SERUM LEVELS OF BONE RESORPTION MARKER C-TELOPEPTIDE OF TYPE-I COLLAGEN FOR PREMENOPAUSAL AND POSTMENOPAUSAL RURAL NIGERIAN WOMEN IN ZUTURUNG DISTRICT, KADUNA STATE, NIGERIA.

ACHIE, L.N.[1]*, IGASHI, J.[2] AND AYEGBUSI, O.[3]

[1]Department of Human Physiology, Ahmadu Bello University, Zaria, Nigeria
[2]Department of Radiology, Ahmadu Bello University Teaching Hospital, Shika, Nigeria
[3]Department of Chemical Pathology, Ahmadu Bello University Teaching Hospital, Shika, Nigeria

ABSTRACT

In postmenopausal women, the two major causes of bone loss are estrogen deficiency after menopause and their advancing age. The objective of this study was to determine serum level of a bone resorption marker C-Terminal telopeptides of Type I Collagen (CTx), serum calcium levels and mean anthropometric parameters for a group of rural postmenopausal women as compared with their premenopausal counterparts. The cross-sectional study was carried out in 38 premenopausal and 75 postmenopausal women in Zuturung, Kaduna State, Nigeria. They were selected based on some inclusion and exclusion criteria. Questionnaires were administered to the participants while their anthropometric parameters were taken. Blood was collected, centrifuged and the sera stored frozen for further analysis using standard methods at the Department of Chemical Pathology, Ahmadu Bello University Teaching Hospital, Shika. Data were analyzed using Student's t-test and a p value of <0.05 was considered to be significant. Menopause had the effect of reduced mean serum calcium (2.30±0.35 mg/dl) and decreased mean serum C-Tx(135.20±42.90 ng/ml) and an increased mean waist circumference (89.63±10.66 cm) for the postmenopausal women as compared with their premenopausal women (2.37±0.15 mg/dl, 155.90±88.70 ng/ml & 83.73±8.00 cm respectively). We conclude that the rural postmenopausal women had lower mean serum calcium levels, lower bone resorption (as evidenced by decrease in mean serum CTx) and also presented with a longer waist circumference as compared to the premenopausal women. We recommend calcium supplementation in the postmenopausal women and screening of women (especially the premenopausal women) for osteoporosis.

Keywords: Bone turnover markers, Calcium, C-Terminal telopeptides of Type I collagen, Menopause, Waist circumference, Northern Nigeria.
***Correspondence:** nzug@yahoo.com, 09091931676

INTRODUCTION

Disorders of bone and mineral metabolism are becoming increasingly relevant in African postmenopausal women. One of such disorders is postmenopausal osteoporosis. Consequently, the interest in, and the need for effective measures to be used in the screening, diagnosis and follow up of such a pathology has increased especially in third world countries with poor resources and facilities [1, 2]. Oestrogen deficiency in menopause causes increases in bone turnover with a higher increase in bone resorption. [3, 4]. Together with clinical and imaging techniques, biochemical tests play an important role in assessment and follow up of subjects with osteoporosis [5]. The study was carried out to evaluate the levels of the mean serum calcium and C-Tx amongst premenopausal and postmenopausal women in Zuturung district, Kaduna State, Nigeria.

MATERIALS AND METHODS

Study participants and area

This cross-sectional study was conducted in 113 women comprising of 38 premenopausal women, and 75 postmenopausal women in Zuturung district, Kaduna State, Nigeria. Zuturung is a community in Zango Kataf Local Government Area of Kaduna State, Nigeria. It is a rural agrarian district made up of five settlements having five village heads. Their population during the 2006 census was estimated to be 4,767 people made up principally two ethnic groups; Bajju and Ikulu.

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) (Daniel, 1999)

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

$$n = 82$$

Participants were selected from the general population according to some inclusion and exclusion

criteria. Postmenopausal women who were diabetics, hypertensive, HIV positive, who smoked cigarette, were amenorrhoeic due to hysterectomy or had cessation of periods other than by a natural cause, having a history of hormone replacement therapy, hysterectomy and fractures were excluded. While postmenopausal women selected were at least 1year amenorrhoeic due to a natural cause. The premenopausal women were regularly menstruating, not pregnant, non-lactating, HIV seronegative with no use of hormonal contraception for at least 1 year.

