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Research Article

Serum calcium levels of premenopausal, perimenopausal and postmenopausal rural women of Zuturung District, Kaduna State, Nigeria.

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Keywords:
Body mass index, calcium, menopause, osteoporosis, waist circumference, Zuturung district

ABSTRACT

Background: Changes in sex hormones during the menopause transition period have an impact on calcium homeostasis. We studied the age at menopause, anthropometric and mean serum calcium levels in a cohort of premenopausal, perimenopausal and postmenopausal women in Zuturung, Kaduna state, Nigeria. **Methods:**135 subjects participated in the crossectional study. They comprised of 38 premenopausal, 22 perimenopausal and 75 postmenopausal subjects. After administering a questionnaire, the height (m), weight (g), and waist circumference (cm) of the subjects were determined using standard methods while the body mass index (BMI, kg/m2) was calculated. 5 milliliters of blood were collected via venipuncture and serum calcium level was determined by utilizing standard laboratory methods. **Results:**The results showed a mean and median age at menopause of 44.23±2.74 years and 44 years, respectively. Postmenopausal and perimenopausal subjects were more likely to be overweight with mean BMI 26.07±5.99 $kg/m^2$and 26.42±7.27kg/m^2 respectively, compared with their premenopausal counterparts with BMI of 25.18±3.48kg/m^2 ($p<0.001$). The postmenopausal and perimenopausal subjects also had a longer waist circumference of 89.63±10.66cm and 92.19±11.91cm respectively compared with the premenopausal women 83.73±8.00cm ($p<0.001$). Only 73.86% of the postmenopausal women had a BMI $\geq$25kg/m^2 whereas the prevalence of central obesity as determined using the waist circumference among the postmenopausal subjects was 79%. Mean serum calcium levels were slightly lower amongst both postmenopausal and perimenopausal subjects, 2.30±0.35mg/dl and 2.36±0.13mg/dl respectively as compared with the premenopausal women 2.37±0.15mg/dl. These differences were not significant ($p>0.05$). **Conclusion:** These findings suggest a lower mean age at menopause, a higher BMI, a longer waist circumference for the postmenopausal subjects (which was significant) with lower mean serum calcium levels (that was not significant) as compared with their premenopausal subjects. We recommend calcium supplementation and screening of postmenopausal women for postmenopausal osteoporosis.

INTRODUCTION

Osteoporosis is a very important health problem worldwide. It is defined as a disease characterized by low bone mass and micro-architectural deterioration of bone tissue, leading to enhanced bone fragility and consequent increase in fracture risk (Shetty *et al*., 2016). Postmenopausal osteoporosis is characterized by an imbalance between increased osteoclast activity and decreased osteoblast function due to estrogen deficiency resulting in increased bone remodeling, bone micro architectural deterioration, and skeletal fragility (Sozen *et al*., 2017). Osteoporosis is a silent disease. The health and financial impact of the disease results from fracture, particularly hip fracture, for which subjects with osteoporosis are at an increased risk. Extracellular calcium ion concentration is determined by the interaction of calcium absorption from the intestine, renal excretion of calcium, and bone uptake and release of calcium, each of these is regulated by parathyroid hormone, vitamin D and calcitonin amongst other hormones (Veldurthy *et al*., 2016). The estrogen deficiency in menopause results in increased bone

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turnover and indirectly may induce calcium loss by indirect effects on extra skeletal calcium homeostasis (Canon *et al*., 2017; NAMS, 2010). The study was carried out to evaluate the calcium status of postmenopausal women in Zuturung district, Kaduna State, Nigeria.

MATERIALS AND METHODS

Subjects

This cross-sectional study was conducted in 135 women comprising of 38 premenopausal, 22 perimenopausal and 75 postmenopausal subjects from Zuturung district, Kaduna state, Nigeria. It is a community in Zango Local Government Area (LGA) of Kaduna State. This LGA consists of predominantly rural farming communities. The only semi-urban town is where the LGA headquarters is sited. Zuturung district is a rural area made up of 5 settlements with a 2006 census estimate of 4,767 people.

