

Correlates of bone resorption marker C-Telopeptide of type-I collagen for rural postmenopausal women in Zuturung, Kaduna State, Nigeria

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Abstract

Bone loss in postmenopausal women is majorly due to estrogen deficiency. The objective of this study was to determine correlates of serum C-Terminal Telopeptides of Type I Collagen levels

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Ethics approval and consent to participate: Ethical Committee on Human Research of the Ministry of Health and Human Services, Kaduna State, Nigeria approved this study (MOH/ADM/744/VOL.1/328). The study is conformed with the Helsinki Declaration of 1964, as revised in 2013, concerning human and animal rights. All patients participating in this study signed a written informed consent form for participating in this study.

Informed consent: Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

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(Ctx), a bone resorption marker, in rural postmenopausal women as compared with their premenopausal counterparts. The study was carried out on 38 premenopausal and 75 postmenopausal women in Zuturung, Kaduna state, Nigeria. Subjects were selected, questionnaires were administered to them, anthropometric parameters were determined and fasting blood samples were collected by venipuncture. The blood samples were centrifuged and the samples stored for further analysis using standard methods in the Department of Chemical Pathology, Ahmadu Bello University Teaching Hospital, Shika. Results were presented as mean \pm SD, data were analyzed using student t test, and a p value of <0.05 considered to be significant while associations between variables were determined by Pearsons' correlation using SPSS version 23. The postmenopausal subjects had reduced mean serum calcium (2.30 ± 0.35 mg/dL), decreased mean serum Ctx (135.20 ± 42.90 ng/mL) and a longer mean waist circumference (89.63 ± 10.66 cm) as compared with the premenopausal women (2.37 ± 0.15 mg/dL, 155.90 ± 88.70 ng/mL & 83.73 ± 8.00 cm respectively). While the waist circumference demonstrated a negative correlation with mean serum Ctx that however was not significant ($p > 0.05$). The mean serum Ctx of postmenopausal women with different educational status of the postmenopausal women showed no significant difference. In conclusion, rural postmenopausal women had a lower mean serum calcium and mean serum C-tx (a bone resorption marker) than premenopausal women. They also presented with a longer waist circumference, a negative correlation of their waist circumference with the mean serum Ctx with an equally no significant difference in mean Ctx level in their respective educational status.

Introduction

Bone is a metabolically active tissue that undergoes continuous remodelling known as bone turnover. Bone turnover is the process of resorption followed by replacement by new bone with little change in shape. Osteoclasts break down bone (bone resorption), osteoblasts are matured bones responsible for bone formation and ossification while osteocytes play a role in maintenance.¹

An imbalance in the remodeling process in menopause impacts on bone strength. An increase in bone turnover, where resorption exceeds formation is observed. It is not only inversely correlated with BMD, but may also alter bone architecture and porosity, increasing the risk of fracture.² Together with clinical and imaging techniques, biochemical tests play an important role in the assessment and differential diagnosis of bone disease.^{3,4} Correlates of bone resorption marker C telopeptide in postmenopausal women were determined in the study.

Materials and Methods

Study site

The study was carried out in Zuturung. It is a community in Zango Kataf Local Government, Area of Kaduna State, Nigeria. Located within Latitude 10.1589593N and Longitude 8.1338558E.

The area is located within the North Guinea Savannah zone with a climate characterized by the wet (rainy) and dry season (dry dusty cool *harmattan* followed by dry and hot season). The population during the 2006 census was estimated to be 4,767 people.⁵ Two major ethnic groups constitute the community; the Bajju and Ikulu tribes. They are an agrarian community, farming mostly for subsistence.⁶ Their source of water includes wells, boreholes and nearby streams. The staple meal for the community is cornmeal usually eaten with a variety of vegetable soups. A few tubers (cassava, yams, potatoes and cocoyams) and legumes are also farmed by the community.

Subjects

The study was a cross-sectional community based study of postmenopausal women in Zuturung district. The first women were randomly selected from a housing list drawn up while mapping the settlement. The women identified were then asked for information of other women who met the eligibility criteria; these women in turn were contacted, screened for eligibility and asked for the names of other women who met our criteria. Eligible participants were then given an appointment to be seen at the community Primary Health Centre for questionnaire administration and data collection.

