INTRODUCTION

Postmenopausal osteoporosis is a major healthcare problem, characterized by low bone mass and increase susceptibility to fragility fractures. It is a silent condition and may remain unnoticed for many years until a fracture occurs. Having sustained an osteoporotic fracture, increases the risk of subsequent fractures. These fractures have a considerable impact on individual health due to a significant increase in overall mortality and also in long-term morbidity due to disabilities. Developing countries like Pakistan continue to be ill-equipped to handle burden of osteoporosis.

For many years, bone mineral density (BMD) measurements have provided the customary method of assessing the risk of fracture. However, to use BMD measurement as a dynamic marker, one must wait for an observation period of 6 months to 1 year before re-measurement. In contrast, BTMs accurately reflect the state of bone metabolism at the point in time of the measurement; and offers a more dynamic, global analysis of the skeleton. Their analysis, repeated at short intervals, also allows a serial assessment of bone turnover. BTMs can be measured in blood or urine and are used in selective combinations of formation and resorption markers that express the metabolic activity of osteoblasts or osteoclasts, respectively. Along with predicting risk of fracture and bone loss, and their correlation with BMD; their other clinical application in osteoporosis is assessment of therapeutic response. The status of bone turnover in Pakistani postmenopausal women is not clearly defined and very few studies have compared osteocalcin (bone formation marker) and β-CTX (bone resorption marker). Osteocalcin (OC) produced by osteoblasts during bone formation is the most abundant non-collagenous matrix protein in bone and forms about 1% of the bone organic component. Carboxy-terminal crosslinked telopeptides (CTX) is cleaved from type 1 collagen by cathepsin-K during bone resorption, and is released into the bloodstream. Currently, β-CTX-I (an isomeric form of CTx) is perhaps the most commonly used crosslink assay. Its quantification serves as a specific marker for the degradation of mature type I collagen. The aim of this study was to determine the clinical utility of BTMs in assessment of osteoporosis in Pakistani postmenopausal females.

ORIGINAL ARTICLE

Bone Turnover Markers for Osteoporosis Status Assessment at Baseline in Postmenopausal Pakistani Females

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ABSTRACT

Objective: To assess bone turnover status in osteopenic and osteoporotic postmenopausal females.

Study Design: Cross-sectional analytical study.

Place and Duration of Study: The Aga Khan University Hospital, Karachi, from January to December 2013.

Methodology: A cross-sectional study was conducted on 203 postmenopausal females undergoing bone mineral density testing (BMD) by DXA scan. Patients with clinical history of any disorder or medications affecting bone turnover were excluded. Bone turnover was assessed with osteocalcin and β-CTX. Data was analyzed by SPSS version 19.

Results: Mean age of the participants was 54 ±4.66 years with a mean BMI of 28.7 ±5.5 kg/m². Mean β-CTX (0.28 ±0.24 ng/ml) and osteocalcin (21.5 ±10.6 ng/ml) levels were within the normal reference range. Subjects were grouped into normal (26.6%), osteopenic (44.8%), and osteoporotic (28.6%) based on the t-scores. Serum levels of osteocalcin and β-CTX between normal, osteopenic, and osteoporotic groups were not significantly different. β-CTX was negatively and significantly associated with only lumber spine BMD (r = -0.13, p=0.04). Positive association (< 0.0001) was noted between both markers in normal, osteopenic, and osteoporotic females. However, association of these markers with BMD in the 3 groups were not found. Multivariate linear regression showed a positive and significant effect of BMI on BMD (β = 0.332, p= < 0.0001). β-CTX had negative but significant effect on BMD (β = -0.155, p= 0.018) of postmenopausal women.

Conclusion: Association between baseline levels of BTM and rate of bone loss is variable and site dependent. β-CTX correlates better with BMD. However, role of osteocalcin in postmenopausal osteoporosis is uncertain and needs further investigation.

Key Words: Bone turnover markers (BTMs). Bone mineral density (BMD). Postmenopausal osteoporosis.
postmenopausal females by using both bone formation and resorption markers.

**METHODOLOGY**

This was a cross-sectional multidisciplinary study conducted at the Sections of Chemical Pathology, Department of Pathology and Laboratory Medicine; and Department of Radiology, The Aga Khan University, between January to December 2013. The study protocol was approved by the Ethics Committee of the Aga Khan Hospital, Karachi.

