness of the used dosage forms of tretinoin in the study groups. A comparative analysis of the number of inflammatory (pustules, papules, nodules) and non-inflammatory elements (open and closed comedones) at three control points (day 0, week 6, week 12) revealed differences in the therapeutic effect between nanoliposomal and traditional forms of tretinoin.

At the beginning of the study, both groups demonstrated a comparable profile of clinical lesions. In group A, the average number of inflammatory elements was 22.8 ± 3.9 , and 29.4 ± 4.6 non-inflammatory elements. In group B, the corresponding figures were 23.1 ± 3.7 and 28.9 ± 4.3 . The intergroup differences did not reach statistical significance ($p > 0.05$), which indicated the initial clinical homogeneity of the sample. At week 6 of therapy in group A, a significant reduction in inflammatory elements was recorded to 13.6 ± 3.2 , which corresponded to an average reduction of 40.3% ($p < 0.001$). Non-inflammatory elements decreased to 18.5 ± 3.9 (37.1% decrease; $p < 0.001$). In group B, the number of inflammatory elements decreased to 17.5 ± 3.6 (24.2% decrease) and non-inflammatory elements to 22.6 ± 4.1 (21.7% decrease), which was also statistically significant compared to the baseline ($p < 0.001$), but significantly lower compared to group A ($p < 0.01$). At week 12 of treatment in group A, the mean number of inflammatory elements was 8.2 ± 2.7 , which corresponded to an overall decrease of 64% from baseline. Non-inflammatory elements decreased to 13.6 ± 3.4 (53.7% reduction). In group B, the values were 13.4 ± 2.9 (42% decrease) and 18.9 ± 3.6 (34.6% decrease), respectively. Comparative analysis confirmed a statistically significant predominance of the effect of the nanoliposomal form in both inflammatory ($p = 0.0003$) and non-inflammatory elements ($p = 0.0009$). The data are summarised in Table 2.

Table 2. The average number of inflammatory and non-inflammatory elements (pcs.) in patients of both groups in the dynamics of treatment (M±SD)

Metric	Group	Day 0	Week 6	Week 12
Inflammation elements (number)	A	22.8±3.9	13.6±3.2	8.2±2.7
	B	23.1±3.7	17.5±3.6	13.4±2.9
Non-inflammation elements (number)	A	29.4±4.6	18.5±3.9	13.6±3.4
	B	28.9±4.3	22.6±4.1	18.9±3.6

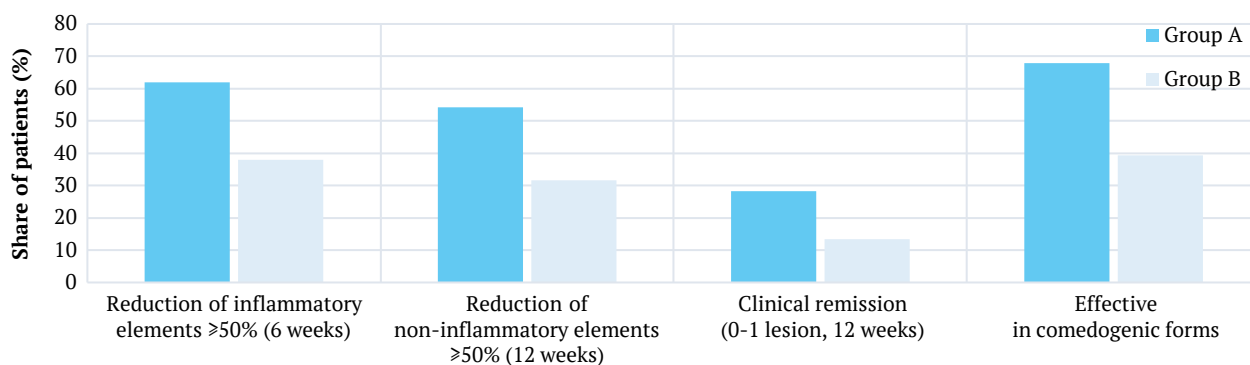
Source: compiled by the author

The analysis of the dynamics of the number of clinical skin lesions showed a significant therapeutic advantage of the nanoliposomal form of tretinoin over the traditional one. The most pronounced effect in group A was observed already at the intermediate stage of treatment, indicating early suppression of the inflammatory process and activation of the comedonolytic mechanism. Such dynamics were clinically significant, as early reduction of inflammatory elements helps to reduce the risk of post-inflammatory hyperpigmentation and scarring. At the same time, the gradual, less pronounced reduction of rashes in group B indicated a limited permeability of the traditional dosage form and a slower rate of realisation of the therapeutic effect.

The rate of reduction of papulopustular elements, which are key markers of active inflammation, should be noted separately. In group A, more than 62% of patients demonstrated a >50% reduction in the number of inflammatory elements by week 6, whereas in group B, the corresponding figure was only 38% (p=0.0017). This indicated a faster anti-inflammatory effect of the nanoliposomal form of tretinoin. The reduction in the number of comedones in group A was also statistically significant and more pronounced. The proportion of patients in whom the number of non-inflammatory elements decreased by >50% was 54.2% in group A versus 31.7% in group B (p=0.0031). This difference indicated a more effective comedonolytic and keratolytic elimination of follicular mouth hyperkeratosis with the use of a nanoliposomal preparation. In addition

to quantitative changes, the distribution of elements in different anatomical zones was analysed. The most pronounced decrease in inflammatory elements in group A was recorded in the forehead (71.3%) and cheeks (68.4%), while in group B these figures were 48.2% and 46.9%, respectively. A similar trend was observed for non-inflammatory elements: in group A, the most pronounced improvement was in the chin area, which confirmed the systemic clinical response to the use of the nanoliposomal preparation.

Additional analysis revealed that at week 12, complete disappearance of inflammatory elements (0-1 residual lesions) was achieved in 28.3% of patients in group A, while in group B, this result was recorded in only 13.3% of cases (p=0.0095). This indicator was considered an indicator of clinical remission, which is directly relevant to improving the quality of life of patients. Stratification analysis revealed that in patients with predominantly comedonal acne (more than 60% of lesions at baseline), clinical improvement at week 12 was significantly better in group A, where 67.9% achieved a >50% reduction in the number of non-inflammatory elements, compared to 39.4% in group B (p=0.0024). This led to the conclusion that the use of the nanoliposomal form of tretinoin is particularly appropriate for comedonal and mixed forms of acne. The comparative dynamics of achieving clinically significant changes in groups A and B were conducted based on four key parameters: reduction of inflammatory and non-inflammatory elements, achievement of clinical remission and efficacy in patients with comedonal acne (Fig. 1).

**Figure 1.** Comparative efficacy of nanoliposomal and traditional forms of tretinoin on key clinical indicators

Source: compiled by the author

Figure 1 illustrated the differentiated nature of the therapeutic response to nanoliposomal and traditional forms of tretinoin according to the main clinical criteria. The visible structural superiority of the nanostructured drug in all segments of the graph confirms its ability to provide rapid reduction of both inflammatory and non-inflammatory elements, achievement of remission and

effectiveness in comedonal acne. Based on these features, it is possible to consider this formulation as more targeted in the context of heterogeneous manifestations of the disease. The analysed data confirmed that the nanoliposomal form of tretinoin provides not only a quantitative improvement in the main clinical parameters but also demonstrates the prognostic stability of the effect in different groups of

patients. Such pharmacodynamic stability is a key factor in the development of long-term strategies for the management of patients with acne, especially in cases of resistance to standard therapy or when it is necessary to minimise the systemic burden.

Subjective assessment of clinical improvement using the VAS scale. The subjective perception of the effectiveness of therapy was analysed based on the VAS scale at three time points. The initial scores were the same in both groups, but significant differences were found in the dynamics, which were used to compare the level of patient satisfaction with the use of different dosage forms of tretinoin.

