



Effect of menopause on bone turn over

Tirthal Rai^{1*}, Mayur Rai², Ashok Kumar³, Mithun Shetty², Srinidhi Rai¹, Prajna⁴

¹Department of Biochemistry, K. S. Hegde Medical Academy, Nitte University, Mangalore-575018, Karnataka, India

²Department of Orthopaedics, A. J. Institute of Medical Sciences, Mangalore, Karnataka, India

³Department of Biochemistry, A. J. Institute of Medical Sciences, Mangalore, Karnataka, India

⁴Vikram Hospital, Mysore, Karnataka, India

Received: 28-06-2014 / Revised: 10-07-2014 / Accepted: 17-07-2014

ABSTRACT

Women of all ethnic groups show an accelerated phase of bone loss, which occurs for about 10 years after the cessation of ovarian function. Biochemical assays reflect the turnover of entire skeleton; can detect early changes in the bone turnover. The present study was undertaken to investigate the role of biochemical markers of bone turnover in postmenopausal women. Fifty premenopausal and fifty early postmenopausal women each were selected after the informed consent and 5ml of venous blood was collected. Serum was separated for estimation of total calcium, phosphorous, serum alkaline phosphatase, osteocalcin, total protein & albumin. Urinary Hydroxyproline and Urinary calcium were estimated. Data was analyzed using one-way ANOVA followed by Tukey's test. P value <0.05 was considered the level of significance. Results were expressed as mean \pm SD and range values. The serum alkaline phosphatase, osteocalcin and Hydroxyproline were significantly increased in early postmenopausal women as compared to that in premenopausal women. From study, we conclude that biochemical markers of bone formation and bone resorption was grossly elevated in early postmenopausal women when compared to premenopausal women indicating an accelerated bone turnover in this age group.

Key words: Premenopausal women, early Postmenopausal women, Bone turnover, Osteoporosis.

INTRODUCTION

The menopause transition, and postmenopause itself, is a natural life change, not a disease state or a disorder in women. Menopause literally means the end of monthly cycle. It is an event that typically but not always, occurs in women in midlife, during their late 40s or early 50s, and it signals the end of the fertile phase of a woman's life [1]

This transition from a potentially reproductive to a non-reproductive state is the result of a reduction in female hormonal production by the ovaries. It is normally not sudden or abrupt, tends to occur over a period of years, and is a natural consequence of aging [2]. However, for some women, the accompanying signs and effects that can occur

during the menopause transition years can significantly disrupt their daily activities and sense of well-being. Postmenopause is a permanent phase that follows perimenopause. It marks the end of fertile years.

Bone is metabolically active throughout life. The remodelling of bone requires the sequential and coordinated actions of the osteoclasts, to remove bone (resorption), and the osteoblasts to replace it. After skeletal growth is complete, remodelling of bone continues and results in an annual turnover of about 10% of the adult skeleton [3]. A change in the balance between bone resorption and bone formation ultimately results in a net loss or gain of bone tissue. High bone turnover, with increased bone resorption, can compromise bone strength, leading to a thinning of the bone structure, resulting

*Corresponding Author Address: Tirthal Rai, Assistant Professor, Department of Biochemistry, K S Hegde Medical Academy, Nitte University, Mangalore-575018, Karnataka, India; Email ID: Tirthalrai1983@gmail.com

in abnormal bone micro-architecture and reduced bone mineralization. This, in turn, leads to a greater propensity to fracture. The life time risk of fracture in white women after 50 years of age is approximately 75%, the risk of hip fracture is 15% [4]. An increased level of bone resorption is the primary cause of age-related bone loss often resulting in osteopenia and is the major cause of osteoporosis.

Women of all ethnic group showed an accelerated phase of bone loss, which occurs for about 10 years after the cessation of ovarian function. Bone grows in size during the first two decades of life, with an accelerated growth during adolescence [5]. Peak adult bone mass is attained at the age of 35 years, after which the bone mass declines with ageing. Approximately 3% of cortical bone is replaced each year and 25% of trabecular bone is resorbed and replaced each year. Hence, the present study was undertaken to investigate the status of biochemical markers of bone turnover in premenopausal and early postmenopausal women.

MATERIALS AND METHODS

This study was conducted in women attending the department of Obstetrics and Gynaecology and Orthopaedics in A J hospital & research centre from 2009 – 2011. The study included 50 premenopausal and 50 early Postmenopausal women. They were given detailed information about the research work being carried out and were included in the research study only after obtaining written consent from them. Ethical clearance certificate was obtained from the institutional Ethical committee.

