VITAMIN K2 AND VITAMIN D: INFLUENCE ON BONE TURNOVER IN WOMEN WITH POSTMENOPAUSAL OSTEOPOROSIS

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Abstract. Introduction: *Vitamins K2 and D play a central role in calcium metabolism and interact synergistically in maintaining bone and cardiovascular health. Vitamin D stimulates the synthesis of proteins that are activated by vitamin K2-dependent carboxylation. The effects of vitamin K2 on calcium metabolism, bone turnover, and its potential role in the prevention and treatment of osteoporosis are not fully understood.* ***Aim:*** *To investigate the levels of vitamins K2 and D in patients with osteoporosis and menopausal women and to study their influence on bone turnover.* ***Materials and Methods:*** *48 menopausal women were studied, divided into two groups after measuring the BMD of the lumbar spine with DXA: a working group, 26 women with osteoporosis aged 65.62±9.2 years, and a control group of 22 women without osteoporosis aged 63.55±8.7 years. Plasma concentrations of calcium, phosphates, ALP, vitamin D, PTH, OC, ucOC were measured. Vitamin K2 status was indirectly assessed by the ucOC/OC ratio.* ***Results:*** *The measured indicators did not show significant differences in the two groups. In both groups ucOC/OC ratio is high and indicates a vitamin K2 deficiency. Vitamin D levels indicate insufficiency. In the OP group, OC showed a positive correlation with vitamin D ($p = 0.015$) and with ALP ($p = 0.025$). UcOC showed a positive correlation with vitamin D ($p = 0.033$). UcOC/OC ratio showed a high negative correlation with OC ($p < 0.001$) and a negative correlation with ALP, ($p = 0.043$).* ***Conclusion:*** *Our data indicate poor vitamin K2 and D status. In the postmenopausal OP group, both vitamins affect bone turnover through the interrelated levels of PTH, ALP and OC, which suppresses osteoblast function and disrupts the balance between bone formation and resorption in favor of resorption.*

Key words: *postmenopausal osteoporosis, vitamin K2, vitamin D, osteocalcin, uncarboxylated osteocalcin*

Introduction

Adequate nutrition plays a major role in preventing and treating osteoporosis (OP). Calcium and vitamin D are the most important nutrients. In many countries, calcium supplementation alone or combined with vitamin D is recommended in prevention and treatment regimens because of their presumed ability to maintain bone health and increase bone mineral density (BMD). But in menopausal women, the simultaneous development of OP and arterial calcification, called the “calcium paradox,” is common [1]. Scientific evidence links calcium supplements to an increased risk of cardiovascular disease, atherosclerosis, angina pectoris, and myocardial infarction, which any potential benefit for bone health [2]. The processes of calcification in atheromatous plaques are similar to those observed in bone remodeling, involve common regulators, and it has been suggested that a single mechanism may be involved in both diseases. Publications in the scientific literature suggest that vitamin K2 and its possible deficiency are the link between calcium supplementation and increased cardiovascular risk [3].

Vitamin K2 is related to regulation of many vital processes, but its physiological effects and mechanisms for influencing calcium metabolism, as well as its potential role in maintaining bone health and the development of OP, are not yet fully understood. There is evidence that this broad involvement is directly related to its essential role in maintaining stable calcium levels in multiple biological pathways and physiological processes [4]. The involvement in calcium metabolism is associated with vitamin K-dependent proteins. Vitamin K is a cofactor of the enzyme γ-glutamyl carboxylase, which catalyzes the carboxylation of glutamic acid (Glu) residues in vitamin K-dependent proteins to γ-carboxyglutamic acid (Gla) and makes them functionally active [5]. The importance of vitamin K for hemostasis is demonstrated from the fact that vitamin K-deficiency is an acute, life-threatening condition due to excessive bleeding. Other members of the Gla-protein family are osteocalcin, matrix Gla-protein (MGP. In bone and blood vessels, osteocalcin (OC) and matrix Gla-protein (MGP) regulate calcium homeostasis.

Osteocalcin is synthesized and secreted almost exclusively by osteoblasts, which are responsible for the synthesis and mineralization of bone matrix during skeletal development and its periodic regeneration [6]. Vitamin K2 is also involved in the activation of 11 or 12 other proteins that play different roles in modulating connective tissue calcification. Since this process is physiologically necessary in bone but pathological in arteries, much research has been initiated to finding a possible link between vitamin K2 and the prevention of OP and cardiovascular disease [7].