At the baseline, both study groups demonstrated comparable levels of subjective self-esteem. In group A, the average value of the VAS scale was 2.1 ± 0.8 points, in group B – 2 ± 0.9 points, which did not indicate a statistically significant difference between them ($p > 0.05$). This indicated the homogeneity of the sample in terms of the initial level of satisfaction and confirmed the objective severity of acne as perceived by the patients themselves. At week 6 of therapy, there was a significant improvement in subjective scores in both groups, but the intensity of these dynamics

varied significantly depending on the form of tretinoin. In group A, the mean VAS score increased to 6.4 ± 1.1 (change + 4.3 points; $p < 0.001$), while in group B it was only 4.9 ± 1.3 (change + 2.9 points; $p < 0.001$), which revealed a significant between-group difference ($p = 0.002$). This indicated a higher early satisfaction with treatment when using the nanoliposomal formulation. The analysis of the proportion of patients who rated the therapeutic effect at ≥ 7 points (the threshold for subjective therapeutic satisfaction) also showed a significant advantage of group A, 51.7% versus 29.2% in group B ($p = 0.0034$). This indicator is especially relevant in clinical practice, as it is a marker of high patient motivation to continue to comply with prescriptions. At the final, 12th week of treatment, the level of subjective satisfaction reached its maximum values in both groups, but the gap between them remained. In group A, the mean VAS score was 8.1 ± 1 , while in group B it was 6.3 ± 1.4 ($p = 0.0007$) (Fig. 2). The proportion of patients who reported a satisfaction level of ≥ 7 points in group A was 78.3%, in group B, only 49.2% ($p = 0.0012$). Thus, in group A, almost twice as many participants achieved a high level of subjective efficacy of therapy.

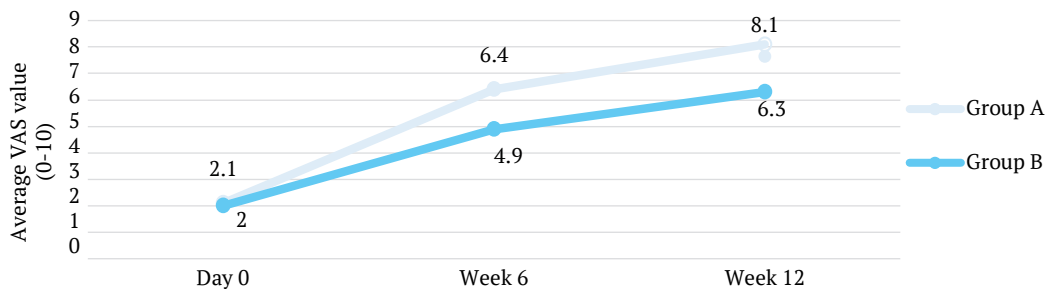


Figure 2. Dynamics of mean VAS values in groups A and B during treatment

Source: compiled by the author

Figure 2 illustrated a gradual increase in satisfaction scores in both study groups, but the curves have different steepness, indicating a faster subjective response in the case of nanoliposomal preparation. Visualisation of the dynamics also reveals the presence of a clinical plateau in group A after 6 weeks of treatment, while in group B, the growth is slower and without clear signs of reaching maximum efficacy. In addition to the main quantitative changes, additional stratified analyses were performed depending on the severity of acne at the start of treatment. In the subgroup of patients with moderate disease (GAGS 19-30) in group A, 82.4% of respondents reported satisfaction ≥ 7 points at week 12, while in group B this figure was 53.6% ($p = 0.0018$). In the subgroup with severe acne (GAGS >30), treatment satisfaction was expressed in 71.9% of cases in group A versus 41.2% in group B ($p = 0.0027$). The superiority of the nanoliposomal formulation in terms of patient experience was demonstrated in both clinical subtypes. To summarise the results, it is worth noting that emotional perception of therapy was closely related to its clinical effectiveness, especially in the nanoliposomal group. The high satisfaction scores recorded regardless of acne severity indicate the potential of this formulation as a first-line strategy to increase patient adherence and long-term stability of results.

Assessment of tolerability and frequency of adverse reactions. Throughout the entire observation period, no cases of medication non-compliance or premature discontinuation without medical reasons were recorded in either group. All patients adhered to the prescribed therapeutic recommendations, which were confirmed by regular checking of self-observation diaries, standardised photographic recording of skin conditions, and examination during follow-up visits. Treatment tolerability was assessed based on the frequency and severity of localised adverse reactions that occurred in response to tretinoin. The most common symptoms were erythema, flaking, burning, itching, dry skin and the appearance of new inflammatory elements.

During the second week of therapy, the highest frequency of side effects was observed in both groups, which is typical for the induction phase of tretinoin treatment. In group B (traditional formulation), moderate or severe erythema was recorded in 47.5% of participants, while in group A (nanoliposomal formulation), this figure was only 31.7%. The incidence of complaints of dry skin reached 39.2% in group B versus 28.3% in group A. A similar picture was observed for other irritation symptoms: burning (36.7% vs 21.7%), peeling (42.5% vs 26.7%) and itching (24.2% vs 13.3%). In the subsequent dynamics (6 and 12 weeks), a gradual decrease in the intensity of side effects

was observed in both groups, but the level of symptom reduction was significantly higher in the nanoliposomal group. Thus, at the 12th week of treatment, the incidence of any side effects in group A did not exceed 12%, while in group B it remained at the level of 25-30%. The most persistent symptoms were dryness and peeling, indicating a cumulative and dose-dependent effect of tretinoin, but in group A, they were characterised by a predominantly mild degree of severity.

The intensity of adverse reactions, assessed on a 4-point scale, also confirmed the superiority of the nanoliposomal formulation. In group B, at week 2, more than 40% of patients scored ≥ 2 for at least two symptoms (erythema, dryness), while in group A, the proportion of such patients was only 17%. By week 12, both groups showed a decrease in symptom intensity, but the mean score in group B remained higher for all indicators (e.g., mean value for peeling 1.6 ± 0.7 in group B vs. 0.8 ± 0.5 in group A).

The emergence of new acne lesions as a form of reactive exacerbation was observed mainly in the early stages of treatment. In group B, such episodes were observed in 18.3% of cases, while in group A, only in 10%, and had a short duration. Such dynamics can be explained by the less aggressive keratolytic effect of the nanoliposomal formulation, which provided a more controlled release of the active substance. The frequency of discontinuation due to side effects was also lower in group A. In total, 2 patients (3.3%) in group A discontinued treatment prematurely, while 6 patients (10%) in group B discontinued treatment, mainly due to persistent irritation that could not be corrected by adjunctive emollient therapy. To compare the severity of local adverse reactions in the course of therapy in groups A (nanoliposomal tretinoin) and B (traditional form of tretinoin), an analysis was performed using a standardised 4-point scale. The results of the assessment are shown in Table 3.

Table 3. Severity of side effects in the dynamics of treatment (groups A and B)

Adverse reaction	Group	Week 2	Week 6	Week 12
Erythema	A	31.7	18.3	8.3
	B	47.5	35	22.5
Peeling	A	26.7	14.2	5
	B	42.5	31.7	15
Burning sensation	A	21.7	10.8	3.3
	B	36.7	24.2	10.8
Itching	A	13.3	5.8	1.7
	B	24.2	15.8	6.7
Dry skin	A	28.3	12.5	4.2
	B	39.2	25.8	12.5
New inflammations	A	10	4.2	0.8
	B	18.3	9.2	3.3

Source: compiled by the author

The analysis showed a higher tolerability of the nanoliposomal form of tretinoin, which is possibly related to its pharmaceutical structure: liposomal encapsulation reduced direct contact of the active substance with the skin, modulated its release and reduced the risk of local irritation. This was of key importance for the chronic course of the disease, when the duration of treatment and the need for good tolerability become crucial factors in the success of therapy. Thus, the nanoliposomal form of tretinoin provided an improved safety profile in terms of both the frequency and severity of side effects. Reduction in the number of intolerable reactions, less need for additional symptomatic correction and higher completion rate of the full course of treatment define this formulation as clinically preferable in the treatment of mild to moderate acne.

