Comparative study of Biochemical bone turnover markers in pre & post-menopausal women

Sri Rekha P, U Venkateswarlu, Sarada U

Abstract

Menopause is associated with adverse changes in bone turnover. When bone resorption exceeds formation, bone loss and osteoporosis will occur. This is explained by Biochemical marker of bone turnover profile. Though Bone Mineral Density (BMD) measurements are the gold standard in calculating bone mass, the changes are usually late and the damage is irreversible. Combined use of biochemical markers of bone turnover helps in early identification of women who are at increased risk of fractures.

Objective: To compare Serum Total Alkaline Phosphatase (ALP), Total Calcium, Urinary OHP in postmenopausal cases and pre-menopausal controls.

Methods: The study included 25 healthy postmenopausal women as cases, and 25 healthy Premenopausal women. Estimation of ALP was done by kinetic method Recommended by International Federation of Clinical Chemistry (IFCC), Serum total Calcium by modification of Ortho- Cresolphthalein Complexone (OCPC) method, Urinary OHP by modified Neumann and Logan method.

Results: Bone formation markers, serum total ALP was significantly increased (p<0.001) and total calcium was significantly decreased (p<0.001) in postmenopausal women compared to premenopausal women. Bone resorption marker, urinary OHP was significantly increased (p<0.001) in postmenopausal women.

Conclusion: This study concluded that, Serum total ALP, total calcium and Urinary OHP combined together provided a fairly useful index of bone resorption in postmenopausal women and these common biochemical parameters can be used to categorize postmenopausal women into rapid and slow bone losers. Preventive measures like calcium supplementation or Hormone replacement therapy can be initiated early in those who are rapid bone losers and prevent the osteoporotic fractures.

Keywords: Postmenopausal women; osteoporosis; Total Alkaline Phosphatase; Total calcium; Urinary hydroxy proline

1. Introduction

Bone is remodeled constantly throughout life. Peak bone mass is around the age of 30 years after which rate of bone resorption is greater than the rate of bone formation (0.3% to 0.5% bone loss per year). Approximately 3% of cortical bone is replaced each year and 25% of trabecular bone is resorbed and replaced each year [1]. Osteoporosis is defined as bone mineral density less than 2.5 SD below the mean peak value in young adults of the same race and sex (t score of -2.5). Women of all ethnic groups show an additional accelerated phase of bone loss, which occurs for about 10 years after the cessation of ovarian function. Total bone loss in osteoporosis may exceed 30 to 40%. In women there is a 15 to 18% risk of hip fracture after the age of 50 years, versus 6% in men. Menopause is associated with adverse changes in bone turnover. When bone resorption exceeds bone formation, bone loss and osteoporosis results. Also changes in Bone Mineral Density (BMD) being late and relatively irreversible, it is important to have a means of identifying high risk individuals and to monitor their treatment before fracture occurs [2]. Bone biochemical markers have been suggested to reflect the menopausal high bone turnover. These markers could be useful in separating women into fast and slow bone losers. Biochemical markers of bone turnover are:

- Markers of bone formation, reflecting the osteoblastic activity are Alkaline Phosphatase: it is measured in the serum or plasma.
- Markers of bone resorption, reflecting osteoclastic activity, are mainly the degradation products of type 1 collagen such as Hydroxy proline, hydroxyypyridinium cross links and Tartrate resistant acid Phosphatase
- Markers of mineralization are Serum Calcium.

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These markers reflect alterations in bone remodeling much earlier than they are apparent radiographically. They have untapped potential in the evaluation of patient at risk for accelerated bone loss especially in postmenopausal women. The occurrence of osteoporosis in women is very common in India.

It is widely accepted that "any single measurement of single biochemical marker of bone turnover has limited value in individual person", so they are often studied in combinations [3].

Hence, in the present study, we intended to measure serum Alkaline Phosphatase, Total calcium and urinary Hydroxyproline in postmenopausal women and evaluate the efficacy of using these biomarkers to assess the bone turnover in postmenopausal women, and the possible risk of developing osteoporosis and fractures.

2. Materials and Methods

A study of biochemical bone turnover markers was conducted in healthy postmenopausal women (cases) and healthy premenopausal women (controls) from Government General Hospital, affiliated to Kurnool Medical College, Kurnool. The study was approved by Ethical and Research Committee of Government General Hospital to use human subjects in the research study. The study was conducted from April 2012 to February 2013.

Subjects: A total number of 50 subjects were selected for the present study based on the inclusion and exclusion criteria.

Cases: Out of 50 subjects, 25 healthy post-menopausal women were considered as cases.

Controls: Out of 50 subjects, 25 healthy pre-menopausal women were considered as Controls.

Inclusion Criteria: For cases healthy women who had attained menopause for 3 to 5 years and not started taking hormone replacement therapy, non-obese and without use of calcium supplementation or any other medications known to affect bone metabolism.

For controls healthy women of reproductive age group 30–40 years with regular menstruation who were non pregnant and not taking oral contraceptive pills.

Exclusion Criteria: Women of known osteoporotic features, Diabetes mellitus, Renal insufficiency, Cirrhosis or those on hormonal replacement therapy and on the steroid treatment for more than 6 months, were all excluded.

