BONE MARKERS IN MONITORING OF ANTIRESORPTIVE THERAPY IN POSTMENOPAUSAL OSTEOPOROSIS PATIENTS

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The aim of this work was to study the effect of two modalities of antiosteoporotic therapy in postmenopausal women at the level of biochemical markers of bone turnover such as bone specific alkaline phosphatase (BALP) and deoxypiridinoline (Dpd) as well as bone mineral density (BMD). The study included 87 patients with postmenopausal osteoporosis (OP). Group A consisted of 48 patients treated with alendronate (AL), whereas group B included 39 patients treated with hormone replacement therapy (HRT). BMD was measured by Lunar DPX 2000 device, on the lumbar spine and the femur, and bone markers (BM) were measured by commercial ELISA assays. There was a statistically significant decrease in the levels of BALP and Dpd after 6 weeks and 8 months of both types of therapy compared to the level of these markers before therapy. There was a statistically significant increase of BMD on both locations after 8 months of both therapies. In addition, there was a statistically significantly higher degree of changes of Dpd values in the group treated with AL than in the group treated with HRT. On the other hand, the changes in the level of BALP were significantly higher in the group treated with HRT. We concluded that the early effect of the two studied antiosteoporotic medications can be monitored by changes in the levels of BM. Dpd as bone resorption marker proved to be a better indicator of the efficiency of applied medications compared to bone formation markers such as BALP. Acta Medica Medianae 2015;54(3):5-11.

Key words: osteoporosis, bone markers, alendronate, hormone replacement therapy

Introduction

Osteoporosis (OP) is the most frequent systemic, progressive, metabolic bone disease which is characterized by lowering the bone mass and its quality as well as by the dysfunction of micro- and macro-architecture of bone tissue (1). As a consequence, the bone is more fragile and at greater risk of fractures. It is estimated that more than 10% of the world population is under such risk (2). Postmenopausal osteoporosis (PmOP) is caused by the loss of estrogen, associated with the early menopause or the surgical removal of ovaries in a woman’s generative period. In the beginning, the bone mass loss is fast and emerges progressively (3). The changes which lead to OP are of low intensity, last for years and are not easily detectable with standard laboratory analysis and osteodensitometry. With the development of tests for measuring the markers of disease at the molecular level, it is possible to track the forming and resorption of bone.

There is a wide range of drugs for OP treatment, used to prevent bone fractures. In clinical practice, monitoring is very important, especially for the early success of therapy, which is not possible to monitor by osteodensitometry. Therefore, the number of studies concerning the significance of bone markers (BM) is rapidly increased. After OP diagnosing, before deciding the model of treatment, it is necessary to evaluate the risk factors involved in its development. Bone formation markers (BFM) evaluate either synthetic activity of osteoblasts or procolagen metabolism after discharging from osteoblasts. Bone resorption markers (BRM) reflect osteoclast activity and/or degradation of collagen (4).

BRM are associated with higher bone loss, regardless of changes in bone mineral density (BMD) (5) and correlate with a two times higher fracture risk (6). The use of deoxypiridinoline (Dpd) was studied in PmOP as well as in the eva-
The concentra-tions of BMs were determined in the collected biological samples. The level of bone specific alkaline phosphates (BALP) was determined in the serum, and the level of Dpd was determined in the urine. The levels of BALP and Dpd were determined by commercial ELISA tests produced by Metra Biosys-tems (USA). The concentra-tion of BALP was expressed in U/L. The concentra-tion of Dpd was corrected by creatinine values and expressed in nmol/mmol of creatinine.

The results of BM and BMD were expressed as mean +/- SD. The changes in studied values after applying therapy were expressed in percents. The results were processed by using appropriate statistical methods (Student’s t-test, Rank sum test and a linear regression model) in GraphPad Prism ver. 5 (GraphPad Software, Inc., SAD).

**Aim**

The aim of our work was to study the effects of two modalities of antiosteoporotic therapy (alendronate and hormone replacement therapy - HRT) at the level of BM and BMD.