The study was undertaken after obtaining consent from the participants and approval from the Ethical Committee on Human Research of the Ministry of Health and Human Services of Kaduna State, Nigeria. The Ethical Approval number is MOH/ADM/744/VOL. 1/328

Anthropometric parameters determination

Questionnaires were administered and anthropometric measurements taken. Height (cm) and weight (kg) of each woman were determined utilizing a stadiometer and the body mass index (BMI) was calculated (kg/m^2). Underweight was defined as a BMI<18.5 kg/m^2, normal BMI as >18.5-24.9 kg/m^2, overweight as BMI between 25-29.9 kg/m^2, obese as BMI>30 kg/m^2 and BMI≥ 35 kg/m^2 was considered as morbid obesity [6]. The waist circumference was measured for the subjects using a flexible metric tape [7].

Blood sample collection and analysis

Five milliliters of venous blood was drawn aseptically from each subject. It was centrifuged at 3,000 rpm for 10 minutes after which serum was separated. The serum was stored at -20°C until used. All subjects had a liver function test performed from the fasting blood sample that was collected. This was done in order to exclude participants with deranged liver function tests.

I. Serum calcium from the fasted subjects was measured. The principle used was based on the metallochromogen Arsenazo III. It combines with calcium ions to form a colored chromophore which was measured at the absorbance of 630 nm. All specimens with haemolysis were excluded.

II. Serum CTx was determined using enzyme linked immunosorbent assay-double antibody sandwich principle to assay CTx in the sample (Melsin Medical Co., Limited, China, CAT. NO: EKHU-0342). The microELISA strip plate 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 while the color change was measured with a spectrophotometer at a wavelength of 450nm (assay range 20 ng/ml – 500 ng//ml, sensitivity of less than 8 ng/ml.

Data analysis

Results were presented as mean±SD and data were analyzed using Students *t* – test, while associations between variables were determined by Pearson's correlation using SPSS version 23. A p value of < 0.05 was considered significant.

RESULTS

A total of 113 women 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 sociodemographic data for the premenopausal and postmenopausal women in Zuturung are in Table 2. The serum CTx and calcium levels for the subjects under study are displayed in Table 3. The parent history of fracture for the postmenopausal women and its effects on mean serum levels of CTx is depicted in Figure 1.

Table 1: Age and the Age at menopause for the premenopausal and postmenopausal women in Zuturung district, Kaduna State.

Parameters	Premenopausal Women (n=38) Mean ± SD	Postmenopausal Women (n=75) Mean ± SD
Age (years)	33.60 ± 4.59	57.67 ± 9.19*
Age at menopause (mean years)	-	44.23 ± 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*

Table 2: Anthropometric and socio-demographic data for the premenopausal and postmenopausal women in Zuturung district, Kaduna State.

Parameters	Premenopausal Women (n=38) Mean ± SD	Postmenopausal Women (n=75) Mean ± SD
Body mass index (kg/m^2)	25.18±3.48	26.07±5.99
Waist circumference (cm)	83.73±8.00	89.63±10.66*
Smoking currently (%)	0%	0%
Drink alcohol (%)	18.31%	36.99%
Family history of fracture (%)	29.58	27.03

P< 0.05*

There was a statistically significant increase in waist circumference (p=0.000) in the postmenopausal women as compared to the premenopausal group. However, the BMI (p=0.120) of the menopausal women was not significantly different from that of the premenopausal group. No subject reported a history of smoking while the 2 groups reported a history of consumption of alcohol.

Table 3: Serum CTx and other biochemical analytes for the premenopausal and postmenopausal women in Zuturung district, Kaduna State.