The sample size (n) was determined as follows:

$$n = \frac{Z^2 P (1 - P)}{d^2}$$

(Z statistics for a level of confidence, P= expected prevalence or proportion, d= precision in proportion of one: if 5% d=0.05).⁷

$$n = \frac{(1.96)^2 \times 0.0571 (0.943)}{0.025}$$

$$n = 82$$

Participants consisted of one hundred and thirteen subjects. They comprised of 38 premenopausal and 75 postmenopausal women. Eligibility criteria were as follows: premenopausal women aged 15–45 years (peak bone mass is attained by age thirty), women resident in the community (>1 year), regularly menstruating, not pregnant, nor on hormonal contraceptive (the last 1 year), not on steroid treatment (>3 months) nor lactating were included in the study. Postmenopausal women selected were women aged between 40–80 years, amenorrhic for 12 months (if amenorrhea is secondary to medication, radiotherapy or surgery they were excluded), non-smokers and ambulatory.⁸

A previous history of a fracture (<5 years), the presence of a skeletal deformity, use of hormone replacement or medication that can affect bone turnover and a history of malignancy served as exclusion criteria.

Informed consent was obtained from the participants after approval of the study by the Ethical Committee on Human Research of the Ministry of Health and Human Services, Kaduna State, Nigeria.

Determination of anthropometric parameters

The questionnaires were filled by an interview and all other parameters determined as described below. The weight, height, waist circumference and hip circumference were determined using a weighing scale, stadiometer and a flexible metric tape respectively. Furthermore, the Body Mass Index (BMI) was computed as kg/m^2 .^{9,10}

Sample collection and analysis

Subjects were asked to fast overnight, while 5mL of venous blood was collected between 8.00 to 12.00 a.m. using a 5mL syringe and 21G needles.¹¹ It was separated by centrifuging and the serum kept under -80°C until further analysis. Then all the samples were thawed at room temperature 30 min before the analysis.

Serum CTx was determined using enzyme linked immunosorbent assay-double antibody sandwich principle to assay CTx in the sample. The microelisa stripplate provided in the kit was coated by purified CTx antibody to make solid phase antibody. CTx was then added to the wells and combined with CTx antibody labeled by HRP to become antibody-antigen-enzyme-antibody complex. After washing, chromogen A and B solutions were added to give a color change (blue to yellow), which was measured with a spectrophotometer at a wavelength of 450nm.

Data analysis

Results were presented as mean \pm SD and data was analyzed using student T test and one way Analysis of Variance (ANOVA) that was followed by Tukey post-hoc test. A p-value of <0.05 was considered statistically significant while associations between variables were determined by Pearsons' correlation using SPSS version 23.

Results

A total of 113 subjects participated in the study. They comprised of 38 premenopausal women and 75 postmenopausal women. The results for the mean age of all the subjects and mean age at menopause for the postmenopausal women are displayed in Table 1. Anthropometric and social data for the premenopausal and postmenopausal women are in Table 2. The educational status of the postmenopausal women is displayed in Figure 1. Figure 2 depicts the mean serum CTx for the subjects under study. The educational status of the postmenopausal women and its effects on mean serum levels of CTX is depicted in Figure 3. The parent history of fracture for the postmenopausal women and its effects on mean serum levels of CTX is represented in Figure 4. Table 3 is the correlation matrix for the variables in the postmenopausal women.

Table 1. Age of premenopausal and postmenopausal subjects.

Parameters	Premenopausal (n=38)	Postmenopausal (n=75)
Age (years)	33.60 \pm 4.59	57.67 \pm 9.19*
Age at menopause (mean years)	-	44.23 \pm 2.74
Age at menopause (median years)	-	44.00
Minimum age at menopause (years)	-	38.00
Maximum age at menopause (years)	-	56.00

p < 0.05* All values are indicated as mean \pm SD.

Results

Table 1 depicts the age and age at menopause characteristics of the premenopausal and postmenopausal women in Zuturing.

Regarding the anthropometric and social data for the premenopausal and postmenopausal women (Table 2), there was a statistically significant increase in waist circumference ($p=0.000$) and waist hip ratio in the postmenopausal women as compared to the premenopausal group. However, their BMI ($p=0.120$) and hip circumference ($p=0.08$) was not significantly different from that of the premenopausal group. No subject reported a history of smoking while all the 2 groups reported a history of consumption of alcohol along with a family/parent history of fracture.

In terms of education (Figure 1), the majority of the women had no form of education (48.5%) while only about 8.8% attained tertiary education.

The premenopausal subjects (who were women in their reproductive age) showed a slightly higher mean serum Ctx as compared with that of their postmenopausal counterparts. This was however not significant ($p>0.05$). There was no significant difference in the mean serum level of CTX among the postmenopausal women irrespective of their educational status ($p>0.05$). There was an apparently slight increase in mean serum CTX among the menopausal women with a parent history of fracture as compared with women without a parent history of fracture. This was however not statistically significant ($p>0.05$; Figures 2, 3 and 4).

