Vitamin D and parathyroid hormone in relation to bone mineral density in postmenopausal women

Vitamin D i paratireoidni hormon i povezanost sa mineralnom gustinom kostiju kod žena u postmenopauzi

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Abstract

Background/Aim. Despite vitamin D insufficiency being widely reported, in Serbia the epidemiological data lack information regarding vitamin D status in the sera of postmenopausal women. The aim of this study was to establish the prevalence of inadequate serum 25-hydroxyvitamin D [25(OH)D] concentrations in postmenopausal Serbian women with seasonal variations of 25(OH)D, in relation to parathyroid hormone (PTH) and bone mineral density (BMD).

Methods. A total of 95 postmenopausal women, mean age 65.1 ± 9.08 years, were examined. Measurements of 25(OH)D and PTH were performed both in the winter and the summer period, using electrochemiluminiscence immunoassays. BMD (g/cm²) was measured by the dual-energy x-Ray absorptimetry (DXA) method on the spine and hip areas.

Results. A decreased value of vitamin D (< 75 nmol/L) in 88.4% of postmenopausal women and an elevated level of PTH (> 65 pg/mL) in 25.3% of the cases were found. Elevated PTH varied individually, but it was mostly increased if 25(OH)D was equal or lower than 37.6 nmol/L. 25(OH)D insufficiency was found in winter in 94.5% and in summer in 80% of the cases (p < 0.01). The mean of the PTH was higher (p < 0.05) in winter than in summer. A significant negative correlation between 25(OH)D and PTH (p < 0.001) was proved. Correlation between 25(OH)D and PTH with BMD at lumbar spine was established in the whole group, but at the femoral neck in women aged over 65 years (p < 0.05).

Conclusion. Our results showed a high prevalence of vitamin D insufficiency (88.4%) among postmenopausal women. The levels of 25(OH)D and PTH changed significantly according to the season.

Key words: vitamin D; parathyroid hormone; bone density; postmenopause.

Zaključak. Rezultati ukazuju na visoku zastupljenost insuficijencije vitamina D kod postmenopauzalnih žena. Vrednosti 25(OH)D i PTH statistički se značajno menjaju u zavisnosti od godišnjeg doba.

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Introduction

The incidence of osteoporosis increases with age and occurs most frequently in postmenopausal women because the decrease in ovarian estrogen associated with the menopause accelerates bone loss and increases bone remodeling. The evidence of vitamin D inadequacy in postmenopausal women is shown in worldwide studies.

In a study of 8,532 postmenopausal, osteoporotic European women, 79.6% were found to have vitamin D insufficiency where the serum 25-hydroxy vitamin D [25(OH)D] threshold was considered to be 80 nmol/L, and 32.1% if the threshold was set at 50 nmol/L.

In a Belgian study, of 1,195 postmenopausal women the prevalence of 25(OH)D inadequacy was 91.3%, 87.5%, 43.1% and 15.9% when considering cut-offs of 80, 75, 50 and 30 nmol/L, respectively.

Vitamin D plays an important role as one of a number of calcium regulating hormones in the pathogenesis of osteoporosis. The two most important forms of vitamin D are vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol). Vitamin D3 is produced in human skin from 7-dehydrocholesterol as a result of sun exposure, and may also be acquired from dietary sources, but vitamin D is rarely found in foods. More than 90% of the vitamin D requirement for most people comes from casual exposure to sunlight.

Cholecalciferol from the skin, together with dietary cholecalciferol and ergocalciferol, are transported to the liver, bound to vitamin-D binding protein where hydroxylation occurs to 25(OH)D. Calcidiol [25(OH)D] is the main circulating form of vitamin D. Further hydroxylation into biologically active 1,25-dihydroxyvitamin D [1,25-(OH)2D] occurs primarily in the kidney by renal 1α-hydroxylase. Hydroxylation in the kidney is stimulated by parathyroid hormone (PTH) and suppressed by phosphate. Calcitriol or 1,25-(OH)2D is the most active metabolite stimulating the absorption of calcium and phosphate from the gut. The free serum 1,25-(OH)2D concentration is very low, as 1,25-(OH)2D is more than 99% bound to vitamin D binding protein (DBP) and albumin with levels approximately 1000-fold less than circulating 25(OH)D. The half-life of 1,25-(OH)2D is only 4–6 h.

