

**BONE TURNOVER MARKERS RELATIONS TO POSTMENOPAUSAL
OSTEOPOROSIS**ODNOSI IZMEĐU MARKERA KOŠTANOG PROMETA
I POSTMENOPAUZALNE OSTEOPOROZE

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Summary: Osteoporosis is a systemic disease characterized by low bone mass and high bone turnover markers in postmenopausal women (PM). The relationship between biochemical bone markers C-telopeptides of type 1 collagen (CTX) and osteocalcin (OC), and bone mineral density (BMD) in the postmenopausal period was examined in 104 PM women divided into three groups according to their BMD: group A – control PM with normal bone density, group B – osteopenic PM and group C – osteoporotic PM. Mean CTX values were highest in group C (0.54 ± 0.24 ng/mL) compared to group B (0.44 ± 0.21 ng/mL) ($p < 0.0001$), and group A (0.33 ± 0.13 ng/mL) ($p < 0.029$). Mean OC levels in group C (26.83 ± 9.91 ng/mL) were significantly higher compared to group A (20.47 ± 7.03 ng/mL) ($p < 0.011$) but not significantly higher compared to group B (24.11 ± 8.38 ng/mL) ($p > 0.05$). Postmenopausal duration was longest in group C (13.1 ± 8.31 yrs) compared to group B (9.6 ± 6.24 yrs), and group A (8.15 ± 6.86 yrs). Postmenopausal women developed osteoporosis with longer menopause duration. PM osteoporotic women were characterized by increased levels of bone turnover markers indicating increased rate of bone remodeling, which resulted in excessive bone resorption, and loss of bone mass. Long-term persistence of high bone resorption marker CTX, insufficiently compensated with bone formation marker OC, enabled osteoporosis development.

Keywords: postmenopausal osteoporosis, CTX, osteocalcin, bone turnover

Kratak sadržaj: Osteoporoza je sistemska bolest koju karakteriše nizak nivo markera koštane mase i visok nivo markera koštanoog prometa kod žena u postmenopauzi (PM). Odnos između biohemijskih koštanih markera, C-telopeptida kolagena tipa 1 (CTX) i osteokalcina (OC), i mineralne gustine kostiju (BMD) u periodu postmenopauze ispitan je kod 104 žene u PM koje su prema BMD podeljene u tri grupe: grupa A – kontrolna grupa žena u PM sa normalnom gustinom kostiju, grupa B – žene u PM sa osteopenijom, i grupa C – žene u PM sa osteoporozom. Prosečne vrednosti CTX bile su najviše u grupi C ($0,54 \pm 0,24$ ng/mL), u poređenju sa grupom B ($0,44 \pm 0,21$ ng/mL) ($p < 0,0001$) i grupom A ($0,33 \pm 0,13$ ng/mL) ($p < 0,029$). Srednje vrednosti OC bile su značajno više u grupi C ($26,83 \pm 9,91$ ng/mL) u poređenju sa grupom A ($20,47 \pm 7,03$ ng/mL) ($p < 0,011$), ali ne i značajno više u poređenju sa grupom B ($24,11 \pm 8,38$ ng/mL) ($p > 0,05$). Trajanje postmenopauze bilo je najduže u grupi C ($13,1 \pm 8,31$ god.) u poređenju sa grupom B ($9,6 \pm 6,24$ god.) i grupom A ($8,15 \pm 6,86$ god.). Žene u postmenopauzi sa dužim trajanjem menopauze dobile su osteoporozu. Žene u PM sa osteoporozom imale su povišene nivoe markera koštanoog prometa što je ukazalo na povišenu stopu koštanoog remodelovanja, čiji je rezultat preterana koštana resorpcija, i gubitak koštane mase. Dugotrajno prisustvo visokih nivoe markera koštane resorpcije CTX, uz nedovoljnu kompenzaciju od strane markera za formiranje kostiju OC, omogućilo je razvoj osteoporoze.

Ključne reči: postmenopausalna osteoporoza, CTX, osteocalcin, koštani promet

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Introduction

Osteoporosis is a systemic disease characterized by low bone mass and increased fracture risk in postmenopausal women. Menopause accelerates the continuous bone loss determined by age (1). Bioche-

mical markers of bone turnover in blood and urine reflect the relative activity of osteoblasts and osteoclasts that are produced or released during bone remodeling (2). The values of all bone turnover markers gradually increase with age, but significantly higher values are obtained in groups of postmenopausal osteoporotic women (3). Menopause induces a 37–52% increase in bone markers formation and 79–97% increase of the markers of bone resorption (4). Higher increase of the markers of bone resorption is a risk factor for osteoporosis in postmenopausal women (5, 6). A high rate of bone turnover is associated with low BMD (bone mineral density) and is strongly linked to fracture risk. Significant negative correlation is found between CTX and BMD in osteoporosis. Bone formation and resorption markers may also be useful as early indicators of response to therapy (7, 8).

The main purpose of this trial was to determine the relationship between biochemical bone markers CTX and osteocalcin, and bone density in postmenopausal women as well as their relationship with menopausal duration. They were estimated in osteoporotic and osteopenic postmenopausal women and compared to postmenopausal women with normal bone density.

