

**Original Research Article**

**Prognostic value of Serum Estrogen, Cortisol, Calcium and Alkaline Phosphatase Activity in Pre and Postmenopausal HIV Women at Nnewi, Nigeria**

**ABSTRACT**

**Background:** Osteoporosis is common in human immunodeficiency virus (HIV) infected persons. The etiology of osteoporosis in HIV-infected patients is likely multifactorial, involving traditional risk factors such as low body weight, hypogonadism, as well as direct effects of chronic HIV infection and antiretroviral therapy.

**Aims of Study:** This study is designed to assess the prognostic value of Serum Estrogen, Cortisol, Calcium and Alkaline Phosphatase Activity in Pre and Postmenopausal HIV infected Women at NAUTH Nnewi, Nigeria.

**Material and Methods:** A total of 80 female participants within the age bracket of 19-45(premenopausal) and 46-80 years (postmenopausal) were randomly recruited for the study from 2013 to 2014 and grouped into: (i) HIV- infected postmenopausal females (n=20). (ii) HIV- infected premenopausal females (n=20). (iii) Control postmenopausal females (n=20). (iv) Control premenopausal females (n=20). Blood samples were collected for determination of estrogen and cortisol using Enzyme linked Immunosorbent Assay (ELISA) techniques. Serum levels of calcium and alkaline phosphatase activity were analyzed using spectrophotometric methods.

**Results:** The result showed that the mean serum alkaline phosphatase and cortisol levels in premenopausal HIV females were significantly higher when compared with the corresponding values in premenopausal control females ( $P=.05$ ). The mean serum estrogen level in postmenopausal HIV females was significantly lower when compared with the value in postmenopausal control female subjects ( $P=.05$ ). However, the serum cortisol level in postmenopausal HIV females was significantly higher compared to the value in the corresponding control female subjects ( $P=.05$ ).

**Conclusions:** The present study demonstrates significant derangement in the alkaline phosphatase activity; estrogen and cortisol levels in HIV infected female subjects thereby raising the incidence of osteoporosis. The clinical implication is discussed.

**Keywords:** HIV Infection; osteoporosis; women; Menopause

**1. INTRODUCTION**

Osteoporosis is derived from a Greek word "*ostoun*" meaning "bone" and "*poros*" meaning "pore". It is a progressive bone disease that is characterized by a decrease in bone mass and density which can lead to an increased risk of fracture [1]. Osteoporosis is an accelerated bone loss which is greater than the physiologically normal loss associated with bone remodeling. The World Health Organization (WHO) has defined osteoporosis as a spinal or hip bone mineral density (BMD) that is 2.5 standard deviations or more below the mean BMD for healthy, young women, measured by dual energy x-ray absorptiometry (DEXA) [2].

Osteoporosis is most common in women after the menopause, when it is called postmenopausal osteoporosis, but may develop in men and premenopausal women in the presence of particular hormonal disorders and other chronic diseases. The common osteoporotic fractures are mostly of the wrist, spine, shoulder and hip. The underlying mechanism in all cases of osteoporosis is an imbalance between bone resorption and bone formation.

Low bone mineral density (BMD) is prevalent in HIV infected subjects. Initiation of antiretroviral therapy is associated with a 2% -6% decreases in BMD over the first 2 years, a decrease that is similar in magnitude to that sustained during the first 2 years of menopause [3]. A previous study has shown that the prevalence of osteoporosis was 3 times higher among HIV infected patients than HIV negative control patients, especially among those receiving antiretroviral therapy (ART) [4].

Hormonal factors affect bone metabolism. For instance lack of estrogen increases bone resorption, as well as decreasing the deposition of new bone that normally takes place in weight-bearing bones [5]. The amount of estrogen needed to suppress this process is lower than that normally needed to stimulate the uterus and breast gland. In addition to estrogen, calcium metabolism plays a significant role in bone turnover, and deficiency of calcium and vitamin D leads to impaired bone deposition; in addition, the parathyroid glands react to low calcium levels by secreting parathyroid hormone (PTH), which increases bone resorption to ensure sufficient calcium in the blood. Estradiol increases production of osteoprotegerin to diminish bone resorption. Glucocorticoids stimulate receptor activator of nuclear factor kappa-B ligand (RANKL) expression while inhibiting osteoprotegerin (OPG) synthesis by osteoblasts to enhance osteoclast proliferation and differentiation, leading to bone resorption [6].

