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# EVALUATION OF BONE TURN OVER MARKERS IN POSTMENOPAUSAL WOMEN

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## ABSTRACT

Biochemical markers of bone turn over have been shown to provide valuable information for the diagnosis and monitoring of metabolic bone disease. These markers include bone resorption markers like urinary hydroxyproline, hydroxylysine, glycosides, pyridinoline, type I collagen telopeptides and bone formation markers like serum calcium, phosphorus, alkaline phosphatase and osteocalcin. Sixty postmenopausal women were taken as cases and forty premenopausal women were taken as controls. Serum calcium, serum alkaline phosphatase and urinary hydroxyproline were measured in both groups. Serum calcium was significantly decreased in postmenopausal women when compared to premenopausal women with a p value <0.01. Serum alkaline phosphatase and urinary hydroxyproline were increased in postmenopausal women compared to premenopausal women with a significant p value <0.01. This study was undertaken to diagnose at the earliest osteoporotic changes in postmenopausal women by the easily available, reliable and cost effective colorimetric methods.

**KEYWORDS:** Menopause, Hydroxyproline , Osteoporosis, Calcium.

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## INTRODUCTION

Bone is a specialised connective tissue consisting primarily of glycoproteins and proteoglycans. The fibres of bone are mostly composed of type-I collagen impregnated with mineral in the form of hydroxyapatite. The functional integrity and strength of the skeleton is maintained by this highly cross-linked structure. There are two types of bone cells - osteoclasts and osteoblasts. Bone cells participate in the growth, modelling and remodelling of bone although they account for only a small fraction of bone volume[1]. Organic matrix consists principally of collagen (90%), other matrix proteins and proteoglycans. It is rapidly mineralized by osteoblasts in close apposition to and throughout the collagen fibrils[2]. Despite its seemingly static appearance, bone is remarkably a labile tissue. Rate of formation or degradation of the bone matrix can be assessed by measuring the enzymatic activity related to the bone forming or resorbing cells. Bone matrix components are released into the circulation, either by the osteoblasts or by the osteoclasts [3]. Bone is metabolically active and is constantly being repaired and remodelled throughout an individual's lifetime. Bone formation is an orderly process in which inorganic mineral is deposited in relation to organic matrix. During bone resorption, first calcium and phosphorus are released into the extracellular fluid and organic matrix is then resorbed. Approximately twenty percent of bone tissue is replaced annually varying by site and type[4]. Remodelling begins before birth and continues until death. After 40 -50 years of age, cortical bone is lost at a rate of about 0.3-0.5% per year in both the sexes. An accelerated loss of cortical bone is superimposed on age related loss around menopause[5]. Menopause is the consequence of the exhaustion of ovarian follicles which results in decreased production of oestradiol and other hormones. Osteoporosis is defined as disease that causes a reduction in the mass of bone per unit volume and is one of the dreaded afflictions of aging[6]. Osteoporosis occurs when bone resorption is the more active resulting in a low bone mass and microarchitectural deterioration of bone tissue, leading to increased bone fragility and consequent increase in fracture risk. There is a close relationship between oestrogen deprivation and development of osteoporosis. Several other factors like muscle bulk, body weight, malabsorption, smoking, alcohol and genetic factors also affect density of the bones . Oestrogen plays an important role in the growth and maturation of bone as well as in the regulation of bone turnover in adult bone. During bone growth, oestrogen is needed for proper closure of epiphyseal growth plates both in females and in males. Also in young skeleton, oestrogen deficiency leads to increased osteoclast formation and enhanced bone resorption. In menopause, oestrogen deficiency induces cancellous as well as cortical bone loss. At cellular level, in bone oestrogen inhibits differentiation of osteoclasts thus decreasing their number and reducing the amount of active remodeling units. This effect is probably mediated through some cytokines, IL-1 and IL-6 being strongest. Oestrogen deprivation is suggested by early development of osteoporosis in women who attained premature menopause either due to natural or surgical cause[6]. Decreased levels of oestrogen in post menopausal women prevents absorption and utilisation of bone calcium and hence there is development of osteoporosis in post menopausal women. The World Health Organisation (WHO) has defined osteoporosis as a bone mineral density (BMD) measured by dual-energy X-ray absorptiometry (DXA) 2.5 standard deviations (SD) or more below the mean peak bone mass of premenopausal females (T-score ≤ −2.5 SD)[7]. Technical developments in the measurement of BMD have led to its adoption as the standard for diagnosis of osteoporosis, however the relatively poor sensitivity contrasting with high specificity means that many potential fractures will be missed if BMD assessment is used alone[8]. In recent years cellular components of the bone matrix have been identified and categorised as either markers of bone formation or resorption. Reliable, rapid, noninvasive, cost effective assays have been developed with improved sensitivity and specificity. Most of the traditional and new markers of the bone resorption measure the collagen degradation products from osteoclast activity and these include urinary Hydroxyproline, Hydroxylysine and its glycosides, total or free pyridinoline crosslinks and crosslinked N or C telopeptides. Hydroxyproline is mainly found in collagen and accounts for 13% of total aminoacid content and derived from proline by post translational hydroxylation. It is used as a marker of osteoclastic bone resorption. Only about 10% Hydroxyproline released during collagen catabolism is excreted in urine. Significant amounts of Hydroxyproline are also found in C1q fraction of complement[9]. C1q, a subcomponent of the first component of complement, has been isolated in a haemolytically active and soluble form by ion-exchange chromatography and gel filtration from human and rabbit sera[10]. Hydroxyproline may be determined in timed fasting samples or 24 hours urine collection .It must be kept in mind that it is influenced by diet. Urinary Hydroxyproline was used as an indicator in assessment of post menopausal women for risk of developing osteoporosis and fractures.