Materials and Methods

Lumbar spine and femoral neck BMD were measured by dual energy X-ray absorptiometry (DXA). Osteoporosis was diagnosed by T-scores ≤ -2.5 and osteopenia was diagnosed by T-scores between -2.5 and -1 .

Bone turnover markers were determined in relation to the bone density of 104 postmenopausal women with mean age 58.65 ± 7.03 yrs. and 11.05 ± 7.59 yrs. postmenopausal duration, divided into three groups: group A consisted of 18 control postmenopausal women with normal bone density; group B consisted of 38 postmenopausal women with osteopenia and group C consisted of 48 postmenopausal women with osteoporosis.

Body mass was determined and expressed in kg. Body mass index (BMI) was calculated as the ratio of body mass and body height (kg/m^2).

The following analyses were performed: β -Cross-Laps, meaning C-telopeptides of type 1 collagen (CTX) as a bone resorption marker and N-MID osteocalcin (OC) as a bone formation marker, both expressed in ng/mL . Their relationship was also assessed. The upper limit of the normal CTX value for healthy young women is $0.299 \text{ ng}/\text{mL}$ and it is $0.556 \text{ ng}/\text{mL}$ for postmenopausal women. Normal O values for PM women are $15\text{--}46 \text{ ng}/\text{mL}$. Bone turnover markers levels were determined by the immunoanalytical in vitro method for quantitative determination ECLIA,

ECL-technology on a Roche Elecsys/2010 analyzer, in the Institute of Biochemistry. Fasting morning blood samples were collected at 8 am.

Statistical analysis was performed by linear correlation (Pearson) and Spearman rank correlation, unifactorial analysis of variance ANOVA, and non-parametric tests Kruskal Wallis and Mann Whitney – U-test of inversion.

Results

Mean CTX values in group A were $0.33 \pm 0.13 \text{ ng}/\text{mL}$, in group B $0.44 \pm 0.21 \text{ ng}/\text{mL}$, and in group C $0.54 \pm 0.24 \text{ ng}/\text{mL}$, and they were significantly different among the groups ($p < 0.001$) (Figure 1). CTX levels in group C were significantly higher compared to group A ($p < 0.0001$), and compared to group B ($p < 0.029$).

Mean OC levels in group A were $20.47 \pm 7.03 \text{ ng}/\text{mL}$, in group B $24.11 \pm 8.38 \text{ ng}/\text{mL}$ and in group C $26.83 \pm 9.91 \text{ ng}/\text{mL}$, and were significantly different among the groups ($p < 0.035$) (Figure 2). OC levels in group C were higher compared to group A ($p < 0.011$), but not significantly different compared

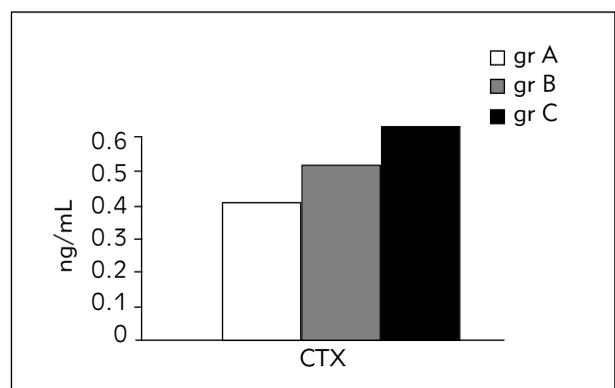


Figure 1 CTX levels in control group A, osteopenic group B and osteoporotic group C.

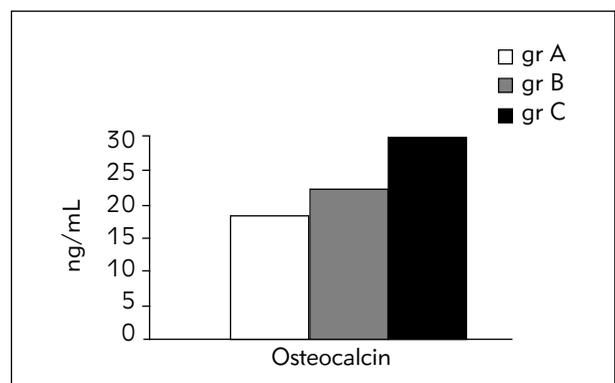


Figure 2 Osteocalcin levels in control group A, osteopenic group B and osteoporotic group C.

to group B ($p > 0.05$). CTX levels correlated significantly with OC levels ($p < 0.0001$) in a highly positive manner.

Postmenopausal women showed significantly different values according to their age ($p < 0.006$). Postmenopausal women in group A were the youngest (54.84 ± 7.75 yrs.), in group B they were older (57.86 ± 6.51 yrs.) and in group C the oldest (60.71 ± 6.53 yrs.). Menopausal duration was significantly different between the groups ($p < 0.027$). It was shortest in group A (8.15 ± 6.86 yrs.), longer in group B (9.6 ± 6.24 yrs.) and longest in group C (13.1 ± 8.31 yrs.).