Cortisol, a steroid hormone produced by the zona fasciculata of the adrenal cortex [7] is released in response to stress and low level of blood glucocorticoids. It has been reported that Cortisol decreases bone formation, favouring long term development of osteoporosis [8] and a condition of overt hypercortisolism also called Cushing syndrome leads to osteoporosis [8].

This study was therefore designed to assess the prognostic importance of some indices such as estrogen, cortisol, calcium and alkaline phosphatase activity in the diagnosis, treatment and monitoring of osteoporosis in postmenopausal and premenopausal HIV infected women.

## 2. MATERIAL AND METHODS

### 2.1. Study Area

The study was carried out at the department of Institute of Human Virology (IHV) in Nnamdi Azikiwe University Teaching hospital, Nnewi (NAUTH), Anambra State Nigeria.

### 2.2. Study population

HIV infected postmenopausal and premenopausal women, within the age bracket of 46-80 and 19-45 years respectively were randomly recruited for the study. The remaining were non HIV-infected premenopausal and postmenopausal women recruited as Control subjects amongst NAUTH hospital staff.

### 2.3. Ethical Consideration

Ethical approval for this study was obtained from the ethics committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi. Informed consent was obtained from the subjects and their participation was voluntary.

### 2.4. Study Design

A total of 80 (eighty) premenopausal and postmenopausal women aged between (19-45) and (45-80) years respectively were randomly recruited for the study from 2013 to 2014. 40 participants were HIV infected and were grouped as (i) HIV infected premenopausal women (n =20), (ii) HIV infected postmenopausal women (n=20). The remaining 40 participants were apparently healthy women which served as control and were grouped as (iii) Control premenopausal women (n= 20) and (iv) Control postmenopausal women (n = 20).

A well structured questionnaire was administered to them to ascertain their menstrual history, age and other biodata.

## 2.5. Sample Processing and Storage

The blood sample was withdrawn from the antecubital vein by means of sterile plastic syringes into plain container, allowed to clot and centrifuged for 5 minutes at 4000 rpm for proper separation of the serum. After centrifugation, the serum samples were separated and stored frozen until analysis of calcium, alkaline phosphatase, estrogen and cortisol.

## 2.6. Methodology

Total serum calcium was estimated as described by Sarkar & Chauhan [9].

Serum alkaline phosphatase activity was estimated using King Armstrong method as described by McComb *et al.*, [10].

Estradiol was determined using Enzyme Immunoassay (EIA) kit as describe by Tsang *et al.*, [11] while Quantitative determination of Cortisol was also done using Enzyme Immunoassay Test kit as described by Engvall & Perlman [12].

## 2.7. Statistical Analysis

Continuous variables and categorical data were analyzed using independent sample *t*-tests and analysis of Variance using version 17 of SPSS package. The variables were expressed as mean ( $\pm$ SD) and post-hoc (LSD) were used to assess significant mean differences. Graph Pad Prism version 5.03 was used for graph presentations. All *p*-values were two-tailed, and a *P*=.05 was considered statistically significant.

## 3. RESULTS

### 3.1. Serum Calcium, Alkaline phosphatase, Estrogen and Cortisol in Premenopausal HIV and control female subjects.

The results showed that there was no significant difference in the mean serum calcium in premenopausal HIV females ( $2.14 \pm 0.08$  mmol/l) when compared with the value in premenopausal Control females ( $2.14 \pm 0.08$  mmol/l) (*P*>.05). The mean serum alkaline phosphatase activity in premenopausal HIV females ( $47.23 \pm 12.02$  mmol/l) was significantly higher when compared with the value in premenopausal Control females ( $31.09 \pm 6.69$  mmol/l) (*P*=.05). There was no significant difference in the mean serum estrogen level in premenopausal HIV females ( $18.50 \pm 9.09$  pg/ml) when compared with the value in premenopausal Control females ( $16.76 \pm 7.99$  pg/ml) (*P*>.05). However, the mean serum cortisol level in premenopausal HIV female was significantly higher ( $28.01 \pm 11.31$  ng/ml) when compared to the value in premenopausal Control females ( $15.21 \pm 6.47$  ng/ml) (*P*=.05) (See Fig 1).