Two hundred and three women, up to 60 years of age with menopause for more than 1 year duration and referred for DXA scanning, were invited to participate in the study. Medical history was obtained from those who consented. Patients with any medical disorder known to affect bone turnover, such as hyperparathyroidism, hyperthyroidism, malabsorption, gastrectomy, diabetes, chronic renal failure and hypogonadism or taking any medications known to affect skeletal metabolism, such as calcium, bisphosphonates, vitamin D, steroids, chemotherapeutic drugs, thyroxine and anti-epileptics, were excluded. BMD was measured at hip, spine and femur, and in few patients also from forearm using DXA (Hologic QDR 1000 Instrument, Hologic, Waltham, MA, USA) and was expressed in g/cm², using standard protocol.

Participants were classified on the basis of T-score according to WHO criteria as normal (T-score of -1.0 or higher), osteopenic (between -1.0 and -2.5), and osteoporotic (-2.5 or lower). Any participant having osteopenia and osteoporosis, even at a single bone site, was labelled as osteopenic and osteoporotic, respectively.

Ten milliliters of blood was withdrawn from the antecubital vein in a vacutainer, using aspetic measures. Samples were centrifuged within 30 minutes at 3000 r.p.m. Required serum was stored at -80°C until assayed. Serum osteocalcin and β-CTx were measured by electrochemiluminescence immunoassay (ECLIA) on the Roche Elecsys 1010/2010 and Modular Analytics E170 (Elecsys module) immunoassay analyzers. Results were expressed as nanogram of bone turnover marker per milliliter of serum (ng/ml). Inter-assay and intra-assay variability for the serum β-CTx assays was 1.6 - 7.6% and 1 - 5.5%, respectively. The range for serum β-CTx levels in postmenopausal women is 0.104 to 1.008 ng/ml with a mean of 0.556 ng/ml. Inter-assay and intra-assay variability for serum osteocalcin is 1.1 to 6.5 % and 0.5 - 4%. The range for serum osteocalcin levels in postmenopausal women was 15 - 46 ng/ml. For quality control, low and high controls were run. All the samples were run in one batch, following standard quality protocol.

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 19 software. Mean and SD were calculated for continuous variables and frequency/percentage was calculated for categorical variables. Chi-square test was applied for distribution of categorical variables like BMI categories and age groups, and Kruskal-Wallis test for continuous variable (osteocalcin and β-CTx levels) across diagnosis categories (normal, osteopenia, and osteoporosis). Spearman Rho-correlation was computed to see the correlation between biochemical markers & BMD. For further analysis, multiple linear regression analysis was applied to find out the correlates of BMD. Initially, variables were subjected to univariate analysis taking BMD as dependent variable. Independent variables with a p-value of < 0.05 were then used in a multiple regression model using the Enter method. Results were reported for the final model as standardized beta-coefficient (β), level of significance, and 95% confidence interval.

**RESULTS**

Mean age of the study subjects was 54 ±4.6 years and mean BMI was 28.7 ±5.4 (Table I). According to Asian BMI classification, 69% of the participants were obese (n=140) and 21.2% were overweight (n=43). Mean β-CTx (0.28 ±0.24 ng/ml) and osteocalcin (21.48 ±10.62 ng/ml) levels were within the normal reference range (osteocalcin 15 - 46 ng/ml, β-CTx 0.104 - 1.008 ng/ml). Prevalence of osteopenia (44.8%) was higher than normal (26.6%) and osteoporosis (28.6%) in postmenopausal females. Both osteopenia and osteoporosis were significantly different (p=0.014) among different age group categories, the highest being in the age group 56 - 60 years (Figure 1).

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**Figure 1:** Distribution of normal, osteopenic, and osteoporotic subjects according to age groups (n=203). Chi-square test (p=0.014).