Mean body weight in group A was 74.46 ± 9.1 kg, in group B 73.32 ± 11.68 kg and in group C it was 65.86 ± 10.25 kg. It was significantly different among the groups ($p < 0.001$). BMI in group A was 29.51 ± 4.36 kg/m², in group B 29.1 ± 4.82 kg/m², in group C 26.69 ± 3.65 kg/m², and it was significantly different among the groups ($p < 0.011$).

Discussion

Osteoporosis is a serious and common chronic condition with very high associated healthcare costs, and is characterized by low bone mass and micro-architectural deterioration of the skeleton leading to enhanced bone fragility and an increased risk of fracture (9). Osteoporosis affects many women after menopause. Its prevalence is especially high among elderly postmenopausal women (10).

The bone resorption and bone remodeling are under tight endocrine control (11). Fertile women have lower CTX values compared to PM women, as well as premenopausal women compared to postmenopausal. In fertile women bone resorption and formation are balanced and there is no bone loss. Postmenopausal osteoporosis is a result of estrogen deficiency, with increased levels of bone turnover markers and increased rate of bone remodeling, excessive bone resorption, and loss of bone mass (12).

Population studies have shown that about 3–5% of perimenopausal women already have osteoporosis (13). In a prospective study Rosenbrock (14) showed that CTX and OC were significantly increased already in the transition period from peri- to postmenopause. Biochemical markers of bone turnover may predict bone loss in women undergoing menopausal transition (15), and may be useful to identify those women losing bone (16, 17), in order to start the treatment on time.

Assessing bone marker levels may be useful in the evaluation of osteoporotic risk (4), and can be used clinically to predict future BMD in postmenopausal women (18, 19). The prediction of bone loss and the risk of fracture in elderly women is improved by using multiple rather than single measurements of bone markers (20). A great number of

studies confirmed lower BMD associated with advanced age, especially in the menopausal period (1). Postmenopausal hip fracture patients show biochemical evidence of decreased bone formation and increased bone resorption compared with healthy postmenopausal subjects (21). Risk of osteoporosis should be determined by a combination of measures of BMD (bone mineral density) and the level of bone markers in each person in early postmenopausal years in order to start the protective treatment and to achieve reduction of the numerous fractures (22).

Many studies confirmed highest bone marker levels and bone resorption during menopause (23). By using ROC curve analysis, very high specificity and sensitivity of the investigated biochemical bone markers CTX and OC in the diagnosis of postmenopausal osteoporosis were proven (4). Increased bone markers by 67% were discovered in women in early menopause compared to premenopausal women (24). CTX determined by the same method Elecsys, ECLIA in the study of Garnero (25) was higher by 39% in perimenopausal women in comparison to premenopausal women and higher by 86% in postmenopausal women. Basal CTX levels correlated negatively with the bone mass changes measured on the middle and distal radius. OC had a high correlation with CTX (18), the same as in our study. In our previous study almost 50% of the examined postmenopausal women had increased CTX levels, and the percentage of women with increased bone turnover markers and risk of osteoporosis increased with age and menopause duration compared to fertile women. OC was not as high as CTX, confirming a disturbed balance with higher bone resorption than bone formation, indicating an increased rate of bone remodeling, and higher negative bone turnover in the postmenopausal period that enabled increased bone mass loss (26, 27).

CTX levels in the osteoporotic group C in our study were significantly higher compared to osteopenic group B, and were highly significantly higher compared to the control postmenopausal group A without osteopenia and osteoporosis. OC levels in the osteoporotic group C were not significantly higher compared to the osteopenic group B, but were higher only compared to the control postmenopausal group A. It indicates an insufficient OC increase compared to CTX increase in osteoporotic women. A conclusion could be made that osteoporosis in group C was a result of a significant increase of CTX and bone resorption and the insufficient increase of osteocalcin levels, indicating insufficient bone formation. Higher bone loss than bone formation enabled the consequent increased bone turnover, osteoporosis development and increased fracture risk.

Osteoporosis as a result of the disturbed balance between osseal resorption and formation was confirmed with a highly significant increase of the bone

resorption marker but not significant increase of the bone formation marker. Postmenopausal osteoporotic women in group C who had higher bone resorption markers compared to bone formation markers resulting in bone loss and osteoporosis development, were characterized by significantly longer menopausal duration. Long-term persistence of high bone resorption marker CTX levels enabled osteoporosis development. All three groups of PM women were overweight, but that did not influence the bone marker values.

The Elecsys beta-CrossLaps serum assay provides a potentially useful tool for assessing bone resorption state, including its response to estrogen replacement therapy (28). This study also confirmed

the automatized assay for quantitative determination of CTX and OC, Elecsys, ECLIA as very precise in predicting the level of bone loss risk and fracture risk in PM women, and treatment follow-up. Serial measurements of CTX may provide a useful diagnostic tool for the early revelation of suboptimal dosing or noncompliance to already optimized therapies with antiresorptive agents (29).

The results of this study led to a conclusion that CTX and OC measurements are useful and non-invasive methods for determining bone remodeling in postmenopausal women, and are also predictors of osteoporosis development that therefore indicate the need for early preventive and therapeutical measures to ward off osteoporosis and its complications.

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