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Conflict of interest. The authors have no conflicts of interest to declare.

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Article received: 12.01.2025.

DOI 10.26724/2079-8334-2026-1-95-28-32
UDC 618.11-008.64; 616.728.3-007.17-073.7:618.173

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RESULTS OF A DENSITOMETRIC STUDY IN WOMEN WITH EARLY MENOPAUSE AND PREMATURE OVARIAN FAILURE

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The study included 50 women divided into two groups: early menopause (n=19) and premature ovarian insufficiency (n=31). A control group consisted of 20 women with preserved menstrual function. Serum estradiol, vitamin D, and ionized calcium were measured, and quantitative ultrasound densitometry was performed (SOS, BUA, BQI, T- and Z-scores). Correlation analysis was used to assess relationships between hormonal, metabolic, and densitometric parameters. Women with premature ovarian insufficiency demonstrated more pronounced bone mineral density reduction compared to those with early menopause. Estradiol levels showed a weak negative correlation with densitometric indices, whereas ionized calcium was positively correlated with BQI and T-score. Hypoestrogenism is a key factor in the reduction of bone mineral density in women with early menopause and premature ovarian insufficiency. A comprehensive assessment of hormonal and metabolic status, combined with densitometry, is essential for the early detection of osteopenia and the prevention of osteoporosis.

Key words: early menopause, premature ovarian insufficiency, estradiol, vitamin D, calcium, ultrasound densitometry, bone mineral density.

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РЕЗУЛЬТАТИ ДЕНСИТОМЕТРИЧНОГО ДОСЛІДЖЕННЯ У ЖІНОК З РАННЬОЮ МЕНОПАУЗОЮ ТА ПЕРЕДЧАСНОЮ ОВАРІАЛЬНОЮ НЕДОСТАТНІСТЮ

Було обстежено 50 жінок, розділених на дві групи: з ранньою менопаузою (n=19) та з передчасною овариальною недостатністю (n=31). До контрольної групи увійшли 20 жінок із збереженою менструальною функцією. Визначали рівень естрадіолу, вітаміну D та іонізованого кальцію, а також проводили ультразвукову денситометрію (показники SOS, BUA, BQI, T- та Z-критерії). Виконано кореляційний аналіз взаємозв'язків між гормонально-метаболічними та денситометричними параметрами. У пацієнток з передчасною овариальною недостатністю виявлено більш виражені зміни мінеральної щільності кісткової тканини порівняно з жінками з ранньою менопаузою. Рівень естрадіолу мав слабку негативну кореляцію з показниками денситометрії, тоді як іонізований кальцій – позитивний зв'язок із BQI та T-критерієм. Гіпоестрогенія є ключовим фактором зниження мінеральної щільності кісткової тканини у жінок з ранньою менопаузою та передчасною овариальною недостатністю. Комплексна оцінка гормонального та метаболічного статусу у поєднанні з денситометрією необхідна для раннього виявлення остеопенічних змін та профілактики остеопорозу.

Ключові слова: рання менопауза, передчасна овариальна недостатність, естрадіол, вітамін D, кальцій, ультразвукова денситометрія, мінеральна щільність кісток.

Funding. *This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. The study was conducted at the authors' primary place of work and was funded from their income there.*

Early menopause and premature ovarian insufficiency (POI) are associated with many complications and problems in women. It is reported that early menopause, which occurs between the ages of 38 and 45, affects up to 12 % of women, and POI, in which loss of ovarian function occurs before the age of 40, affects about 2–4 % of women [6].

Long-term health problems associated with early menopause and POI include an increase in cardiovascular diseases, cognitive impairment, an increased risk of autoimmune diseases, and a

decrease in bone mineral density (BMD), as well as a decrease in quality of life and life expectancy [5]. Women with early menopause and POI have an increased risk of osteoporosis, which is associated with prolonged estrogen deficiency, which causes these conditions, accelerates bone loss, which can lead to a decrease in bone mineral density and an increased risk of fractures compared to women with natural menopause. An increase in the duration of estrogen deficiency leads to an increased risk of osteoporosis [7]. It is noted that osteoporosis affects up to 15 % of women with POI [5]. Studies show lower BMD in women with early menopause with a corresponding increased risk of fractures [3, 7, 14].