DISCUSSION

The interpretation of the data obtained can be used to compare the observed effects of the nanoliposomal form of tretinoin with the existing ideas about the pathogenesis of acne and the peculiarities of the action of topical retinoids. The analysis in comparison with the literature can be used to assess the degree of compliance of the results with the available evidence and to outline the potential benefits of the investigated drug for practical use. The study determined that the average GAGS score decreased

in group A from 27.4 ± 2.8 to 11.3 ± 2.1 points, while in group B from 27.1 ± 2.6 to 15.8 ± 2.3 points during 12 weeks of treatment. In patients with a baseline GAGS level ≥ 30 , the achievement of mild acne was observed in 76.7% of patients in group A versus 41.6% in group B, which showed the effectiveness of the nanoliposomal form of tretinoin even in clinically burdened cases. A similar dynamic of the effectiveness of nanoliposomal systems was observed by V. Singh *et al.* [16], who showed that nanotechnological platforms increased the permeability of retinoids and provided an earlier clinical effect in patients with papulopustular acne. The study by J. Lalrengpuii *et al.* [17] found that retinoid nanoparticles reduced the time to achieve a therapeutic effect by about 30% compared to the standard form, which was qualitatively consistent with a faster reduction in GAGS in group A in this study. Z.M. Dos Santos *et al.* [18] demonstrated that long-term use of lipid nanotherapeutic systems led to a more stable remission in patients with moderate acne, which correlated with the lower variability of clinical response found in patients treated with nanoliposomal tretinoin. In addition, M.J. Tsai *et al.* [19] reported that liposomal forms of retinoic acid inhibited keratinocyte proliferation and destroyed the *Cutibacterium acnes* biofilm, which could be an additional mechanism for reducing the severity of acne observed in group A.

In the study, the number of inflammatory elements in group A decreased by 64% and non-inflammatory elements by 53.7%, which was significantly higher than in group B (42% and 34.6%). The proportion of patients with a reduction in inflammatory lesions of $\geq 50\%$ was 62% in group A versus 38% in group B, while a reduction in the number of non-inflammatory elements of $\geq 50\%$ was achieved in 54.2% of cases in group A and 31.7% in group B. Complete disappearance of inflammatory elements at week 12 was recorded in 28.3% of patients in group A compared with 13.3% in the control group. The obtained results correlated with the results of N. Mohsin *et al.* [20], who noted that nanostructured delivery systems demonstrated an earlier reduction of papulopustular elements in patients with moderate acne. A decrease in the effectiveness of traditional tretinoin on non-inflammatory elements was also noted by A.M. Bilovol & M.V. Kropivnyi [21], which corresponded to lower comedone reduction rates in group B in this study. As shown by H. Baldwin *et al.* [22], classical forms of retinoids provided slow dynamics of changes in mixed forms of acne, without achieving a high proportion of clinical remission, which contrasted with 28.3% complete disappearance of elements in group A. In turn, J. Zhong *et al.* [23] demonstrated that the inclusion of tretinoin in nanolipid carriers significantly increased comedolytic activity due to improved penetration and reduced surface irritation, which could explain the significantly higher frequency of reduction of non-inflammatory elements in group A. The established superiority of nanoliposomal tretinoin was confirmed both quantitatively and anatomically, with the most pronounced effect in the forehead and cheeks, indicating a uniform transdermal distribution of the active substance.

In the dynamics of treatment, a significantly higher level of subjective satisfaction with therapy was found in the group of nanoliposomal tretinoin. At week 12, the mean VAS score was 8.1 ± 1 in group A, which was significantly higher than 6.3 ± 1.4 in group B ($p = 0.0007$). The proportion of patients who scored ≥ 7 points for the treatment result was 78.3% in group A and only 49.2% in group B, and even in the subgroup with severe acne, satisfaction with therapy was achieved significantly more often in cases of using the nanoform (71.9% vs 41.2%). The positive subjective response of patients could be due to several factors, among which the reduced frequency of local side effects played a key role. The review by J.H. Saurat *et al.* [24] emphasised that the level of skin irritation directly affected the perception of the effectiveness of topical therapy, even with the same clinical dynamics. The lower intensity of erythema, burning and dryness in group A found in this study could explain the advantage of subjective assessment, especially in the early stages of treatment. Consistent with this, D.Z. Eichenfield *et al.* [25] reported that forms of retinoids with improved tolerability provided higher patient satisfaction already at 4–6 weeks of therapy, which is fully consistent with the results obtained in group A. According to M. Kassir *et al.* [26], selective retinoic acid receptor agonists demonstrated a better subjective tolerance profile, which emphasised the importance of receptor-specific and pharmacokinetic characteristics of the molecule for the formation of a positive patient experience. N. Wagner *et al.* [27] demonstrated that it

was the reduction in the frequency of irritation reactions that was a leading factor in the formation of adherence to therapy and an increase in patient confidence, which is fully consistent with the high VAS scores in group A, even in patients with severe clinical forms of acne.

In the group treated with nanoliposomal tretinoin, a significantly lower frequency and intensity of local adverse reactions were recorded compared to the traditional formulation. Already at 2 weeks of treatment, the incidence of erythema, burning, dryness and peeling was 12–20% lower in group A, and at the final stage of therapy, side effects were recorded in less than 12% of patients compared to 25–30% in the control group. The mean value of peeling severity in group B was twice as high as in group A (1.6 ± 0.7 vs 0.8 ± 0.5), which was accompanied by a higher frequency of early discontinuation of therapy (10% vs 3.3%). A similar reduction in the irritant potential of nanostructured retinoids was demonstrated by A. Otlewska *et al.* [28], who reported that encapsulation of the active substance in liposomes reduced contact toxicity and provided a gradual release without peak concentrations on the skin surface. A systematic review by J. Kapała *et al.* [29] noted that a reduction in the incidence of side effects to $< 15\%$ was associated with a better adherence profile, which was consistent with fewer treatment discontinuations in group A. The positive effect of lipid carriers on reducing irritation was also confirmed by the study by M. Pawłowska *et al.* [30], which showed that liposomal systems with retinol and peptides mitigated transepidermal stress and maintained physiological pH, which increased tolerability even with prolonged use. In addition, İ. Eroğlu *et al.* [31] showed that the combination of liposomes with moisturising components further neutralised cumulative dryness, which potentially explains the lower severity of this symptom in group A in the present study.

The efficacy of the nanoliposomal form of tretinoin observed in the study could be due not only to the physicochemical advantages of the delivery platform but also to its effect on key pathogenic mechanisms of acne. As noted by C. Mias *et al.* [32], chronic inflammation in acne lesions was mediated by the activation of Th17 lymphocytes, with intensive involvement of the IL-17-dependent pathway. Controlled delivery of tretinoin in the follicular zone could modulate this response by reducing the local expression of proinflammatory genes. In addition, the study by I. Cavallo *et al.* [33] demonstrated that *Cutibacterium acnes* biofilms, characteristic of recurrent acne, increased microbial resistance and reduced the effectiveness of standard topical agents. Liposomal carriers, due to their ability to penetrate the extracellular matrix of biofilms, provided targeted drug delivery and increased antimicrobial activity. According to M. Deng *et al.* [34], disruptions in intercellular interaction in the inflammatory microenvironment were important, due to overexpression of inflammatory regulatory genes in keratinocytes. Nanostructured platforms could potentially compensate for these mechanisms by prolonging the release of the active substance and avoiding local concentration peaks. A. Sedighidarijani *et al.* [35] additionally confirmed that lipid carriers with retinoids demonstrated efficacy in experimental acne modelling both *in vitro* and *in vivo*, which confirmed the universality of the mechanisms underlying the clinical results.

The results of the study confirmed the clinical feasibility of using the nanoliposomal form of tretinoin in patients with moderate acne. Convincing evidence of its advantages over the traditional formulation was obtained both in terms of objective efficacy (reduction of GAGS scores, reduction of inflammatory and non-inflammatory elements) and subjective assessment of patients. Reduced frequency and intensity of local adverse reactions resulted in better tolerance and higher adherence to treatment. Given the stable dynamics of the therapeutic response, especially in subgroups with more severe disease, nanoliposomal tretinoin can be considered an effective and safe alternative for long-term topical acne therapy with a high potential for widespread clinical use.