## MATERIALS AND METHODS

The present study was conducted on sixty(60) outpatients in King George hospital , Andhra medical college , Visakhapatnam, India with prior approval from Andhra medical college vide Rc no 409/A9/Disser/PG(MM)11-12 and registered with number 46/8/12. Informed consent was taken from patients. The mean age group was 46 to 75 years. Forty (40) healthy premenopausal women were taken as controls. Their mean age group was 25 to 45 years. Most of cases and controls are vegetarians. Inclusion criteria were women who attained menopause. Exclusion criteria comprises pregnant women, women on hormone replacement therapy and other conditions which interfere with results like liver diseases, renal pathology, thyroid diseases,etc. Estimation of urinary Hydroxyproline was done by modified Newmann and Logan method[11]. The principle of this method includes : Oxidation of Hydroxyproline with hydrogen peroxide in presence of alkaline copper sulphate, Destruction of excess of peroxide by heat. Reaction of oxidation product with p- dimethyl amino benzaldehyde by heating in presence of dilute sulphuric acid to produce red

colour and the absorbance is measured colorimetrically at 540 nm. Serum calcium was also measured by Arsenazo's method colorimetrically[12]. Serum alkaline phosphatase was measured by para nitrophenyl phosphate ( p-NPP) method[13].

# RESULTS

**Table 1**
***Estimation of Serum calcium, Alkaline phosphatase and Urinary Hydroxyproline in controls***

| Particulars | Calcium | Alkaline phosphatase | Hydroxyproline |
|---|---|---|---|
| **Mean of cases** | 8.55 | 240 | 26.2 |
| **SD of cases** | 1.02 | 45.7 | 4.65 |
| **Mean of controls** | 9.15 | 170 | 16.1 |
| **SD of controls** | 0.643 | 30.6 | 1.48 |

**Table 2**
***Comparision between mean of cases and controls***

| | Serum calcium (mg/dl)- | Serum Alkaline phosphatase (IU) - | Urinary Hydroxyproline mg/24 hrs |
|---|---|---|---|
| **Controls** | 9.15 | 170 | 16.1 |
| **Cases** | 8.55 | 240 | 26.2 |
| **P value** | 0.01 | 0.01 | 0.01 |
| **Inference** | Significant | Significant | Significant |

***In the present study, mean value of Hydroxyproline in cases is 26.2 mg/24 hrs ± 4.65 mg/24 hrs (MEAN ± SD ) when compared to controls 16.1 mg/24 hrs ± 1.48 mg/24hrs (MEAN ± SD) with a statistically significant' p' value of < 0.01. Mean value of serum calcium among controls is 9.15 mg/dl ± 0.643 mg/dl (MEAN ± SD) when compared to cases 8.55 mg/dl ± 1.02 mg/dl (MEAN ± SD) with a statistically significant p value of <0.01. Mean value of serum alkaline phosphatase among controls is 170 IU/L± 30.6 when compared to cases 240 IU/L ± 45.7 with a statistically significant p value of < 0.01.***

