The use of Serum & Urinary Biochemical Markers of Bone turnover in Post Menopausal Women.

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Abstract:

Background: The period during which the female sexual cycle ceases and female sex hormones diminish rapidly to almost none at all is called Menopause. Low bone mass and micro architectural deterioration of bone tissue, is most common in postmenopausal women which leading to enhanced bone fragility and a consequent increase in fracture risk. Menopause is the single most important cause of osteoporosis.

Materials & Methods: The study group 60 postmenopausal women in the age groups of 46-66 years were taken as case from our OP. As control, 60 healthy volunteers with age group of 25 to 45 years were recruited. The investigations carried out were Serum alkaline phosphatase, calcium, phosphorus, Total protein, Serum 25-hydroxy vitamin D3 (25-OHD), Serum estradiol. In 24 hours urine sample urinary calcium, Urinary phosphate & Urinary hydroxy proline.

Results: There were increased Serum alkaline phosphatase, Total protein, Urinary calcium, Urinary phosphate, Urinary hydroxy proline & decreased serum calcium, 25-hydroxy vitamin D3 (25-OHD), estradiol levels in postmenopausal women comparatively premenopausal women

Conclusion: estrogen deficiency induces bone resorption in cancellous bone leads to general bone loss and reduces bone strength resulting in osteoporosis. Hence, estrogen replacement therapy may reduce the risk of osteoporosis. The 25-hydroxy vitamin D3 (25-OHD) insufficiency was also been found with calcium imbalance in postmenopausal women which is an important in follow up of therapy. The results also suggests that simple, easy, common biochemical markers such as urinary hydroxyproline, total serum ALP, serum calcium, Urinary calcium and phosphate could be used as a tool to assist health care professionals to predict bone turnover to minimize fracture due to osteoporotic changes

Keywords: 25-hydroxy vitamin D3 (25-OHD); osteoporosis; estrogen; Post menopause.

Introduction:

The period during which the female sexual cycle ceases and female sex hormones diminish rapidly to almost none at all is called Menopause. It occurs between 45-55 years of age. [1]. Due to menopause ovarian follicles lose its function, which results in decreased production of estradiol and other hormones. Decreased levels of estrogen leads to increased osteoclast formation and enhanced bone resorption, which intern leads to loss of bone density and destruction of local architecture resulting in osteoporosis [3]. The prevalence of osteoporosis increases with age, by WHO definition up to 70% of women over the age 80 years have osteoporosis [2]. In India experts groups peg the number of osteoporosis to increase by 36 million by 2013 [3]. During the first five years after menopause a women can lose as much as 25% of her bone

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mass. If left untreated this condition can cause painful, debilitating fractures [3]. Osteoporosis is related to well-characterized secondary causes (endocrine, renal, inflammatory, malignant diseases, etc.). Other causes such as genetic (parental history), anthropometric (low body weight), reproductive (hypogonadism, premature menopause, lactation, etc.), nutritional (low calcium intake, vitamin D insufficiency, high sodium or protein intake) or lifestyle (smoking, sedentariness) conditions can also affect the acquisition of peak bone mass or lead to excessive bone loss after peak bone mass was attained [4]. Approximately 30% of postmenopausal women sustain at least one osteoporotic fracture. However, the true incidence of these fractures is difficult to assess because a large number of vertebral fractures remain asymptomatic. Several studies reported Osteoporotic hip fractures occur in the third and fourth decades after menopause; they are twice as common in women as in men. By 90 Years of age, about 33% of women and at least 17% of men sustain a hip fracture [6-9]. Life time losses may reach 30% to 40% of the peak bone mass in women. The occurrence of Osteoporosis in postmenopausal women is very common problem especially in India who is exposed to many of the risk factors like prolonged amenorrhea, low calcium diet, lack of exercise, Vitamin D deficiency. But there are very few Indian studies regarding the prevalence of osteoporosis in postmenopausal women and also regarding the biochemical markers which indicate bone turnover[10-13].

With the above background we aimed the assessment of biochemical markers of bone turnover which will provide valuable information for the clinical utility of diagnosis and monitoring of metabolic bone disease to assess the risk of osteoporoses in postmenopausal women.