✦ CONCLUSIONS

During 12 weeks of treatment, both forms of tretinoin demonstrated a reduction in the clinical severity of acne, but the nanoliposomal form provided a significantly more pronounced effect. A 58.8% reduction in GAGS scores in group A was accompanied by less variability in response and a higher frequency of mild acne, especially in the subgroup of patients with more severe initial courses. This indicates the increased therapeutic activity of the nanostructured form and its ability to provide a clinical response even in difficult clinical cases. Both forms of tretinoin helped to reduce the number of inflammatory and non-inflammatory lesions, but in patients of group A, the reduction was significantly higher. This was true for both inflammatory elements (64% vs 42%) and non-inflammatory elements (53.7% vs 34.6%), indicating the universal efficacy of the nanoliposomal formulation. The frequency of achieving a reduction of $\geq 50\%$ was higher in group A, and the complete disappearance of inflammatory elements was recorded almost twice as often. Such dynamics indicate the simultaneous effect of the nanoparticle form on the key pathogenetic links of comedogenesis and inflammation.

The VAS score confirmed high subjective satisfaction with nanoliposomal tretinoin treatment. In the dynamics of therapy, this formulation was consistently associated

with higher scores (8.1 ± 1 vs 6.3 ± 1.4 at week 12) and with a higher proportion of patients who perceived the results positively (78.3% with VAS ≥ 7 in group A). This was especially noticeable in patients with severe disease, where satisfaction scores were 30% higher. This indicates a close relationship between clinical improvement, tolerability and positive patient assessment of therapy when using the nanoformulation. The safety profile of the nanoliposomal formulation of tretinoin was significantly better than that of the traditional formulation. A reduction in the incidence of side effects such as erythema, flaking, burning and dryness was evident already at week 2 of treatment. By the end of the course of therapy, adverse reactions in group A were observed in less than 12% of patients, which is almost twice as low as in group B. The average severity of peeling was two times lower, and the frequency of treatment discontinuation due to intolerance was three times lower. Such indicators indicate better dermatological tolerance and potentially higher adherence to long-term therapy when using the nanoformulation. The main limitation of the study is the limited period of observation (12 weeks), which excludes conclusions on the duration of clinical remission, the frequency of relapse and the long-term safety of the nanoliposomal form of tretinoin in chronic acne. Further studies should include long-term prospective follow-up to assess the durability of the therapeutic effect, the inclusion of inflammatory and microbiota biomarkers to better determine the mechanisms of action, and the expansion of the sample by age and acne phenotypes to increase the extrapolation value of the results.

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✦ CONFLICT OF INTEREST

None.

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Оцінка ефективності наноліпосомальних форм ретиноїдів у лікуванні акне

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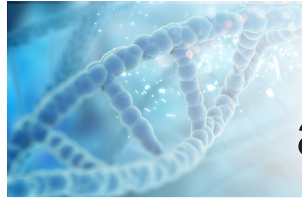
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Анотація. Метою дослідження було визначення клінічної ефективності та профілю переносимості наноліпосомальної форми третиноїну у пацієнтів з акне середнього ступеня. Дослідження проводилося як рандомізоване контрольоване дослідження за участю 120 пацієнтів з акне середнього ступеня на кафедрі дерматовенерології, алергології, клінічної та лабораторної імунології Національного університету охорони здоров'я України імені П. Л. Шупика; протягом 12 тижнів порівнювали клінічну динаміку, суб'єктивну оцінку ефективності та частоту побічних реакцій з наноліпосомальною та традиційною формами третиноїну. У пацієнтів, які отримували наноліпосомальний третиноїн, спостерігалось зниження тяжкості клінічного акне на 58,8 %, що свідчить про вищу терапевтичну ефективність порівняно з традиційною формою, де зниження становило лише 41,7 %. У цій групі кількість запальних елементів зменшилася на 64 %, а незапальних елементів – на 53,7 %, тоді як у контрольній групі відповідні показники становили 42 % та 34,6 %, що підтверджує здатність наноформи впливати як на комедогенез, так і на запальні процеси. Повного зникнення запальних уражень було досягнуто у 28,3 % пацієнтів основної групи, що більш ніж удвічі перевищувало показники групи традиційного третиноїну. Суб'єктивна оцінка ефективності лікування була значно вищою серед користувачів наноформ (8,1 проти 6,3 балів), що свідчить про кращу задоволеність результатом. Побічні реакції виникали менш ніж у 12 % учасників основної групи, тоді як у групі порівняння їх частота перевищувала 25 %, що свідчить про вищу дерматологічну переносимість наноліпосомального препарату. Отримані результати можуть бути використані дерматологами для обґрунтованого вибору місцевої терапії акне, зокрема при призначенні сучасних форм третиноїну пацієнтам з гіперчутливістю шкіри або низькою переносимістю до традиційних препаратів

Ключові слова: шкіра; комедони; запалення; третиноїн; лущення; побічна реакція; непереносимість



Correlation of systolic blood pressure and pulse pressure with albuminuria in patients of hypertension without diabetes

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Abstract. Hypertension and microalbuminuria (MAU) are independent yet interconnected markers of cardiovascular and renal dysfunction. While MAU is an early indicator of renal impairment, its relationship with blood pressure (BP) components, particularly systolic blood pressure (SBP) and pulse pressure (PP), remains underexplored in non-diabetic hypertensive populations. The purpose of this study was to investigate the independent association between these BP components and MAU, identify which parameter exhibits a stronger correlation, and enhance the understanding of early renal dysfunction in hypertension management. For this analytical cross-sectional study was conducted at Hind Institute of Medical Sciences, Safedabad, Barabanki, Uttar Pradesh, over 12 months, involving 120 hypertensive patients attending outpatient and inpatient departments. Patients with comorbidities like diabetes mellitus, renal failure, or overt proteinuria were excluded. BP was measured in two separate readings using a standard sphygmomanometer, and microalbuminuria was assessed via immunoturbidometric assay. The urine albumin-to-creatinine ratio (ACR) served as the primary indicator of MAU. SBP and PP demonstrated significant positive correlations with MAU ($\rho = 0.25$, $p = 0.032$; $\rho = 0.30$, $p = 0.015$, respectively), while diastolic BP (DBP) showed a negative yet non-significant association ($\rho = -0.20$, $p = 0.065$). Among anthropometric parameters, body mass index (BMI) and waist-hip ratio exhibited no significant differences between groups. Urine ACR was markedly higher in the MAU group (182.5 ± 156.5 mg/L) compared to the non-MAU group (17.6 ± 7.1 mg/L; $p < 0.0001$). Lipid profiles, fasting blood glucose, and renal function markers like serum creatinine and blood urea nitrogen were comparable between groups, highlighting BP components as primary predictors of albuminuria. The strong positive correlations between SBP, PP, and MAU highlighted the need for precise BP management in non-diabetic hypertensive patients. Regular monitoring and treatment to optimise SBP and PP levels could mitigate renal damage and reduce cardiovascular risks

Keywords: renal dysfunction; cardiovascular disease; urine albumin-to-creatinine ratio; nephropathy

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INTRODUCTION

Hypertension is a major public health concern and a leading cause of cardiovascular and renal complications. It is estimated to affect over 1.28 billion adults worldwide and remains a key risk factor for end-organ damage, particularly involving the heart, brain, and kidneys [1]. The relationship between blood pressure components and early markers of kidney dysfunction, such as microalbuminuria (MAU), has gained significant attention. Identifying hypertensive patients with early renal function changes is critical for preventing the progression of chronic kidney disease (CKD). Systolic blood pressure (SBP) and pulse pressure (PP) have been suggested as independent predictors of renal damage, yet their correlation with albuminuria remains underexplored in non-diabetic hypertensive patients.

W. Zhang *et al.* [2] reported that intensive control of both blood glucose and SBP (<130/80 mmHg) significantly reduced the prevalence of albuminuria in patients with coexisting diabetes and hypertension, suggesting the role of SBP in renal protection. Similarly, S. Coşkun *et al.* [3] found that in patients with isolated systolic and diastolic hypertension, SBP and left atrial diameter were independent predictors of microalbuminuria, although PP was not significant in multivariate analysis. Conversely, L. Liu *et al.* [4] conducted a large community-based study in pre-diabetic individuals and demonstrated a significant positive association between PP and albuminuria. The highest PP quartile showed a more than twofold increase in the odds of albuminuria compared to the lowest quartile, independent of antihypertensive treatment and other covariates. In another study, J. Sołtysiak *et al.* [5] found that nocturnal diastolic BP, diastolic load, and non-dipping BP status were significantly associated with increased urinary albumin excretion in adolescents with type 1 diabetes, pointing to the importance of circadian BP rhythm and vascular stiffness.

