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## Comparison of Urinary Bone Resorption Markers with Bone Densitometry in Elderly Individuals in Port Harcourt, Rivers State

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**ABSTRACT:** *This study concentrated on urine bone markers, N and C- telopeptide, and their concentrations evaluated in the urine of elderly participants and compared with that of the young of the same gender. Markers for bone resorption are proteins, and usually measured in urine, where they serve to assess bone turnover. A cross-sectional prospective study conducted at the orthopaedic and Family Medicine Departments of the University of Port Harcourt Teaching Hospital, a public tertiary healthcare facility located in Choba community of Obio/Akpor Local Government Areas of Rivers state, Nigeria, among elderly patients aged 60 years and above. Considering the age of the test population, all the females were post-menopausal and no statistical difference was observed in relation to the results for gender in the study. More females participated, while the control group had more males. And there is no statistically significant difference in mean bone resorption markers among the two age groups ( $p < 0.005$ ). Creatinine values were used to normalize the urine N and C Telopeptide. while stemming calcium imbalance among the elderly individuals arise from severe chronic illness, malabsorption syndrome and poor socio-economic factor such as malnutrition. Urine values of NTX and CTX were generally increased in the elderly. Dual x-ray absorptiometry also corresponded with values of the urinary markers of bone resorption and may be useful for diagnosing BMD of normal, osteopaenia and osteoporosis. However, it is worthy to note that urine values are not completely reliable owing to possible interference, unlike serum, thus, correction with creatinine is required. The study has public health importance owing to the fact that bone pain is a common complaint among older individuals. It also revealed that at the elderly age, most people experience osteopaenia, with some already having osteoporosis, thus, it is recommended that elderly patients that are likely to have resorption should be enlightened on markers of bone resorption, and can serve diagnostic purposes.*

**KEYWORDS:** Bone markers; urine creatinine; bone densitometry; metabolism.

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## INTRODUCTION

Humans, like every life form, are known to age with time. However, the environment the individual lives in, also plays some role in the ageing process. Occasionally, ageing can be expedited by certain factors such as disease and lifestyle, other than the natural process that comes with the environment and time. As age increases, metabolic processes in the host begin to decline in the host, and in humans, one of such is the decline in bone and mineral metabolism. Disorders of bone and minerals among the ageing population is becoming relevant to everyday medical practice (Lunenfeld and Stratton, 2013; Seibel, 2005) and is peculiar to every climate, (Cadmus *et al.*, 2014). Studies have shown that metabolic loss of bone and minerals in the ageing population is associated with the median rise in age of countries, specifically, as the distribution of its population tends towards older age (Juni, 2015). Ageing is defined as the chronological, changes in social role and capabilities respectively by some organizations of international repute, with change in social role predominating the features and considered to commence at the age of 60 years, with same cut off adopted by most developing countries (Glascock and Feinmen, 1980). The adopted cut off age of 60 years for the elderly, as postulated by some organizations, however, does not sit well with other bodies. Thus, some developed countries have pegged theirs at 65 years (Juni, 2015; Krueger *et al.*, 2015), while the United Nations is yet to set a standard numerical criterion but rather adopted 60 years as its benchmark (Gesinde and Adekeye, 2011). In Africa, old age is usually linked to retirement from active service, thus, no existing consensus, at the moment, regarding the global standard for defining old age (Freitas *et al.*, 2010).

In medical practice, there are several methods used to assess the age of bones, those of humans inclusive. Some of the commonly used methods include Tanner White house, Bone Xpert, Greulich, Pyle and X-ray (Ewa, Arkaduz *et al.*, 2001; Bull *et al.*, 1998). Each method comes with its peculiar characteristics, ranging from assessment of skeletal maturity to prediction of adult height, but there could be occasional overlapping features between some methods. The importance of assessing bone age lies in the fact that it can be used to diagnose growth disorders or failures, rule out congenital adrenal defects, some forms of amenorrhea and hypothyroidism (Ewa *et al.*, 2001; Bull *et al.*, 1998).

Observations by James (2011) reports that the decrease in bone mass that come with ageing has an early female preponderance, with the onset observed to begin around 50 years in males, but a little earlier in females. The author further observed that the percentage of loss of bone mass is approximately 1% and 2% per year in males and females respectively, while the variation in annual magnitude is thought to be due to factors such as menopause and the early progression due to menstruation, with the fraction among males being consistent. For instance, from menopause to five years after menopause, the rate is between 2%-3% and 1% afterwards, and cumulatively, about 53% of bone mass is lost by the age of 80 years, as compared to the cumulative loss of about 18% in males at that same age (James, 2011).