**Methodology**

This study included patients diagnosed with PmO, treated at the Department for Osteoporosis at the Military Medical Academy. The inclusion criteria for the study were: period of menopause lasting 5 or more years, patients who did not receive any therapy for OP and did not have any other systemic diseases or took drugs which could induce bone mass loss. All patients were divided into two groups, according to the therapy they received. The first group (A) included 48 women diagnosed with PmOP, and after diagnosing the therapy included alendronate (AL) 70 mg/daily. The second group (B) included 39 women diagnosed with PmOP, and after diagnosing they were treated with continuous HRT (2 mg of 17 β estradiol and 1 mg of noretisteron acetate, daily). This group of women, along with proven OP, had postmenopausal climacteric symptoms. All patients received the supplement of calcium lactate gluconate and calcium carbonate, in doses of 1 g daily and alphacalciodole, analogue of vitamin D, in doses of 0.25 μg, three times a day.

Bone density was measured by double-energy absorptiometry of X rays (DXA) on Lunar D PX 2000, on the location of lumbar spine, L1-4, and femur. Received values for BMD were expressed in g/cm², and as T score. Measuring was made before treatment and after 8 months of therapy.

Biological materials (blood and urine) were taken from all patients before starting the therapy, after 6 weeks, and after 8 months of therapy. Blood was taken in the early morning (before 10 a.m. because of circadian variations) by punctation from the cubital vein without anticoagulants. The prepared serum samples were kept at -20°C until their use for BM detection by ELISA tests. Urine samples were collected in the early morning, before 10 a.m., and then kept at -20°C until use.

We assessed the correlation between BM and BMD before the beginning of AL and HRT therapy. Before starting therapy in the group A, BMD was correlated with BALP (L1-4 R=0.65, p<0.001; femur R=0.766, p<0.001) and Dpd (L1-4 R=0.402, p<0.01; femur R=0.508, p<0.001). In the group B, BMD was correlated with BALP (L1-4 R=0.367, p<0.05; femur R=0.435, p<0.01), while it did not correlate with Dpd (L1-4 R=0.244, p>0.05; femur R=0.281, p>0.05).

After the first 6 weeks and after 8 months of both types of therapy, there was a statistically significant decrease of BALP value, compared to the
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**Figure 1.** Levels of BALP before therapy, 6 weeks and 8 months after alendronate (AL) and hormone replacement therapy (HRT). Values are given as mean ± SD. *** p<0.001 compared to the corresponding levels before therapy.

**Figure 2.** Levels of Dpd before therapy, 6 weeks and 8 months after alendronate (AL) and hormone replacement therapy (HRT). Values are given as mean ± SD. *** p<0.001, compared to the corresponding level before therapy. p<0.01 level after 6 weeks of therapy compared to the level after 8 months of therapy.

The comparison of the BMD before and after 8 months of AL therapy showed that there had been a statistically significant increase of BMD after 8 months therapy on both localizations (Figure 3). During the 8 months of AL therapy, there was a 3.58±2.83% increase of BMD at L1-4 level and 5.5±7.5% on the femur. After 8 months of HRT, increase of BMD was also statistically significant (Figure 3). In the group B there was a 2.97±2.43% increase of BMD at L1-4 level and 3.34±3.57% on the femur. The differences in the medication efficiency between the groups resulting in an increase of BMD were statistically insignificant.

After 8 months of AL therapy, the correlation between BMD and BALP (L1-4 R=0.633, p<0.001; femur R=0.607, p<0.001) and Dpd (femur R=0.331, p<0.05) levels was detected, while 8 months after HRT the measured BMD correlated only with the level of BALP (L1-4 R=0.326, p>0.05 and femur R=0.449, p<0.05).

Figure 4 shows the improvement in BM levels (expressed as percentages) after treatment with AL and HRT. The results show that the changes in Dpd levels compared to the changes of BALP were significantly higher than the changes in BALP levels in both groups.

