Snežana Marić Krejović[*], Danijela Marković Knežević[**], Božidar Petrović[***], Ana Dević[****], Sreten Knežević[*****]

EFFECTS OF THE ANTIRESORPTIVE THERAPY ON BONE METABOLIC ACTIVITY IN POSTMENOPAUSAL WOMEN

Abstract: *Introduction*: Osteoporosis, a systemic disease of bones, represents a serious health and socio-economic problem because of its consequences - bone fractures. It is believed that 10% of the world population suffers from osteoporosis and it affects mostly postmenopausal women.

Methods: The survey was conducted within a group of 80 postmenopausal women with osteopenia treated with antyresorptive therapy. The control group included 40 postmenopausal women who did not take any kind of therapy. Bone metabolic activity was evaluated by using osteocalcin as a parameter of bone formation.

Blood analysis were made before therapy introduction and 3 months after initial therapy. The average value of osteocalcin three months after tibolone and HRT therapy was lower compared to the average value of osteocalcin before the treatment.

Results: During implementation of tibolone and HRT therapy, osteocalcin serum concentrations were significantly lower than those before the therapy. Achieved results showed high efficiency of tibolone and HRT on bone resorption and suppressive effects on bone formation, what arises from connections between bone formation and resorption.

[*] General Hospital Uzice- Health Center Arilje, Serbia; e-mail: snezana.krejovic@gmail.com

[**] General Hospital Uzice- Health Center Užice, Serbia.

[***] General Hospital Uzice- Health Center Arilje, Serbia.

[****] Clinical Center of Zemun- *Department* of Obstetrics and *Gynecology*, Serbia.

[*****] General Hospital Uzice- *Department* of Radiology, Serbia

Conclusion: Monitoring parameters of bone metabolic activity is also very useful diagnostic tool in assessing the effects of tibolone on bone metabolic activity and possibly forecast the final outcome on bone mass.

Key words: Osteoporosis, postmenopausis,tibolone, HST

Introduction

Osteoporosis is a bone disease characterized by an abnormal bone density which causes increased predisposition to fractures. Bone strength means the bone quantity (bone mass - density, the size of the bone) and bone quality (microarchitecture, matrix mineralization, bone metabolism, accumulated microscopic damage, trabecular and cortical damages) [1]. The consequence of these disorders are fractures, most commonly on the dorsal spine, hip and distal part of forearm [2]. Risk of spine fractures on women is 20%, of hip fracture 18% and 15% fracture of the distal forearm. Mortality is not negligible, especially in case of hip fractures, because approximately 20% of patients older than 50 years die in the first year after the occurrence of fractures [3]..

Early diagnosis of osteoporosis is possible only by measuring bone mineral density (BMD-Bone Mineral Density), and recommended method is based on the application of low-energy X rays (dual energy X-ray absorptiometry DXA), which measures bone density in the vertebral bodies and femoral neck. Normal bone density finding shows 1 standard deviation (SD) lower in comparison to the average maximum bone mass of young, healthy women aged between 20 and 30 (T score).

Osteopenia is defined as a bone density that is 1-2,5 SD (10-25%) lower than average maximum bone mass of young, healthy women aged between 20 and 30. (T score).

Osteoporosis is defined as a bone density that is 2, 5 SD (25%) lower than maximum bone mass of young, healthy women.

Osteoporosis prevention and treatment agents are classified in 2 groups:

1. Antiresorptive agents (hormonal therapy – HRT, bisphosphonates, selective estradiol receptor modulators, calcitonin, estrogen replacement therapy) working to prevent further loss of bone mass
2. Anabolic agents (made on the basis of parathyroid hormone) working to enhance the construction of bone matrix and stimulate bone anabolism

Bone biomarkers or in other words bone changes markers are bone metabolism indicators originating from the bone marrow and bone cells. In addition to the diagnosis of osteoporosis history, risk factor assessment, clinical examination and certain laboratory studies, the most important is to determine the mass, more precisely bone density and to assess bone metabolic turnover [4]. Information about the dynamic state of bone metabolism could also indicate early pathological changes in

the bones and the risk of some diseases of the locomotors apparatus. Measuring the concentration of bone markers provides more reliable information about response to therapy in relation to the measurement of bone density. Significant changes in value of bone markers can be detected quickly, three months after the introduction of therapy, while the change of bone density can be estimated one year after the introduction of therapy [5, 6].