Premature ovarian insufficiency (POI), defined as loss of ovarian function before the age of 40, is a life-changing diagnosis with numerous long-term consequences. Most cases of POI have an unknown etiopathology, but some causal factors have been identified. At first glance, the diagnosis of osteopenia and osteoporosis in patients with POI may seem simple and straightforward, but the broader clinical situation often turns out to be more complicated. Numerous factors and tools commonly used to diagnose BMD reduction and stratify fracture risk are of limited utility in POI [9]. Complications from the musculoskeletal system, including osteoporosis and fractures, are a key problem for women with POI.

Thus, women with early menopause and POI have an increased risk of osteoporosis and fractures, while data on risk factors for bone density loss, as well as associations with osteoporosis, are limited.

The purpose of the study was to assess bone mineral density in women with early menopause and premature ovarian insufficiency, using estradiol, vitamin D, and ionized calcium levels as markers.

Materials and methods. The study was conducted between October 2023 and May 2025. 50 women with reproductive disorders who met the criteria for early menopause or POI were examined. All the patients signed an informed consent to participate in the study, which was conducted at the Family Planning Center (Baku, Azerbaijan).

19 women were diagnosed with early menopause (group I), 31 women with POI (group II). Criteria for inclusion in the study: early menopause (group I): 38–45 years of age, with confirmed amenorrhea for ≥12 months to 45 years of age and elevated FSH levels (>25 IU/l) when measured twice with an interval of at least 4 weeks; POI (group II): women under 40 years of age with amenorrhea or oligoamenorrhea, a twofold increase in FSH >25 IU/l and a decreased level of AMH (<1.1 ng/ml).Exclusion criteria**:** pregnancy, lactation, use of combined oral contraceptives or other hormonal drugs within the past 3 months, concomitant diseases associated with osteoporosis: type 1/2 diabetes mellitus, thyroid pathology in a state of decompensation, liver or kidney failure, oncopathology, acute infectious processes. The control group consisted of 20 healthy women with regular menstrual cycles and no signs of endocrine disorders, comparable in age (33–42 years). The examination of women in the control group was conducted at the Scientific Research Institute of Obstetrics and Gynecology (Baku, Azerbaijan).

The serum levels of estradiol, vitamin D, and calcium were determined in all participants. At the same time, blood sampling from patients with early menopause and POI was performed on any day, and in women of the control group, in the early follicular phase (days 2–5 of the cycle). The levels of estradiol and vitamin D were determined by immunochemiluminescence analysis using an automated Roche Cobas e411 immunoanalyzer (Roche Diagnostics, Germany). Ionized calcium was measured by potentiometric analysis using ion-selective electrodes on a biochemical analyzer with an AVL 9180 ionometry module (Roche Electrolyte Analyzer 9180 (Roche Diagnostics, Germany). All patients underwent ultrasound densitometry using the Osteosys Sonost 2000 device (OsteoSys Co., Ltd., Korea). The object of the study was the calcaneus. The scan was performed on either the right or left leg. The following indicators have been determined**:** SOS (Speed of Sound), which measures the speed at which an ultrasonic wave passes through a bone; BUA (Broadband Ultrasound Attenuation) – reflects the degree of attenuation of the ultrasound wave as it passes through the bone; BQI (Broadband Quantitative Ultrasound Index) is a combined index that uses SOS and BUA data for a comprehensive assessment of bone quality; T is a criterion that compares bone density with the normative values of a healthy young person. The Z-index reflects the deviation of bone density from the standard values typical for people of the same age, weight, and gender.

The statistical analysis was performed using the Statistics 16.0 program. The data is presented as figures and percentages. To assess differences between groups, the Student's t-test and $\chi 2$ test were used. To assess the relationships among hormonal, metabolic, and densitometric parameters, a correlation analysis was performed using the Pearson correlation coefficient (r). Correlations between the levels of estradiol, vitamin D, and ionized calcium, as well as ultrasound densitometry (SOS, BUA, BQI, T- and Z-criteria) were considered. The values of $p<0.05$ were considered statistically significant.