**Table I:** Bone turnover status of postmenopausal females (n=203).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal (n=54)</th>
<th>Osteopenia (n=91)</th>
<th>Osteoporosis (n=58)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-CTx</td>
<td>0.20 (0.3 - 0.11)</td>
<td>0.19 (0.26 - 0.13)</td>
<td>0.25 (0.52 - 0.13)</td>
<td>0.10</td>
</tr>
<tr>
<td>Osteocalcin</td>
<td>17.62 (23.5 - 12.8)</td>
<td>18.6 (30.17 - 14.1)</td>
<td>21.4 (29.5 - 14.2)</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Kruskal Wallis Test
Both markers were higher for women with osteoporosis than for normal and osteopenic, but the results were not significantly higher (Table I).

There was a significant positive correlation between osteocalcin and β-CTx levels. However, there was no significance of osteocalcin levels with BMD at different skeletal sites. The correlation of β-CTx levels with BMD was negative but was significantly correlated only with lumbar spine BMD (Table II).

Multivariate linear regression analysis was performed to find out correlates of BMD (Table III). There was a positive and significant effect of BMI on BMD which reflects that obese females tend to have higher BMD. β-CTx had negative and significant effect on BMD of postmenopausal women.

**DISCUSSION**

In many cases in osteoporosis, the degree of bone resorption is more prominent than formation as evaluated by BTMs. Therefore, prior to treatment of patients with a definitive osteoporosis diagnosis, the status of bone metabolism can be more clearly ascertained by simultaneous measurement of both bone formation and resorption markers.3 Relationships between BTMs and fracture risks have been investigated, first in retrospective studies comparing bone marker levels in patients with and without osteoporotic fractures, then in prospective studies in which BTMs were measured before the occurrence of fracture. The risk of fractures is 2 times higher in people who have high levels of BTM than in those with normal levels, and can rise up to 4 to 5 times as high in people who have both high marker levels and low bone density.12,13 Increased levels of BTMs, more consistently bone resorption ones, in postmenopausal women can be considered as an independent risk factor for future fracture. A meta-analysis of reference BTMs showed a significant association between s-CTx and risk of fracture, unadjusted for BMD.14

In this study, both osteocalcin and β-CTx levels were high in osteoporotic females than normal; but the difference was non-significant, though previous studies have reported significant difference for both CTx and osteocalcin levels.11,15,16 Comparing osteopenic patients to those with normal BMD, results have been very discordant between studies; and the differences studies on postmenopausal females in Pakistan.7-10

We found a significant negative correlation of BMD with age (r = -0.198, p = 0.005) which correlates well with literature as age is the most important risk for predicting low BMD; and hence, the increase risk of osteoporosis fracture. Latif et al. also reported negative correlation of BMD with age, in postmenopausal females with and without osteoporosis as compared to premenopausal.11

Majority of the study subjects were identified to be obese, based on BMI. In addition, prevalence of osteopenia and osteoporosis were significantly different among subjects. Multivariate linear regression analysis showed BMI to be a significant predictor of BMD with obese females having higher BMD. However, no association was observed between BMI and turnover markers. An increase in systemic bone turnover reflected by high BTM value is associated with future bone loss, independent of bone mass, and other osteoporosis risk factors.3

**Table II:** Correlation of biochemical markers with average BMD and BMD at different sites (n=203).

<table>
<thead>
<tr>
<th>Biochemical markers</th>
<th>Spearman's rho Correlation</th>
<th>β-CTx</th>
<th>BMD (average)</th>
<th>Lumbar Spine</th>
<th>Left hip</th>
<th>Femoral neck</th>
<th>Left forearm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteocalcin</td>
<td>Correlation coefficient</td>
<td>0.607</td>
<td>-0.116</td>
<td>-0.06</td>
<td>-0.12</td>
<td>-0.08</td>
<td>-0.01</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>&lt;0.0001</td>
<td>0.10</td>
<td>0.34</td>
<td>0.06</td>
<td>0.25</td>
<td>0.93</td>
</tr>
<tr>
<td>β-CTx</td>
<td>Correlation coefficient</td>
<td>1</td>
<td>-0.121</td>
<td>-0.13</td>
<td>-0.07</td>
<td>-0.05</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>-</td>
<td>0.086</td>
<td>0.04</td>
<td>0.31</td>
<td>0.47</td>
<td>0.81</td>
</tr>
</tbody>
</table>

**Table III:** Multiple regression model showing determinants of BMD of postmenopausal women.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Standardized β</th>
<th>p-value</th>
<th>95% Confidence Interval for β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.010</td>
<td>0.976</td>
<td>Lower boundary: Upper boundary</td>
</tr>
<tr>
<td>Duration since menopause</td>
<td>-0.154</td>
<td>0.636</td>
<td>-0.029</td>
</tr>
<tr>
<td>BMI</td>
<td>0.332</td>
<td>&lt;0.0001</td>
<td>0.006</td>
</tr>
<tr>
<td>β-CTx</td>
<td>-0.155</td>
<td>0.018</td>
<td>-0.175</td>
</tr>
</tbody>
</table>
between the levels of bone markers do not appear to be relevant.