1. Biochemical markers of bone metabolic turnover are molecules that are released during bone development and degradation or represent enzymes or active osteoblasts. Biochemical markers of bone can be classified into two groups:
2. Biochemical markers of bone formation (total and bone-specific alkaline phosphatase, osteocalcin, carboxyl -terminal propeptide of type I collagen, amino-terminal propeptide of type I collagen)

Biochemical markers of bone resorption (tartrate-resistant acid phosphatase and free piridinolin and deoksipiridinolin, N-or C-terminal telopeptide of type I collagen (CTX and NTX I).

For this work osteocalcin was used as biochemical marker of bone turnover. Osteocalcin is a marker of bone changes, which as a bone-specific protein binds calcium and is released during bone resorption and formation. Osteocalcin is a small peptide consisted of 49 amino acids, also known as bone glutamic acid protein (Gla) because it contains three residues of y-carboglutamic acid. By the effects of vitamine K these residues of glutamic acid are carboxylated to gamma-translational position of the remains. Osteocalcin is primarly synthetisized by osteoblasts, odontoblasts and hypertrophic hondrociti. After synthesis osteocalcin releases and builts into the extracellular matrix (> 80%). One part (10-30%) of synthetisiezed osteocalcin is released into the blood flow system where its concentration can be measured by immunochemical methods [7].

In 2004. National Committee for Clinical Laboratory Standards (NCCLS, USA, www. NCCLS. org) published The guidelines for the use of bone biomarkers. Recommendations for the use of bone biomarkers are following:

– The identification of individuals with increased bone metabolism
– Risk assesmenet of bone fracture in postmenopausal women
– in assessing response to treatment of patients with osteoporosis or assesing risk of osteoporosis in case when treatment can be carried out by using anti-resorptive or anabolic agents

The objective of this research was to determine if tibolon and HRT have impact on bone metabolic turnover in postmenopausal women with decreased bone marrow density.

Methods and materials

This prospective clinical study included 120 postmenopausal patients with lower bone mineral density (osteopenia) who were under the treatment of tibolone and hormone replacement therapy. The study also included 40 women (control group) in postmenopausal period with reduced bone density who were not treated by any kind of therapy.

- 1st group consisted of 40 patients treated with estrogen replacement steroid (tibolone 2,5 mg) – 1 pill once a day
- 2nd group consisted of 40 patients treated by HRT (2mg estradiol and 1mg noretisteron acetate) – 1 pill once a day
- 3rd group consisted of 40 patients (control group) in postmenopausal with normal value of bone mineral density

Selection criteria for participation in the research study are:

1. Natural or surgical (hysterectomy) postmenopause (one year after the last menstrual period to five years after menopause)
2. Low bone mineral density (T<1) diagnosis confirmed after dual energy x-ray absorptiometry (DXA)

Venous blood samples for determination of bone markers were performed early in the morning (between 7h and 8 h), after avoiding night eating and before taking therapy. Considering the fact bone markers can provide information about success of therapy very quickly, 3 months after introduction of therapy, measuring value of serum bone markers was repeated at all patients.

Results

Osteocalcin value has been measured in all patients before and 3 months after the treatment. Descriptive startistics for osteocalacin before the therapy in groups and in all patients is shown in the Table 1.

Table 1.Ostecalcin value before therapy

GROUP	N	Average value	Minimum	Maximum	SD
Tibolone	40	29.66	20	39	3.343
HRT	40	29.91	21	37	3.257
Control	40	17.84	11	23	4.003
Total	120	26.86	11	39	6.175

The table shows that the average value of ostecalcin before the treatment in patients treated with tibolone and HRT is very similiar, while control group has significantly lower average value of osteocalcin than the other groups. After data analysis according ANOVA test, it has been determined that statisticaly there were significant differencies among groups in regard to the average value of osteocalcin before the treatment (F = 131. 436; p = 0. 000).

Additional anlysis by Bonferroni test showed that the difference is statistically significant only between control group and other groups. Then 3 months after the treatment osteocalcin has been analized and descriptive statistics results of osteocalcin within each group and in all patients together as well are shown in Table 2.