D. Suzuki *et al.* [6], in their analysis of over 4,000 participants in the J-HOP study, found a significant association between average home SBP and urinary albumin-to-creatinine ratio (UACR), regardless of diabetes status. However, day-to-day BP variability metrics were inconsistently associated with albuminuria, with only average real variability (ARV) of morning SBP showing a significant correlation in diabetic patients. B.A. Durak *et al.* [7] further supported this link in a study utilising ambulatory BP monitoring (ABPM). They observed that non-dipper and reverse-dipper BP profiles, along with elevated 24-hour and nocturnal SBP and DBP, were strongly associated with proteinuria. This suggests that altered BP patterns and nocturnal hypertension may accelerate renal microvascular injury in hypertensive individuals. Moreover, in the context of secondary and resistant hypertension, G.P. Rossi *et al.* [8] and G. Lamirault *et al.* [9] emphasised the need for early identification and targeted treatment of high-risk hypertensive profiles to prevent renal sequelae such as albuminuria. These reviews highlighted that resistant and secondary hypertension were often underdiagnosed yet accounted for a significant portion of patients with progressive renal damage.

Despite these findings, several gaps remain in the literature. While the association between diabetes and albuminuria has been extensively studied, the specific correlation between different blood pressure components and

MAU in non-diabetic hypertensive patients has not been thoroughly examined. Moreover, existing studies often focus on broad hypertensive populations without distinguishing the independent effects of SBP and PP. The relative contribution of these parameters to renal dysfunction remains unclear, necessitating further investigation. The objective of the present study was to assess the independent associations between microalbuminuria and blood pressure components, specifically SBP and PP, after adjusting for potential confounders.

MATERIALS AND METHODS

The present study was conducted at the Department of Medicine, Hind Institute of Medical Sciences, Safedabad, Barabanki, Uttar Pradesh, over a period of 12 months, from May, 2023 to May, 2024. It was designed as an analytical cross-sectional study to evaluate the correlation between SBP, PP, and albuminuria in hypertensive patients. The study included patients attending both the outpatient and inpatient departments. Ethical clearance was obtained from the Institutional Ethics Committee (HIMS/IHEC/MD/MS/0011/2023), and written informed consent was obtained from all participants. The study adhered to the ethical principles outlined in the Declaration of Helsinki [10].

A total of 120 patients diagnosed with hypertension or undergoing treatment for hypertension, defined as having SBP >140 mmHg and DBP >90 mmHg, were enrolled in the study. To maintain the specificity of the study, patients with confounding conditions such as overt proteinuria, congestive cardiac failure, renal failure, diabetes mellitus, urinary tract infections, obstructive uropathy, nephrolithiasis, or pregnancy were excluded. The total study population consisted of 120 hypertensive patients, with 57 participants in GROUP-NA and 63 in GROUP-MA. The mean age of the total cohort was 66.07 ± 13.25 years, with the majority of patients falling within the 60-69 age group (62 out of 120), followed by 50-59 years (30 patients), and 70-79 years (28 patients). The gender distribution was relatively balanced, with 63 males and 57 females.

Blood pressure measurements were performed using a standard sphygmomanometer. Participants were advised to avoid smoking, tea, and coffee for at least 30 minutes prior to measurement to prevent acute fluctuations. Two BP readings were taken 10 minutes apart in a seated position, and the higher value was recorded for analysis. Body mass index (BMI) was calculated using the standard formula: $\text{weight (kg)}/\text{height (m)}^2$, with both parameters measured using calibrated instruments. A detailed cardiovascular examination was conducted, including palpation of peripheral pulses – specifically carotid and femoral arteries – to assess early signs of atherosclerosis. Fundoscopic examination was performed to evaluate hypertensive retinopathy.

Comprehensive laboratory investigations were conducted to assess renal and metabolic parameters. Routine urine analysis included albumin, sugar, and sediment evaluation. Fasting and postprandial blood glucose levels, serum urea, and creatinine tests were conducted to rule out underlying metabolic or renal dysfunction. Lipid profiles, including total cholesterol, low-density lipoprotein (LDL),

high-density lipoprotein (HDL), and triglycerides, were measured using an enzymatic colorimetric method to examine their potential association with microalbuminuria. Blood urea nitrogen (BUN) was quantified using a kinetic UV assay to evaluate renal function.

Urine albumin levels were determined using an immunoturbidometric assay performed on early morning urine samples. The urine albumin-to-creatinine ratio (UACR) was calculated from spot urine samples, with laboratory analysis completed on the same morning, to ensure consistency and accuracy. All collected data were systematically recorded in Microsoft Excel and analysed using SPSS version 26 (SPSS Inc., Chicago, IL, USA). Continuous variables, such as SBP, PP, BMI, lipid levels, UACR, and BUN, were presented as mean \pm standard deviation (SD). Categorical variables, including gender, smoking status, and hypertensive retinopathy, were expressed as frequency distributions. To compare group means for normally distributed continuous variables, the Student's t-test was utilised, while categorical variables were analysed using the Chi-square test. The relationship between blood pressure components (SBP and PP) and albuminuria was assessed using Spearman's correlation coefficient. Statistical significance was set at a p-value <0.05 , with values <0.001 considered highly significant.

RESULTS AND DISCUSSION

The age distribution between GROUP-NA and GROUP-MA did not show any statistically significant differences, as indicated by the p-values of 0.2739 for the categorical age ranges (50-59, 60-69, 70-79) and 0.1262 for the mean age comparison. While GROUP-NA had a greater proportion of younger participants in the 50-59 age range (25 vs 5), GROUP-MA had a higher representation in the older age brackets, specifically 60-69 years (40 vs 22) and 70-79 years (18 vs 10). The mean age in GROUP-MA was slightly higher (68.2 ± 8.9 years) than in GROUP-NA (65.5 ± 10.3 years). However, this difference was not statistically significant, suggesting that age alone was not a confounding factor in determining differences in clinical outcomes between the two groups. Gender distribution was also comparable between the groups, with GROUP-NA consisting of 28 males and 29 females, while GROUP-MA had 35 males and 28 females. The p-value for gender distribution was 0.7047, further confirming no significant difference in gender proportions between the groups. This suggests that any observed variations in clinical parameters or outcomes are unlikely to be attributed to differences in age or gender, allowing for a more focused assessment of hypertension-related factors and albuminuria levels in both cohorts (Table 1).

Table 1. Demographical profile of the enrolled patients among the groups

Variable	GROUP-NA (n = 57)	GROUP-MA (n = 63)	TOTAL (n = 120)	p-value
Age (years)				
50-59	25	5	30	X = 2.590 p = 0.2739
60-69	22	40	62	
70-79	10	18	28	
Mean \pm SD	65.5 ± 10.3	68.2 ± 8.9	66.07 ± 13.25	t = 1.540 p = 0.1262
Gender				
Male	28	35	63	X = 0.4966 p = 0.7047
Female	29	28	57	

Source: compiled by the authors

Blood pressure parameters showed that systolic blood pressure (SBP) was significantly higher in GROUP-MA (149.6 ± 18.4 mmHg) compared to GROUP-NA (142.9 ± 17.2 mmHg), with a t-value of 2.528 and a p-value of 0.0057. This indicates that individuals in GROUP-MA had a greater elevation in SBP, which is a well-established risk factor for cardiovascular events and renal impairment. The higher SBP in GROUP-MA suggests a potential association with the presence of microalbuminuria, as increased systolic pressure has been linked to endothelial dysfunction and glomerular damage, which contribute to albuminuria. Diastolic blood pressure (DBP) was slightly higher in GROUP-MA (86.3 ± 11.7 mmHg) compared to GROUP-NA (85.5 ± 10.1 mmHg), but this difference was not statistically significant ($t = 0.3989$, $p = 0.6907$). The lack of a significant difference in DBP suggests that elevated DBP alone may not be a strong predictor of microalbuminuria in this study population. However, the combination of high SBP and widened pulse pressure (PP)

is likely to contribute more to vascular damage and renal complications than DBP alone.