In current scientific literature, there is increasing evidence of poor vitamin K2 status and associated adverse effects on bone and vascular metabolism. Direct measurement of vitamin K levels in the blood has limitations, both for research purposes and in clinical practice, because its molecule is small and is detected only in high concentrations [8]. In vitamin K2 deficiency, the undercarboxylated fractions of OC (ucOC) and MGP (ucMGP) in serum are increased, which better represent its functional status [9]. The ucOC/OC ratio is now considered a better indicator of vitamin K2 status than ucOC and is more widely used in clinical settings due to the better possibility of comparing the obtained data. Based on studies conducted on the dietary intake of vitamin K2, ucOC/OC values ≥ 20% are an indicator of deficiency [10].

The aim of our study was to obtain data on vitamin K2 and vitamin D status of menopausal women with OP and to investigate their influence on bone turnover assessed with some biomarkers.

Materials and Methods

Participants

Forty-eight postmenopausal women were studied. All participants signed informed consent. The diagnosis was made after measurement of BMD of the lumbar spine by dual-energy X-ray absorptiometry (DXA). Grouping was done according to WHO standards, and BMD was determined as follows:

- Normal bone mineral density: T-score ≥ -1.0 SD;
- Osteopenia: T-score between -1.0 SD and -2.5 SD;
- Osteoporosis: T-score ≤ -2.5 SD;
- Severe osteoporosis: T-score ≤ -2.5 SD + fracture.

The participants were divided into study and control group.

Inclusion criteria of participants in the study group

- Age ≥ 45 years.
- Onset of menopause.
- Anamnestic and clinical evidence of absence of diseases leading to secondary OP.
- Demonstrated postmenopausal OP by BMD examination with DXA.
- Lack of osteoporosis treatment until diagnosis, including supplementation with vitamin D, K2, and calcium preparations.

Inclusion criteria of participants in the control group

- Age ≥ 45 years.
- Onset of menopause.
- Demonstrated absence of OP by DXA BMD testing.
- Supplementation with vitamin D, K2, and calcium preparations was not performed until the time of the study.

All participants underwent anthropometric measurements. Body weight and height were measured with a calibrated scale and a standard height gauge. From the body weight in kilograms and height in square meters, BMI was calculated using the formula BMI = kg/m^2.

Laboratory tests

All tests were conducted between October and March. Venous blood samples were collected in the morning between 8.00 and 9.30 am to avoid circadian variability and after a 12-hour fasting period, and were tested in a certified medical diagnostic clinical laboratory.

Calcium, phosphates, alkaline phosphatase – 5 ml of venous blood taken in sterile vacutainers for serum separated by centrifugation was used. Samples were analyzed on the day of collection with a Cobas C 311 biochemical analyzer, Roche Diagnostics.

Parathyroid hormone, vitamin D, osteocalcin – venous blood 5 ml taken in sterile serum vacutainers separated by centrifugation was used. Samples were examined on the day of collection by electrochemiluminescence immunoassay on a Cobas E 411 immunology analyzer, Roche Diagnostics. Elecsys PTH with a sensitivity of 1.20 – 5.00 pg/ml or 0.127 – 530 pmol/ and Elecsys Vitamin D total Roche Diagnostics kits with a sensitivity of 3.00 – 100 ng/ml or 7.50 – 250 pmol/L, and Elecsys N-MID Osteocalcin Roche Diagnostics kit with a sensitivity of 0.500-300 ng/ml were used, respectively.

Vitamin D levels are interpreted according to the criteria of the Bulgarian Society of Endocrinology:

- Severe deficiency of vitamin D – below 25 nmol/l
- Insufficiency of vitamin D – 25-50 nmol/l
- Normal level of vitamin D – 50 120 nmol/l.

Undercarboxylated osteocalcin: 3 ml venous blood in sterile hematology vacutainers was used. Samples for analysis after centrifugation and plasma separation were frozen and stored at -80°C. Concentrations were measured using the Human Undercarboxylated Osteocalcin (ucOC) ELISA Kit (Abbexa, UK), with a test range of 6.25 pg/ml – 400 pg/ml and a sensitivity of 3.75 pg/ml. Assay was performed according to the manufacturer's instructions.

Vitamin K2 status was assessed indirectly by the percentage ucOC/OC ratio. In a 2017 paper, Accepted ucOC/OC values > 20% were accepted as an indicator of dietary vitamin K2 deficiency.

Statistical analysis: the data were entered and processed using the statistical software SPSS v23.0 and Microsoft Office Excel 2003.