Biochemical Analytes	Premenopausal Women (n=38) Mean ± SD	Postmenopausal Women (n=75) Mean ± SD
Calcium (mg/dl)	2.37±0.15	2.30±0.35
Phosphorus (mg/dl)	1.09±0.14	1.09±0.19
Albumin (g/dl)	39.00±4.49	38.48±7.05
CTx (ng/ml)	155.90±88.70	135.20±42.90

p<0.05=*

There was an apparent decrease in mean serum calcium and CTx in the postmenopausal women as compared with their premenopausal counterparts that was however not significant (p>0.05).

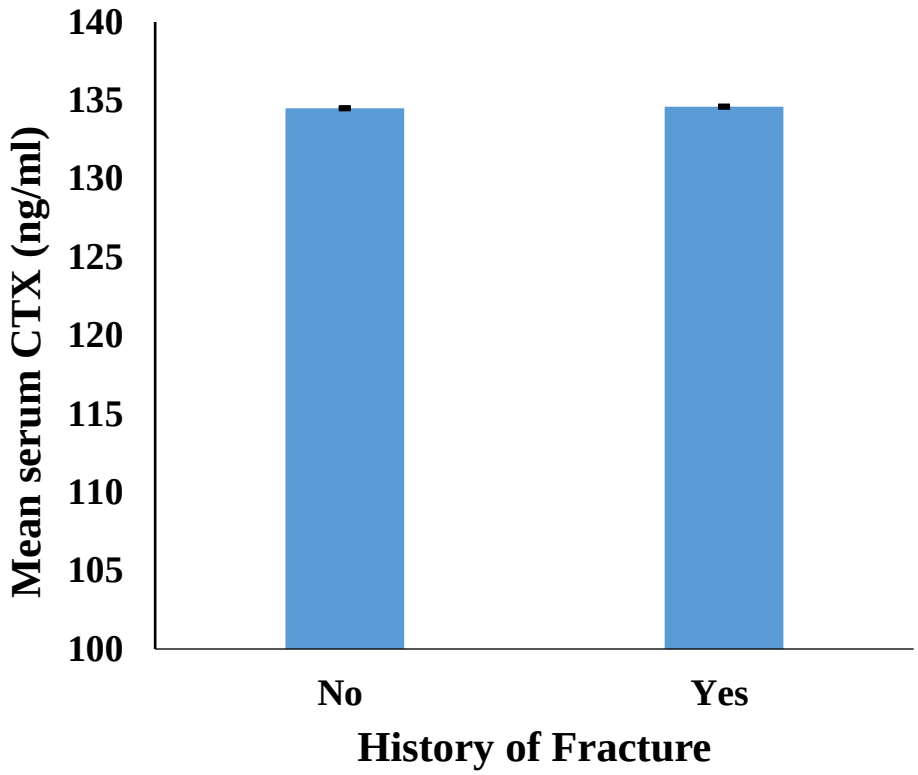

Figure 1. The parent history of fracture for the postmenopausal women and its effects on serum levels of CTX.

There was no significant difference in serum CTx between the groups according to the parent history of fractures ($p>0.05$).

DISCUSSION

Although menopause is a universal phenomenon among women, the timing of the onset is not. The age at menopause is more sensitive to varying rates of atresia of ovarian follicles than to the absolute number of oocytes depleted, but menopause is reached when depletion of follicles reaches approximately 1000 [8]. The mean and median age at menopause for the postmenopausal women in Zuturung was 44.23±2.74 years and 44 years respectively (Table 1). These findings are similar to the previous studies by other authors on Nigerian postmenopausal women. The mean and median age at menopause for Nigerian women in Zaria, Ibadan and Saganmu was found to be lower than as obtained in developed countries; 46.2 and 46 years respectively; 48.4 and 48.0 years respectively; 48.91 years and 49.00 years respectively [9, 10, 11]. The age at menopause holds intrinsic clinical and public heath interest because it serves as a marker of aging and health [12] Later age at menopause has been associated with a longer life expectancy, reduced risk of cardiovascular disease and less loss of bone density [12, 13]. While a lower age at menopause (as recorded in our participants) is associated with an earlier decline in cognitive function and an increased risk of cardiovascular diseases (14). The factors leading to this earlier onset of menopause include racial/ethnic, geographical, mother's age at menopause, lower BMI and socioeconomic status [15, 16, 17]. The mean age of the premenopausal women was significantly lower than that of the postmenopausal subjects (Table 1). That is understandably so because women attain the later phase with advancing age.