Pulse pressure (PP), which represents the difference between SBP and DBP, was also significantly higher in GROUP-MA (62.3 ± 16.2 mmHg) compared to GROUP-NA (56.1 ± 14.6 mmHg), with a t-value of 1.684 and a p-value of 0.0154. This finding suggests that widened PP, often indicative of arterial stiffness and reduced vascular compliance, may play a critical role in the pathogenesis of microalbuminuria. Elevated PP has been associated with increased cardiovascular morbidity and mortality, and renal impairment due to increased glomerular pressure fluctuations. Overall, these findings highlight that GROUP-MA, which had significantly higher SBP and PP, may be at an elevated risk for microvascular and renal complications compared to GROUP-NA. This underscores the importance of early detection and management of elevated SBP and PP in hypertensive patients to prevent the progression of albuminuria and its associated complications (Fig. 1).

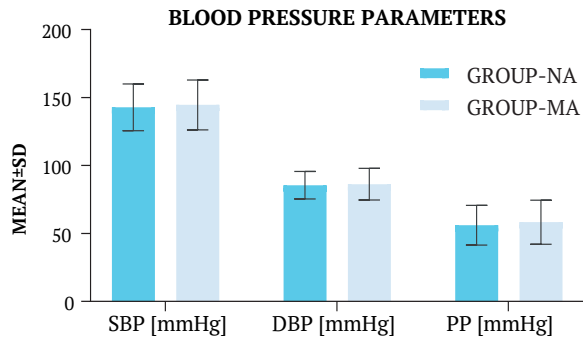


Figure 1. Graphical representation of blood pressure parameters

Source: compiled by the authors

Anthropometric measurements showed no significant differences, with the BMI averaging 25.1 ± 3.7 kg/m² for GROUP-NA and 25.4 ± 4 kg/m² for GROUP-MA ($p = 0.6715$). The waist-hip (W-H) ratio was identical for both groups at 0.92 ± 0.07 for GROUP-NA and 0.92 ± 0.06 for GROUP-MA ($p > 0.9999$). Additionally, the mean duration until hypertension diagnosis was slightly longer in GROUP-MA (9.65 ± 3.88 years) compared to GROUP-NA (8.63 ± 2.71 years), showing non-significant differences ($t = 1.653$, $p = 0.1010$). The use of antihypertensive medications was comparable between groups, with 36 participants in GROUP-NA and 41 in GROUP-MA using Amlodipine (5 mg), 42 in GROUP-NA and 56 in GROUP-MA using Losartan (50 mg), and 19 in GROUP-NA and 14 in GROUP-MA using Hydrochlorothiazide (25 mg). The Chi-square test for

medication distribution yielded a non-significant difference ($p = 0.3414$)

A number of lifestyle characteristics, including smoking, alcohol intake, and levels of physical exercise, were likewise comparable amongst the groups. Smoking was reported by 18 participants in GROUP-NA and 21 in GROUP-MA, while 39 and 42 participants in GROUP-NA and GROUP-MA, respectively, were non-smokers ($p = 0.2049$). Alcohol consumption was reported by 13 participants in GROUP-NA and 17 in GROUP-MA, while 44 and 46 participants, respectively, did not consume alcohol ($p = 0.5977$). Regular physical activity was noted in 28 participants in GROUP-NA and 36 in GROUP-MA, while 29 and 37 participants in GROUP-NA and GROUP-MA, respectively, were sedentary ($p = 0.9826$).

Fasting blood glucose (FBG) levels were not significantly different between GROUP-NA (5.2 ± 1.1 mmol/L) and GROUP-MA (5.6 ± 1.8 mmol/L, $p = 0.1497$). Urine ACR was significantly higher in GROUP-MA (182.5 ± 156.5 mg/L) compared to GROUP-NA (17.6 ± 7.1 mg/L, $p < 0.0001$). Total protein levels were similar between GROUP-NA (71.52 ± 5.85 mg/L) and GROUP-MA (72.09 ± 5.67 mg/L, $p = 0.5890$), as were albumin levels (45.51 ± 3.76 mg/L vs 45.37 ± 3.78 mg/L, $p = 0.8394$). Creatinine levels were 76 ± 24.8 μ mol/L for GROUP-NA and 79.9 ± 40.7 μ mol/L for GROUP-MA ($p = 0.5327$), and blood urea nitrogen (BUN) levels were 5.31 ± 1.69 mmol/L for GROUP-NA and 5.51 ± 2.07 mmol/L for GROUP-MA ($p = 0.5657$), with no significant differences (Table 2). Lipid profile markers such TC, TG, LDL-C, and HDL-C were likewise similar between groups (all p -values > 0.05) (Fig. 2).

Table 2. Comparison of the metabolic parameters in enrolled patients among the groups

METABOLIC PARAMETERS	GROUP-NA (n = 57)	GROUP-MA (n = 63)	TOTAL (n = 120)	p-value
FBG [mmol/L]	5.2 ± 1.1	5.6 ± 1.8	5.4 ± 1.5	$t = 1.450$ $p = 0.1497$
Urine ACR [mg/gm]	17.6 ± 7.1	182.5 ± 156.5	99.7 ± 135.7	$t = 7.944$ $p < 0.0001$
Total protein [mg/L]	71.52 ± 5.85	72.09 ± 5.67	71.8 ± 5.77	$t = 0.5417$ $p = 5890$
Albumin [mg/L]	45.51 ± 3.76	45.37 ± 3.78	45.44 ± 3.77	$t = 0.2031$ $p = 0.8394$
Cr [μ mol/L]	76 ± 24.8	79.9 ± 40.7	77.5 ± 33.1	$t = 0.6258$ $p = 0.5327$
BUN [mmol/L]	5.31 ± 1.69	5.51 ± 2.07	5.41 ± 1.89	$t = 0.5761$ $p = 0.5657$

Source: compiled by the authors

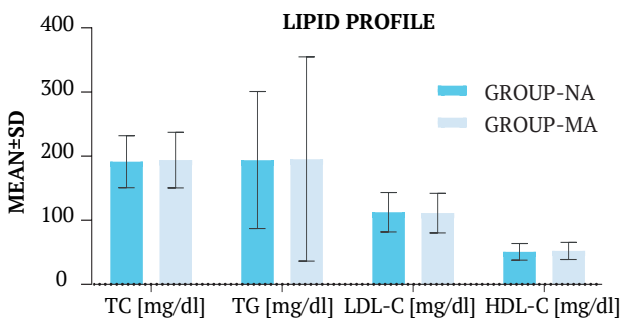


Figure 2. Graphical representation of lipid profile in enrolled patients

Source: compiled by the authors

Correlational analysis revealed that SBP had a significant positive correlation with albuminuria ($\rho = 0.25$), as did PP ($\rho = 0.30$), indicating that increased systolic pressure and widened pulse pressure are associated with greater urinary albumin excretion. This reinforces the idea that SBP and PP contribute to endothelial dysfunction and heightened glomerular permeability, which can lead to renal damage in hypertensive patients. Conversely, DBP exhibited a negative but non-significant correlation ($\rho = -0.20$), suggesting that DBP alone may not have a substantial direct impact on albuminuria. In addition, total protein ($\rho = 0.35$, $p = 0.009$) and albumin ($\rho = 0.28$) showed significant positive correlations with albuminuria, indicating that variations in plasma protein levels could influence albumin excretion

in hypertensive patients. This aligns with the hypothesis that systemic inflammatory responses and alterations in endothelial function may be linked to renal impairment.

Other metabolic parameters, including uric acid ($\rho = -0.12$) and fasting blood glucose (FBG) ($\rho = 0.08$), did not exhibit significant correlations with albuminuria, suggesting that these factors may not independently contribute to increased urinary albumin excretion in the studied cohort. Similarly, creatinine ($\rho = -0.05$) and blood urea nitrogen (BUN) ($\rho = 0.20$) showed non-significant correlations,

indicating that kidney function, as measured by these markers, was not directly associated with the degree of albuminuria in this population. Ultimately, the most significant associations were noted for SBP, PP, total protein, and albumin, highlighting the importance of these parameters in predicting early renal impairment in hypertensive patients. These findings support the need for targeted blood pressure management strategies focusing on reducing SBP and PP to mitigate the risk of albuminuria and subsequent renal complications (Table 3).