Table 2. Osteocalcin value 3 months after the treatment

GROUP	N	Average value	Minimum	Maximum	SD
Tibolone	40	26.32	21	31	3.312
HRT	40	27.63	20	35	3.193
Control	40	18.98	12	24	3.495
Total	120	25.25	12	35	4.821

The table shows that osteocalcin value in both groups is similar, approximately 27ng/mL, while osteocalcin value in control group is much lower in comparison to the other 2 groups. After data analysis according ANOVA test, it has been determined that statisticaly there were significant differencies among groups in regard to the average value of osteocalcin 3 months after the treatment (F = 73. 550 and p = 0. 000).

Additional anlysis by Bonferroni test showed that the difference is statistically significant only between control group and other groups. All diferencies are statistically significant. Then the impact of group membership on osteocalcin change has been anlized. In fact, statistical test examined whether the different treatment affect changing values of osteocalcin 3 months after starting the treatment. This analysis was performed according ANOVA test and repeated measurement results are shown in Table 3.

Table 3. ANOVA – osteocalcin results after repeated measuremets

Greenhouse – Geisser

	F	p=	Ета^2
osteocalcin	50.679	.000	.323
*osteocalcin * group*	15.184	.000	.301

Results of ANOVA test shows statistically high differencies of average osteocalcin value before and after the treatment in all patients and that the difference in terms of quantity is small but not negligible. Also, it was determined that there was statistically high effect on changes of osteocalcin value depending of treatment type (group), and that effect in terms of quantity is low, but not negligible.

Further analysis examined changes of osteocalcin with each of the groups. Test results of subsequent tests are shown in table 4.

Table 4. Subsequent tests of osteocalcin changes by groups

GRUPA	Average difference before-after	SE	p=	95% IP za razliku	
				Lower limit	Upper limit
Tibolone	3.34(*)	.455	.000	2.494	4.299
HRT	2.30(*)	.455	.000	1.469	3.274
Control	-1.14(*)	.558	.023	-2.391	-.181

The table shows that all differencies are statistically significant, with the greates difference of osteocalcin average value in group of patients treated by tibolone, while the smallest changes are registered in the control group.

Discussion

The study shows analyzed results of clinical application of HRT and tibolone in the treatment of postmenopausal women with osteoporosis. Determination of parameters of bone metabolic activity, which indicates the degree of bone formation (osteocalcin), were used for the assessment of the therapy effects. The key point of our research study was monitoring results of this parameter during the treatment and comparison with results measured before the therapy. Our results showed significant effectiveness of HRT and tibolone on bone resorption, which was highly statistically significant. According to our results, this effect was registered 3 months after introduction of therapy. This fact is very important for doctors since monitoring of this metabolic parameters can quickly provide data necessary to predict the final desired treatement effect in terms of increasing the bone mass.

Results also show the marker values of the control group, but since the markers themselves are exclusively used as indicators of the therapy effects, this group is shown only for comparison with other groups.

The average value of osteocalcin in our study was the lowest in the control group, while other groups of patients had approximately equal average values. Applied ANOVA test showed that the difference in average values is statistically significant, but subsequent testing showed significant difference only between control group and other groups.

Analysis of osteocalcin average values 3 months after strating therapy showed that the average values of bone markers are very similar within the treated groups, exluding the control group, where the average value was significantly lower than in other groups. By analysis of variance it was determined that there is a significant difference, but that difference applies only to the control and other groups. Since it was mentioned before that the control group was used only in order to compare results, it could be concluded that 3 months after, value of osteocalcin within the groups was not significantly different, as well as it was the case at the beginning of the therapy. However, interest point for this study was to determine the change of osteocalcin within each of the groups. Previous analysis showed that the average values of osteocalcin before the treatment and after the treatment were approximately equal within each group, but the key question is if the changes of osteoklacina within each group was whether positive or negative and if it was statistically significant. By analysis of variance of the repeated measurements it was determined that there is statistically significant effect of therapy application on osteocalcin changes. During analysis of osteocalcin average difference within each of the groups, it was found that the greatest difference was registered in the patients treated with tibolone and then in the patients treated with HRT. Changes of osteocalcin average value in the control group were not considered and served only for comparison purposes. Many studies indicate that decrease of osteocalcin is expected in the case of efficient therapy.