Bone resorption occurs when osteoclasts, which are bone macrophages, breakdown bone tissues and release minerals located in such bone tissues into the circulation (blood). These minerals include calcium and phosphorus. These minerals can thus, be detected at increased levels in body fluids such as the urine and serum of older people, and is indicative of increased bone resorption among them. Thus, in older people, the remodelling process of bones (formation and loss of bone minerals and architecture) is skewed in favour of bone mass loss. In contrast, adolescents (10-19 years), bone remodelling favours bone formation, leading to an increased bone mass among young people (formation exceeding resorption), with the peak bone mass formation in humans attained between 15-25 years of age, while equilibrium bone formation and resorption remains constant for about ten years (Kloss and Gassner, 2006). With ageing, and especially, after attaining peak bone mass formation, a gradual but constant loss of bone minerals ensues, accounting for less dense bones with increased tendency of fragility and susceptibility to osteoporosis (Clark, 2008).

In addition to old age, other factors, which could be intrinsic or extrinsic, can also expose bones to high tendency of fracture (Ferruci *et al.*, 2014). The intrinsic factors include genetics, alterations in cellular composition of the bone, hormonal changes, and vascular and biochemical status of the individual (Demontiero *et al.*, 2012), while extrinsic factors include physical activity, nutrition, drugs and co-morbid medical conditions (Pereira *et al.*, 2008). Feng and McDonald (2011) and Seibel (2005) also opines that hormones such as vitamin D, parathyroid hormones, steroid hormones, growth factors and cytokines play vital roles in the remodelling of bones (Ginaldi *et al.*, 2005), with their hyper-production observed to aid demineralization, while optimal production serves equilibrium remodelling status (De-Martinis *et al.*, 2005).

Several markers have been observed as effective, thus, employed for the assessment of bone resorption in humans. Majority of the markers are proteins, which are determined and their concentrations measured in body fluids such as the urine and serum. These proteins include type-1 collagen, sulphated proteoglycans, acidic glycoproteins and osteocalcin (Florencio-Silva *et al.*, 2015). Their measurement is premised on their turnover. Some markers commonly used for the assessment of bone mass include N-telopeptide (type-1 collagen), C- telopeptide (type- 1 collagen), pyridinoline cross links, hydroxyproline, bone sialoprotein and tartrate resistant acid phosphatase (TRAP)- 5b (Swaminathan, 2001), while another form of TRAP, subset 5a is reported to originate from macrophages like dendritic cells (Halleen *et al.*, 2006).

There is current body of knowledge regarding the relationship between the pathogenesis of osteoporosis and ageing. This stimulated the interest of researchers to ascertain the association between bone resorption and the process of ageing (Boskey and Coleman, 2010). Experimentally, the pathogenesis of bone resorption, can be observed on the surface of the bone (Clark, 2008) and involves the attachment of osteoclasts to the osteon (Van Oers *et al.*, 2014; Clark, 2008), which then leads to infolding of the cell membrane of the osteoclast to produce collagenase and other

enzymes (Suda and Takahashi, 2008) and further excavation into the bone and release of minerals, such as calcium, magnesium, phosphate and collagen into the extracellular fluid (Moe, 2008). Other than the osteoclasts (bone resorption), other cells found in bone are osteoblasts (bone formation), osteocytes (bone maintenance) and stromal cells (Florencio-Silva *et al.*, 2015; Clark, 2008), with each a distinct function but collectively perpetrating bone remodelling.

Human bone is composed of organic and inorganic matter in a ratio of 33:67. The organic matter component is 28% collagen and 5% non-collagen protein, while the inorganic matter comprises of 57% hydroxyapatite crystals and 10% calcium carbonate and other trace elements (Palacios . *et al.*, 2012). Bone collage is composed of 95% type- 1 and 5% type- V, while its non-collagenous component is formed by osteocalcin, osteopontin, saloprotein and osteonectin.

This study focussed on urine bone resorption markers, N and C- telopeptide and densitometry, and their concentrations were evaluated in the urine of elderly participants and compared with that of young individuals of same gender. This is based on the fact that bone matrix is mainly composed of collagen, which is responsible for its integrity and strength, and these markers are mostly composed of type- 1 collagen, while densitometry determines the bone mass. The study was necessitated by the fact that, in the environment of the study (Port Harcourt), elderly individuals frequently complain of bone pain, thus, their urine levels of these bone resorption markers (N and C telopeptide) was estimated, believing that its outcome can help the clinical diagnosis and management of increased bone resorption, and initiate interventions before bone becomes too fragile and also help in predicting osteoporosis.