Group-I included healthy pre-menopausal women whereas; **Group-II** included Healthy post-menopausal women within 2yrs of attaining menopause. Females with Chronic illness like diabetes mellitus, renal insufficiency, malignancy, liver disease, on treatment with anticonvulsants and glucocorticoids, immobilized patients, fractures, Surgically induced menopause and Patients on hormone replacement therapy were excluded. 5ml of venous blood was collected aseptically from antecubital vein using vacutainers. Serum was separated for estimation of total calcium, phosphorous, serum alkaline phosphatase, osteocalcin, total protein & albumin. The early morning urine sample was collected between 2 to 9 AM and taken after adding a preservative (Hcl: 5ml/l of urine) for the estimation of Urinary Hydroxyproline and urinary calcium.

Estimation of serum calcium by Arsenazo method [6]. In brief, at neutral pH calcium ions

form with Arsenazo III a complex, the color intensity which is directly proportional to the concentration of calcium in the sample. This was estimated by using commercially available kit on an autoanalyser CAFA 200 at a Wavelength of 650nm. **Urinary calcium** was also estimated by the same method.

Estimation of serum inorganic phosphorous by phosphomolybdate method [7]. This was estimated by using commercially available kit on an autoanalyser CAFA 200 at a Wavelength of 340nm. The principle of this reaction is based on the formation of phosphomolybdic complex when Ammonium molybdate reacts with sulphuric acid in the presence of Phosphorous.

Estimation of serum alkaline phosphatase by IFCC / kinetic method [8]. This was also estimated by using commercially available kit on an autoanalyser CAFA 200 at a wavelength of 405nm. Kinetic determination of alkaline phosphatase based on the formation of p – nitro phenol and inorganic phosphate by the reaction of p nitrophenylphosphate with H₂O in the presence of alkaline phosphatase.

Estimation of serum Total Protein by Biuret method [9]. This estimation was done using commercially available kit on an autoanalyser CAFA200 at a Wavelength of 546nm. The Colorimetric determination of total protein based on the principle of the Biuret reaction (copper salt in an alkaline medium). Protein in plasma or serum sample forms a blue colored Complex when treated with cupric ions in alkaline solution. The intensity of the blue color is Proportional to the protein concentration.

Estimation of serum Albumin by Bromocresol Green method [10]. This estimation was done using commercially available kit on an autoanalyser CAFA 200 at a wavelength of 630nm. This is based on the reaction between albumin from serum or plasma and the dye bromocresol green produces a change in color that is proportional to the albumin concentration.

Estimation of serum Osteocalcin by chemiluminescent method [11]. This estimation of serum osteocalcin was done using commercially available kit on an autoanalyser IMMULITE 1000. In short, Osteocalcin is a solid phase, two site chemiluminescent immunometric assay. The solid phase (bead) is coated with monoclonal murine anti- osteocalcin antibody. The liquid phase consists of alkaline phosphatase conjugated to polyclonal rabbit anti – osteocalcin antibody in buffer. The patient sample and the reagent are

incubated together with the coated bead for 30 minutes. During this time, osteocalcin in the sample forms an antibody sandwich complex with monoclonal murine anti – osteocalcin antibody on the bead and enzyme conjugated polyclonal rabbit anti-osteocalcin antibody in the reagent. Unbound patient sample and enzyme conjugate are then removed by centrifugal washes. Finally chemiluminescent substrate is added to the reaction tube containing the bead and the signal is generated in proportion to the bound enzyme.

Estimation of urinary Hydroxyproline by modified Neuman and Logan method [12].

This is based on the principle that, Hydroxyproline is treated with CuSO_4 and H_2O_2 in an alkaline solution; this results in the formation of a pyrroline-4-carboxylic acid, which upon acidification is converted to pyrrole-2-carboxylic acid. The latter condenses with p-dimethyl aminobenzaldehyde to give the colored complex which is measured at 540 nm.

Statistical Analysis

Results are presented as mean \pm standard deviation value. Data was analyzed using one way ANOVA followed by Tukey's test. P value <0.05 was considered the level of significance.

RESULTS

Our study showed that mean values of serum osteocalcin was increased in early postmenopausal women when compared to premenopausal women. The Mean Urinary hydroxyproline level increased in early postmenopausal women when compared to the premenopausal women. The mean serum alkaline phosphatase was (95.25 ± 22.75) in early post menopausal women as compared to premenopausal women (74.24 ± 11.33) . This increase in serum alkaline phosphatase in early postmenopausal women as compared to premenopausal women was statistically highly significant ($p < 0.001$, $(t-6.041)$, Table-1). The mean serum osteocalcin in early postmenopausal women was (12.08 ± 4.85) when compared to premenopausal women (5.55 ± 2.92) . The serum osteocalcin levels were found to be increased early postmenopausal women, which was statistically highly significant ($p < 0.0001$), $(t-8.017)$, Table-2)