Subjects were selected from the general population according to some inclusion and exclusion criteria. Subjects who were diabetics, hypertensive, who smoked, amenorrhoeic due to hysterectomy or cessation of periods other than by a natural occurrence, having a history of hormone replacement therapy, hysterectomy and fractures were excluded. The premenopausal subjects were regularly menstruating, non-pregnant, non-lactating with no use of hormonal contraception for at least 1 year. Postmenopausal women selected were at least 1 year amenorrhoeic due to a natural occurrence. All subjects were required to fast for 8 hours (no water or food after dinner) and were assessed alongside with sample collection between the hours of 06.00 and 10.00am.

The study was undertaken after obtaining consent from the subjects and approval from the Ethical Committee on Human Research of the Ministry of Health and Human Services of Kaduna State, Nigeria.

Determination of anthropometric parameters

Questionnaires were administered and anthropometric measurements obtained. Height (cm) and weight (kg) of each woman were determined utilizing a stadiometer while the body mass index was calculated (BMI = 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>30-39.9 kg/m^2 and BMI≥35 kg/m^2 was considered as morbid obesity (Guyton and Hall, 2006).

The waist circumference was measured for the subjects using a flexible metric tape (Visscher *et al*., 2001)

Sample Collection and Analysis

Five milliliters (5ml) of venous blood was drawn aseptically from each fasting subject. It was centrifuged at 3,000 rpm for 10 minutes after which serum was separated (Baird & Taitlock London limited, England).The serum was stored at -20°C until ready for use.

Serum calcium from the fasted subjects was measured. The principle used was based on the metallochromogen Arsenazo III. As described by the instruction manual, it combines with calcium ions to form a colored chromophore, which was measured at the absorbance of 630nm (Reckon Diagnostics P. LTD, India). All specimens with hemolysis were excluded. The specimens (0.01ml) were mixed with the reagent (1ml), incubated for 5 minutes at room temperature and read at the specified absorbance. The result of the test absorbance was calculated with a standard absorbance and expressed as mg/dL.

Serum phosphorus was measured based on the method using Ammonium Molybdate. Inorganic phosphorus reacts with Ammonium molybdate in the presence of sulphuric acid to form unreduced phosphomolybdate complex. One milliliter of the reagent was mixed with 0.01ml of the test sample, mixed well and incubated at room temperature for 5 minutes. The color change was then measured at the absorbance of 340nm. The absorbance of the test divided by the absorbance of the standard, multiplied by 5 gave the phosphorus concentration in mg/dL as described in the instruction manual (Reckon Diagnostics P. LTD, India).

Serum albumin was determined using the principle of dye binding in an acidic medium (Reckon Diagnostics P. LTD, India). The sample (10µL) was mixed with the reagent (1mL), incubated for 10 minutes at room temperature and the product was measured at the absorbance of 630nm as recommended on the instruction manual. Albumin binds with bromocresol green (BCG) causing a shift in the absorption spectra of the yellow BCG dye. The blue green color formed is directly proportional to the albumin present and was then measured. The absorbance of test and standard after 10 minutes was read against reagent blank (600-650nm or with RED filter). The concentration of albumin in patient specimens as calculated from a standard known albumin concentration was expressed as mg/dL

Data Analysis

Results were presented as mean ± SD and data were analyzed using one way analysis of variance (ANOVA), while $p < 0.05$ was selected as the level of significance.

RESULTS

A total of 135 subjects participated in the study. They comprised of 38 premenopausal, 22 perimenopausal and 75 postmenopausal subjects. The results for the mean age of all the subjects and mean age at menopause for

Table 1: Mean age and age at menopause for the premenopausal, perimenopausal and postmenopausal subjects.

Parameters	Pre-Menopausal (N=38)	Peri-Menopausal (N=22)	Post-Menopausal (N=75)
Age (years)	33.60 ± 4.59	46.00 ± 5.80*	57.67 ± 9.19*
Age at menopause (mean years)	-	-	44.23 ± 2.74
Age at menopause (median)	-	-	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 ± SD

Table 2: Anthropometric and sociodemographic data of premenopausal, perimenopausal and postmenopausal subjects.

Parameters	Pre-Menopausal (N=38)	Peri-Menopausal (N=22)	Post-Menopausal (N=75)
BMI (kg/m^2)	25.18±3.48	26.42±7.27	26.07±5.99
Waist circ. (cm)	83.73±8.00	92.19±11.91*	89.63±10.66*
Smoking currently	0%	0%	0%
Drink alcohol (%)	18.31	22.72	36.99

p< 0.05* BMI-Body mass index, Waist circ-Waist circumference, All values are indicated as mean ± SD

Table 3: Mean serum albumin, calcium and phosphorus for the premenopausal, perimenopausal and postmenopausal subjects.