### 3.2. Serum Calcium, Alkaline phosphatase, Estrogen and Cortisol in postmenopausal HIV and Control female subjects.

The results showed that there was no significant difference in the mean serum calcium level in postmenopausal HIV females ( $2.17 \pm 0.12$  mmol/L) when compared with the value in postmenopausal Control females ( $2.22 \pm 0.06$  mmol/L) (*P*>.05). There was no significant difference in the mean serum alkaline phosphatase between postmenopausal HIV females ( $51.22 \pm 21.74$  mmol/L) and postmenopausal Control females ( $49.09 \pm 14.48$  mmol/L) (*P*>.05). However, there was a significant decrease in the mean serum estrogen level in postmenopausal HIV females ( $8.42 \pm 6.58$  pg/ml) compared with the value in corresponding Control females ( $8.42 \pm 6.58$  pg/ml) (*P*=.05). The mean serum cortisol in postmenopausal HIV females ( $27.77 \pm 11.93$  ng/ml) was significantly higher when compared with the value in corresponding Control females ( $18.29 \pm 16.20$  ng/ml) subjects (*P*=.05). (See fig 2).

### 3.3. Serum Calcium, Alkaline phosphatase, Estrogen and Cortisol in premenopausal HIV and postmenopausal HIV female subjects.

The results showed that there was no significant difference in the mean serum calcium level premenopausal HIV females ( $2.14 \pm 0.08$  mmol/L) when compared with the value in postmenopausal HIV females ( $2.17 \pm 0.12$  mmol/L) (*P*>.05). Similarly, there was no significant difference in the mean serum

alkaline phosphatase in premenopausal HIV females ( $47.23 \pm 12.02$  mmol/L) when compared with value in postmenopausal HIV females ( $51.22 \pm 21.74$  mmol/L) ( $P > .05$ ). The mean serum estrogen in premenopausal HIV females ( $18.50 \pm 9.09$  pg/ml) was significantly higher when compared with value in postmenopausal HIV females ( $8.42 \pm 6.58$  pg/ml) ( $P = .05$ ). However, the mean serum cortisol was not significantly different between premenopausal HIV females ( $28.01 \pm 11.31$  ng/ml) and postmenopausal HIV females ( $27.77 \pm 11.93$  ng/ml) ( $P > .05$ ). There was no significant difference in the CD4<sup>+</sup> count of premenopausal HIV females ( $427.752 \pm 26.88$ ) and the CD4<sup>+</sup> count of postmenopausal HIV females ( $528.50 \pm 181.43$ ) ( $P > .05$ ). (See fig. 3).