The mean urinary hydroxyproline was (16.10 ± 3.19) in early postmenopausal women and (5.71 ± 2.55) in premenopausal women. There was a statistically high significant increase in urinary hydroxyproline levels in early postmenopausal women as compared to premenopausal women ($p < 0.0001$), $(t-17.560)$, Table-3). The mean urinary

calcium is (163.72 ± 17.93) mg/dl in early postmenopausal women and (111.96 ± 9.17) in premenopausal women. The urinary calcium levels were found to be increased in early postmenopausal women as compared to pre-menopausal women, which was statistically highly significant ($p < 0.0001$) $(t-18.454)$, Table-4). The mean total serum calcium was (9.24 ± 0.69) in early postmenopausal women and (9.26 ± 0.59) in premenopausal women. The serum calcium levels showed no statistical significant difference among the two groups. ($p < 0.880$), $(t-0.152)$, Table-5). The serum phosphorous, total protein and serum albumin was not statistically significant among the 2 groups ($p < 0.846$), ($p < 0.900$) and ($p < 1.000$) respectively as shown in Table 6.

DISCUSSION

A variety of biochemical assays that reflect the activity of osteoblasts and osteoclasts have been developed for clinical use. Markers of bone formation are excellent indices of disease activity in osteomalacia, rickets, osteoblastic bone metastases, and to a lesser extent in renal osteodystrophy. In a study on markers of bone turnover in postmenopausal women and premenopausal women, there showed an increased excretion of Hydroxyproline in postmenopausal women as compared to the premenopausal women. Serum alkaline phosphatase, urinary excretion of hydroxyproline and Calcium was found to be significantly increased in postmenopausal women reflecting the increased bone activity both osteoclastic and osteoblastic as compared to premenopausal women [13].

Garnero et al, 2003 evaluated long term variability of markers of bone turnover in post-menopausal women and their implications for clinical use in a total of 268 untreated post-menopausal women aged 50 – 81 years of age from a population-based cohort. Morning fasting blood samples were collected every year for 4 years to measure serum intact osteocalcin and serum carboxy-terminal type I collagen telopeptide (CTX) as bone formation and resorption markers. 20 – 30 % of these women with high turnover at baseline turned to be false positives 4 years later. This suggested that further investigation would be required to reduce the number of false positive patients who would be treated unnecessarily if the decision was made on a single bone marker measurement [14].

In a study on biochemical markers of bone metabolism and prediction of fracture risk in elderly women, 1040 women 75 years of age were randomly recruited for over a period of 4 years, 178 of the women sustained at least one fracture.

Serum alkaline phosphatase and 4 different forms of osteocalcin were analyzed as formation markers. Serum CTX, TRACP and urinary free DPD were used as resorption markers. Results showed for the first time that, in elderly women, biochemical markers of bone turnover can predict fracture and in particular, clinical vertebral fractures [15]. In another study to determine the value of measurements of biochemical markers for the prediction of rates of bone loss, sixty postmenopausal women (aged 49-62 years), 43 of whom had gone through a natural menopause 1-20 years previously and 17 of whom had undergone hysterectomy 3-22 years ago were studied. They found significant negative correlations (Spearman rank) between all measured biochemical markers and rate of change in bone density [16]. Later in a study on years since menopause on bone mineral metabolism in south Indian women. They concluded that the risk of bone resorption is greater in early years than late years of menopause. The decreased bone resorption risk in late menopause could be due to increased FSH levels [17]. Jasmina.M et al showed that postmenopausal osteoporotic women had increased levels of bone turnover markers indicating increased rate of bone remodelling, which resulted in excessive bone resorption, and loss of bone mass. Long term persistence of high bone resorption marker CTX insufficiently compensated with bone formation marker osteocalcin, enabled osteoporosis development. The results of this study led to a conclusion that CTX and OC measurements are useful and non-invasive methods for predicting osteoporosis [18].

In the present study, we found that the serum levels of alkaline phosphatase and osteocalcin were significantly increased in early postmenopausal women as compared to that in premenopausal women. This was accompanied by significant increase in urinary calcium and Hydroxyproline in early postmenopausal women. This may be due to oestrogen deficiency after menopause. The bone turnover increases to high levels and oestrogen deficiency may induce calcium loss by indirect effects on extra skeletal calcium homeostasis. The present study indicates that in postmenopausal women declining ovarian function during menopause is accompanied by an accelerated bone turnover which is reflected by increase in both resorption and formation markers.

CONCLUSION

In our study serum osteocalcin, serum alkaline phosphatase and urinary hydroxyproline level were found to be elevated in early postmenopausal women when compared to the premenopausal group. Our study showed that in early post menopausal group, biochemical markers of bone formation and bone resorption were grossly elevated. This indicates that there is an accelerated bone turnover during early menopause which is reflected by increase in both bone formation markers and bone resorption markers. Hence, we conclude that biochemical markers of bone turnover are valuable tools of detecting the activity level of the entire skeleton.