The active metabolite 1,25-(OH)2D acts through the vitamin D receptor which is present in the intestine, bone, kidney and parathyroid gland. In addition to the classic target organs, vitamin D receptor are also present in tissues and organs that are not directly involved in the regulation of calcium homeostasis, such as the brain, breast, immune cells, muscle tissue, cardiomyocytes, vascular endothelial and vascular smooth muscle cells, endothelial cells of colon mucosae, as well as malignant colon cells. This suggests the possibility of a broad range of functions of vitamin D, as seen in hypertension, immunoregulation, embryogenesis and tumorigenesis.

Circulating 25(OH)D should be measured in the blood to determine the overall status of vitamin D, because it is the major storage form of vitamin D in the human body. This primary circulating form of vitamin D is biologically inactive. The half life of 25(OH)D is at least 2–3 weeks.

Vitamin D deficiency leads to a decrease in calcium absorption and secondary hyperparathyroidism resulting in bone loss, mineralization defect and increasing fracture risk.

For a full understanding of the dynamic of secretion, should first be appreciated the bifunctional relationship between PTH and serum calcium, because the serum calcium concentration controls PTH secretion while simultaneously PTH regulates serum calcium concentrations. Serum calcium concentration is maintained at a very constant level, which is supersaturating with respect to bone mineral.

The suppression of PTH by hypercalcemia acts to restore serum calcium to normal by increasing renal excretion of calcium through both the effect of reduced PTH values and activation of the calcium-sensing receptor in the loop of Henle. A reduced PTH value also decreases calcium efflux from bone, renal phosphorus excretion, and calcitriol production, all of which act to restore the serum calcium concentration to normal. Conversely, when hypocalcemia develops, the resulting increase in PTH restores the serum calcium value to normal by increasing calcium efflux from bone, serum calcitriol production, renal reabsorption of calcium, and renal phosphorus excretion. The effect of the last is mediated through the reduction of the serum phosphorus concentration.

Despite the fact that vitamin D insufficiency is widely reported, in Serbia we do not have the epidemiological data considering vitamin D status in the sera of postmenopausal women. The aim of this study was to establish the prevalence of inadequate serum 25(OH)D concentrations in postmenopausal Serbian women with seasonal variations of 25(OH)D, in relation to PTH and axial bone mineral density (BMD).

Also, we evaluated serum and urinary calcium, ionized calcium, serum and urinary phosphate.

Methods

The study was approved by the Ethics Committee of the Institute for Rehabilitation, Belgrade.

A total of 95 postmenopausal women with low BMD were recruited for this study, from November 2008 to March 2010. The patients were divided into the winter group (n = 55) and the summer group (n = 40). Summer was defined as May through October, winter as November through April. No women had received vitamin D supplements before the study.

Following an overnight fast, all the patients brought a 24 h urine collection and gave a blood sample for biochemical analyses. Biochemical measurements were performed in daily routine assays. 25(OH)D and PTH were measured using electrochemiluminescence immunoassays (Roche Diagnostics, Elecsys 2010). Ionized calcium was determined by ion-selective electrode (ILyte, Instrumentation Laboratory), while serum and 24 h urinary calcium and phosphate were measured by colorimetric assays (Cobas Integra 400, Roche Diagnostic). BMD was measured by dual-energy x-ray absorptiometry (DXA) at the lumbar spine – LS (L1-L4) and the hip. We assessed the lowest two vertebra BMD and the lower value on the hip (femoral neck or total hip). A spine

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phantom was scanned each morning as a quality control and instrument calibration.

The values were expressed as mean ± standard deviation. Statistical tests were performed by the statistical package Statistic for Windows (Stat for Windows, R. 7.0) choosing the parametric or nonparametric methods in accordance to coefficient of variability. The difference between groups was determined by the Mann-Whitney U-test or Student’s t-test for independent samples. The correlation was analyzed by the Pearson linear regression test or Spearman nonparametric correlation test. Values of \( p < 0.05 \) were taken as statistically significant 18.

**Results**

Participants were aged 65.1 ± 9.08 years (43–86 years) with years since menopause 15.15 ± 10.0. The mean serum concentration of 25(OH)D, PTH, ionized calcium, serum calcium, urinary calcium, serum and urinary phosphate as well as descriptive characteristics were shown in Table 1.