Biochemical parameters	Pre-Menopausal (N=38)	Peri-Menopausal (N=22)	Post-Menopausal (N=75)
Albumin (g/dl)	39.00±4.49	41.07±3.71	38.48±7.05
Calcium (mg/dl)	2.37±0.15	2.36±0.13	2.30±0.35
Phosphorus (mg/dl)	1.09±0.14	1.10±0.12	1.09±0.19

P<0.05= *All values are indicated as mean ± SD.

the postmenopausal women are displayed in Table 1. Anthropometric and sociodemographic data for the premenopausal, perimenopausal and postmenopausal subjects in Zuturung are in Table 2. The mean serum calcium levels and other biochemical analytes for the 3 groups of women are displayed in Table 3. There was a statistically significant increase in waist circumference (p=0.000) amongst the perimenopausal and menopausal subjects as compared to the premenopausal subjects. However, the BMI (p=0.120) of the perimenopausal and menopausal was not significantly different from that of the premenopausal group. No subject had a positive history of smoking while some subjects in all the 3 groups reported a positive history of consumption of alcohol as indicated in Table 2. There was no statistically significant difference in the biochemical analytes for both the perimenopausal and postmenopausal subjects as compared to their premenopausal subjects. However, the mean serum calcium showed a declining trend across the 3 groups with a lowest value in the postmenopausal women which was not significant as indicated in Table 3.

DISCUSSION and CONCLUSION

In our previous study, Achie *et al*., (2011), we reported the mean age at menopause for postmenopausal women in Zaria to be 46.2years. Zaria is a cosmopolitan town also located in Northern Nigeria. In this study, the mean age at menopause for the rural women in Zuturung district was 44.23±2.74 years. This age is lower than that of the aforementioned study.

Reasons given for the earlier age at natural menopause in rural women are said to be due to the interplay of genetic and environmental factors. Previous reports indicate that the lower age at menopause for the mothers of rural women, (menopausal age in mothers and daughters being heritable and associated with higher FSH levels in their daughters), later age at menarche for rural postmenopausal women and lower life expectancy of the women (living in a developing country) contributed to the lower age at menopause in our rural postmenopausal women (Bjelland *et al*., 2018; Gold *et al*., 2013; Steiner *et al*., 2008; van Asselt *et al*., 2004; Thomas *et al*., 2001). Other factors that have been suggested as contributing to the lower age at menopause for the rural postmenopausal women were their lower nutritional status, higher infection rates, higher parity, lower body mass index (due to their subsistence agricultural practices) and their being from a developing country (Saw and Resni, 2020; Parsaeian *et al*., 2017; WHO, 1996). However, there are reports of an inverse association of parity with risk of early menopause (Langton *et al*., 2020). Rural women in developing countries are reported to experience menopause about eight years earlier than in developed countries; reasons being suggested include stress. However, eliciting the age at natural menopausal in this study was by recall which is generally 2 years earlier (recall bias), this could have contributed to our findings of an earlier age at menopause in the rural women under study.

There were differences in the BMI and the waist circumference of the postmenopausal women as compared with that of the premenopausal and perimenopausal women which was significant for waist circumference but not significant in the for BMI (Table 2). The increases in overweight and obesity in menopausal women are important public health concerns. Reasons for the increase in body weight in menopause include higher dietary intake of food as evidenced by hyperphagia demonstrated in ovariectomised rats (Witte *et al*., 2010). The hypothalamus coordinates food intake, satiety and energy expenditure (Timper and Bruning, 2017). Estrogens and estrogen depletion (as found in menopause) plays a role in this regulation (Xu and Lopez, 2018). Cyclic estradiol treatment has been proved to restore physiological patterns of feeding and alongside that, normalize body weight in ovariectomised rats (Riviera and Stincic, 2017; Asarian and Geary, 2002). These effects are also observed on injection of 17β estradiol into various brain regions. Estrogens produce rapid responses which include activation of kinase, phosphatase and phospholipase. They ultimately mediate physiological responses such as effects on cell cycle, cell survival and energy metabolism (Lu *et al*., 2020; Liu *et al*., 2018).