# DISCUSSION

Majority of the controls (80%) and the cases (88%) were vegetarians. Diet has also been proven to be an independent risk factor for the development of osteoporosis. High protein diet (non-vegetarian diet) particularly leads to excessive acid formation which may contribute to "dissolution" of bones as the body tries to buffer the extra acid. Acidosis may also increase osteoclastic function directly. A total of 100 subjects were included in the study. It included forty (40) premenopausal women (controls) and sixty (60) postmenopausal women ( cases ). The estimated mean serum calcium level in controls was in range of 9.15 mg/dl ± 0.643mg/dl (MEAN ± SD) and in postmenopausal women was in range of 8.55 mg/dl ± 1.02 mg/dl( MEAN ± SD) . Table 2 indicates that serum calcium level was significantly decreased in postmenopausal women than in controls with a statistically significant p value of < 0.01. The present study correlated with study of Indumathi .v .vidya s patil[14]and steven[15]. In the Study of Vidya patil, total and ionized calcium were significantly decreased and urinary Hydroxyproline was significantly raised in postmenopausal women compared to premenopausal women and urinary Hydroxyproline was significantly raised in postmenopausal women compared to premenopausal women . The estimated mean value of urinary Hydroxyproline in controls is 16.1 mg/ 24 hrs ± 1.48mg/24hrs and in cases is 26.2 mg/ 24 hrs ± 4.65mg/24hrs. The result showed a significant increase in urinary Hydroxyproline value in cases compared to controls with a statistically significant p value of < 0.01. The results correlated with studies of Indumathi.v[14], ashuma sachadeva, shashi seth[16] and Ren XH,XD[17.] Increased level of Hydroxyproline was due to excessive bone loss in postmenopausal women. Study of Ashuma Sachadeva, shashi seth was that urinary Hydroxyproline was significantly raised in post menopausal women compared to premenopausal women. Study of Ren XH was that urinary Hydroxyproline was significantly raised in postmenopausal women compared to premenopausal women. Thus measure of urinary Hydroxyproline is an useful index of bone resorption in postmenopausal women. Hence it's measure will play an important role in early diagnosis of osteoporosis and in decreasing the incidence of fractures commonly observed in elderly women. Accordingly, we can supplement calcium or put the patient on HRT. Any single measurement of a single biochemical marker of bone turnover has limited utility in the individual person. Thus increase in urinary excretion of Hydroxyproline and decrease in serum calcium together were used and were found to be significant in postmenopausal women reflecting the increased bone activity (osteoclastic and osteoblastic) as compared to premenopausal women[18.] The estimated value of mean alkaline phosphatase level in controls was 170 IU/L +/- 30.6 and in postmenopausal women was 240 IU/L +/- 45.7. Table 2 showed that alkaline phosphatase was significantly increased in cases, p value <0.01. The present study correlated with the study of Indumathi .v, ashuma sachadeva, kimHJj iLeeH[19] and garner P. Serum alkaline phosphatase was the most commonly used marker of bone formation and played an important role in osteoid formation and mineralization. Much of the knowledge gained in the last decade on osteoporosis and other metabolic bone diseases has come from three different approaches: Bone density, Bone biopsy and Biochemical assays. Each approach has advantages and disadvantages. Bone density measurements are noninvasive, site specific and are sufficiently sensitive to measure changes in bone density. However they are expensive, have a limited availability and are unable to identify early changes resulting from therapy[20.] Bone biopsy is an invasive procedure, hence biochemical assessment of skeletal metabolism holds great

importance. These markers reflect alterations in bone remodeling much earlier than they are apparent radiographically. In addition, these markers now make it possible to determine the efficacy of anti-resorptive drugs, their optimum dosage in a time frame that is reasonably much less as compared to months before there is a radiographic evidence of a therapeutic response. They have untapped potential in the evaluation of patient at risk for accelerated bone loss e.g. in postmenopausal women[21.] Measurement of urinary and serum osteocalcin may provide important insights into the metabolic derangements in osteoporosis and other bone disorders[22].

## CONCLUSION

Change in the levels of serum calcium, serum alkaline phosphatase and urinary Hydroxyproline show that bone remodelling is accelerated at menopause. Increased levels of urinary Hydroxyproline can be used as a marker to assess bone turn over in post menopausal women. Thus assessing bone markers is useful in evaluation of osteoporosis.

## CONFLICT OF INTEREST

Conflict of interest declared none.

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