A decreased value of 25(OH)D (< 75 nmol/L) was found in 84 (88.4%) of postmenopausal women (45.65 ± 14.4 nmol/L). An elevated level of PTH (> 65 pg/mL) was found in 24 (25.3%) of all investigated cases (91.7 ± 28.5 pg/mL). In the group with increased PTH, the mean level of 25(OH)D was 37.6 ± 11.6 nmol/L (Table 1).

The insufficiency of 25(OH)D in the subgroup aged ≥ 65 years was found in 49 (92.5%) women. Also, this number represents 58.3% of all cases with a low level of 25(OH)D (46.3 ± 15.19 nmol/L; min.19.55 nmol/L; max.71.6 nmol/L). In this subgroup, elevated PTH was found in 13 (54.2%) pg/mL (91.52 ± 16.3 pg/mL; min 67.8 pg/mL; max 128.7 pg/mL) of all the patients with elevated PTH levels.

A serum level of 25(OH)D ≤ 50 nmol/L was recorded in 51 (53.7%) patients and 20 (39.2%) of them had elevated PTH. Also a serum level of 25(OH)D ≤ 30 nmol/L was found in 11 (11.6%) patients and 6 (54.5%) of them had increased value of PTH.

The mean of 25(OH)D in the winter period was lower \( (p < 0.01) \) than in the summer one (44.32 ± 16.8 nmol/L vs 60.12 ± 24.48 nmol/L). Conversely, the mean of the PTH was higher \( (p < 0.05) \) in winter than in summer (65.03±32.24 pg/mL, vs 49.36 ± 20.62 pg/mL). In addition, 25(OH)D insufficiency was found in the winter period in 94.5% and in the summer in 80% of the cases. In Tables 2A and 2B seasonal variations of PTH in regard to 25(OH)D insufficiency were presented.

Moreover, the mean of the serum calcium, ionized calcium and serum phosphate, urinary calcium and urinary phosphate were in accordance with the reference values (Table 1). It should be noted that the ionized calcium was above the upper reference limit in 1 (1.1%) and below the lower limit in 5 (5.3%) of investigated patients. Also, se-

Table 1

| Characteristics              | whole (n = 95) | Groups of women with decreased 25(OH)D (n = 84) | with increased PTH (n = 24) |
|------------------------------|---------------|--------------------------------|----------------------------|
| Age (year)                   | 65.1 ± 9.08   | 65.5 ± 9.22                    | 65.2 ± 9.63                |
| Years since menopause        | 15.15 ± 10.00 | 15.66 ± 10.12                 | 13.33 ± 7.85               |
| T-score LSP (SD)             | -2.61 ± 0.973 | -2.62 ± 0.981                 | -2.84 ± 0.888              |
| BMD LSP (g/cm²)              | 0.821 ± 0.117 | 0.822 ± 0.118                 | 0.784 ± 0.125              |
| T-score hip (SD)             | -1.86 ± 0.785 | -1.88 ± 0.812                 | -1.94 ± 0.704              |
| BMD hip (g/cm²)              | 0.757 ± 0.106 | 0.756 ± 0.109                 | 0.753 ± 0.107              |
| T-score neck (SD)            | -1.94 ± 0.803 | -1.94 ± 0.840                 | -1.99 ± 0.643              |
| BMD neck(g/cm²)             | 0.713 ± 0.119 | 0.714 ± 0.122                 | 0.701 ± 0.118              |
| 25(OH) D (nmol/L)            | 50.97 ± 21.55 | 45.65 ± 14.4                  | 37.6 ± 11.6                |
| PTH (pg/mL)                  | 58.8 ± 29.1   | 62.3 ± 29.1                   | 91.7 ± 28.5                |
| Ionized-Ca (mmol/L)          | 1.18 ± 0.063  | 1.18 ± 0.069                  | 1.15 ± 0.079               |
| Serum-Ca (mmol/L)            | 2.40 ± 0.11   | 2.40 ± 0.11                   | 2.39 ± 0.13                |
| Urinary-Ca (mmol/24h)        | 4.30 ± 2.36   | 4.12 ± 2.30                   | 3.80 ± 2.26                |
| Serum-P (mmol/L)             | 1.15 ± 0.15   | 1.15 ± 0.15                   | 1.11 ± 0.13                |
| Urinary-P (mmol/24h)         | 20.53 ± 6.56  | 19.77 ± 6.26                  | 20.85 ± 7.32               |