The Study of Fenkci V and his associates [8] which monitored the effects of tibolone therapy on postmenopausal women indicates that the three-month tibolone treatment reduces the concentration of serum osteocalcin by 50%. It is important to mention that this study included women without osteoporosis and thus the effect of the treatment was significantly higher within shorter period. The study of Rymer J and his associates [9] included 110 women in the early postmenopausal period (6-36 months after the last menstrual period) without osteoporosis or ostepenia diagnosed and they were monitored during 8 years. Two groups were followed up, group of women treated with Tibolone and control group of women (without any kind of therapy). During 8 year monitoring period, it was determined that average values of osteocalcin varied, but there was not significant variations. However, the values of osteocalcin were lower in group of women treated with tibolone than in the control group.

The reason for this is small change of bone mineral density (BMD) in women who were on tibolone therapy, while the control group recorded continuous decerease of BMD. Based on the results of this study we can conclude that tibolone therapy is very effective even with the healthy women and has a protective effect on bones. The

same conclusion was made by Gambacciani M and his associates [10], indicating that tibolone decreases average values of osteocalcin even with the healthy postmenopausal women. The research study Ederven AG and HJ Klosterboer [11], performed on the experimental animals showed that ovariectomy rats who received tibolone had much better BMD values in comparison to the control group, and that the decrease of osteocalcin was higher in animals that received tibolone.

Another study examing the effect of tibolone in early postmenopausal women [12] indicates that in addition to the protective effect on the bone, this drug has a favorable effect on some cardiovascular risk factors, such as lipids in the blood. Specifically, this effect is reflected in the decrease of the total cholesterol, triglycerides and Lp (a) in postmenopausal women, so in addition to effects on bone, this medicament has a positive effect on the cardiovascular system.

The study of Delmas PD and his associates l [13] which covered postmenopausal women with osteopenia, indicates that 12 weeks after the starting therapy osteocalcin value significantly decreased by 17,8%. In our study the decrease of osteocalcin in 12th week amounts 11,3%, and the main reason for this difference lies in the fact that state of bones in our study was worse than in Study of Delmas and his associates.

Our research results regarding the effect of tibolone on osteocalcin are matching with results of other studies, which means that there is a positive effect of tibolone on bone metabolism in postmenopausal women with osteopenia.

Many studies also indicate positive effect of HRT on bone metabolism. Monitoring of bone turnover marker within certain period is the best indicator of bone metabolism state during application of specific therapy. The Study of Tuppurainen and his associates [14] which monitored women with osteopenia during 5 years, shows that Kliogest reduces osteocalcin in menopausal women with osteopenia by 55%, while the combination of Kilogest and Clodronate reduces ostecalcin approximately by 70%. The Clodronate has similiar effect as Kilogest and reduces osteocalcin by 54%. Considering the fact that our study was related to the shorter period, we could not expect such a great reduce of osteocalcin, but our results clearly shows that there was a significant decrease of bone markers after 3 months. The study of Arrenbrecht S and his associates [15] which monitored postmenopausal women indicates that decrease of osteocalcin average value in the 1st year is significantly higer in women treated by HRT than in women receiving placebo treatment.

The Study of Albrecht W. E. and his associates [16] also confirms positive effects of HRT on bone metabolism and opens a new question if the conventional HRT therapy should be replaced with longlasting therapy. Warming L. And his associates examined the effect of hormon therapy on bone density and metabolism in postmenopausal women with osteopenia.

The results of this study indicate that in postmenopausal women with osteoporosis, HRT has a positive effect on bone metabolism and bone mineral density. There is

a significant difference in the decrease of osteocalcin average values and other bone markers within the group of patients treated with HRT in relation to placebo treatment, where the values of osteocalcin recorded a slight increase. Comparing these results with our study, the control group also recorded a slight increase in the average value of osteocalcin, while the group of patients treated with HRT recorded decrease of osteocalcin after 3 months.

Conclusion

Since the Tibolone and HRT are considered as antiresorptive therapy, they significantly reduce the level of bone resorption in postmenopausal women with osteopenia. This kind of effect of Tibolone and HRT may be considered as favourable metabolic process for the bone mass increase and reduction of fracture risk. The effect of Tibolone and HRT on bone metabolism could be registered 3 months after introduction of therapy. Monitoring parameters of metabolic activity is very usefull diagnostic tool in assesing the effect of the antiresorptive therapy on bone metabolic activity, as well as in assesing the final outcome on bone mass.