Human adipose tissue express estrogen receptor alpha and beta (ESRα & ESRβ) which play a role on the activity of adipocytes and sexual dimorphism of fat distribution (Palmer and Clegg, 2015). Acute activation of ESRα has been reported to decrease food intake, meal size and body weight in ovariectomised rats. Implying that estrogens via their receptors cause satiety (suppress feeding behaviour) and indirectly regulate body weight. Over the period of the menopause transition a decrease in physical activity has been observed which contributes to the reduced energy expenditure associated with menopause (Leeners *et al*., 2017). Along with the alteration in energy homeostasis in menopause is a decrease in subcutaneous fat, an increase in visceral fat (explaining the increased waist circumference) and a decrease in fat oxidation (Palmer and Clegg, 2015; Oliveira *et al*., 2012). Alterations in mitochondrial fatty acid oxidation in the heart during menopause is associated with lipotoxicity and increased cardiovascular risk in menopausal women. These are all mediated by the decline in estrogen in menopause. Other factors affecting energy homeostasis mediated via estrogens are neuropeptide Y, ghrehlin, resistin and the inflammatory cytokines IL1, TNFα and CD68 (Clegg *et al*., 2007

Only 73.86% of the postmenopausal women had a BMI ≥25 kg/m2 whereas the prevalence of central obesity (as measured using the waist circumference) was 79%. This finding supports the study by Lear *et al* (2009), where waist circumference is reported to be a better measure of cardiovascular risk than the BMI especially in the Asian population.

The mean serum calcium levels were lower in the postmenopausal subjects compared with the premenopausal and perimenopausal women (Table 3). Bhattarai *et al*., (2014) also observed a similar lower serum calcium in postmenopausal women. Khadka *et al*., (2017), Kalita and Choudhury (2017) 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* (2007) 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. Dairy products, meat and some vegetables serve as sources of calcium. The dietary history of the subjects revealed their staple food was mostly from grains. They predominantly fed on gruels, corn meals, fresh/ dry vegetables for soups and sparingly could afford fish, meat and milk. In developing countries these foods are expensive and hence cannot be afforded by individuals belonging to a low socioeconomic class. Our postmenopausal subjects were elderly rural women who were predominantly farmers involved in subsistence farming hence belonged to a low socioeconomic class. While some of them were retired from active farming and thus entirely dependent on their children for sustenance. This definitely determined the quality of their diet.

Other factors reported to influence calcium homeostasis in menopause include increased urinary excretion of calcium, which is both menopause and age related. This occurs in the proximal tubule and can be corrected with oestrogen replacement (Bansa *et al*., 2013; Dick and Prince, 1997; Pedrosa and Mandel, 1992).

A decrease in intestinal absorption of calcium is also reported in menopause and declining levels of vitamin D is implicated (Veldurthy *et al*., 2016; NAMS, 2010; Nordin et al., 2004; Wishart *et al*., 2000). Though one study suggests that oestrogen has a direct effect on calcium absorption by reducing the level of 1, 25 $(OH)_2$ D, another study showed that oestrogen may work by directly altering the intestinal responsiveness to 1, 25 $(OH)_2$D (Fleet and Schoch, 2010; Gennari *et al*., 1990). The reduction in intestinal calcium absorption was observed to be much higher in older postmenopausal subjects above 75 years (Nordin *et al*., 2004).

In conclusion, the rural postmenopausal subjects experienced menopause at an earlier age, had a higher BMI and longer WC as compared to the premenopausal subjects however they (postmenopausal subjects) had lower mean serum calcium levels as compared with their premenopausal and perimenopausal subjects which was not significant

The use of calcium supplements have been advocated in postmenopausal women so they can meet their recommended dietary allowance for calcium (Supriyatiningsih, 2018). This is especially needed in our subjects from a rural community with a decreased dietary calcium intake, high parity and longer lactation duration. The use of these supplements are not devoid of its attendant gastrointestinal side effects and the risk of kidney stones. However, there is a report suggesting the lack of evidence supporting the beneficial effect of calcium and vitamin D supplementation (Chiodini and Bolland, 2018; Aggarwal, 2013).

We also recommend implementing strategies to prevent falls and studies on assays of hormones involved in calcium homeostasis for rural Nigerian postmenopausal women.

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