The results are described by tables, graphs and numerical values. Continuous variables are expressed as mean (X) ± standard deviation (SD). The data were tested for normality of the distribution using the Kolmogorov–Smirnov and Shapiro–Wilk tests. The statistical processing is tailored to the type of data (nominal, ordinal, interval) and the distribution of variables. For statistical significance in the various analyses, $p < 0.05$ values were accepted. Descriptive, comparative and correlation analysis methods were used. When comparing the groups, the mean value of the quantities (X) ± standard deviation (SD) was most often used as an indicator of the variability of the studied factor or the median values depending on the type of distribution. In case of normal distribution of data, the significance of differences between groups was determined by one-way analysis of variance (ANOVA) and post-hoc Tukey test, T-test for independent samples. In case of data deviating from normal distribution, non-parametric Kruskal–Wallis tests for more than two samples and Mann–Whitney tests for two independent samples were used for comparison. Depending on the distribution of data, Pearson correlation analysis and Spearman's rho rank correlation were used. The degree of correlation was assessed according to the correlation coefficient (r) on a 5-point scale, as: weak ($r < 0.3$), moderate ($0.3 < r < 0.5$), significant ($0.5 < r < 0.7$), high ($0.7 < r < 0.9$) and very high ($0.9 < r < 1$).

Results

After DXA measurement of the BMD of the lumbar spine, participants with postmenopausal OP and a T-score equal to or lower than -2.5 SD were included in the working group (n = 26). The participants without OP and T-score greater than -2.5 SD were included in the control group (n = 22). The mean age, concentrations of calcium, phosphates, ALP, OC, ucOC, ucOC/OC, vitamin D, PTH, BMI, and BMD are presented in Table 1.

The levels of calcium, phosphates, ALP and OC in the study and control groups are within the reference ranges of the kits used. The ucOC/OC ratio shows values above the accepted norm of 20% in all studied women, with the lowest value in the OP group being 30.04% and in the control group 36.31%. The results show poor vitamin K2 status in both groups. The average levels of vi-

Table 1. Concentrations of calcium, phosphates, OC, ucOC, ucOC/OC, vitamin D, PTH, BMI, and BMD expressed by T-score in the working and control groups

	Osteoporosis	Controls	Statistical significance
Age	65.62±9.2	63.55±8.7	p=0.670
Ca – mmol/l	2.47±0.11	2.51±0.12	p=0.220
Pi – mmol/l	1.19±0.19	1.17±0.14	p=0.701
ALP – U/l	86.77±48.78	95.5±50.48	p=0.383
OC – ng/ml	23.41±10.08	23.09±6.94	p=0.605
ucOC – ng/ml	17.33±3.38	17.69±3.92	p=0.700
ucOC/OC – %	77.36±22.27	83.63±29.27	p=0.709
Vitamin D – nmol/l	48.09±19.41	48.34±23.1	p=0.943
PTH – pg/ml	67.89±39.56	64.99±25.12	p=0.911
BMI – kg/m^2	24.56±3.88	29.84±5.45	p<0.001***
BMD – T – score	-2.98±0.52	-0.21±0.85	p<0.001***

Results are presented as mean ± SD. Statistically significant difference p<0.001***.

tamin D show insufficiency in both groups. PTH in the OP group is in elevated values normal up to 65 pg/ml/. The comparison between the groups showed no statistically significant differences ($p > 0.05$). The patients with OP have normal body weight, compared to the controls, who are overweight, and the differences in BMI of the two groups are statistically significant ($p < 0.001$). An expected statistically significant difference is also observed in BMD ($p < 0.001$).

The concentrations of OC, ucOC and ucOC/OC of women from the work and control groups distributed by decade are shown in Table 2.

The comparison of parameters by decade in the OP group showed statistically significant differences in OC concentrations ($p = 0.01$) and no statistically significant differences in ucOC concentrations ($p = 0.906$) and ucOC/OC ratio ($p = 0.376$). Post-hoc tests showed statistically significantly higher OC concentrations in the group of women up to 59, compared to women aged 60-69 years ($p = 0.006$) and over 80 ($p = 0.017$). In the controls, no statistically significant differences were found in OC concentrations ($p = 0.172$), ucOC ($p = 0.085$) in women in different age groups.

An increase in the ucOC/OC ratio with advancing age was observed in both the work and control groups, but the differences were statistically significant only in the control group. Women aged up to 59 years have a significantly lower ucOC/OC ratio than women aged 60-69 ($p = 0.031$) and 70-79 years ($p = 0.048$). The comparison in different age groups between women from the study and control groups did not reveal statistically significant differences in the concentrations of OC, ucOC and ucOC/OC, ($p > 0.05$). The mean concentrations of OC, ucOC and the ucOC/OC ratio at different levels of vitamin D in the OP group are shown in Figure 1. The concentrations of OC at different levels of vitamin D gradually decrease, and the difference is statistically significant ($p = 0.036$). Post-hoc tests showed statistically significantly higher concentrations of OC in women with normal levels of vitamin D compared to women with deficiency ($p = 0.015$).