Menopause is also accompanied by pronounced changes in body composition which includes loss of bone and muscle, overall increased adiposity and fat redistribution towards central-type obesity [18, 19]. This was depicted among our study subjects; even though it was a cross sectional study (Table 2). There was a statistically significant increase in the length of the waist circumference ($p<0.05$) of the postmenopausal women as compared to their premenopausal group (Table 2). Their BMI ($p>0.05$) was not significantly higher than that of the premenopausal control participants. Obesity as determined by BMI and waist circumference

measurement is associated with diseases like hypertension, atherosclerosis, diabetes mellitus type II and metabolic syndrome [20, 21]. However, a beneficial effect of increased adiposity was reported by Mazocco and Chagas, [22]. They reported a lower prevalence of osteopenia and osteoporosis in obese postmenopausal women as compared with normal weight subjects. Higher BMI are associated with a higher body weight that imposes a greater mechanical load on bone which enhances increase in bone formation in order to accommodate the load. It does this by decreasing apoptosis and increasing proliferation and differentiation of osteoblasts and osteocytes [23, 24]. Adipocytes from such an increase in weight, are a source of production of estrogen, leptin, and adiponectin which act directly or indirectly on osteoblast and osteoclast activity, resulting in the development of bone mass [25]. However, visceral fat promotes systemic inflammation, which can lead to bone loss, besides having an association with increased levels of pro-inflammatory cytokines such as TNF and IL-6, which increase bone resorption [25, 26]. Although, there is a report of a stronger correlation of waist circumference in males than in females [27].

Mean serum CTx was determined in the 2 groups and a report of a slightly lower mean serum CTx (for bone resorption activity) in the postmenopausal women (135.20±42.90) as compared to the premenopausal women (155.90±88.70) was observed (Table 3). Even though this was not significant in our study ($p>0.05$), this could infer a higher bone turnover in the control premenopausal women group as compared with the postmenopausal women. The typical report on bone turnover is that of higher bone turnover in growing children due to their skeletal growth velocity [28]. Peak bone mass is usually established in the 30s while a higher bone resorption with advancing age and in menopause is reported [8, 29, 30]. Differences in population characteristics may at least in part explain these inconsistencies. One cannot also exclude the fact that some of the premenopausal women investigated may include women with osteoporosis [31]. These findings could be due to the premenopausal women being younger women (high bone turnover in the young), rural women (energetic farmers with higher mechanical loading), in their reproductive age (modulation by lactation and pregnancy; though we selected not lactating, not pregnant participants) or it might be contributions from their race [32, 33, 34]. Confounding factors like estimates of renal and liver function have to be taken into account when evaluating menopause dependent changes in bone turnover. We did not completely exclude such factors in both groups.

There was no significant difference in serum CTx among the postmenopausal women with a parent history of fracture as compared with women without a parent history of fracture (Figure 1). This is contrary to what was reported in another study [35]. Mean serum calcium levels were slightly reduced in the postmenopausal women as compared with the control premenopausal women which was not significant (Table.3). Bhattarai *et al*. [36] also observed a similar significant decrease in serum calcium in menopausal women. Khadka *et al.* [37], also reported a marked significant decrease in serum calcium in postmenopausal women as compared with their premenopausal subjects. However, another study in Nigerian menopausal women by Usoro *et al.* [38] reported a significantly higher mean serum calcium levels in the postmenopausal women as compared to the premenopausal women. Inferring that menopause alters the metabolism of calcium. Some of the mechanisms include diminished dietary intake of calcium containing foods, decrease in intestinal absorption of calcium and increased urinary excretion of calcium [39, 40]. Hence the use of supplements is advocated in postmenopausal women in order for them to meet the recommended dietary allowance for calcium. This also serves as a strategy for the prevention of osteoporosis. It is especially needed in these subjects from a rural community with a decreased dietary intake of calcium and high parity.