Table 3. Correlation analysis of the enrolled patients' parameters with the albuminuria

Parameters vs Albuminuria correlation analysis	Spearman's Rho	p-value
SBP vs Albuminuria	0.25	0.032
DBP vs Albuminuria	-0.20	0.065
PP vs Albuminuria	0.30	0.015
Uric acid vs Albuminuria	-0.12	0.252
FBG vs Albuminuria	0.08	0.421
Total protein vs Albuminuria	0.05	0.529
Albumin vs Albuminuria	0.28	0.026*
Cr vs Albuminuria	-0.05	0.654
BUN vs Albuminuria	0.20	0.071

Source: compiled by the authors

CVD risk is increased by arterial hypertension, which is characterised by high systolic and/or diastolic blood pressure. A drop in blood pressure reduced cardiovascular events. MAU is a sensitive and specific diagnostic for early renal impairment. MAU is strongly linked to diabetes or metabolic syndrome. There is insufficient evidence linking MAU and blood pressure components. After controlling for possible variables, the present study investigated whether pulse pressure (PP) and SBP/DBP were independently linked with MAU. While analysing the correlation between SBP and microalbuminuria, the present study found that SBP has a positive correlation with albuminuria ($\rho = 0.25$, $p = 0.032$), indicating a significant relationship. DBP shows a negative but non-significant correlation ($\rho = -0.20$, $p = 0.065$). Pulse pressure (PP) is positively correlated with albuminuria ($\rho = 0.30$, $p = 0.015$), indicating significance. Uric acid has a negative and non-significant correlation ($\rho = -0.12$, $p = 0.252$). FBG also shows a non-significant correlation ($\rho = 0.08$, $p = 0.421$). Total albumin has a significant positive correlation with albuminuria ($\rho = 0.28$, $p = 0.026$). Creatinine (Cr) and blood urea nitrogen (BUN) have non-significant correlations with albuminuria ($\rho = -0.05$, $p = 0.654$ and $\rho = 0.20$, $p = 0.071$, respectively). Significant correlations are noted with SBP, PP, total protein, and albumin. J. Song *et al.* [11] found inconsistent findings on fasting blood glucose (FBG) and albuminuria. S. Yimthiang *et al.* [12] found a 5-fold increase in albuminuria risk as blood glucose levels approached 180 mg/dL. P.K. Chandie Shaw *et al.* [13] found no significant correlation between FBG and albuminuria in South Asian type 2 diabetics. B. Lei *et al.* [14] found no indication that lowering blood glucose levels could prevent albuminuria. There is also controversy about whether rigorous glycaemic therapy improves albuminuria. In another trial, S.G. Coca *et al.* [15] found that intense blood glucose management reduced microalbuminuria and macroalbuminuria. Intensive glycaemic management did not significantly slow clinical albuminuria progression.

Conventional and aggressive glycaemic therapy had no statistically significant longitudinal effect on clinical albuminuria progression.

According to K.M. Ahmed Aziz [16], spot urine protein excretion is 67.5 mg/dL at 150 mmHg SBP. If SBP is 110 mmHg, spot urine protein is 12 mg/dL. A MAP of 110 yields 63 mg/dL spot urine protein. If MAP is 120, spot urine protein excretion is 81.6 mg/dL. According to the data, pulse pressure can estimate spot urine protein. Thus, spot urine protein is 20 mg/dL if PP is 40, which is normal. Spot urine protein excretion is 82 mg/dL at PP 80. The present study's mathematical calculations and connections between blood pressure and renal protein excretion are noteworthy. These mathematical formulae estimate kidney protein excretion under specified blood pressure values to monitor proteinuria and diabetic kidney disease. Long-term HTN patients had a greater rate of microalbuminuria than recently diagnosed patients, according to K.R. Bhusal *et al.* [17]. Patients without controlled blood pressure showed a greater rate of microalbuminuria.

In the current study, fasting blood glucose (FBG) levels were comparable between GROUP-NA (5.2 ± 1.1 mmol/L) and GROUP-MA (5.6 ± 1.8 mmol/L), with a p-value of 0.1497, indicating no significant difference. Urine ACR, however, was significantly elevated in GROUP-MA (182.5 ± 156.5 mg/L) compared to GROUP-NA (17.6 ± 7.1 mg/L), with a p-value of <0.0001 . Total protein (71.52 ± 5.85 mg/L vs 72.09 ± 5.67 mg/L, $p = 0.5890$), albumin (45.51 ± 3.76 mg/L vs 45.37 ± 3.78 mg/L, $p = 0.8394$), creatinine (76 ± 24.8 μ mol/L vs 79.9 ± 40.7 μ mol/L, $p = 0.5327$), and blood urea nitrogen (5.31 ± 1.69 mmol/L vs 5.51 ± 2.07 mmol/L, $p = 0.5657$) levels showed statistically non-significant differences.

While previous studies, such as by K. Thieme & M. Oliveira-Souza [18], found that SBP and PP were independently associated with MAU after adjusting for confounders, the current study did not identify such a relationship. An increase in SBP typically elevates glomerular pressure and

filtration rate (GFR), potentially causing proteinuria due to trans-membrane protein leakage, as seen in animal models with angiotensin II infusion. E. Lee *et al.* [19] reported higher GFR in MAU patients compared to normoalbuminuric individuals. Unlike earlier research, this study focused on newly diagnosed, treatment-naïve hypertensive patients, avoiding medication-related confounding factors. Findings suggest that PP is a meaningful biomarker for assessing the renal impact of elevated blood pressure, with SBP and PP showing comparable sensitivity and specificity for predicting MAU based on ROC analysis.

In this study, SBP demonstrated a significant positive correlation with albuminuria ($\rho = 0.25$), while diastolic blood pressure (DBP) had a negative but non-significant correlation ($\rho = -0.20$). Pulse pressure (PP) showed a significant positive correlation ($\rho = 0.30$). Uric acid and fasting blood glucose (FBG) exhibited non-significant correlations with albuminuria ($\rho = -0.12$, and $\rho = 0.08$ respectively). Significant positive correlations were observed for total protein ($\rho = 0.35$, $p = 0.009$) and albumin ($\rho = 0.28$). Creatinine (Cr) and blood urea nitrogen (BUN) showed non-significant correlations ($\rho = -0.05$, and $\rho = 0.20$, respectively). Overall, significant associations were found between albuminuria and SBP, PP, total protein, and albumin.

According to N. Xie *et al.* [20], patients with microalbuminuria (MAU) exhibited greater SBP and PP than those with normo-albuminuria ($p < 0.05$), whereas DBP was identical. Significantly higher serum levels of FBG, total protein, Cr, BUN, and HDL-C were seen in the MAU group ($p < 0.05$). Urine ACR was significantly greater in the MAU group (182.5 ± 156.5 mg vs 17.6 ± 7.1 mg, $p < 0.001$). In this study, systolic blood pressure (SBP) averages were similar between GROUP-NA (142.9 ± 17.2 mmHg) and GROUP-MA (144.6 ± 18.4 mmHg), with no significant difference ($p = 0.6032$). Diastolic blood pressure (DBP) was also comparable, with GROUP-NA at 85.5 ± 10.1 mmHg and GROUP-MA at 86.3 ± 11.7 mmHg ($p = 0.6907$). Pulse pressure (PP) showed no significant difference either, with GROUP-NA at 56.1 ± 14.6 mmHg and GROUP-MA at 58.3 ± 16.2 mmHg ($p = 0.4379$). Therefore, blood pressure indicators were not significantly different across groups.

B.J. Kim *et al.* [21] found that even in prehypertension, SBP of 120-129 mmHg or DBP of 80-84 mmHg posed a lower risk than higher thresholds. Y.H. Jung *et al.* [22] also noted a higher incidence of albuminuria in patients with SBP below 150 mmHg compared to those above, suggesting that elevated SBP affects albuminuria, particularly in patients not on medication. The researchers concluded that less stringent controls (SBP < 150 mmHg, DBP < 90 mmHg) are appropriate for those over 60 years old, balancing the risk of cardiovascular disease and albuminuria. Conversely, S. Bangalore *et al.* [23] highlighted the benefits of stricter blood pressure control, noting fewer cardiovascular events and strokes in adults with SBP < 140 mmHg compared to those with SBP < 150 mmHg. In adults without diabetes, stricter control (SBP < 120 mmHg) reduced total mortality and vascular disease compared to less strict targets (SBP < 140 mmHg).

