THE USE OF BIOCHEMICAL MARKERS OF BONE TURNOVER IN THE DIAGNOSIS OF POSTMENOPAUSAL OSTEOPOROSIS

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ABSTRACT

Osteoporosis is a systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture. Postmenopausal women due to the various hormonal factors are at increased risk. The aim of this study was to assess bone turnover using biochemical markers, such as beta crosslap (\(\beta\)-CTX) and osteocalcin (OC) and determine their relationship with bone density and age in postmenopausal women. A total of 80 postmenopausal Albanian women participated in the study. Subjects were divided into three groups according to their bone mineral density (BMD): group A – control with normal bone density, group B – osteopenia and group C – osteoporosis. All subjects completed a questionnaire on lifestyle factors. Height and weight were measured. Bone density was scanned using Quantitative Ultrasound (QUS). Serum samples were collected and beta crosslaps and osteocalcin levels were measured by electrochemiluminescence (ECL) using Elecsys 2010. A positive correlation was found between age and bone turnover markers osteocalcin (r=0.316, p=0.004) and \(\beta\)-CTX (r=0.281 p=0.012). \(\beta\)-CTX levels correlated significantly with OC levels (r=0.732 and p<0.0001) in a highly positive manner. Postmenopausal 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 \(\beta\)-CTX, insufficiently compensated with bone formation marker OC, enabled osteoporosis development.

KEYWORDS: Bone Turnover Markers, Osteocalcin, Beta Crosslaps, Osteoporosis, Postmenopausal Women

INTRODUCTION

Osteoporosis is defined as a skeletal disorder characterized by compromised bone strength, which predisposes the patient to develop fractures [Brown & Josse, 2002]. Postmenopausal women due to the various hormonal factors are at increased risk. Bone loss occurs in
postmenopausal women as a result of an increase in the rate of bone turnover and an imbalance between the activity of the osteoclasts and osteoblasts [Khosla & Riggs, 2005]. Bone mineral density (BMD) measurements are gold standard in calculating bone mass, the changes are usually late and the damage is irreversible. Biochemical markers of bone turnover may be of value for prediction of individual bone loss and can also be used to provide information about future bone loss of that individual [Lofman et al., 2005]. Combined use of biochemical markers and BMD screening may provide a better prediction of osteoporosis than BMD measurements alone [Melton et al., 1997]. Biochemical markers of bone turnover in blood and urine reflect the relative activity of osteoblasts and osteoclasts that are produced or released during bone remodeling [Allende-Vigo, 2007]. Bone markers are usually classified as markers of bone formation –serum alkaline phosphatase (ALP), osteocalcin (OC) and procollagen type-I extension Peptides (PINP) and markers of bone resorption are urinary hydroxylysine glycosides, hydroxyproline, plasma tartrate resistant acid phosphatase (TRAP) and collagen pyridinium cross-links (NTX and CTX). [Eastell, 2009]. Osteocalcin is a small specific bone matrix protein produced primarily by osteoblastic cells during the late phases of bone formation [Hauschka et al., 1989]. The structure of osteocalcin is characterized by three Gla-residues which provide the protein with a high affinity to bone hydroxyapatite [Hoang et al., 2003]. Although osteocalcin is primarily deposited into the developing bone matrix, a small amount of it enters the circulation where it can be detected by immunoassays [Price et al., 1980; Brown et al., 1984]. The epitope for the CTX assay is an eight-amino-acid peptide EKAHDGGR located at the C-terminal segment of collagen chain and produced by the action of osteoclastic enzyme cathepsin K during bone resorption [Garnero et al., 2003]. Asp (D) residue in CTX can undergo isomerization (α/β) and racemization (L/D) giving rise to four forms of CTX detected in serum: αL, αD, βL, and βD [Cloos & Fledeius, 2000]. Especially relevant collagen type I fragments are the β-isomerized C-terminal telopeptides (β-CTX). These isomerized telopeptides are highly specific for the degradation of type I collagen dominant in bone.

The aim of this study was the assessment of biochemical markers of bone turnover, such as beta crosslap and osteocalcin and determine their relationship with bone density and age in postmenopausal women.

MATERIALS AND METHOD
To evaluate the serum levels of osteocalcin and beta crosslaps (β-CTX) we studied 80 postmenopausal women. Subjects were divided into three groups according to their bone mineral
density (BMD) : group A – control with normal bone density, group B – osteopenia and group C – osteoporosis. Their mean age was 57 years (44-74) years. Height, weight, BMI, age at menarche, years since menopause in case of postmenopausal women, history of disease, and fracture, if any, were recorded. BMD assessment was done using Quantitative Ultrasound (QUS) and T-scores were calculated. According to World Health Organization (WHO) diagnostic guidelines:

• T-score -1.0 or greater is “Normal”.
• T-score between -1.0 and -2.5 is “Osteopenia”.
• T-score -2.5 or below is “Osteoporosis”.