## **METHODOLOGY**

This cross-sectional prospective study was conducted at the orthopaedic and Family Medicine Departments of the University of Port Harcourt Teaching Hospital, a public tertiary healthcare facility located in Choba and Alakahia communities of Obio/Akpor Local Government Areas of Rivers state, Nigeria. The facility undertakes management of patients, training of health professionals and medical and allied research, while serving as referral medical facility for health facilities within the state and its neighbouring states; Bayelsa, Imo, Abia and Akwa-Ibom. The participants were healthy elderly (60 years old and above) males and females, who presented at the recruitment points in the departments earlier mentioned, while a second group served as controls and were aged 15-25 years old and 30-45 years old respectively, taking into cognizance the fact that that is when peak bone mass is achieved in individuals and when bone mass begins to decline respectively (Kloss and Gassner, 2006; Teegarden *et al.*, 1995). A total number of 220 individuals participated (110 test and controls each), with ethical approval obtained from the Ethical Committee of the same facility.

The reagents that were used for the study are human cross-linked N- telopeptide type- 1 collagen, human cross-linked C- telopeptide type- 1 collagen and Randox creatinine assay kit for calcium and albumin. Other materials that were used include assay microplate, adhesive strips, microplate reader, water bath, automated microplate washer, absorbent paper towels, deionized/distilled water, pipette and pipette tips, test tubes and cuvettes, graduated cylinders, calibrated micropipettes, centrifuge, spectrophotometer, timer, laboratory coats, sterile gloves, glass pipette filter, waste disposal bin and the instruction manuals from the manufactures of the various reagents. Laboratory methods for conducting the assays, as prescribed by the manufacturers were adhered to, while the prevalence was based on the 2014 Nigeria Population Census that reported the total elderly in the population as 5.2%.

## RESULTS

A total of 220 respondents participated in the study (110 participants and 110 controls). They were in two major groups (first group with age ranges of 60-85 years, mean age was  $66.6 \pm 6.6$  years and a second group of two subgroups with age of 15-25 years and 30-45 years respectively). There were 44 (40.0%) males and 66(60.05) females. 46(50.6%) had history of hypertension, while 13(14.3%) had co-morbidities of hypertension and diabetes mellitus, but none had history of only diabetes mellitus, while 78(80.0%) had bone pain and 32(20.0%) did not have bone pain.

Considering the age of the test population, all the females were post-menopausal and no statistical difference was observed with regards to the results for gender in the study. Urine N- Telopeptide and C – Telopeptide results were corrected for creatinine. Urine N Telopeptide was higher in the participants ( $p= 0.03$ ) compared to the controls. Urine C Telopeptide was also higher in the participants compared to controls ( $p = 0.04$ )

**Table 1: Table of numbers**

|              |   | Age group (years) | Frequency (n) | Percentage (%) |
|--------------|---|-------------------|---------------|----------------|
| Controls     | 1 | 15 - 25           | 50            | 45.5           |
|              | 2 | 30 – 45           | 60            | 54.5           |
| Participants |   | 60 and above      | 110           | 100            |

**Table 2. Urine N–Telopeptide in participants and controls**

| Group                                      | N Tel nm/mmolcr  |
|--|------------------|
| Participants                               | $152.7 \pm 20.2$ |
| Controls (15 – 25 years)                   | $141.3 \pm 45.2$ |
| Controls (30 – 45 years)                   | $140.2 \pm 44.1$ |
| Total number of controls                   | $143.6 \pm 36.9$ |
| p value of participants and total Controls | 0.03             |

**Table 3: Urine C - Telo peptide in participants and controls**

| Group                                      | C Tel $\mu\text{g}/\text{mmolcr}$ |
|--|-----------------------------------|
| Participants                               | 41.1 $\pm$ 12.6                   |
| Controls (15 – 25 years)                   | 24.4 $\pm$ 11.2                   |
| Controls (30 – 45 years)                   | 23.9 $\pm$ 9.9                    |
| Total number of Controls                   | 24.8 $\pm$ 10.5                   |
| P value of Participants and Total Controls | 0.04                              |

**Table 4: Comparison of Bone Densitometry and Urine Bone Resorption Markers**

| T Score range                 | N Tel nm/mmolcr | C Tel $\mu\text{g}/\text{mmolcr}$ |
|-------------------------------|-----------------|-----------------------------------|
| Normal ( $> -1.0$ )           | 125.8 – 156.5   | 5.2 – 37.4                        |
| Osteopaenia ( $-1.0 - -2.5$ ) | 156.7 – 180.5   | 37.5 – 59.2                       |
| Osteoporosis ( $< -2.5$ )     | $> 181$         | $>60$                             |

## DISCUSSION

The study recruited a total of 220 participants (110 subjects and 110 controls, with the male population being slightly lower than the females). The subjects had more females than males, while the controls had more males than females, with the control group stratified into two subgroups, 15–25 years (45.5%) when peak bone mass is achieved and 30–45 years (54.5%) when total bone mass begin to decrease (Kloss *et al.*, 2006; Teegarden *et al.*, 1995). There is no statistically significant difference in the mean value of bone resorption markers between the two age groups in the study.