The research was approved at a meeting of the Ethics Committee of the Azerbaijan State Advanced Training Institute for Doctors named after A. Aliyev (ASATID) on February 10, 2023, protocol No. 3.

Results of the study and their discussion. The average age of patients in group I was 41.0±2.54 years, in group II – 31.6±4.09 years, and in the control group – 36.5±2.78 years. The analysis of the age distribution showed that patients in group I were significantly older than patients in group II and the

control group. The largest proportion of women in group I (57.9 %) was in the 41–45-year age range, whereas in the control group, this age range accounted for only 10.0 % of patients (χ^2=10.06, p=0.002). In addition, significant differences between groups I and II were observed in the 37–40-year range, with the proportion of patients in group I (42.1 %) significantly higher than in group II (12.9 %; χ^2=5.507, p=0.019). Group II was dominated by patients aged 31–36 years (54.8 %), which differed statistically from the distribution in group I (χ^2=15.587, p<0.001). Table 1 shows the average levels of estradiol, vitamin D, and ionized calcium in the blood of the examined patients.

Table 1

Average concentration of estradiol, vitamin D, and ionized calcium in the patients of the study groups

Indicator	Group I (n=19)	Group II (n=31)	Control (n=20)	p1	p2	p1-2
Estradiol, pg/ml	9.99±5.62	7.13±4.14	77.03±27.56	0.023	0.016	0.684
Vitamin D, ng/ml	30.11±10.46	26.81±5.70	68.01±10.60	0.015	0.001	0.783
Calcium, mmol/l	1.14±0.29	1.03±0.04	1.24±0.05	0.736	0.002	0.709

Note: p1, p2, p1-2 – intergroup differences (p1- I group-control; group II – control; p1-2 – I – II groups) calculated using the Student's t-test.

A significant decrease in estradiol concentration in both groups (p1=0.023; p2=0.016) confirms severe estrogen deficiency. Low estradiol levels are an important pathophysiological factor underlying the clinical manifestations of hypoestrogenism – menstrual cycle disorders, vasomotor symptoms, and early metabolic changes. The concentration of 25(OH)D in the blood, which is the main marker of vitamin D status, in patients of group I ranged from 11.9 to 56.7 ng/ml, which averaged 30.11±10.46 ng/ml; In group II patients, it ranged from 15.8 to 40 ng/ml, with an average concentration of 26.81±5.70 ng/ml. In the control group, vitamin D levels ranged from 44.5 to 82.3 ng/ml, with an average of 68.01±10.60 ng/ml. The patients in groups I and II showed a significant decrease in 25(OH)D concentration compared with the control group (p=0.015 and p=0.001), with the most pronounced deficiency in group II (11.6 % lower than in group I, p=0.783).

Ultrasound densitometry of the right foot was performed in 42 (84.0 %) patients, and of the left foot in 8 (16.0 %) patients. Given that only a small number of women have ultrasound densitometry of the left foot, and that there is a slight difference in the indicators between the left and right feet, we used the average of these indicators. The parameters of ultrasonic densitometry are presented in Table 2.

Table 2

Ultrasound densitometry parameters in patients with early menopause (group I) and POI (group II)

Indicator	Groups	N	Average	SD	Minimum	Maximum	P
SOS, meter/sec	I	19	1506.57	24.31	1446.8	1549.7	0.834
	II	31	1515.15	32.78	1446.8	1560.0	
BUA, dB/MHz	I	19	42.39	8.71	30.6	55.6	0.727
	II	31	46.75	8.88	30.6	62.9	
BQI, dB/MHz	I	19	73.38	11.03	49.0	88	0.765
	II	31	78.63	13.52	49.0	89.3	
T-criterion	I	19	-1.69	0.60	-3.0	-0.9	0.768
	II	31	-1.41	0.73	-3.0	-0.8	
Z-criterion	I	19	-1.0	0.56	-2.6	-0.3	0.491
	II	31	-0.43	0.60	-1.2	0.5	

