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## Biochemical Bone Turnover Markers in Postmenopausal Women in Calabar Municipality

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**Abstract:** This study is aimed at estimating the serum levels of calcium, inorganic phosphate, total protein, albumin, globulin, alkaline and acid phosphatase activities in postmenopausal and premenopausal women and the effect of Years Since Menopause (YSM) on these parameters. Serum calcium, inorganic phosphate, total protein, albumin, globulin, BMI, alkaline and acid phosphatase activities were determined in 50 premenopausal and 50 postmenopausal women in Calabar, Nigeria using colorimetric methods. Based on YSM, the postmenopausal women were divided into 3 groups; 1-5 YSM, 6-15 YSM and >15 YSM. The mean Age, BMI, total proteins, albumin and calcium levels were significantly ( $p < 0.05$ ) higher in postmenopausal women when compared to premenopausal women. Other parameters were not significantly ( $p > 0.05$ ) different in both groups. No significant variation ( $p > 0.05$ ) was observed in the levels of all the parameters in the various YSM groups. A significant negative correlation ( $p < 0.05$ ,  $r = -0.286$ ) was observed between BMI and acid phosphatase activity in postmenopausal women of the study. Menopause and ageing alters the metabolism of serum calcium, inorganic phosphate total proteins, albumin, alkaline phosphatase and acid phosphatase activities by increasing their serum levels.

**Key words:** Menopause, calcium, inorganic phosphate, alkaline phosphatase, acid phosphatase, total proteins

### Introduction

Menopause and ageing is associated with accelerated loss of cortical bone. Bone loss occurs when the balance between formation and resorption is upset and resorption is excessive resulting in a negative remodeling balance (Ashuma *et al.*, 2005; Dogan and Posaci, 2002). Despite its seemingly static appearance, bone is a remarkably labile tissue and bone turnover is a dynamic process which increases in postmenopausal period as a consequence of oestrogen deficiency (Uemura *et al.*, 2000). The rate of formation and degradation of the bone matrix can be assessed by measuring the enzymatic activity related to the bone forming or reabsorbing cells. Numerous studies have observed increased bone resorption in postmenopausal women and many markers of bone resorption and formation have been identified (Edward and Cooper, 2001; Suresh and Naidu, 2006; Mazzuoli *et al.*, 2000; Garcia-Perez *et al.*, 2004; Harimarayan, 2005; Plehwe, 2003). These markers produced by osteoclasts during bone resorption and measured in urine includes hydroxyproline, free and total deoxypyridinoline and pyridinoline, whereas bone formation markers measured in serum includes collagen propeptide, bone alkaline phosphatase, acid phosphatase, calcium, inorganic phosphates, total proteins and albumin (Ashuma *et al.*, 2005). Adverse changes in plasma calcium, inorganic phosphates, alkaline phosphatase, acid phosphatase, total proteins and albumin due to oestrogen deficiency have been implicated in the increased incidence of osteoporosis in postmenopausal women (Recker *et al.*, 1998).

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Measurements of some of these markers in women have an untapped potential in the evaluation of patients at risk of accelerated bone loss especially in postmenopausal women. They may also be of potential use in prediction of bone mass, prediction of bone loss and risk of fractures, selection of patients for antiresorptive therapy and monitoring effectiveness of therapy.

## **Materials and Methods**

### *Selection of Subjects*

This case control study was carried out in the out patient clinic of University of Calabar Teaching Hospital (UCTH). The ethical committee of University of Calabar Teaching Hospital UCTH approved the study protocol. Informed consent was sought and obtained from each subject before recruitment into the study. Subjects included both premenopausal (aged between 25-45 years) and postmenopausal (aged between 45-70 years) women in Calabar metropolis. The subject consisted of 100 healthy women (50 premenopausal and 50 postmenopausal) between the ages of 25 to 70 years.

### *Sample Collection*

Blood samples were collected into clean, dry sterile, plain sample bottles from both premenopausal and post menopausal women by venepuncture using 21SWG needles and syringes. Blood samples were allowed to clot and spun at 3000 rpm for 10 min to aid proper separation of cells from serum. The serum was transferred into dry well-labeled specimen plastic tubes and analysed within 48 h. The serum samples were analyzed for calcium, inorganic phosphates, total proteins, albumin, acid phosphatase and alkaline phosphatase activities. The height and weight of subjects were measured and used in calculating the Body Mass Index (BMI). Blood pressure of the subjects was also obtained using a sphygmomanometer. Other relevant data of the subjects like age, diet and menstrual cycle were obtained through a comprehensive questionnaire.

### *Methods*

Serum calcium, inorganic phosphate, total protein, albumin and globulin were estimated using colorimetric methods, while alkaline phosphatase and acid phosphatase activities were determined using enzymatic methods.