Creatinine values were used to normalize the urine N Telo peptide and C Telo peptide. With an average elderly person having negative calcium balance (Heanery *et al.*, 1982), with this stemming from calcium imbalance among this category of individuals arising from severe chronic illness, malabsorption syndrome and poor socio-economic factor such as malnutrition<sup>93</sup>. The calcium imbalance in the elderly is usually ameliorated by placing them calcium and vitamin D-containing supplements; however, none of the participants was on any supplement.

The urine N- telo peptide (NTX) among the study participants were compared and it establish that urine NTX levels were higher in the elderly participants than controls. This finding is similar with other previous reports of high levels of N Telo peptide in elderly (Baxter *et al.*, 2012; Scariano *et al.*, 2009). In comparison with serum, studies by the same author have shown than the urine values were higher. This points to bone resorption. Higher values of NTX in the elderly subjects suggest higher loss of bone mineral density.

Similarly, the urine C- telopeptide (CTX) was found to follow the same pattern between the subjects and controls. The levels were higher in the elderly subjects than controls and the same explanation applies as for urine N- telopeptide.

Elevated levels of CTX in the urine of the elderly subjects are a predictor of risk of osteopaenia or osteoporosis, since it indicates depreciation in the BMD. This finding is similar to that of a study by Ivaska *et al.* (2010) and the authors predicted that individuals with high biomarkers in urine had increased risk of pathological fractures. However, a study by Nguyen *et al.* (2007) showed that CTX was not sufficiently sensitive to predict the rate of change in BMD, but this study revealed the opposite (CTX is a sensitive predictor). The difference in the findings may be due to the fact that Nguyen *et al.* (2007) used Caucasians whereas our study used elderly African.

Many studies have suggested that low bone mineral density is an important determinant of increased bone resorption which was evaluated using bone markers like NTX and CTX (Mc Chun, 2005; Sarka *et al.*, 2004; Ross and Knowlton, 1998). Ross and Knowlton (1998) reported that markers of bone resorption were significant predictors of bone loss rate, in their study of osteoporotic fracture among elderly post-menopausal women (Ross and Knowlton, 1998).

Bone densitometry was done by employing dual X- ray absorptiometry in this study and the T – score used to interpret the outcome. A previous study of bone mass density (BMD) using bone densitometry on proximal femur and radius among elderly men and women showed that BMD was significantly related to age in both sexes (Hannan *et al.*, 1992). Its importance lies in the fact that it can be used to determine osteopaenia among the elderly population, while its combination with the indices of bone resorption improves the prediction of fracture risk (Sornay–Rendu *et al.*, 2005; Granero *et al.*, 1996). In the environment of this study, majority of the health facilities that do not have instruments to perform dual X– ray absorptiometry, thus, they go for reagents to estimate the bone resorption markers, which is affordable.

This study has shown that reference ranges of the markers of bone resorption could be used to estimate BMD. For instance, an elderly individual with urine NTX of 130nm/mmolcr have normal T –score of > -1.0. A normal T –score means the individual has normal BMD. On the contrary, elderly individuals with urine NTX of 190nm/mmolcr are estimated to have a T- score of < -2.5 and are considered to have osteoporosis. Without having the dual X–ray absorptiometry equipment, the markers could be used to estimate the BMD of elderly individuals. Linear relationship exists between the markers and bone densitometry in both the subjects and the controls. It is, thus, suggested that employing more than one resorption marker will improve diagnostic ability bone markers.

## **SUMMARY/CONCLUSION**

This study established that urine values of NTX and CTX were increased in the elderly participants. Dual X-ray absorptiometry readings corresponded with suggested values of the urinary markers of boneresorption and may be useful for diagnosing BMD of normal, osteopaenia and osteoporosis. These urine markers can also be used to monitor patients on treatment. However, it is worthy to note that urine values are not completely reliable owing to possible interference, unlike what is reported for serum by some investigators, including one by this same author, thus, correction with creatinine is required.

### **Recommendations**

Findings from this study have public importance owing to the fact that bone pain is a common complaint among older individuals. This study helped to understand that at this age, most people experience osteopaenia, with some already having osteoporosis, thus, it is recommended that elderly patients that are likely to have resorption should be enlightened on markers of bone resorption, that can be used to diagnose possible osteoporosis in them.

### **Consent**

Written and informed consent was obtained from the participants.

### **Ethical approval**

Ethical approval for the study was obtained from the Ethical Committee of the orthopaedic and Family Medicine Departments of the University of Port Harcourt Teaching Hospital.

### **CONFLICTING INTEREST**

Authors have declared that no competing interests exist.

### **ACKNOWLEDGEMENT**

None.

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Author's contributions:

This work was carried out in collaboration among all authours. Kiyesi Adekemi designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Victor Chukwuma Wakwe assisted in designing and supervising the work, made inputs in the manuscript and modification of the study. All authours read and approved the final manuscript.