The mean concentrations of ucOC gradually decrease, and the ucOC/OC ratio increases, but the differences are not statistically significant ($p > 0.05$).

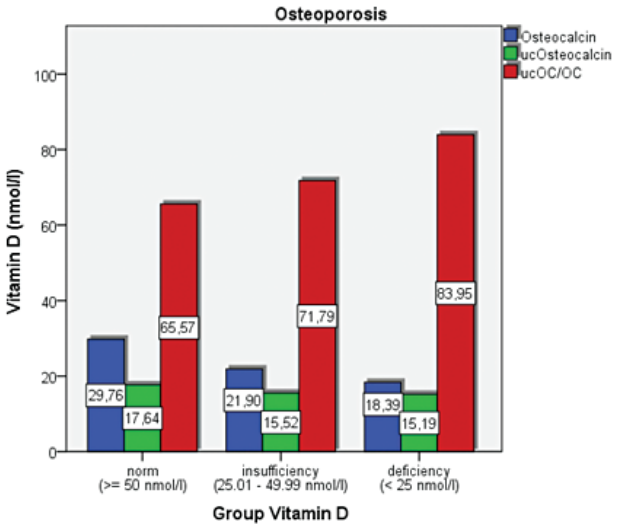


Fig. 1. Mean serum OC, ucOC and ucOC/OC values in women of the study group with different vitamin D levels. Statistically significant differences in OC concentrations were observed in women with normal and vitamin D deficiency, p < 0.05.

The mean concentrations of OC, ucOC, and the ucOC/OC ratio at different levels of vitamin D in the control group are shown in Figure 2. In the control group, we found no statistically significant differences in the concentrations of OC and ucOC ($p > 0.05$) at different levels of vitamin D. Statistically significant differences were observed in the ucOC/OC ratio ($p = 0.047$), and post-hoc tests showed statistically significantly lower levels in the ratio ($p = 0.020$) in women with normal compared to those with vitamin D deficiency.

Table 2. Concentrations of OC, ucOC, and percentage ucOC/OC of women in the treatment and control groups distributed by decade

Age	OC		ucOC		ucOC/OC	
	PMO	Controls	PMO	Controls	PMO	Controls
≤ 59	29.7±13.55	27.8±5.23	17.5±2,74	16.14±3.32	66.5±22.11	59.1±11.71
60-69	20.58±3.03	20.85±7.01	15.15±2.87	16.7±2.69	73.95±10.6	88.61±30.62
70-79	26.49±9.85	24.57±4,67	16.94±2.46	23.17±5.77	68.24±16.01	96.6±29.9
≥ 80	16.92±0	21.92±0	16.82±0	15.46±0	99.4±0	70.5±0

Results are presented as mean ± SD. Concentrations of osteocalcin (OC) and uncarboxylated osteocalcin (ucOC) are presented in ng/ml, and the ucOC/OC ratio in percentage.