Table 3 shows the data relative to the correlation matrix. The waist circumference demonstrated a positive correlation with mean serum PINP ($p<0.05$). However, the waist circumference showed a negative correlation with the mean serum Ctx that was not significant ($p>0.05$).

Discussion and Conclusions

Menopause is an important milestone in women signalling the

Table 2. The anthropometric and sociodemographic data for the premenopausal and postmenopausal subjects.

Parameters	Premenopausal (n=38)	Postmenopausal (n=75)
BMI (kg/m^2)	25.18 \pm 3.48	26.07 \pm 5.99
Waist circ. (cm)	83.73 \pm 8.00	89.63 \pm 10.66*
Hip circ. (cm)	98.97 \pm 8.38	100 \pm 9.8
Waist-Hip ratio	0.85 \pm 0.04	0.89 \pm 0.07*
Smoking currently (%)	0	0
Family history of fracture (%)	29.58	27.03
Drink alcohol (%)	18.31	36.99

$P<0.05$ *. BMI: Body Mass Index; Waist circ.: Waist circumference; Hip circ.: Hip circumference. All values are indicated as mean \pm SD.

Table 3. A correlation matrix for bone turnover markers and other variables among the postmenopausal subjects.

Variables	PINP	CTX	WC	IL-1
PINP	1			
CTX	0.038	1		
WC	0.303*	-0.139	1	
IL-1	0.043	0.196	0.103	1

$p<0.05$ Ctx - C-Terminal Telopeptides of Type I Collagen, PINP – Procollagen I N-Terminal Propeptide, IL-1- Interleukin 1, WC-Waist circumference.

end of a woman's reproduction life. Throughout the last decades interest in the timing of natural menopause has increased.^{12,13} Median age at menopause in developed countries is approximately 51 years and differs in different populations due to a combination of genetic and environmental factors.¹⁴ Our subjects had a mean and median age at menopause of 44.23 \pm 2.74 years and 44 years respectively (Table 1). These findings are similar to the previous study by Wang *et al.*,¹⁵ where a mean age at menopause for a rural Chinese community of about 17,076 postmenopausal women was recorded as 48.94 years. Early or late menopause is linked with a variety of chronic diseases. Early menopause, as determined in our study in Zuturing women, is associated with a higher risk of cardiovascular disease, osteoporosis and all-cause mortality.¹⁶

There was a statistically significant increase in waist circumference ($p=0.000$) and waist hip ratio in the postmenopausal women as compared to the premenopausal group (Table 2).

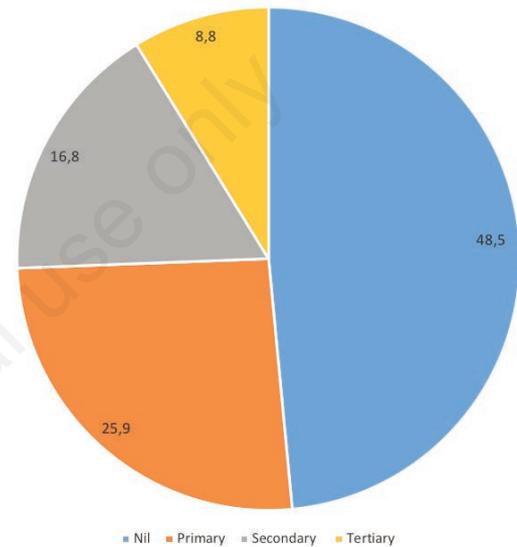


Figure 1. The educational status of the postmenopausal women.

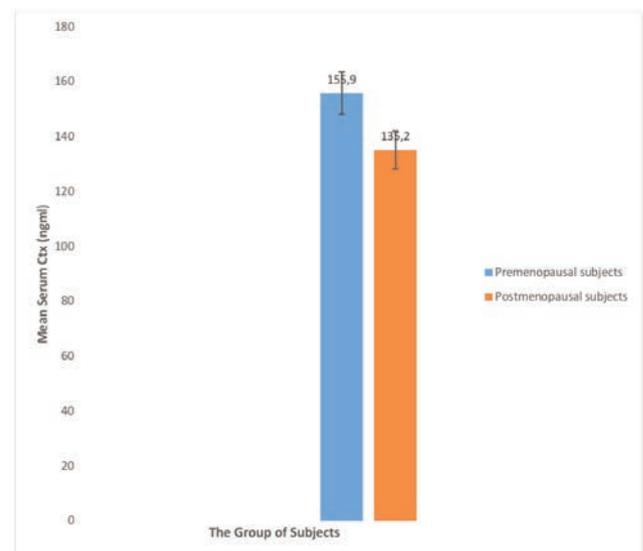


Figure 2. Mean serum levels of Ctx for the premenopausal and postmenopausal subjects.