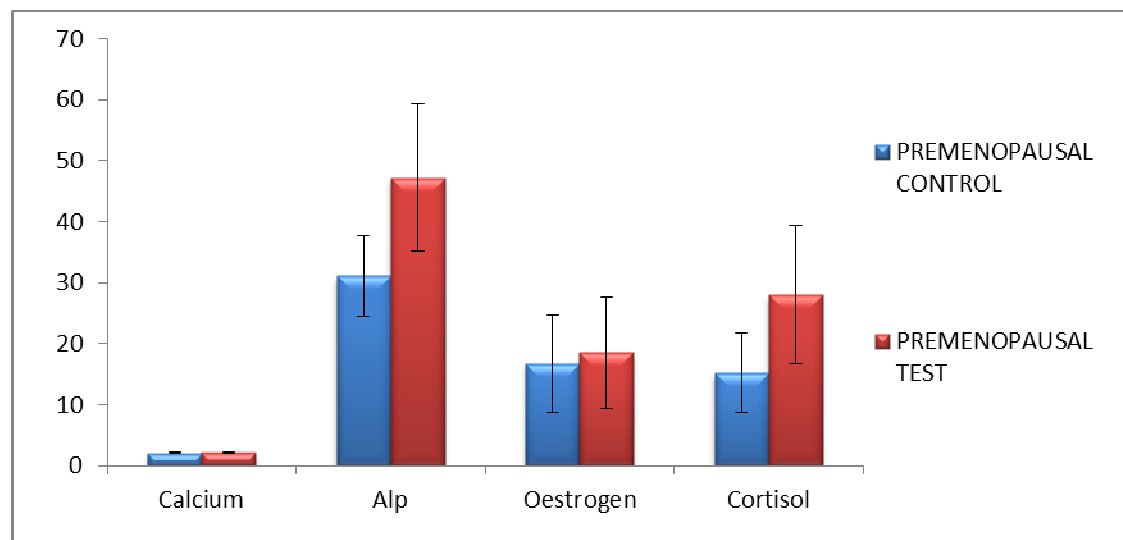


Fig 1: Mean serum Calcium, Alkaline Phosphatase, Estrogen and Cortisol in Premenopausal HIV females and Premenopausal Control female subjects.

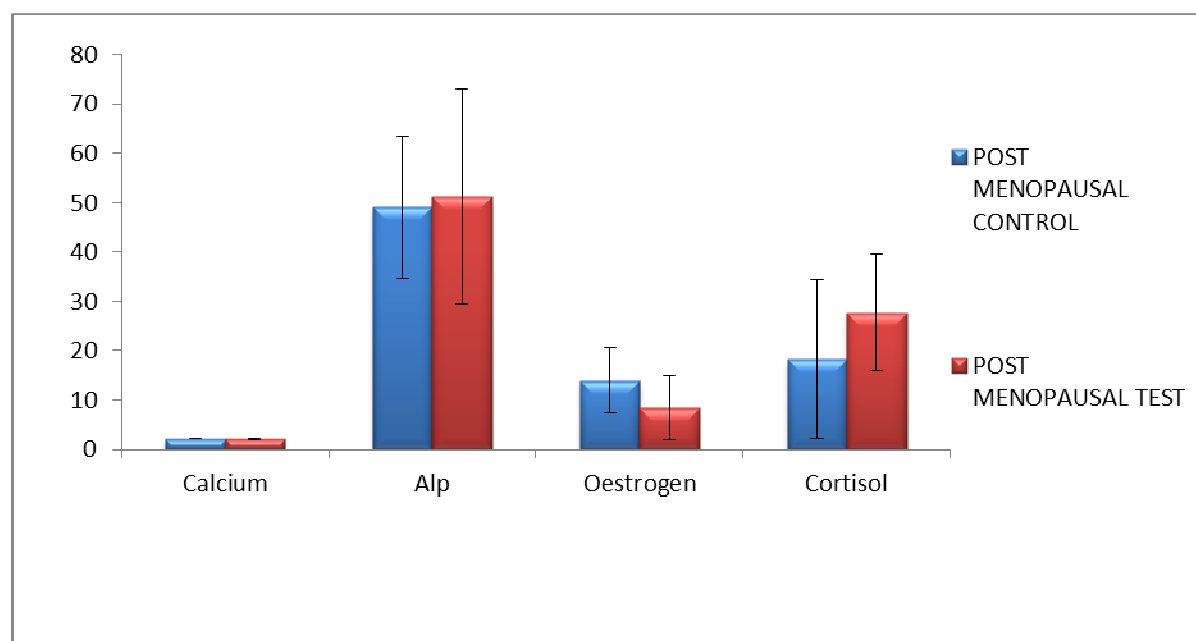


Fig .2: Mean serum Calcium, Alkaline Phosphatase, Estrogen and Cortisol in Postmenopausal HIV females and Postmenopausal Control female subjects.