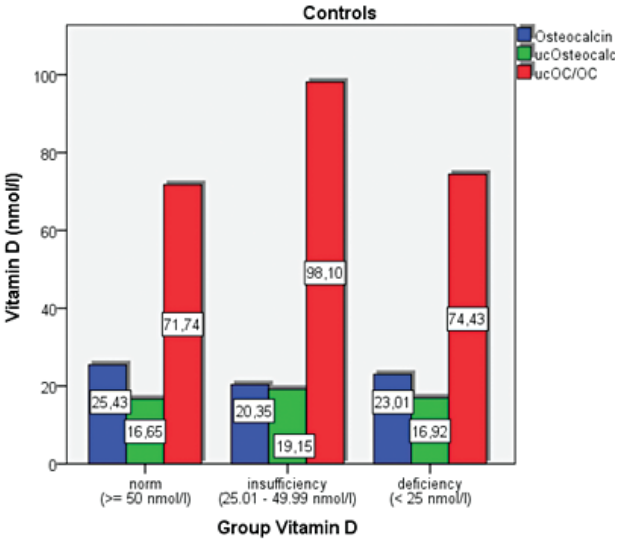


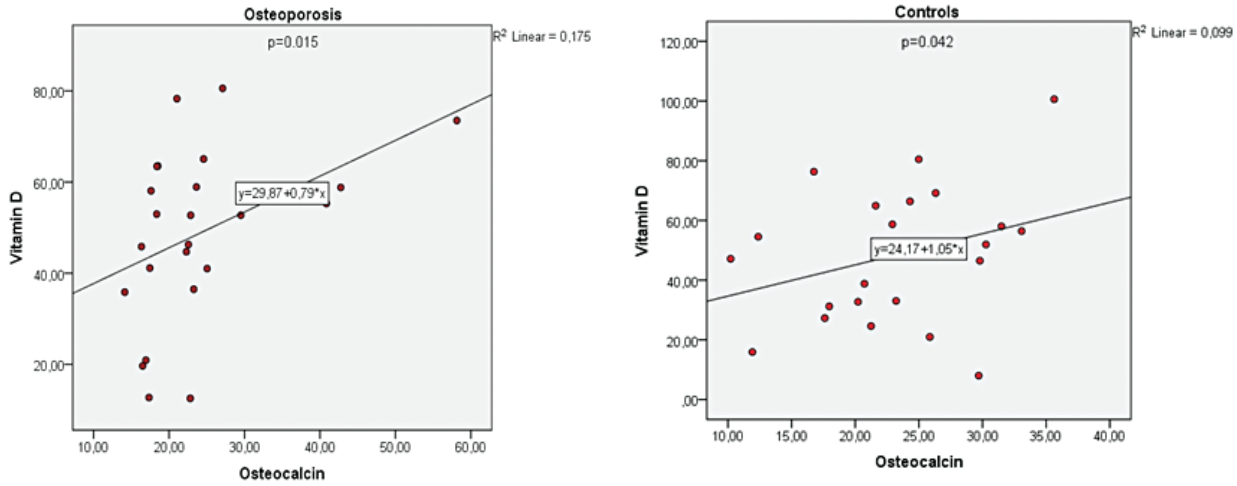
Fig. 2. Mean serum OC, ucOC and ucOC/OC values in women of the control group with different vitamin D levels. Statistically significant ucOC/OC ratio differences were observed in women with normal and vitamin D deficiency, p < 0.05

We found a moderate negative correlation between vitamin D and PTH in the OP group (r = -0.421; p = 0.004) and a significant correlation in the control group (r = -0.618; p = 0.003). Vitamin D levels and OC showed a moderate positive correlation both in the OP group (r = 0.492; p = 0.015), and in the control group (r = 0.418; p = 0.042) (Fig. 3 and 4).

In the OP group, vitamin D levels also showed a moderate positive correlation with ucOC concentrations (r = 0.437; p = 0.033) (Fig. 5).

In the OP group, serum OC concentrations showed moderate negative correlations with decreasing concentrations with increasing age (r = -0.440; p = 0.025) and BMI (r = -0.496; p = 0.010). We also found moderate positive correlations between OC and ALP (r = 0.457; p = 0.025) (Fig. 6), negative correlations between ucOC/OC and ALP (r = -0.416; p = 0.043) (Fig. 7), and significant positive correlations with BMI (r = 0.557; p = 0.011).

The ucOC/OC ratio showed a high negative correlation with OC in both the OP group (r = -0.772; p < 0.001) and the control group (r = -0.839; p < 0.001).



Figs. 3 and 4. Positive correlation between serum vitamin D concentrations and OC in the study and control groups