CONCLUSION

The study shows that the rural women experienced menopause at an earlier age, the postmenopausal women had significantly longer WC as compared to the premenopausal women however they (postmenopausal women) had lower serum calcium and serum CTx as compared with their premenopausal subjects which was however not significant. Hence, we recommend studies with a higher sample size, screening subjects to reduce confounders and assays of hormones involved in calcium homeostasis for Nigerian postmenopausal women.

ACKNOWLEDGEMENT

We wish to acknowledge the District head of Zuturung, the research assistants for the study, and all the women who participated in the study.

REFERENCES

1. BURCH, J., RICE, S., YANG, H., NEILSON, A., STRIK, L., FRANCIS, R., HOLLOWAY, P., SELBY, P. & CRAIG, D. (2014). Systematic review of the use of bone turnover markers for monitoring the response to osteoporosis treatment: the secondary prevention of fractures, and primary prevention of fractures

in high-risk groups. *Health Technology Assessment*, **18**(11): 1-180.

2. FLORENCIO-SILVA, R., RODRIGUES, G., SASSO-CERRI, E., SIMÕES, M.J. & CERRI, P.S. (2015). Biology of bone tissue: Structure, function, and factors that influence bone cells. *BioMed Research International*, - https://doi.org/10.1155/2015/421746.
3. WEITZMANN, M.N. & PACIFICI, R. (2006). Estrogen deficiency and bone loss: An Inflammatory tale. *The Journal of Clinical Investigation*, **116**(5): 1186-1194.
4. STREICHER, C., HEYNY, A., ANDRUKHOVA, O., HAIGL, B., SLAVIC, S., SCHULER, C., KOLLMAN, K., KANTNER, I., SEXL, V., KLEITER, M., HOFBAUER, L.C., KOSTENUIK, P.J. & ERBEN, R.G. (2017). Estrogen regulates bone turnover by targeting RANKL expression in bone lining cells. *Scientific Reports*, **7**: 6460. https:/doi.org/10.1038/s41598-017-06614-0.
5. SEIBEL, M.J. (2001). Biochemical Markers of Bone Turnover Part I: Biochemistry and variability. *Clinical Biochemistry Review*, **26**(4): 97–122.
6. NORTON, K., WHITTINGHAM, N., CARTER, L., KERR, D., GORE, C. & MARFELL-JONE, M. (1996). Measurement techniques in anthropometry, chapter 2. In: Norton K, Olds T (eds) Anthropometrica. University of New South Wales Press, Sydney, Australia, 1996: 25–75.
7. VISSCHER, T.L.S., SEIDELL, J.C., MOLARIUS, A., VAN DER KUIP, D., HOFMAN, A. & WITTEMAN, J.C.M. (2001). A comparison of body mass index, waist-hip ratio and waist circumference as predictors of all-cause mortality among the elderly: the Rotterdam study. *International Journal of Obesity*, **25** (11): 1730 – 1735.