Serum osteocalcin (OC) and beta crosslaps (β-CTX) levels were measured on the fasting sample. Blood was collected by Vein puncture in the morning and serum was separated by centrifugation (10 minutes at 3000 rpm / rotate for minute). Serums were stored at -20°C until analysis. We use the electrochemiluminescence assay (ECL) on Elecsys 2010 from Roche Diagnostics, which is a sandwich immunoassay. Normal values for postmenopausal women are: 15–46 ng/ml for osteocalcin, and 0.112–1.018 ng/ml for β-CTX Statistical analysis was performed using one-way analysis of variance, Pearson correlation and student’s unpaired t-test. For the statistical analysis we used SPSS. 19 programm. Significance limits was p<0.05.

RESULTS AND DISCUSSION

A total of 80 postmenopausal women, aged 44–74, with no menses for at least 12 months were recruited. Mean age was 57 ± 6.54 years. Group A consisted of 11 control postmenopausal women with normal bone density; group B consisted of 55 postmenopausal women with osteopenia and group C consisted of 14 postmenopausal women with osteoporosis. Mean serum of osteocalcin and β-CTX in postmenopausal women were 22.7 ng/ml ±11.7 and 0.336 ng/ml ±0.226 respectively. (Table 1)

Table 1. Descriptive Statistics

<table>
<thead>
<tr>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>80</td>
<td>44.00</td>
<td>74.00</td>
<td>57.437</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>80</td>
<td>144.00</td>
<td>175.00</td>
<td>159.587</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>80</td>
<td>45.00</td>
<td>102.00</td>
<td>71.385</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>80</td>
<td>18.15</td>
<td>39.43</td>
<td>28.068</td>
</tr>
<tr>
<td>BMD (T-score)</td>
<td>80</td>
<td>-3.00</td>
<td>-0.10</td>
<td>-1.813</td>
</tr>
<tr>
<td>Calcium (ng/ml)</td>
<td>80</td>
<td>8.10</td>
<td>10.10</td>
<td>9.051</td>
</tr>
<tr>
<td>β-CTX (ng/ml)</td>
<td>80</td>
<td>0.02</td>
<td>1.28</td>
<td>0.336</td>
</tr>
<tr>
<td>Osteocalcin (ng/ml)</td>
<td>80</td>
<td>6.26</td>
<td>63.61</td>
<td>22.795</td>
</tr>
</tbody>
</table>
β-CTX levels correlated significantly with OC levels ($r=0.732$ and $p<0.0001$) in a highly positive manner. Elevated serum concentration of β-CTX was accompanied by elevated OC serum concentrations and vice versa.

Mean β-CTX values in group A were $0.31\pm0.18$ ng/mL, in group B $0.32\pm0.18$ ng/mL, and in group C $0.43\pm0.36$ ng/mL. (Figure 1). β-CTX levels in group C were significantly higher compared to group A, and compared to group B ($p=0.006$). But β-CTX levels in group A were not significantly different from β-CTX levels of group B ($p>0.05$)

Figure 1. Beta crosslap levels in normal (Gr. A), osteopenic (Gr. B) and osteoporotic (Gr.C) postmenopausal women.

Mean OC levels in group A were $17.25 \pm 5.16$ ng/mL, in group B $22.64 \pm 10.97$ ng/mL and in group C $27.73 \pm 16.15$ ng/mL, and were significantly different among the groups ($p<0.05$) (Figure 2). OC levels in group C were higher compared to group A ($p=0.014$). And also OC levels in group B were higher compared to group A ($p=0.005$). Postmenopausal osteoporotic women were characterized by increased levels of bone turnover markers indicating increased rate of bone remodeling.

OC levels in osteoporosis group increase with 18.3% and β-CTX levels with 25.2%. It indicates an insufficient OC increase compared to β-CTX increase in osteoporotic women. A conclusion could be made that osteoporosis in group C was a result of a significant increase of β-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.
Using one way analysis of variance we found that postmenopausal women showed significantly different values according to their age ($F=7.63$, df=$[2, 77]$, $p<0.001$). Postmenopausal women in group A were the youngest (54.18±7.67 years.), in group B they were older (56.72 ± 5.70 years.) and in group C the oldest (62.78±5.57 years.). BMD generally decreased with age. Using linear regression analysis with Pearson correlation coefficients we found a negative correlation between age and BMD ($r = -0.388$, $p=0.004$). As the age increases, osteoporotic cases increase, especially after 60 years, this is consistent with other studies where osteoporosis is prevalent in women after 60. Several reasons were reported age as a risk factor due to estrogen deficiency after menopause [Kanis et al., 2000; McNamara, 2010; Li et al., 2010]. Also a positive correlation was found between age and bone turnover markers osteocalcin ($r=0.316$, $p=0.004$) and β-CTX ($r=0.281$ $p=0.012$). The increased bone turnover in older subjects from our study is consistent with previous studies in White, Black and Chinese women [Adachi, 1996; Kleerekoper et al., 1994; Tsai et al., 1996; Yan et al., 2002]. It is also consistent with studies conducted in both male and female subjects, in which women after menopause present much higher levels of bone markers compared with older men [Krall et al., 1997]. It can also be concluded from the present study that bone turnover, which is significantly elevated after menopause, remains at a high level over the years. [Garnero & Delmas, 1996; Guerrero et al., 1996; Han et al., 1997]. Serum levels of OC and β-CTX in the analysed population strongly depended of both bone mineral density and age. The results of this study led to a conclusion that β-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 toward off osteoporosis and its complications.
References