In patients with early menopause (group I), the average velocity of ultrasound wave propagation (SOS) was 1506.57±24.31 m/s, while in women with POI (group II) it was 1515.15±32.78 m/s, which indicates a slightly higher bone density in the second group, although the differences were statistically unreliable (p=0.834). The broadband ultrasound attenuation index (BUA) in the group with early menopause was 42.39±8.71 dB/MHz, and in the group with POI, 46.75±8.71 dB/MHz, reflecting a trend toward better acoustic properties of bone tissue in women with POI. The values of the bone quality index (BQI) in groups I and II were 73.38±11.03 and 78.63±13.52 dB/MHz, respectively, confirming a similar trend to that observed for other densitometric parameters. The average T-criterion values, which characterize the degree of deviation of bone mineral density from the peak of bone mass in healthy young women, were -1.69±0.60 in group I and -1.41±0.73 in group II, indicating osteopenic changes in the majority of the examined individuals in both groups. The Z-criterion reflecting the deviation from the age norm was -1.0±0.56 in early menopause and 0.43±0.60 in POI, which suggests that in women with POI, a decrease in bone mineral density is less pronounced than in patients with early menopause.

Despite the more pronounced estradiol deficiency in women with premature ovarian insufficiency, their ultrasound densitometry rates were slightly higher than in patients with early menopause. This may be due to a shorter duration of the hypoestrogenic state, lower age, and preservation of partial ovarian or peripheral estrogen production. Thus, the decrease in bone mineral density in patients

of both groups reflects the effect of estradiol deficiency, aggravated by vitamin D deficiency and impaired calcium metabolism.

The correlation analysis showed that in women with early menopause (group I), the level of estradiol has a weak negative relationship with bone mineral density (r from -0.15 to -0.30), which indicates a tendency to decrease the rate of ultrasound and acoustic attenuation with a more pronounced estrogen deficiency. In the group of patients with premature ovarian insufficiency (group II), a similar dependence persisted, but the correlation was even weaker (r from -0.21 to -0.05), which is probably due to pronounced and prolonged hypoestrogenism and the equalization of indicators against the background of chronic hormone deficiency.

The relationship between vitamin D and ultrasound densitometry parameters was weakly negative in both groups, which may reflect secondary changes in calcium metabolism associated with decreased estrogen levels. On the contrary, the level of ionized calcium showed positive correlations with the BQI, T-, and Z-criteria (r = 0.08–0.45), with the correlation particularly pronounced in patients with POI, thereby confirming the role of calcium as a compensatory factor that partially stabilizes bone structure.

In general, the analysis indicates that the leading factor driving the decrease in bone mineral density in women with early menopause and POI is hypoestrogenism, with changes in calcium and vitamin metabolism exerting an additional but less significant effect.

The revealed correlations confirm the need for an early examination of the condition of bone tissue in women with signs of hypoestrogenism, especially with a decrease in estradiol and vitamin D levels. Ultrasound densitometry, in combination with a hormonal and biochemical profile, enables the timely detection of the initial manifestations of osteopenia and the determination of the risk group for developing osteoporosis. This highlights the clinical feasibility of an integrated approach to assessing mineral metabolism in early menopause and premature ovarian insufficiency.

The analysis of hormonal and metabolic parameters showed a pronounced estradiol deficiency in women of both groups compared with the control group. The average estradiol values in patients with early menopause and POI had no significant differences, indicating a comparable degree of hypoestrogenism. At the same time, the examined patients showed a significant decrease in vitamin D concentration and a tendency toward decreased ionized calcium levels, reflecting a disturbance in calcium-phosphorus metabolism and contributing to the development of osteopenia.

Despite similar hormonal profiles, ultrasound densitometry results showed slightly better indicators of bone mineral density in patients with POI than in women with early menopause. This multidirectionality may be associated with a shorter duration of the hypoestrogenic state and a younger age of patients with POI, as well as with the possible preservation of residual ovarian activity or peripheral estrogen production. In both groups, T-test values within the osteopenic range were recorded, confirming the negative effect of sex hormone and vitamin D deficiency on bone tissue.