Results are given as mean ± standard deviation

25(OH)D – 25-hydroxyvitamin D; PTH – parathyroid hormone; LS – lumbar spine; BMD – bone mineral density; Ca – calcium; P – phosphate

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A strong negative linear correlation between 25(OH)D and PTH ($r = -0.508, p < 0.001$) was found (Figure 1). Serum levels of 25(OH)D were significantly correlated to T score at the lumbar spine ($r = 0.227, p < 0.05$), but not at the total hip and femoral neck ($r = 0.093, p = 0.373; r = 0.110, p = 0.290$) in the whole group. In addition, PTH correlated only to BMD at lumbar spine ($r = -0.258, p < 0.05$). It was noticed that in the subgroup aged $\geq 65$ years, correlations between 25(OH)D and T score LS ($r = 0.315, p < 0.05$), BMD of at the neck ($r = 0.321, p < 0.05$), and PTH with BMD of the neck ($r = -0.352, p < 0.05$) were found. (Figures 2 and 3).

### Table 2A

| 25(OH)D values (nmol/L) | Women (n) | PTH (pg/mL) $\bar{X} \pm SD$ | Women (n) | 25(OH)D (nmol/L) $\bar{X} \pm SD$ |
|-------------------------|-----------|-------------------------------|-----------|-------------------------------|
| $< 75$                  | 42        | 67.31 ± 32.1                  | 52        | 42.22 ± 13.98                 |
| $< 50$                  | 28        | 76.28 ± 34.4                  | 36        | 34.63 ± 8.66                  |
| $< 30$                  | 10        | 85.43 ± 39.6                  | 10        | 22.92 ± 3.08                  |

### Table 2B

| 25(OH)D values (nmol/L) | Women (n) | PTH (pg/mL) $\bar{X} \pm SD$ | Women (n) | 25(OH)D (nmol/L) $\bar{X} \pm SD$ |
|-------------------------|-----------|-------------------------------|-----------|-------------------------------|
| $< 75$                  | 22        | 54.69 ± 21.5                  | 32        | 51.23 ± 13.5                 |
| $< 50$                  | 10        | 59.07 ± 24.1                  | 15        | 38.83 ± 5.42                 |
| $< 30$                  | –         | –                             | 1         | 29.5 ± 0                     |

### Table 3

| T score   | Lumbar spine (L1-L4) $\bar{X} \pm SD (n)$ | Total hip $\bar{X} \pm SD (n)$ | Femoral neck $\bar{X} \pm SD (n)$ |
|-----------|--------------------------------------------|---------------------------------|-----------------------------------|
| $<-2.5$ SD| $-3.12 \pm 0.582 (59)$                     | $-2.85 \pm 0.369 (23)$          | $-2.87 \pm 0.316 (24)$            |
| $<-1.0$ and $>-2.5$ SD | $-1.92 \pm 0.340 (27)$ | $-1.70 \pm 0.40 (59)$          | $-1.82 \pm 0.390 (60)$           |
| $> -1.0$ SD | $0.125 \pm 1.158 (4)$ | $-0.554 \pm 0.326 (11)$ | $-0.561 \pm 0.745 (10)$ |

$n$ – number of women
Discussion

Recent publications suggest that for bone and overall health desirable 25(OH)D concentration should be up to 75 nmol/L. In accordance with other authors, we found a high prevalence of vitamin D insufficiency in postmenopausal women (88.4%).