### *Statistical Analysis*

The significance of difference between the groups was tested using the t-test analysis, variation within and among groups was determined using one way analysis of variance (ANOVA), while association between variables was determined using the Pearson's correlational analysis on Microsoft excel and SPSS soft ware 10.0 version (California Inc.). A two sided p-value <0.05 was considered statistically significant for the t-test, ANOVA and correlational analysis.

## **Results**

The mean age, BMI, systolic and diastolic blood pressure, total proteins and albumin were significantly ( $p < 0.05$ ) higher in postmenopausal women when compared to those of premenopausal women. There was no significant difference ( $p > 0.05$ ) in the globulin values of both groups (Table 1).

The mean serum calcium was significantly ( $p < 0.05$ ) higher in postmenopausal women compared to premenopausal women. No significant differences ( $p > 0.05$ ) were observed in inorganic phosphate, alkaline phosphatase and acid phosphatase activities of both groups (Table 2).

No significant variation ( $p > 0.05$ ) was observed in the levels of all the parameters in the various YSM groups (Table 3).

There was no significant variation in serum calcium, inorganic phosphate, total proteins, albumin, alkaline phosphatase and acid phosphatase in normal weight, preobese and obese subjects (Table 3). The BMI do not seem to exert any significant effect on the levels of these parameters.

A significant negative ( $p < 0.05$ ,  $r = -0.286$ ) correlation was observed between BMI and acid phosphatase in postmenopausal women. No significant correlation ( $p > 0.05$ ) was observed between BMI and other parameters (Fig. 1).

Table 1: Mean age, body mass index (BMI), blood pressure, total proteins, albumin, globulin and albumin/globulin (A/G) ratio in premenopausal and postmenopausal women

Subjects	Age (years)	BMI ( $\text{kg m}^{-2}$ )	Blood systolic pressure (mm Hg)	Pressure diastolic pressure (mm Hg)	Total proteins ( $\text{g L}^{-1}$ )	Albumin ( $\text{g L}^{-1}$ )	Globulin ( $\text{g L}^{-1}$ )	A/G ratio
Premenopausal women (n = 50)	29.52 ± 5.78	23.31 ± 3.52	122.80 ± 8.82	82.80 ± 8.09	78.04 ± 28.03	43.97 ± 11.07	35.06 ± 23.88	2.39 ± 2.80
Postmenopausal women (n = 50)	57.80 ± 9.69	25.97 ± 5.97	128.90 ± 15.16	86.00 ± 8.08	98.08 ± 27.51	53.49 ± 27.51	44.57 ± 24.63	2.25 ± 3.89
p-value	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p > 0.05$	$p > 0.05$

Table 2: Mean serum calcium, inorganic phosphate, alkaline phosphatase (ALP) and acid phosphatase (ACP) activity in premenopausal and postmenopausal women

Subjects	Calcium ( $\text{mmol L}^{-1}$ )	Inorganic phosphate ( $\text{mmol L}^{-1}$ )	ALP ( $\text{IU L}^{-1}$ )	ACP ( $\text{IU L}^{-1}$ )
Premenopausal women (n = 50)	1.79 ± 0.41		63.11 ± 29.54	1.19 ± 0.53
Postmenopausal women (n = 50)	2.29 ± 0.39	1.18 ± 0.25	63.69 ± 29.59	1.21 ± 0.58
p-value	$p < 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$

Table 3: Influence of years since menopause (YSM) on serum calcium, inorganic phosphate, total proteins, albumin, alkaline phosphatase (ALP) and acid phosphatase (ACP) activity on all subjects of the study

YSM	Calcium ( $\text{mmol L}^{-1}$ )	Inorganic phosphate ( $\text{mmol L}^{-1}$ )	Total proteins ( $\text{g L}^{-1}$ )	Albumin ( $\text{g L}^{-1}$ )	ALP ( $\text{IU L}^{-1}$ )	ACP ( $\text{IU L}^{-1}$ )
1-5 (n = 24)	2.34 ± 0.44	1.27 ± 0.30	94.43 ± 28.70	56.33 ± 14.78	62.89 ± 29.07	1.44 ± 0.70
6-15 (n = 10)	2.33 ± 0.13	1.05 ± 0.14	90.70 ± 15.45	46.90 ± 9.19	47.90 ± 19.79	1.31 ± 0.35
>15 (n = 16)	2.19 ± 0.41	1.14 ± 0.13	106.63 ± 28.47	53.36 ± 14.94	74.33 ± 29.69	1.26 ± 0.53
p-value	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$