Results of both β-CTx and osteocalcin in the present study were scattered, indicating the presence of considerable inter-individual variability. This variability is linked to the time of blood sampling due to diurnal and seasonal variation, food intake, and level of physical activity etc. The samples in this study were not taken in fasting condition, and at a specified time. These can be the reasons of the insignificant difference in the levels of both the markers in those 3 groups.

The correlations between BTM and BMD have been analyzed in many studies, with high heterogeneity. In this study, there was a correlation between levels of BTMs and BMD in postmenopausal women, with varying degrees of correlation coefficients at different skeletal regions (Table II). In general, BTMs correlate better with BMD at large skeletal sites such as the spine, than with BMD at regions such as the femoral neck and the total hip. Though even at spine, there have been large disparities between studies in terms of intensity and direction of the correlation.

The β-CTx levels in the present study were inversely and significantly correlated with BMD at lumbar spine while it was not significant at left hip and femur, as measured on DXA (Table II). For postmenopausal women, negative and significant correlations have been frequently reported between CTx and lumbar spine BMD. Multivariate linear regression analysis showed significantly negative effect of β-CTx on BMD of postmenopausal women (Table III). These findings are in accordance with other studies showing an inverse correlation between CTx and BMD, and would suggest that β-CTx assay could be of clinical utility when added to BMD measurement to better define bone status and future risk for osteoporosis-related fractures.

Serum osteocalcin did not have significant correlation with BMD and age. Conflicting results have been reported with some studies showing significant correlation. A possible explanation for these conflicting findings along with the pre-metrologic sources of variability could be the differences in methodology used for measuring osteocalcin.

Though the clinical usefulness of BTMs in the contemporary management of postmenopausal osteoporosis remains a controversial issue, correlations between BTMs and rate of bone loss becomes stronger when serial measurements of BTM are used. That is where the best established clinical use of BTMs and the control of the therapeutic efficacy, steps in. Baseline measurement, if done before commencement of anti-resorptive treatment, can be checked 3 - 6 months later to verify response and adherence to treatment. Several studies have shown that after initiating anti-resorptive therapy there is a significant decrease in both resorption (within 4 - 6 weeks), and in formation (2 - 3 months) markers. Changes in BTM levels depend on the therapeutic agent employed and the marker analyzed. Showing patient the changes in these values may increase treatment compliance. However, the optimal threshold for each marker is not well established; and long-term data when the biomarkers are reduced below the reference range is lacking.

A limitation of this study is that it was not longitudinally designed (serial measurements of BTM), which is needed to confirm the findings obtained in this study. It is difficult to perform such a study in our setting as it takes a lot of time and is very expensive.

Secondly, blood specimens were taken from subjects without strict control over sampling time or meals, i.e. not taken in fasting state, which minimizes pre-analytical variation in BTMs. It is best to measure the BTMs in as similar set of circumstances as possible.

Lastly, the reference ranges currently reported fail to account for many of the pre-analytical variables that are known to affect measures of BTMs. Therefore, it is essential to establish reference values for each BTM from a representative sample of the healthy young adult population, which will help in assessment of the bone turnover status of subjects of varying ages and conditions. It is also important for each laboratory to investigate the transferability of the quoted reference intervals to its own patient population based on equivalent standardized collection conditions.

CONCLUSION

The diagnostic value of BTMs at baseline in osteoporosis is fairly low from this study in the sample studied. But there is indeed a moderate relationship between levels of bone markers and bone loss. β-CTx can be used in the assessment of postmenopausal osteoporotic patients but the role of osteocalcin is uncertain and needs further investigation.

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REFERENCES