Given that sunshine exposure is the most important source of vitamin D, one should expect that vitamin D status depends on geographic location and seasonal variation. In addition, time spent outdoors, clothing habits, skin type and pigmentation, as well as diet, may influence differences in vitamin D status among countries. We found a high prevalence of vitamin D insufficiency among postmenopausal women in Belgrade (45 degree latitude North). This may be due to the fact that the elderly population has a higher prevalence of vitamin D insufficiency between 25(OH)D and PTH levels. Vitamin D insufficiency is often associated with secondary hyperparathyroidism. In our study, in accordance with other investigators, we found a high prevalence of vitamin D insufficiency among patients. Consequently, the prevalence of secondary hyperparathyroidism was higher (28.6%) among subjects with vitamin D insufficiency. PTH is not always high. Despite of increases in serum PTH associated with vitamin D insufficiency, PTH values were usually within the normal reference ranges. It should be noted that the prevalence of elevated PTH (54.2%) in women aged ≥ 65 years is similar to those with severe 25(OH)D insufficiency (55.5%). Literature data confirmed that PTH rises with age, but we have not found relationship between PTH and age (p > 0.05). In addition, many studies have reported that PTH values are higher in older than in younger adults. Contributing to the higher PTH values in the elderly are several factors that are intrinsic to aging, such as decreased renal function, age related changes in the dermis which diminish the capacity for cutaneous synthesis of vitamin D, less efficient intestinal absorption of vitamin D status among countries. Our results of seasonal variation among postmenopausal women in Belgrade (45 degree latitude North) confirmed significant difference in 25(OH)D and PTH levels. Vitamin D insufficiency is often associated with secondary hyperparathyroidism. In our study, in accordance with other investigators, we found secondary hyperparathyroidism in 25.3% of all investigated patients. Consequently, the prevalence of secondary hyperparathyroidism was higher (28.6%) among subjects with vitamin D insufficiency. PTH is not always high. Despite of increases in serum PTH associated with vitamin D insufficiency, PTH values were usually within the normal reference ranges.

It should be noted that the prevalence of elevated PTH (54.2%) in women aged ≥ 65 years is similar to those with severe 25(OH)D insufficiency (55.5%). Literature data confirmed that PTH rises with age, but we have not found relationship between PTH and age (p > 0.05). In addition, many studies have reported that PTH values are higher in older than in younger adults. Contributing to the higher PTH values in the elderly are several factors that are intrinsic to aging, such as decreased renal function, age related changes in the dermis which diminish the capacity for cutaneous synthesis of vitamin D, less efficient intestinal absorption of calcium, resistance to the calcemic action of PTH, a greater prevalence of vitamin D insufficiency, and perhaps the acidic tendency of old age.

In the group of patients with elevated PTH, the mean of vitamin D was 37.6 nmol/L. This practically means it is a cut point value below which appears elevated PTH. This result was similar or equal with other authors. The modifying factor of the relationship between serum 25(OH)D and PTH is the calcium intake. The 24 h pattern of PTH secretion decreases markedly after an increase in calcium intake.

Our study shows that vitamin D insufficiency is a common risk factor for osteoporosis in ambulatory postmenopausal women.

It is of relevance, in the elderly population, that chronic vitamin D insufficiency leads to osteoporosis or gradual loss of bone, which results in the impaired structural integrity of trabecular bones, with thinner and more porous cortical bones, thereby making the bones weaker and more likely to fracture.

A statistical analysis confirmed a significant correlation between 25(OH)D insufficiency and low BMD at lumbar spine in the whole group, and at femoral neck in the subgroup aged over 65 years. The pathogenic role of vitamin D insufficiency in decreased bone mass is shown by the significant correlation between 25(OH)D and T-score at lumbar spine. Vitamin D status appeared to be less related to proximal femur than to lumbar spine BMD. This may be caused by the fact that other mechanical factors (i.e., physical activity and weight) could influence proximal femur BMD to a higher extent. Moreover, the sex hormone deficiency makes lumbar spine bone more susceptible to vitamin D insufficiency. As suggested previously, oestrogen deficiency potentiates the effect of PTH excess because of vitamin D insufficiency. Furthermore, hyperparathyroidism predisposes to cortical rather than cancellous bone loss, which would be more obvious at femoral neck compared with lumbar spine and, also may explain why PTH was a significant predictor of BMD at femoral neck as confirmed by our results.

Conclusion

Our results showed a high prevalence of 25(OH)D insufficiency among postmenopausal women (88.4%) with seasonal variation in serum levels of 25(OH)D and PTH. Secondary hyperparathyroidism appeared in 25.3% of the patients. A very significant inverse correlation between 25(OH)D and PTH was established by BMD at lumbar spine in the whole group, and femoral neck in the subgroup of postmenopausal women aged more than 65 years. In patients with 25(OH)D insufficiencies, elevated PTH varied individually, but it was most often increased if 25(OH)D was equal or lower than 37.6 nmol/L.

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