Previous research is inconclusive as to whether mid-life body weight gain in women is related to aging *per se* or hormonal changes at menopause.¹⁷ The studies used a number of methods for assessing WC making cross-study comparison difficult. In a longitudinal study of middle-aged women, there was no difference in weight gain of women who remained premenopausal compared with those who became postmenopausal over follow-up. However, the difference in weight gain was as a result of decreased physical energy expenditure in postmenopausal women who were becoming more sedentary.¹⁸ Similarly, the Study of Women's Health Across the Nation (SWAN), a survey of ~13 000 multi-ethnic women in the United States, reported that BMI in women who had undergone natural menopause was not significantly different from premenopausal women.¹⁹ Contrary to the above finding, researchers²⁰ observed an increase in subcutaneous abdominal fat in middle-aged women, while menopause was associated with increase in visceral abdominal fat and total body fat. The lower estrogen (affecting fat distribution), the change in circulating follicle-stimulating hormone, increased appetite and lower energy expenditure in menopause are among the factors implicated in the change in fat mass leading to the conclusion that ovarian aging does play a role in body composition changes at menopause.^{18,21,22}

The majority of the postmenopausal women had no form of education (48.5%), while 25.9% and 16.8% had attained primary school and secondary school education respectively. Only 8.8% of the postmenopausal women had tertiary education (Figure 1). Those with tertiary education worked as teachers in the nearby primary and secondary schools. This is the typical picture in a rural community in a developing nation like Nigeria. Most of the women were therefore housewives of farmers. Farming was mostly in the form of subsistence farming, thus the women could be classified based on their educational attainment and occupational status as women within a low socioeconomic class.

We measured serum Ctx in the 2 groups and report a slightly lower mean serum Ctx (bone resorption marker) in the postmenopausal subjects ($135.20 \pm 42.90 \text{ ng/mL}$) as compared with the premenopausal women ($155.90 \pm 88.70 \text{ ng/mL}$; Figure 2). It infers a higher bone turnover in the premenopausal subjects as compared with the postmenopausal women. This was however not significant ($p > 0.05$). Together with clinical and imaging techniques, biochemical tests play an important role in the assessment and differential diagnosis of metabolic bone disease.²³ The isolation and characterisation of cellular and extracellular components of the skeletal matrix have resulted in the development of molecular markers that are considered to reflect either bone formation or bone resorption.²⁴ These biochemical indices are non-invasive, comparatively inexpensive and, when applied and interpreted correctly, helpful tools in diagnostic and therapeutic assessment.^{25,26}

One cannot also exclude the fact that some of the premenopausal women investigated could include women with osteoporosis. Could this findings also be due to the premenopausal women subjects being younger women (high bone turnover in the young) or rural women (the younger women are the energetic farmers with higher mechanical loading)?²⁶⁻²⁸ Alongside that, confounding factors like estimates of renal and liver function were not taken into account in our study.

Mean serum CTX showed no significant difference irrespective of the educational status of the postmenopausal women (Figure 3). The educational status of individuals have been linked to their socioeconomic status. In women this also translates to the level of their knowledge in handling their homes. This includes their knowledge on good nutrition. A higher income would suggest

a better diet and probably that would infer such women might take supplements or ingest more calcium containing meals. Reports by other authors^{29,30} suggest that a higher dietary calcium intake would enhance bone formation and reduce bone resorption.

There was an apparently slight increase in serum CTX among the menopausal women with a parent history of fracture as compared with women without a parent history of fracture (Figure 4). This is similar to what is reported in another study.³¹ In our study it was however not statistically significant ($p > 0.05$).