8. SOWERS, M.R., ZHENG, H., GREENDALE, G.A., NEER, R.M., CAULEY, J.A., ELLIS, J., JOHNSON, S. & FINKELSTEIN, J.S. (2013). Changes in bone resorption across the menopause transition: Effects of reproductive hormones, body size, and ethnicity. *Journal of Clinical Endocrinology & Metabolism*, **98**(7): 2854 – 2863.
9. ACHIE, L.N., OLORUNSHOLA, K.V & MABROUK, M. (2011). Age at natural menopause among Nigerian women in Zaria, Nigeria. *Asian Journal of Medical Sciences* **3**(8): 151-153.
10. OKONOFUA, F.E, LAWAL, A. & BAMGBOSE, J.K. (1990). Features of menopause and menopausal age in Nigerian women. *International Journal of Gynecology & Obstetrics*, **31**(4): 341 – 345.
11. ADEFUYE, P.O., ADEFUYE, B.O., LAMINA, M.A., SHORUNMU, T.O. & AKINDELE, A. (2017). Menopause in Sagamu, southwest Nigeria. *East African Medical Journal*, **94**(12): 986-997.
12. GOLD, E.B. (2011). The timing of the age at which menopause occurs. *Obstetrics and Gynecology Clinics of North America*, **38**(3): 425 – 440.
13. GOLD, E.B., BROMBERGER, J., CRAWFORD, S., STEVE, S., GREENDALE, G.A., HARLOW, S.D. & SKURNICK, J. (2001). Factors Associated with Age at Natural Menopause in a Multiethnic Sample of Midlife Women. *American Journal of Epidemiology*, **153**(9): 865–874.
14. KUH, D., COOPER, R., MOORE, A., RICHARDS, M. & HARDY, R. (2018). Age at menopause and lifetime cognition. *Neurology*, **90**(19): e1673 – e1681.
15. HARLOW, B.L. & SIGNORELLO, L.B. (2000). Factors associated with early menopause. *Maturitas*, **35**(1): 3 – 9.
16. MCKNIGHT, K.K., WELLONS, M.F., SITES, C.K., ROTH, D.L., SZYCHOWSKI, J. M., HALANYCH, J.H., CUSHMAN, M. & SAFFORD, M.M. (2011). Racial and regional differences in age at menopause in the United States: findings from the reasons for geographic and racial differences in stroke (REGARDS) study *American Journal of Obstetrics and Gynecology*, **205**(4): 353.e1–353.e8.
17. CEYLAN, B. & OZERDOGAN, N. (2015). Factors affecting age of onset of and determination of quality of life in menopause. *Journal of Turkish Society of Obstetrics and Gynecology*, **12**(1): 43 – 49.
18. HO, S.C., WU, S., CHAN, S.G. & SHAM, A. (2010). Menopausal transition and change of body composition: A prospective study in Chinese perimenopausal women. *International Journal of Obesity*, **34**(8): 1265 – 74.
19. GREENDALE, G.A., STERNFELD, B., HUANG, M., KARVONEN-GUTIERREZ, C., RUPPERT, K., CAULEY, J.A., FINKELSTEIN, J.S., JIANG, S. & KARLAMANGLA, A.S. (2019). Changes in body composition and weight during the menopause transition. *JCI Insight*, **4**(5): e124865.
20. BOSELLO, O. & ZAMBONI, M. (2003). Visceral obesity and metabolic syndrome. *Obesity Reviews*. **1**(1): 47 – 56.