Collection of Samples: About 5 ml of venous blood from all subjects was collected aseptically from antecubital vein. Serum was separated immediately by centrifugation and kept at 4 degrees C until analysis was carried out. A random urine sample was collected at the same time in a clean plastic container.

Parameters Measured: Serum Alkaline Phosphatase, Serum Calcium, Urinary Hydroxyproline.

Serum Alkaline Phosphatase was measured by Kinetic method recommended by the International Federation of Clinical Chemistry. Using appropriate reagents and appropriate assay parameters [4, 5]. Serum Calcium was measured by Orthocresolphthalein complexone method [5, 6]. Urinary Hydroxyproline was estimated by Modified Neumann and Logan method [7, 8, 9].

Statistical Analysis: Statistical analysis of biochemical parameters done by unpaired t test

3. Observations & Results

Table 1: Age Wise Distribution of Premenopausal Controls and Postmenopausal Cases:

<table>
<thead>
<tr>
<th>Age</th>
<th>Premenopausal cases</th>
<th>Postmenopausal cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-30</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>31-40</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>41-50</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>51-60</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>61-70</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>&gt;70</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>54.84 ± 7.1</td>
<td>31.96 ± 7.1</td>
</tr>
</tbody>
</table>

Table 2: Comparison of BMI, Levels of Serum Albumin, Total Alkaline Phosphatase Calcium and Urinaryhydroxyprotilne/Creatinine Ratio between Premenopausal Controls and Postmenopausal Cases

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>BMI (kg/m²)</th>
<th>Alb (g/dl)</th>
<th>ALP (ukat/L)</th>
<th>CA (mmol/L)</th>
<th>OHP/Cr ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-menopausal cases</td>
<td>25</td>
<td>27.23±2.05</td>
<td>4.34±1.12</td>
<td>5.41±3.8</td>
<td>1.03±0.03</td>
<td>27.51±6.63</td>
</tr>
<tr>
<td>Pre-menopausal controls</td>
<td>25</td>
<td>23.87±2.30</td>
<td>4.48±1.27</td>
<td>1.09±0.4</td>
<td>3.50±0.60</td>
<td>19.64±2.94</td>
</tr>
<tr>
<td>'t' value(unpaired t test)</td>
<td></td>
<td>5.44</td>
<td>0.41</td>
<td>6.86</td>
<td>2.50</td>
<td>5.42</td>
</tr>
<tr>
<td>'P' value</td>
<td></td>
<td>&lt;0.001</td>
<td>0.68</td>
<td>&lt;0.001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Statistical significance</td>
<td></td>
<td>HS</td>
<td>NS</td>
<td>HS</td>
<td>HS</td>
<td>HS</td>
</tr>
</tbody>
</table>

The mean serum calcium in post-menopausal women was 1.03 mmol/L and 3.50 mmol/L in pre-menopausal women which is statistically significant. Mean ALP was 5.4 in postmenopausal women and 1.09 in premenopausal women which is statistically significant. Mean urinary hydroxy proline was 31.64 in postmenopausal women and 19.61 in premenopausal women which is statistically significant. The statistical analysis by unpaired t-test shows that BMI and levels of serum total ALP, and urinary hydroxy proline creatinine ratio is increased in postmenopausal women.

Table 3: Relationship of Various Biochemical Parameters of Bone Turnover in Postmenopausal Cases with Age and BMI

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>Relation with age</th>
<th>Relation with BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r-value *</td>
<td>P-level</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>-0.41</td>
<td>0.03</td>
</tr>
<tr>
<td>ALP(ukat/L)</td>
<td>0.23</td>
<td>0.25</td>
</tr>
<tr>
<td>Serum total calcium (mmol/L)</td>
<td>0.1</td>
<td>0.61</td>
</tr>
<tr>
<td>Hydroxyproline(mg/dl)</td>
<td>-0.06</td>
<td>0.75</td>
</tr>
<tr>
<td>Creatinine (gm/day)</td>
<td>0.45</td>
<td>0.02 S</td>
</tr>
<tr>
<td>OH P/Cr</td>
<td>-0.44</td>
<td>0.02 S</td>
</tr>
</tbody>
</table>
Statistical analysis using Pearson's correlation co-efficient shows that the correlation of age with serum albumin, total ALP, total calcium Hydroxyproline (mg/dl), is not significant with p-values of 0.03, 0.25, 0.61, 0.75 respectively and with urinary hydroxy proline/creatinine ratio is significant. Correlation of BMI with serum albumin, total ALP, total calcium and Hydroxy proline (mg/dl), urinary hydroxy proline/creatinine ratio is not significant with p-values of 0.24, 0.003, 0.4, 0.5, 0.04, 0.01 respectively.

In the present study the biochemical marker of bone formation is decreased and biochemical markers of bone resorption are increased in Post-menopausal women. The present study also indicates that urinary hydroxyproline excretion increased with increasing age. We thus conclude that biochemical markers of bone turnover are valuable tools in detecting osteoporosis as they reflect activity level of entire skeleton.

6. References