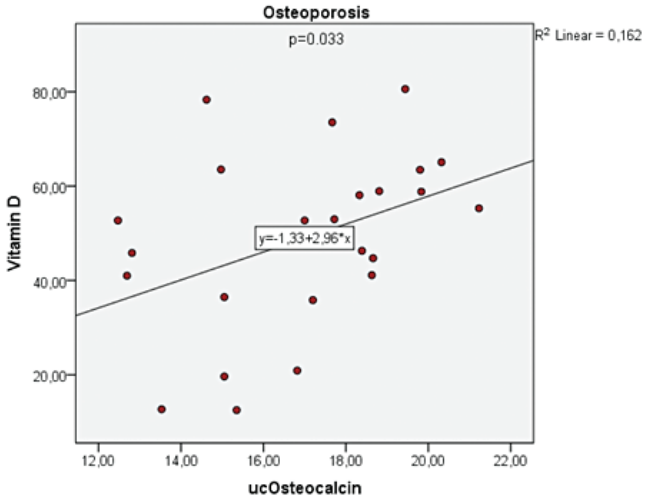
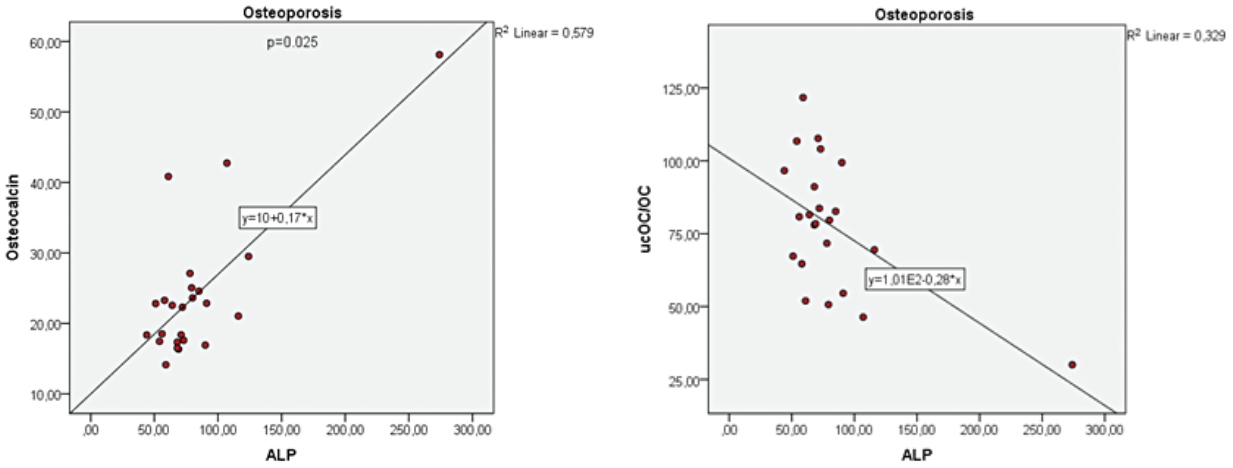


Fig. 5. Moderate positive correlation between serum vitamin D concentrations and ucOC in the study group



Figs. 6 and 7. Moderate positive correlation between OC and ALP and moderate negative correlation between ucOC/OC and ALP in the women with OP

In the study group, no significant correlations were found between vitamin D and BMI, BMD, calcium, phosphates, ALP, ucOC/OC ($p > 0.05$). In the control group, no significant correlations were found between vitamin D and BMI, BMD, calcium, phosphates, ALP, ucOC, ucOC/OC ($p > 0.05$).

Discussion

The maintenance of calcium-phosphate homeostasis is based on a complex, tightly regulated system involving many ions and hormones. Vitamin D and PTH are the two main regulators of mineral metabolism and form a tightly controlled feedback loop, with PTH being the main stimulator of vitamin D synthesis in the kidneys, and vitamin D exerting a negative feedback on PTH secretion [12]. The serum calcium and phosphate concentrations we found were within reference rangesin both groups, with increased PTH concentrations only in the OP group. Calcium concentrations in the OP group were lower and phosphate concentrations were higher compared to the control group, without the differences reaching statistical significance. It is known that PTH levels often increase in old age, which can be explained by the age-related gradual decrease in serum calcium levels, and the increase in PTH in the elderly is defined as secondary hyperparathyroidism. It seems that the maintenance of normal plasma calcium levels in women with OP is associated with chronically increased PTH secretion with adverse effects on bone resorption.

Vitamin D stimulates intestinal calcium absorption and is involved in bone growth and bone remodeling by osteoblasts and osteoclasts [13]. Vitamin D is more than just a fat-soluble vitamin – it is a true hormone with major importance for the musculoskeletal system and for many other tissues and biological processes in the human body [14]. A deficiency below 50 nmol/l accelerates bone turnover, bone loss and is still considered a risk factor in the development of OP and fractures [15]. It is not yet fully understood whether optimal vitamin D levels increase BMD and reduce fracture risk, as well as whether they exert additional musculoskeletal effects, as some meta-analyses of randomized clinical trials question these previously proposed effects, and data obtained in different geographical regions are contradictory [16]. We found vitamin D levels indicating insufficiency without a statistically significant difference in both the OP group and controls (Table 1) and confirmed the negative correlation between vitamin D and PTH values reported by other authors [17, 18, 19].

In adults, bones are continuously remodeled to repair micro-damage that has occurred, to maintain its mechanical strength and durability, and to maintain calcium homeostasis. Bone turnover markers (BTM) provide dynamic information regarding skeletal status independent of BMD and are recommended to accompany and complement its measurements. In Bulgaria, BTM testing has not yet entered routinely in clinical practice and so far there is no experience of their use in women with postmenopausal OP. Published data on their levels are scarce. We examined serum concentrations of alkaline phosphatase, one of the oldest but available markers of bone formation, and osteocalcin, which is considered a marker of osteoblast function and bone quality.