One of our aims was to study the possible differences between the efficiency of AL and HRT, measured by an increased BMD and normalization of BM after 8 months of therapy protocols. Comparing the improvements in BMD after applying
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**Discussion**

During the first two years of menopause, degradation of bones is higher compared to their formation, while osteoblast activity slowly starts to grow from the third to the fifth year of menopause, followed by changes of BALP and hydroxiproline values (11). The medications have a different influence on stabilizing the existing bone mass and its improvement, as well as preventing new fractures. Such a wide variety of antiresorptive medications enables us to choose an adequate and individual treatment model for each patient. The HRT, bisphosphonates or combination of both therapies for the treatment of PmOP have been used today, because these drugs decrease bone mass loss and prevent fractures (12, 13).

Our patients with PmO were treated with AL and HRT, and their effect was monitored by tracking the changes of BM values. Before starting the treatment, the level of measured markers in both groups of patients was significantly increased. In addition, there was a correlation between BM and

### Figure 3

**Figure 3.** Bone mineral density (g/cm²) before therapy and 8 months after alendronate (AL) and hormone replacement therapy (HRT). Values are given as mean ± SD. *** p<0.001 compared to the corresponding levels before therapy

### Figure 4

**Figure 4.** Percentage of BM normalization 6 weeks and 8 months after alendronate (AL) and hormone replacement therapy (HRT). Values are given as mean ± SD. *** p<0.001 Dpd compared to BALP in each group for both studied periods p<0.001, p<0.01 compared to group B for both studied periods

Both of the chosen medications did not give any statistically significant differences in both measured localizations - L1-4 (p>0.05) and femur (p>0.05). The results of changes of BM in percents after 6 weeks of therapy show that there was a significantly higher degree of changes of Dpd values in the group of patients treated with AL, compared to the group treated with HRT. On the other hand, the changes of BALP were significantly higher in the group treated with HRT, compared to the group treated with AL. The degree of changes of BM after 8 months of the treatment had the same trend as the results obtained after 6 weeks (Figure 4).
BMD. Similar results were also obtained by other authors (14, 15).

In contrast, the values of Dpd and BDM, in the group of patients treated with HRT, did not have any correlation, which could be explained by the fact that our group of women had a lower average age and the menopausal period was shorter. In that period, the changes in BM values were higher than the simultaneous bone mass loss, which could be an answer for the obtained results. The results showing a large increase in the levels of pyridinoline and telopeptides during the first five years of menopause, followed by a significant decrease in the following five years, are in line with this assumption (16).

Alendronate therapy efficiency studies showed that after only 6 weeks this medication significantly decreased the values of BALP and Dpd. The decrease of these parameters after 8 months of treatment was also significant. Further-more, the level of Dpd was significantly decreased compared to the level measured after 6 weeks of therapy. After 8 months of treatment, alendronate also contributed to a significant increase of BMD. Our results are in accordance with the results obtained by many other authors who used BM for monitoring the efficiency of alendronate therapy. For example, after 3 months of alendronate therapy, there was a significant decrease of BALP values, and after 6 months its levels were decreased by about 25% in as much as 95% of patients (17). Bell et al. showed the same effects of alendronate therapy applied in Afro-American women − a significant increase in BMD and reduction of BM levels, compared to the placebo group (18). Our results are also in line with the results obtained by Ravn et al. (19) who demonstrated that BM is a good indicator of alendronate antiresorptive therapy response. Similarly, Prinsloo and Hosking (20) pointed out that alendronate leads to BMD increase, which correlates with the decrease of BRM (Pd, PICP), and the effect depends on the dosage. There were also correlations between the changes of values of PICP, PINP and OC after 6 months of alendronate therapy and changes in BMD after 4 years (19).