Conflict of interest statement: The authors declare that there is no conflict of interest.

Table 1: Comparison of SERUM ALKALINE PHOSPHATASE (mμ/ml) between premenopausal women and postmenopausal women; Values are expressed as Mean ± SD. N= 50 each.

Groups	N	MEAN±SD	p value
Premenopausal women	50	74.24 ±11.33	<0.001
Post-menopausal women	50	95.25 ±22.75	

Table 2: Comparison of SERUM OSTEOCALCIN (ng/ml) between premenopausal women and postmenopausal women . Values are expressed as Mean ± SD. N= 50 each.

Groups	N	MEAN ± SD	p
Postmenopausal women	50	12.08 ± 4.85	<0.001
Premenopausal women	50	5.55 ± 2.92	

Table 3: Comparison of URINARY HYDROXYPROLINE (μg/ml) between premenopausal women, postmenopausal women . Values are expressed as Mean ± SD. N= 50 each.

	N	MEAN ± SD	p
Postmenopausal women	50	16.10 ± 3.19	<0.001
Premenopausal women	50	5.71 ± 2.55	

Table 4: Comparison of URINARY CALCIUM (mg/dl) between premenopausal women and postmenopausal women . Values are expressed as Mean ± SD. N= 50 each.

	N	MEAN ± SD	p
Postmenopausal women without fracture	50	163.72 ± 17.93	<0.001
Premenopausal women	50	111.96 ± 9.17	

Table 5: Comparison of SERUM CALCIUM (mg/dl) between premenopausal women, postmenopausal women . Values are expressed as Mean ± SD. N= 50 each.

	N	MEAN ± SD	p
Postmenopausal women	50	9.24 ± 0.69	<0.880
Premenopausal women	50	9.26 ± 0.59	

Table-6: Comparison of serum Phosphorous, Albumin and total protein between premenopausal women and postmenopausal women . Values are expressed as Mean ± SD. N= 50 each.

	N	S.PHOS	Alb	TP
Postmenopausal women	50	2.77±.621	4.02±.176	6.50±.344
Premenopausal women	50	2.74±.605	4.02±.176	6.49±.424
P value		p<0.846	p<1.000	p<0.900

REFERENCES:

1. Kanis JA et al. Risk of hip fracture according to the world health organization criteria for osteopenia and osteoporosis. Bone 2003; 27 (5): 585 – 590.
2. Neer RM et al. Effect of parathyroid hormone on fractures and bone mineral density in postmenopausal women with osteoporosis. Endocrinal Metab Clin North Am 2006; 32 (1): 115-134.
3. Pierred D, Delmas. Biochemical markers of bone turnover. J Bone Miner Res 1993;8: S549–S555.
4. Delmas PD et al. The use of biochemical markers of bone turnover in osteoporosis. Osteoporos Int 2000; 11(6): S2 17.
5. Ashuma S et al. Study of some common biochemical bone turnover markers in postmenopausal women. Ind J Clin Biochem 2005; 20(1):131– 134.
6. Bauer PJ. Estimation of calcium. Ann Biochem 1981; 1:10-16.
7. Teitz N. Estimation of serum inorganic phosphorous. Clinical Guide to Laboratory Tests 1983; 95:52.
8. Schledusch H, Gomall A. Alkaline phosphatase liquicolor. Dtsch Med Wschr 1974; 1(9):7-14.
9. Gomall A. Estimation of total protein. J Biol chem 1949 ;(751):177.
10. Dounasa BT. Estimation of serum albumin. Cain Chim Acto 1971; 87:31-36.
11. Merle B, Delmas PD. Normal carboxylation of circulating osteocalcin. Bone Miner 1990; 11:237- 245.
12. Leach aa. Notes on a Modification of the Neuman & Logan Method for the Determination of the Hydroxyproline. Biochem J 1960; 74(1): 70–71.
13. Indumathi V, Vidhya S. Hospital based preliminary study on osteoporosis in postmenopausal women. Ind J Clin Biochem 2007; 22:96 – 100.
14. Garnero P et al. Long term variability of bone turnover in post menopausal women and implications for their clinical use. J Bone Miner Res 2003; 18:1789 -1794.
15. Gerdhem P et al. Biochemical markers of bone metabolism and prediction of fracture risk in elderly women. J Bone Miner Res 2004; 19:386 – 93.
16. Rogers A et al. Biochemical markers as predictors of rates of bone loss after menopause. J Bone Miner Res 2000; 15:1398-1404.
17. Suresh M, Dhananjaya M. Influence of years since menopause on bone mineral metabolism in south Indian women. Ind J Med Sci 2005; 60:190 – 198.
18. Jasmina M et al. Bone turnover markers relations to postmenopausal osteoporosis. J Bone Miner Res 2009; 28:161 – 165.