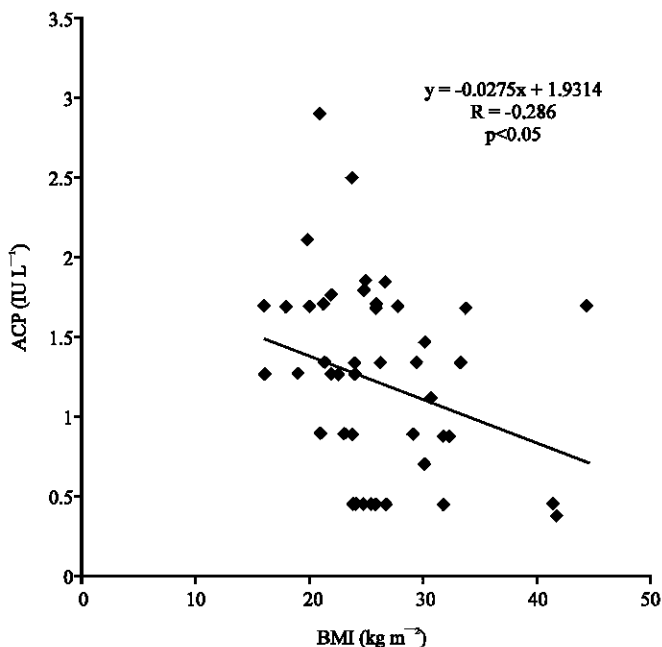


Fig. 1: Correlation plot of BMI and ACP in postmenopausal women

## **Discussion**

Menopause is associated with numerous physiological and biochemical changes affecting bone mineral metabolism. Results from this case control study on estimation of serum calcium, inorganic phosphate, total proteins, albumin, alkaline phosphatase and acid phosphatase activities in pre and postmenopausal women have shown that the serum calcium levels of postmenopausal women were significantly higher than those of the premenopausal women; the inorganic phosphate though higher in postmenopausal women is not statistically significant. This is in accordance with the findings of Ashuma *et al.* (2005), Suresh and Naidu (2006) and Masse *et al.* (2005) who also reported higher levels of these parameters in postmenopausal women. Ageing and menopause, which leads to decline in estrogen and progesterone production has been implicated in the increased levels of calcium and inorganic phosphates in postmenopausal women (Ashuma *et al.*, 2005). It is well known that oestrogen deficiency induces synthesis of cytokines by the osteoblasts, monocytes and T-cells and thereby stimulates bone resorption by increasing osteoclastic activity (Esbrit, 2001; Riggs *et al.*, 1998; Kurland *et al.*, 2000). This results in modification of the reabsorption, excretion and resorption of calcium and phosphate ion leading to increased circulating levels of these ions.

No significant variation was observed in serum levels of calcium, inorganic phosphate, total proteins, albumin, alkaline phosphatase and acid phosphatase activities in the various years since menopause (YSM) groups. However, contrary to this finding, higher calcium, phosphates and ALP activity have been demonstrated in early postmenopausal women ( $\leq 10$  YSM) compared to late menopausal women ( $\leq 10$  YSM) (Suresh and Naidu, 2006; Kato *et al.*, 1995; Christiansen *et al.*, 2002; Nilas and Christianse, 1998; Roger *et al.*, 2000). Heterogeneity of older adults, variabilities in body chemistry and their unique rate of ageing may be responsible for the disparity in these results.

Total proteins, albumin, globulin, albumin/globulin ratio were also elevated in postmenopausal women when compared to premenopausal women. This agrees with the findings of Eastell *et al.* (2001) who reported same and attributed this to ageing and menopause. However, Ragno and Delmas (1999) reported lower serum albumin, total proteins and globulin levels in postmenopausal women compared to premenopausal women. This was attributed to number of years since menopause and advancing age, since ageing is often associated with loss of height, weight and the development of stooped posture. Hypoalbuminaemia does not play a significant role in the pathogenesis of bone density reduction. It may be related to the reduction of bone mass only in subjects affected by diseases associated with a significant albumin reduction (Harmarayam *et al.*, 2004; Ragno and Delmas, 1999).

Alkaline phosphatase, acid phosphatase activities were also elevated in postmenopausal women when compared to premenopausal women. This is in accordance with studies by Dogan and Prosaci (2002) and Suresh and Naidu (2006), that in early postmenopausal women, there is high alkaline phosphatase and acid phosphatase activity when compared to those of premenopausal women as a result of the inhibitory effects of estrogen on bone turn over rate which is dependent on age and Body Mass Index (BMI).

Body mass index was found to be higher in postmenopausal women when compared to premenopausal women. However, no significant variation was observed in serum calcium, inorganic phosphate, total proteins, albumin, alkaline phosphatase and acid phosphatase activities in normal weight, preobese and obese women of the study. Bone resorption has been reported to be decreased in obesity and there is an inverse relationship between body mass index and bone resorption (Lindsay, 1993). Low BMI has been known to be a risk factor for osteoporosis and fractures (Ashuma *et al.*, 2005). The negative correlation seen between BMI and acid phosphatase activity in the present study is in agreement with previous findings.

## **Conclusion**

This study has shown that serum calcium inorganic phosphate, total proteins, albumin, alkaline phosphatase and acid phosphatase activities are higher in elderly and postmenopausal women. Years since menopause do not seem to affect the serum levels of these parameters. Menopause and ageing therefore increases the risk for bone loss and fractures.

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