Osteocalcin plays an important role in metabolic regulation, bone mineralization, and calcium ion homeostasis. It is considered a specific marker of

osteoblast function, as its levels have been shown to correlate with the rate of bone formation [20]. Osteoblast-specific OC expression is controlled at the transcriptional level by vitamin D through the respective vitamin D-dependent promoters of osteocalcin genes [21]. The content of OC in the bone matrix increases until the age of 45, then reaches a plateau of a relatively stable level for the next 10 years. After the age of 55, a decrease in OC is observed. Considering that OC reflects the activity of osteoblasts, a significant decrease in its level indicates reduced bone formation [22]. Our study showed that OC concentrations in women up to the age of 59 were statistically significantly higher than the concentrations of OC in women aged 60-69 and over 80 years only in the OP group, but not in the control group (Table 2). In the OP group, we also found a positive correlation between OC and ALP levels (Fig. 6). Osteoblasts produce and secrete large amounts of collagen, which forms a fibrillar network, together with other non-collagenous proteins. These proteins play a role in the subsequent deposition of minerals in the form of hydroxyapatite crystals. The main stages of osteoblastogenesis are proliferation, matrix maturation and mineralization, and are characterized by consistently expressed distinctive osteoblast markers. Early differentiation of osteoblasts is determined by the expression of high levels of ALP, and late differentiation is characterized by the expression of osteocalcin and osteopontin [23]. The higher concentrations of OC in women with OP up to 59 years of age, as well as the positive correlation between the two markers, reflect increased osteoblastic activity, increased bone turnover, which is associated with accelerated trabecular bone loss in the perimenopausal period. The negative correlations we found with a decrease in serum OC levels with increasing age in the OP group, but not in the control group, are a sign of a decrease in osteoblastic function with a decrease in bone formation and deterioration of bone quality in women with OP.

Despite the apparent relationship between vitamin D and OC and the great interest of researchers, very few results from randomized clinical trials in humans have proven it, especially in women with postmenopausal OC. Guney et al. reported a significant positive correlation between vitamin D levels and OC in healthy menopausal women [24]. Alissa et al. investigated the relationship between serum OC and vitamin D status in postmenopausal women and reported a significant negative correlation between OC and dietary vitamin D intake in all subgroups studied [25]. In women with OP, we found statistically significantly higher concentrations of OC in the subgroup with normal vitamin D levels compared to the subgroup with deficiency (Fig. 1). The positive correlations we found with increasing OC levels with increasing vitamin D in both the study and control groups confirm the relationship between them (Fig. 3 and 4).

Vitamins D and K2 have synergistic effects on bone and the cardiovascular system. Both vitamins are fat-soluble and play a central role in calcium metabolism. Vitamin D stimulates gene expression and synthesis of the immature and functionally inactive form – ucOC, and vitamin K2 is required for the transformation of ucOC into mature, functionally active carboxylated OC. Adequate levels of vitamin D are necessary to stimulate adequate synthesis of OC. In the OP group, we found a moderate positive correlation between vitamin D and ucOC (Fig. 5), a scenario in which increased bone turnover and OC production are not coupled with adequate carboxylation due to vitamin K2 deficiency or other factors.

Vitamin K2 is a multifunctional vitamin that has attracted much attention due to its efficacy in improving bone turnover. It promotes bone formation by stimulating osteoblast differentiation and increasing the levels of some bone formation markers, such as ALP and insulin-like growth factor-1 [26], and regulates the mineralization of the extracellular matrix through γ-glutamyl carboxylation of OC [27]. In addition, vitamin K2 reduces bone resorption through its anti-catabolic effects, namely reducing osteoclast differentiation and inhibiting osteoblast apoptosis [26]. These effects are considered to be confirmed in animal models and cell cultures, but have yet to be confirmed in randomized clinical trials in humans [23]. Results from various clinical studies show that in modern diets, vitamin K consumption is gradually decreasing, and even a well-balanced diet cannot provide vitamin K in quantities sufficient to meet the body's needs [28]. There is insufficient data in the scientific literature to assess vitamin K2 levels in postmenopausal women and those with osteoporosis. It is known that vitamin K2 requirements increase with age, and the mechanism is not precisely established, but it is probably related to the reduced number of osteoblasts and the reduced activity of γ-glutamyl carboxylase. This suggests an existing deficiency in the elderly. The reduced activity of γ-glutamyl carboxylase leads to incomplete carboxylation of OC. Uncarboxylated OC cannot be deposited in the bone matrix and enters the bloodstream. Increased concentrations of ucOC are an indirect indicator of vitamin K2 deficiency and there are scarce published data that they are associated

with poor bone status and an increased risk of fractures. The content of ucOC in bone adversely affects bone strength, with a greater effect on bone quality than on BMD [29].