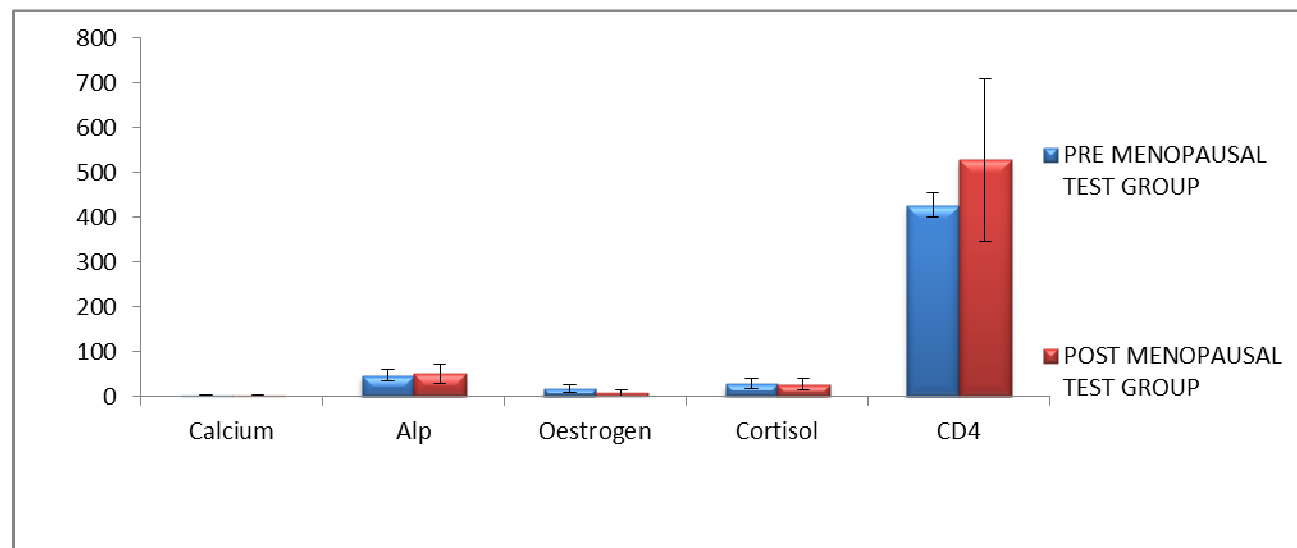


Fig 3: Mean serum Calcium, Alkaline Phosphatase, Estrogen and Cortisol in Premenopausal HIV females and Postmenopausal HIV female subjects.

## DISCUSSION

Human immunodeficiency virus (HIV) is associated with the derangement in the body's physiological function, alteration in bone homeostasis and production of some biochemical metabolites. Derangement in these biochemical parameters arises from the involvement of the viral infection in the metabolism of bone cells. Several studies have used Dual Energy X-ray Absorptiometry (DEXA) to study bone mineral status during osteoporosis. However, relatively, studies have used bone homeostatic parameters (e.g calcaemia, magnesemia, phosphatamia, and bone-ALP) to monitor derangement in bone homeostasis in patients with osteoporosis. Altered serum levels of bone minerals and ALP activities is not fully appreciated in literature because the few studies that have tried to explore bone impairment have usually used Dual Energy X-ray Absorptiometry (DEXA) [13].

The present study showed that mean total serum estrogen level of HIV infected postmenopausal females was significantly decreased compared to the value in HIV infected premenopausal females. This finding is in accordance with a similar research by [14]. The authors explained that estrogen deficiency is the most important cause of postmenopausal bone loss in women. Also similar research by Zaidi and colleagues showed that estrogen deficiency causes both early and late forms of osteoporosis in postmenopausal females [15]. This, he associated with large increase in bone resorption caused by increased osteoclast activity. The lower levels of estrogen accelerate bone loss for a period of about five to eight years postmenopausal females. Women normally loose annually an average 3% of their bone mass in the years after menopause. A recent study done by Sinnessael [5] showed that hormonal factors strongly determine the rate of bone resorption. Lack of estrogen increases bone resorption as well as decreasing the deposition of new bone that normally takes place in weight bearing bones. The mean total serum level of estrogen in HIV-infected premenopausal females showed an insignificant increase when compared with premenopausal Control group. Low bone mineral density is a recognized complication associated with HIV infection. The viral infection induces proinflammatory cytokines eg TNF- $\alpha$  and OPG which affects osteoblast and osteoclast development [16]. Research done by Waugh *et al.*, [17] showed that estrogen suppresses osteoclastogenic cytokine production in T- cells and osteoblast. It plays a role in bone metabolism focusing on proinflammatory cytokines and prostaglandin. These factors increase bone resorption. Therefore estrogen induces apoptic death of osteoclasts, increases osteoprogesterin production thus leading to bone resorption [17].