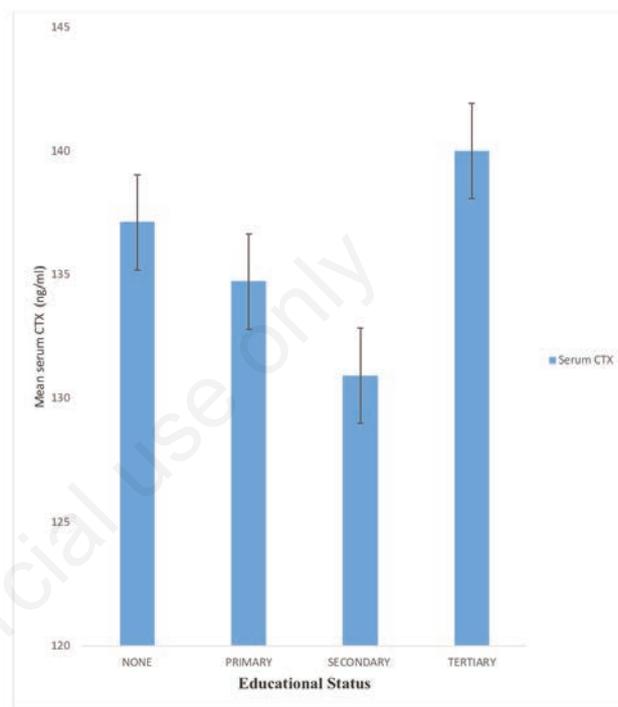


Figure 3. The effect of the educational status of the postmenopausal women on their mean serum levels of CTX.

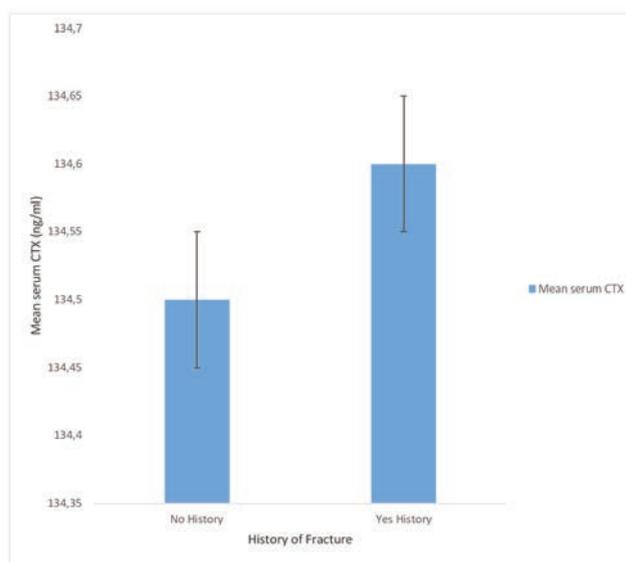


Figure 4. The parent history of fracture for the postmenopausal women and its effects on mean serum levels of CTX. Ctx: C-Terminal Telopeptides of Type I Collagen.

The waist circumference demonstrated a positive correlation with mean serum PINP (Table 3). Obesity is reported to possibly affect bone metabolism through several mechanisms. Obesity may increase adipocyte differentiation and fat accumulation while decreasing osteoblast differentiation and bone formation. Obesity is also revealed to be associated with chronic inflammation. The increased circulating and tissue proinflammatory cytokines in obesity may promote osteoclast activity and bone resorption through modifying the receptor activator of NF- κ B (RANK/RANK ligand/osteoprotegerin pathway). Furthermore, the excessive secretion of leptin and/or decreased production of adiponectin by adipocytes in obesity may either directly affect bone formation or indirectly affect bone resorption through up-regulated proinflammatory cytokine production. Implying a negative correlation between bone formation and obesity which can be defined by an increased waist circumference.³² However, in our study the negative correlation was between bone resorption and waist circumference in postmenopausal women, as suggested also in another study³³ where it was observed that the waist circumference of their subjects was independently and inversely associated with their bone mineral density. Which by inference implies that the waist circumference was a predictor of osteoporosis in their subjects.

In conclusion, the rural women experienced menopause at an earlier age, the postmenopausal women had a higher BMI and WC as compared to the premenopausal women however they (postmenopausal women), had a lower mean serum Ctx as compared with their premenopausal subjects. The study also revealed a higher Ctx in subjects whose parents had a history of fracture, with no difference in mean serum Ctx, increasing educational status and a negative correlation of Ctx and the waist circumference of the postmenopausal women; this correlation, however, was not considered significant.

Hence, the use of calcium supplements, dietary adjustment to enhance dairy products ingestion, and maintenance of a normal BMI is advocated in both the premenopausal and postmenopausal women. This is in order to alter the bone turnover as well as to meet the recommended dietary allowance for calcium in the postmenopausal subjects (for peak bone mass attainment in premenopausal women). We also recommend assays of hormones involved in calcium homeostasis for Nigerian postmenopausal women.

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