21. SILVEIRA, E.A., KLIEMANN, N., NOLL, M., SARRAFZADEGAN, N. & OLIVEIRA, C. (2020). Visceral obesity and incident cancer and cardiovascular disease: An integrative review of the epidemiological evidence. *Obesity Reviews*, **22**(1): e13088.
22. MAZOCCO, L. & CHAGAS, P. (2017). Association between body mass index and osteoporosis in women from northwestern Rio Grande do. *Revista Brasileira de Reumatologia*, **57**(4): http://dx.doi.org/ 10.1016/j.rbre.2016.10.002.
23. NELSON, L.R. & BULUN, S.E. (2001). Estrogen production and action. *Journal of the American Academy of Dermatology*, **45**(3): S116 – 24.
24. HOU, J., HE, C., YANG, M., LUO, X. & LI, C. (2020). Obesity and Bone Health: A Complex Link. *Frontiers in Cell and Developmental Biology*, https://doi.org/10.3389/fcell.2020.600181
25. CAO, J.J. (2011). Effects of obesity on bone metabolism. *Journal of Orthopaedic Surgery and Research*, https://doi.org/10.1186/1749-799X-6-30.
26. KIM, D., LIM, H., CHANG,S., KIM, J., ROH, Y. & CHOI, M. (2019). Association between body fat and bone mineral density in normal-weight middle-aged Koreans. *Korean Journal of Family Medicine*, **40**(2): 100–105.
27. LIAN-HUA, C., MIN-HO, S., SUN-SEOG, K., JIN-SU, C., JUNG-AE, R., YOUNG-HOON, L., HAE-SUNG, N., SEUL-KI, J., KEONG-SOO, P., SO-YEON, R. & SEONG-WOO, C. (2014). Sex-related differences in the association between waist circumference and bone mineral density in a Korean population. *Musculoskeletal Disorders*, **15**: 326. doi: 10.1186/1471-2474- 15-326. PMCID: PMC4193133.
28. HUANG, Y., EAPEN, E., STEELE, S. & GREY, V. (2011). Establishment of reference intervals for bone markers in children and adolescents. *Clinical Biochemistry*, **44**(10-11): 771-8.
29. GARNERO, P., SORNAY-RENDU, E., CHAPUY, M.C. & DELMAS, P.D. (1996). Increased bone turnover in late postmenopausal women is a major determinant of osteoporosis. *Journal of Bone & Mineral Research*, **11**(3): 337-49. doi: 10.1002/jbmr.5650110307. PMID: 8852944.
30. SZULC, P., MONTELLA, A. & DELMAS, P.D. (2008). High bone turnover is associated with accelerated bone loss but not with increased fracture risk in men aged 50 and over: the prospective MINOS study. *Annals of the Rheumatic Diseases*, **67**: 1249-1255.
31. PEPE, J., BODY, J., HADJI, P., MCCLOSKEY, E., MEIER, C., OBERMAYER-PIETSCH, B., PALERMO, A., TSOURDI, E., ZILLIKENS, M.C., LANGDAHL, B. & FERRARI, S. (2020). Osteoporosis in premenopausal women: A clinical narrative review by the ECTS and the IOF, *The Journal of Clinical Endocrinology & Metabolism*, **105** (8): 2487–2506, https://doi.org/10.1210/clinem/dgaa306
32. GREENBLATT, M.B., TSAI, J.N. & WEIN, M.N. (2017). Bone Turnover Markers in the Diagnosis and Monitoring of Metabolic Bone Disease. *Clinical Chemistry* **63**(2): 464–474.
33. DAI, Z., WANG, R., ANG, L.W., YUAN, J.M. & KOH, W.P. (2016). Bone turnover biomarkers and risk of osteoporotic hip fracture in an Asian population. *Bone*, **83**:171- 177.
34. LESLIE, W.D. (2012). Ethnic differences in bone mass—Clinical implications. *Journal of Clinical Endocrinology & Metabolism*, **97**: 4329 – 4340.
35. DEY, M. & BUKHARI, M. (2019). Predictors of fragility fracture and low bone mineral density in patients with a history of parental fracture. *Osteoporosis and Sarcopenia*, **5**(1):6-10. doi:10.1016/j.afos.2019.03.001.
36. BHATTARAI, T., BHATTACHARYA, K., CHAUDHURI, P. & SENGUPTA, P. (2014).Correlation of Common Biochemical Markers for Bone Turnover, Serum Calcium, and Alkaline Phosphatase in Post-Menopausal Women. *Malaysian Journal of Medical Science*, **21**(1): 58–61.
37. KHADKA, B., TIWARI, M.L., GAUTAM, R., TIMALSINA, B., PATHAK, N.P., KHAREL, K., SHARMA, S. & ACHARYA, D. (2018). Correlates of Biochemical Markers of Bone turnover among Post-Menopausal. *Journal of Nepal Medical Association*, **56**(212): 754 –758.
38. USORO, C.D.O., ONYEUKWU, C.U. & NSONWU, A.C. (2007). Biochemical bone turnover markers in postmenopausal women in Calabar municipality. *Asian Journal of Biochemistry*, **2**(2): 130 – 135.
39. Position statement of The North American Menopause Society. (2010). Management of osteoporosis in postmenopausal women. *Menopause*, **17**(1): 23–24.DOI: 10.1097/gme.0b013e3181cdd4a7.
40. WISHART, J.M., SCOPACASA, F., HOROWITZ, M., MORRIS, H.A., NEED, A.G., CLIFTON, P.M. & NORDIN, B.E. (2000). Effect of perimenopause on calcium absorption: a longitudinal study. *Climacteric*, **3**(2): 102-8.