Materials & Methods:

The study group 60 postmenopausal women in the age groups of 46 to 66 years were taken as case from our OP. As control, 60 healthy volunteers with age group of 25-45 years were recruited. None of the subjects were receiving any form of drugs. Subjects with the habit of smoking and taking alcohol were also excluded from the study, pregnant women and patients on oral contraceptive pills, subjects with fractures in previous one year and women on hormone replacement therapy or any other medications are excluded from this study because that may affect serum and urinary profile. All the experimental procedures were approved by the Institute Human Ethics Committee and informed consent was obtained from all the participants.

After an overnight fast of 12 to 14 hrs, blood is collected by Vein puncture in the morning. It is and serum was separated by centrifugation and Serum alkaline phosphatase, calcium, phosphorus, Total protein, Serum 25-hydroxy Vitamin D3 (25-OHD) was estimated by optimized standard method, Serum estradiol was estimated by immune enzymatic assay.

24 hour urine samples were collected in clean plastic containers. The pH of urine was adjusted to > 2 by adding 2ml HCl and mixed thoroughly. Total volume measured and aliquots were stored at -20ºC until analysis. Twenty four hour urinary calcium & Urinary phosphate was estimated by the spectrophotometric method. Urinary hydroxy proline was estimated by modified neuman and logan method. Height and weight was measured in meters and kilograms respectively and BMI was calculated by using the formula Wt/Ht² (weight in kilograms divided by height in (meters)²

The data obtained was analyzed, and the differences in the mean of various parameters were compared using student’s t-test. Statistical analysis was performed using software SPSS windows.
Table 1 Clinical characteristics variables in Study group and controls (mean±SD) Age, BMI, Serum & Urinary biochemical markers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Study group</th>
<th>Control group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>56.09 ± 9.91</td>
<td>35.44 ± 9.66</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BMI</td>
<td>25.10 ± 2.12</td>
<td>25.38 ± 4.59</td>
<td>NS</td>
</tr>
<tr>
<td>S.Ca mmol/l</td>
<td>2.10 (+/- .12)</td>
<td>2.32 (+/- .11)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>S.Phos mmol/l</td>
<td>1.17 (+/- .18)</td>
<td>1.18 (+/- .20)</td>
<td>NS</td>
</tr>
<tr>
<td>S.ALP IU/L</td>
<td>161.4 (+/- 33.66)</td>
<td>129.9 (+/- 13.20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Estradiol pg/ml</td>
<td>50.33 ±11.42</td>
<td>75.76 ±12.38</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>U.Ca (mg/gm Creatinine)</td>
<td>206.05 (+/-18.13)</td>
<td>146.8 (+/-16.79)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>U.Phos (mg/gm Creatinine)</td>
<td>520.2 (+/-13.3)</td>
<td>458.85 (+/-14.72)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>S. Protein gm/dl</td>
<td>6.28 (+/-)</td>
<td>6.30(+/-)</td>
<td>NS</td>
</tr>
<tr>
<td>U. Protein mg/day</td>
<td>128.04 (+/-12.31)</td>
<td>110.4 (+/-29.96)</td>
<td>NS</td>
</tr>
<tr>
<td>U. Hydroxyproline mg/day</td>
<td>107.75 (+/-10.45)</td>
<td>61.66 (+/- 9.58)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>S. 25 OH D3 ng/ml</td>
<td>17.75 (+/-10.45)</td>
<td>28.61 (+/- 10.58)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Table 1 shows the main demographic characteristics variables in study group and controls. Subjects and controls have differed with respect to age. Body height, weight and BMI were showing a little variation even though it was statistically not significant. There was a significant decrease in serum calcium, estradiol, 25 OH D3 levels in study group comparatively with controls. Women with postmenopausal had significantly higher serum alkaline phosphatase, urinary calcium, urinary phosphorus, urinary hydroxyproline when compared with control group. There was a variation in serum phosphorus, serum total protein & urinary protein of the study and control groups but it was not significant.

**Discussion:**

BMI was not significant in postmenopausal women, in comparison to premenopausal women. Literature says that a low BMI is one of the risk factors for increased bone turnover(14). However, we could not find such a correlation in present study. We found a higher proportion of women with low BMI in postmenopause comparative controls.