The discrepancy between the two studies can be interpreted in light of their differing patient populations. The present study evaluated hypertensive individuals without confirmed ischaemic heart disease, in whom the influence

of smoking and other lifestyle factors on renal microvascular injury may be less pronounced or more multifactorial. In contrast, A. Basu & J.S. Jhala [24] highlighted the exacerbating effect of smoking on endothelial dysfunction in patients with established cardiovascular disease, which may amplify the risk and severity of microalbuminuria. Therefore, while lifestyle factors did not significantly influence microalbuminuria status in the present hypertensive cohort, they appear to play a more critical role in populations with advanced atherosclerotic disease. A. Mimarm [25] made similar observations. However, the present study did not show any significant difference.

According to A. Basu & J.S. Jhala [24], BMI was categorised as > 25 or < 25 , with 26 cases having BMI > 25 . Among these, microalbuminuria was observed in 21 cases (80.76%), while 5 (19.24%) did not have it. This aligns with the Gubbio population study by M. Cirillo *et al.* [26], which also reported an association between high BMI and microalbuminuria. The study further noted that among controls with BMI > 25 , 5 out of 10 (50%) had microalbuminuria, showing a significant association. In the study group of 36 individuals with BMI > 25 , 26 (72.22%) were cases, and 10 (27.77%) were controls. Microalbuminuria was observed in 26 participants in this group, with 21 being cases and 5 controls, suggesting a higher prevalence of microalbuminuria among cases with BMI > 25 . However, non-significant differences, indicating that microalbuminuria could be an independent risk factor for ischaemic heart disease (IHD). The chance of microalbuminuria positivity was higher among cases (26.58%) compared to controls (0.65%) despite similar BMI values, emphasising the potential role of microalbuminuria in IHD risk. In contrast, the current study found no significant difference in BMI between GROUP-NA (25.1 ± 3.7 kg/m²) and GROUP-MA (25.4 ± 4 kg/m², $p = 0.6715$). Similarly, the waist-hip (W-H) ratio was identical between the groups (0.92 ± 0.07 for GROUP-NA and 0.92 ± 0.06 for GROUP-MA, $p > 0.9999$). These findings indicate that anthropometric measurements (BMI and W-H ratio) showed non-significant differences.

In this study, lipid profile parameters show non-significant differences between GROUP-NA and GROUP-MA. Total cholesterol (TC) levels were 193 ± 40.6 mg/dL for GROUP-NA and 195.5 ± 43.3 mg/dL for GROUP-MA ($p = 0.7455$). Triglycerides (TG) levels were 195.1 ± 607.8 mg/dL for GROUP-NA and 196.8 ± 160.7 mg/dL for GROUP-MA ($p = 0.9464$). LDL-C levels were 113.2 ± 31 mg/dL for GROUP-NA and 111.9 ± 31.2 mg/dL for GROUP-MA ($p = 0.8193$). High-density lipoprotein cholesterol (HDL-C) levels were 51.1 ± 13.1 mg/dL for GROUP-NA and 52.5 ± 13.5 mg/dL for GROUP-MA ($p = 0.5662$). These findings indicate non-significant differences in lipid profiles between the two groups. J.S. Jensen *et al.* [27] also reported a significant association between lipid abnormalities and microalbuminuria, with cases showing higher TC levels (188.54 ± 29.25 mg/dL) compared to controls (174 ± 17.90 mg/dL). However, no significant variations in TC, TG, LDL-C, or HDL-C levels were found between the two groups in this investigation, demonstrating diversity between populations.

This study highlighted the significant correlation between systolic blood pressure (SBP), pulse pressure (PP), and albuminuria in hypertensive patients without diabetes, suggesting that elevated SBP and widened PP were key contributors to early renal impairment. The findings

emphasised that SBP and PP, rather than diastolic blood pressure (DBP), are stronger predictors of microalbuminuria, likely due to their role in endothelial dysfunction and glomerular pressure fluctuations. Additionally, the significant correlation between albuminuria and total protein levels suggests potential systemic inflammatory or vascular mechanisms contributing to renal damage. These results underscore the importance of early and aggressive blood pressure management, particularly targeting SBP and PP, to mitigate the risk of albuminuria and subsequent renal complications.

◆ CONCLUSIONS

The findings of this study reinforce the crucial role of blood pressure regulation in preventing renal and cardiovascular complications in hypertensive patients without diabetes. The significant positive correlation between systolic blood pressure (SBP), pulse pressure (PP), and albuminuria suggests that elevated SBP and widened PP contribute to microvascular damage and increased glomerular permeability, leading to early renal impairment. Clinically, this underscores the necessity of stringent blood pressure control strategies, including lifestyle modifications,

regular monitoring, and the use of appropriate antihypertensive therapies to maintain optimal SBP and PP levels. Effective blood pressure management not only reduces the risk of albuminuria and slows the progression of chronic kidney disease but also mitigates the risk of cardiovascular events such as stroke and heart failure, given the well-established link between hypertension and vascular dysfunction. Future research should aim to validate and expand upon the observed statistically significant correlations between albuminuria and systolic blood pressure ($\rho = 0.25$, $p = 0.032$), pulse pressure ($\rho = 0.30$, $p = 0.015$), and serum albumin ($\rho = 0.28$, $p = 0.026$), to better elucidate their predictive value for early renal impairment in hypertensive patients without diabetes.

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◆ CONFLICT OF INTEREST

None.

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Кореляція систолічного артеріального та пульсового тиску з альбумінурією у пацієнтів з артеріальною гіпертензією без діабету

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Анотація. Артеріальна гіпертензія та мікроальбумінурія (МАУ) є незалежними, але взаємопов'язаними маркерами серцево-судинної та ниркової дисфункції. Хоча МАУ є раннім індикатором ураження нирок, її зв'язок з компонентами артеріального тиску (АТ), зокрема систолічним артеріальним тиском (САТ) та пульсовим тиском (ПТ), залишається недостатньо вивченим у популяції гіпертензивних пацієнтів без діабету. Метою даного дослідження було встановити незалежний зв'язок між цими компонентами АТ і МАУ, визначити, який з параметрів має сильнішу кореляцію, та поглибити розуміння раннього ниркового ураження в контексті ведення пацієнтів з артеріальною гіпертензією. Це аналітичне поперечне дослідження проводилося в Хіндському інституті медичних наук, Сафедабад, Барабанкі, Уттар-Прадеш, протягом 12 місяців і включало 120 пацієнтів з гіпертензією, які звернулися до амбулаторного або стаціонарного відділень. Пацієнтів із супутніми захворюваннями, такими як цукровий діабет, ниркова недостатність або явна протеїнурія, було виключено. Артеріальний тиск вимірювався двічі за допомогою стандартного сфігмоманометра, а мікроальбумінурія визначалась імунотурбідиметричним методом. Основним показником МАУ був співвідношення альбумін/креатинін у сечі (ACR). САТ та ПТ продемонстрували значущу позитивну кореляцію з МАУ ($\rho = 0,25$, $p = 0,032$; $\rho = 0,30$, $p = 0,015$ відповідно), тоді як діастолічний тиск (ДАТ) мав негативний, але незначущий зв'язок ($\rho = -0,20$, $p = 0,065$). Серед антропометричних параметрів індекс маси тіла (ІМТ) та співвідношення талія/стегна не виявили значущих відмінностей між групами. Середній АCR був суттєво вищим у групі з МАУ ($182,5 \pm 156,5$ мг/л), ніж у групі без МАУ ($17,6 \pm 7,1$ мг/л; $p < 0,0001$). Показники ліпідного профілю, глюкози натще та функції нирок (сироватковий креатинін, сечовина) були подібними в обох групах, що підкреслило важливість компонентів АТ як основних предикторів альбумінурії. Сильна позитивна кореляція між САТ, ПТ і МАУ підкреслила необхідність точного контролю АТ у гіпертензивних пацієнтів без діабету. Регулярний моніторинг і терапія, спрямована на оптимізацію рівнів САТ і ПТ, можуть допомогти зменшити ниркові ушкодження та знизити серцево-судинні ризики

Ключові слова: ниркова дисфункція; серцево-судинні захворювання; співвідношення альбумін/креатинін у сечі; нефропатія

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