In our patients, the HRT significantly decreased BM after 6 weeks of treatment. The decrease of BM level was also significant after 8 months of therapy, the levels of Dpd were significantly lower compared to the levels after 6 weeks of therapy, while the values of BALP were not significantly changed. The normalization of Dpd values was observed after 6 months of estrogen application, compared to the group without therapy (21). The 30% - 40% decrease of Pyd and Dpd by estrogen therapy was obtained in the study of Garnero et al. (22). Also, there was a significant increase in BMD after 8 months of treatment with HRT (12). The correlation between BMD and values of Dpd after 8 months of treatment with HRT could be explained by the fast inhibitory effect of estrogen on osteoclasts, and thereby on bone resorption. The results of mutual comparison of percentage decrease of studied BM values show that Dpd is a significantly better indicator of therapeutical effects than BALP. BRM respond more quickly to medications such as alendronate (during 1 month) compared to BFM which respond after 3 months. The degree of BM decrease correlates with BMD increase after 24 months of alendronate therapy (23). A fact which also confirms the significance of BRM is the finding that tartrat resistant acid phosphatase of isofrom 5b is the most sensitive and most specific marker for monitoring the efficiency of alendronate therapy (24). By studying the mutual connection between Pyd and telopeptide, Gerrits et al. showed that these two types of BM provide similar information about the degree and kinetics of bone resorption during menopause (increase during the first five years and slow decrease during the next five years) (16). The results of this study also showed that BRM are good indicators of bone metabolism disorder and the degree of its loss (16). According to these results we can say that BM, especially Dpd, as BM, responded relatively quickly to the applied therapy before they become measurable by BMD changes.

Our results showed that alendronate led to a significant decrease of Dpd, compared to HRT 6 weeks and 8 months after applying the therapy protocols. BALP was significantly lower in patients treated with HRT, compared to patients treated with alendronate, but the percentage of decrease was less 8 months after the beginning of treatment than after 6 weeks of therapy. This indicated that this marker is not a very reliable parameter in evaluating the efficiency of this type of therapy. Such finding could be explained by the effect of estrogen on osteoblasts (25). Hosking et al. (26) showed that alendronate has a better effect compared to residronate measured by decreased values of PICP as soon as after 3 months, while BALP was significantly better only after 12 months of therapy. Better efficiency of alendronate compared to calcitonine therapy, reflected in the increase of BMD followed by significant decrease values of BALP, OC and Pyd. According to these results, the usage of BM in evaluating the efficiency of different therapy modalities could be justified. After 8 months of treatment there were not any significant differences between BMD in patients whose underwent different modalities of therapy (27).

**Conclusion**

Our results showed that the early effect of two studied antiosteoerotic medications can be monitored by changes in the levels of BM. Dpd is has proved to be a better indicator of the efficiency of applied medications compared to BALP.
References

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Cilj rada bio je praćenje efekata antiresorptivne terapije kod žena u postmenopauzalnom periodu na osnovu nivoa markera koštanog metabolizma – koštane specificne alkalne fosfataze (ALP) i deoksipiridinolina (Dpd), kao i mineralne gustine kostiju (BMD). Ispitivanje je obuhvatio 87 bolesnica sa osteoporozom u postmenopauzi (OP). Grupu A je činilo 48 bolesnica na terapiji alendronatom (AL), dok je u grupi B bilo 39 na hormonskoj terapiji (HRT). Na aparatu Lunar DPX 2000 meren je BMD lumbalnog dela kičme i femura, a nivo markera određivan komercijalnim ELISA testovima. Analizom rezultata utvrđen je statistički značajno smanjen nivo ALP-a i Dpd u obe grupe ispitivane nakon 6 nedelja i 8 meseci terapije u poređenju sa vrednostima pre terapije. Zapažen je porast BMD-a nakon osmomesečne terapije pomoću oba preparata. Uzrokan je značajno veći nivo Dpd u grupi tretiranih AL-om nego kod lećenih HRT-om. Sa druge strane, promene u vrednostima ALP-a bile su znatno više u grupi na terapiji HRT-om. Iz navedenog se zaključuje da se efekti terapije dva ispitivana antiosteo-
porotična preparata mogu pratiti preko nivoa markera. Pokazano je da je Dpd, kao
marker koštane resorpcije, bolji indikator efikasnosti terapije nego ALP, marker

Ključne reči: osteoporoz, markeri koštanog metabolizma, alendronat, hormonska terapija

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