Bone is a connective tissue that provides mechanical support to the body vital organs and act as reservoir of calcium and phosphate as 99% of calcium and 85% of phosphate are present in skeleton. Peak bone mass is achieved during the third decade of life which gradually declines leading to osteopenia which predisposes to osteoporosis.(15) Various risk factors are involved for the decline in Bone Mineral Density (BMD) including dietary deficiency of calcium, phosphorus and vitamin D. We found the subjects with postmenopausal had 25-hydroxy vitamin D3 (25-OHD) insufficiency which is not different than that found in the control group and this also been reported in the general French adult urban population [20].

Some studies [21] reported that bone resorption markers raises when serum 25-OHD levels falls down, it is unlikely that vitamin D insufficiency was the main cause of osteoporosis in our population. The 25-hydroxy vitamin D3 (25-OHD) insufficiency seen with calcium imbalance in postmenopausal women. This might because of decrease in estrogen levels. Estrogen deficiency at menopause increases the rate of bone remodeling which results in high turnover bone loss. In this study there is a decrease serum estradiol levels in postmenopausal women when compared to premenopausal women. Hence present study supports the view that estrogen deficiency induces bone resorption by which releases calcium into the extracellular space and which in turn suppresses PTH secretion, calcitriol synthesis, and intestinal absorption of calcium in cancellous bone leads to general bone loss and destruction of local architecture and reduced bone strength resulting in osteoporosis.(16-19)

Serum alkaline phosphatase (ALP) is the most commonly used marker of bone formation that plays an important role in osteoid formation and mineralization. Normality of serum ALP levels is thus an important diagnostic criterion in the diagnosis of osteoporosis (20). There is a significant increase in mean total serum alkaline phosphatase activity when compared to controls. This shows that the bone mass continues to decline with menopausal age. ALP is a ubiquitous enzyme that plays an important role in osteoid formation and mineralization. It is observed that ALP levels were high in early postmenopausal women when compared to those of premenopausal women as a result of the inhibitory effects of estrogen on bone turnover which is dependent on age and body mass index.(22) The concentration of total serum protein increases in post menopausal women non significantly as compared to controls.

There was a significant correlation of urinary calcium and phosphate were found in the postmenopausal group.
and phosphate were found in the postmenopausal group compared to the premenopausal group. Similar results are reported in a study by George BO (24) which has highlighted the fact that biochemical parameters can be used to monitor bone loss in elderly persons. Baseline levels of bone markers is not only helpful in diagnosing osteoporosis in early stage but also for predicting future bone loss in postmenopausal women. This also been reported by Deutschmann et al (23) who has found association of hypercalciuria with severe osteoporosis. The significant correlation suggests that the factors that increase the urinary excretion of calcium and phosphate would affect the quality of bones both in males and females. Calcium salts in bone are embedded in collagen fibrils, 13% of which is mainly hydroxyproline. During bone loss, collagen fibrils are broken down thus calcium and hydroxyproline is excreted in the urine. Urinary hydroxyproline is thus considered as an index of bone resorption and a major determinant of bone status (20). In the present study there was a significantly increased urinary excretion of calcium & hydroxyproline in postmenopausal women when compared to control. Sachdeva. et al, also reported same observations in their study (19). Similar observations were reported by a number of other studies (20, 21). By taking into consideration of various criteria, methodology availability and feasibility we have chosen these parameters. Our study also amply demonstrating the usefulness of urinary profile and its significance in predict Osteoporosis in post menopausal women.

**Conclusion:** estrogen deficiency in postmenopausal women induces bone resorption in cancellous bone leads to general bone loss and reduces bone strength resulting in osteoporosis. Hence, estrogen replacement therapy may reduce the risk of osteoporosis. The 25-hydroxy vitamin D3 (25-OHD) insufficiency was also been found with calcium imbalance in postmenopausal women, which is an important area in the follow up of therapy. The results also suggests that simple, easy, common biochemical markers such as urinary hydroxyproline, total serum ALP, serum calcium, urinary calcium and phosphate could be used as a tool to assist health care professionals to predict bone turnover to minimize fracture due to postmenopausal changes.

**References:**


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