The interpretation of published data on ucOC concentrations is hampered by the lack of sufficient studies in patients with OP. There is evidence that postmenopausal women with serum ucOC levels ≥ 4.0 ng/ml have lower serum vitamin K2 concentrations, higher markers of bone resorption, and an increased incidence of vertebral fractures. To facilitate discussion of guidelines for vitamin K2 deficiency, ucOC values measured by different assay systems will need to be standardized, as there is considerable variation [30]. For the Japanese population of OP patients, a cut-off value of less than 4.5 ng/ml has been adopted [31]. Results from different studies show a wide range of OC and ucOC concentrations, but this is likely due to the different methods used to determine them [32, 34]. We found higher concentrations of ucOC in both study groups, without a significant difference between them, which could be associated with low dietary intake and poor vitamin K2 status, but in our country there are still no defined reference ranges for the indicator for different groups of individuals (Table 1).

Indirect assessment of vitamin K2 status, through the ucOC/OC ratio, facilitates the interpretation of data obtained from various clinical studies. McKeown et al. studied 837 men and women of different ages and found poor vitamin K status in 44% of men and 54% of women [10]. Population-based studies of vitamin K status conducted among Caucasians showed poor vitamin K status in 25-33% of the studied individuals [35]. Our results show a very high ucOC/OC ratio in the study and control groups, without statistically significant differences. The gradual increase in ucOC/OC in different decades was observed in both groups, but the differences were statistically significant only in the control group (Table 2). The lowest values of ucOC/OC in both groups were above the accepted norm, which determines poor vitamin K2 status and its deterioration with age in the women studied by us. Similar results were reported by Theuwissen et al., who examined the vitamin K2 status of 22 healthy Belgian women aged 18-45 years and found a high ucOC/OC ratio of 95% [33]. A study published by Shea et al. of vitamin K status in a multiethnic group of 438 adults (60-80 years, 59% women) in the USA showed deficiency or insufficiency in 97% of the subjects [36].

Vitamin K2 deficiency may adversely affect bone quality more than bone mass. In our study, we found a significantly stronger negative correlation between the ucOC/OC ratio, respectively vitamin K2, and ALP in women from the working group, but not in controls (Fig. 7). The ucOC/OC ratio also shows a high negative correlation with OC in both studied groups. High levels of ucOC/OC, which are associated with vitamin K2 deficiency, reduce ALP and OC with an adverse effect on osteoblast function and bone formation. This disrupts the balance between bone formation and bone resorption in favor of resorption and in women with OP, worsens the quality of the formed bone and may increase fracture risk, regardless of BMD.

Today, the synergistic effect of vitamins D and K2 on bones and blood vessels is considered indisputable, due to their central role in calcium metabolism. We studied Bulgarian women who did not take calcium, vitamin D, and K2 supplements and were not treated for OP. The data we obtained confirm that poor vitamin D status is more likely to be expected in menopausal women and women with OP, and the data on possible vitamin K2 deficiency are very worrying and emphasize the importance of their examination in these fracture risk groups. It can be assumed that older age with higher needs for vitamin K2, inadequate and incomplete nutrition, increased alcohol consumption and the widespread use of antibiotics that destroy the intestinal flora are factors that determine the possible vitamin K2 deficiency in the studied groups of Bulgarian women. Disadvantages of our study are: the small number of participants from both groups and the inability to assess the dietary intake of vitamin K2 due to the lack of a validated and adapted questionnaire for our country.

Conclusion

In the postmenopausal women studied, we found no correlations between vitamin D and K2 levels and lumbar spine BMD. However, in the postmenopausal OP group, we found that both vitamins affect bone metabolism through several interrelated mechanisms. Vitamin D deficiency, through elevated PTH levels, increases bone resorption, decreases bone formation, and slows bone turnover. Furthermore, high levels of ucOC/OC associated with vitamin K2 deficiency decrease alkaline phosphatase and osteocalcin, which negatively affects osteoblast function and disrupts the balance between bone formation and resorption in favor of resorption.

Acknowledgments: This research is supported by the Bulgarian Ministry of Education and Science under the National Program "Young Scientists and Postdoctoral Students – 2"

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Постъпил за печат: 24.07.2025 г.

Submitted: 24.07.2025

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