The results indicate that estrogen deficiency in women with early menopause and premature ovarian insufficiency is associated with impaired vitamin D metabolism and reduced ionized calcium levels, which together contribute to decreased bone mineral density. The revealed osteopenic changes confirm the high vulnerability of the bone system in hypoestrogenic conditions and the need for early diagnosis of bone metabolism disorders. Timely administration of correction – optimization of calcium and vitamin D intake and, if indicated, hormone therapy – can play a key role in preventing osteoporosis and related complications in this patient group.

The results of the study demonstrate that women with early menopause and premature ovarian insufficiency have marked violations of hormonal and metabolic homeostasis, accompanied by a decrease in bone mineral density. Our results are consistent with data from other studies. [2, 4, 12, 15].

The data from this study are consistent with modern ideas about the leading role of estrogens in regulating bone metabolism. A decrease in estradiol levels leads to increased bone resorption due to osteoclast activation and decreased osteoblastic activity, creating the prerequisites for the development of osteopenia and osteoporosis in the early post-reproductive period. [1, 8, 10, 11, 13].

The examined patients showed a significant decrease in estradiol levels in both groups, with the decrease most pronounced in premature ovarian insufficiency. This is accompanied by a moderate vitamin D deficiency and a decrease in ionized calcium, which indicates secondary metabolic disorders of calcium and phosphorus. It is known that vitamin D deficiency increases bone loss, reducing the efficiency of calcium metabolism and its absorption in the intestine. Thus, the combined deficiency of estrogens, vitamin D, and calcium forms the pathogenetic basis for the early development of osteopenic syndrome.

Ultrasound densitometry data confirmed a decrease in bone mineral density in women of both study groups compared with the control group. The most pronounced changes were revealed in the BUA and BQI indices, reflecting a violation of the microstructure of the trabecular bone. Negative values of the T- and Z-criteria in the majority of the examined indicate the presence of osteopenia, and in some cases, borderline signs of osteoporosis.

The correlation analysis showed that estradiol levels have a weak negative relationship with ultrasound densitometry parameters, which is explained by decreased ultrasound speed and acoustic attenuation in pronounced hypoestrogenism. At the same time, in patients with POI, the relationship between hormonal and densitometric parameters was less pronounced, probably due to the

duration and stability of estrogen deficiency, during which bone changes reach a “plateau”.

It is interesting to note that the level of ionized calcium showed a positive correlation with the BQI and T-criteria, particularly in the POI group, suggesting compensatory mechanisms in calcium metabolism. On the other hand, the negative, albeit weak, relationship between vitamin D and densitometry parameters in both groups is probably due to secondary metabolic rearrangements with pronounced hypoestrogenism.

Thus, the results obtained confirm that a decrease in estrogen activity is a leading factor in the pathogenesis of osteopenic changes in women with early menopause and premature ovarian insufficiency. A timely assessment of the hormonal profile, combined with the determination of vitamin D, calcium levels, and ultrasound densitometry indicators, makes it possible to identify early disorders of mineral metabolism and identify patients at risk for developing osteoporosis.

Limitations. This study is limited by its relatively small sample size (n=50), which may reduce statistical power and restrict the external validity of the findings.

Conclusions

1. In women with early menopause and premature ovarian insufficiency, there was a significant decrease in the levels of estradiol, vitamin D, and ionized calcium compared with the control group, indicating marked hormonal and metabolic imbalance.

2. Ultrasound densitometry indicators (SOS, BUA, BQI, T- and Z-criteria) reflect a decrease in bone mineral density in both groups, especially in patients with POI.

3. Correlation analysis revealed a weak negative relationship between estradiol levels and densitometry parameters, which confirms the leading role of estrogen deficiency in the formation of osteopenic changes.

4. The calcium concentration showed a weak positive relationship with bone mineral density, which may indicate a partial compensatory effect of calcium metabolism.

5. The results obtained confirm the need for early screening of bone tissue in women with hypoestrogenism, including the determination of estradiol, vitamin D, ionized calcium levels, and ultrasound densitometry for the prevention of osteoporosis.

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Conflict of interest. The authors have no conflicts of interest to declare.

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Article received: 30.01.2025.