The mean serum cortisol in HIV-infected premenopausal females was significantly higher compared to the value in premenopausal Control females. Also the mean serum level of cortisol in HIV-infected postmenopausal females was statistically higher compared to their corresponding Control females. Cortisol is released in response to stress and low levels of blood glucocorticoids. The finding above is in accordance with the research done by Berk *et al.*, [18] which showed that cortisol decreases bone formation. The authors reported that cortisol reduces bone formation favouring long term development of osteoporosis. Cortisol suppresses many aspects of the immune system and response including the proliferation of the lymphocytes, the activities of natural killer cells, macrophages and the production of certain cytokines [19]. It has also been shown that the condition of overt hypercortisolism also leads to osteoporosis and fractures in up to 70% cases [19]. A recent study done by Bolland *et al.*, [20] showed that high cortisol levels are seen in individuals with many types of severe acute or chronic illness and HIV/AIDS is no exception. Wester *et al.*, [21] also showed that adrenal insufficiency occurs in Human immunodeficiency virus (HIV)/ Acquired immunodeficiency syndrome (AIDS) causing morbidity and mortality in HIV patients. Elevated cortisol levels inhibit osteoblast formation and cell proliferation. This dramatically decreases bone building and lowers bone density.

The mean serum level of calcium in HIV infected premenopausal females was found to be statistically decreased than in those of premenopausal control. Conversely, the mean serum level of calcium in HIV-infected postmenopausal females showed an insignificant difference when compared to their control counterparts. Calcium is an essential nutrient and it is the most abundant in the body. The finding above is similar with the research done by Heaney, [22], which showed that low calcium intake may be a risk factor in the development of osteoporosis. Calcium deficiency causes mobilization of bone and leads sooner or later to osteoporosis. Loss of bone starts in women at the time of menopause and in men at about age 55 and leads to an increase in fracture rates in both sexes. Nordin, [23], explained that there is a rise an obligatory calcium excretion at menopause which increases the theoretical calcium requirement in postmenopausal women to about 25 mmol (1000 mg). In the same vane, Hellman *et al.*, [24], showed that there is impaired parathyroid hormone secretion in HIV infected patients even in the absence of parathyroid infiltrative disease thus decreasing intestinal absorption of calcium.

The mean serum alkaline phosphatase activity of HIV infected premenopausal females was significantly higher than in those of their Control counterparts. There was an insignificant difference in the mean serum levels of alkaline phosphatase in HIV infected postmenopausal females than in those of their control counterparts. These findings were in accordance to the research work done by Dinesh, [25]. The authors explained that there was a slight but significant positive correlation indicating an increasing alkaline phosphatase activity with decreased bone mass. They also said that a slight increase in alkaline phosphatase in women with more severe osteoporosis is related to bone resorption. The insignificant difference observed in the mean serum alkaline phosphatase activity between HIV-infected postmenopausal females and their Control counterpart was similar to the research work carried out by Gibellini *et al.*, [26]. The authors explained that the magnitude of bone alkaline phosphatase decreases in population of postmenopausal females with normalization to premenopausal levels. Also that several HIV protein may affect the functionality and maturation of osteoblasts, inducing apoptic stimuli in mesenchymal stem cells which are precursors of osteoblast. In particular, HIV- 1-gp120 and p55gag can reduce bone alkaline phosphatase activity and calcium deposition by osteoblast.

There was a moderate positive correlation between CD4 counts, estrogen, cortisol, calcium and alkaline phosphatase in both premenopausal and postmenopausal women. In other words, increased CD4 count is associated with concomitant increase in the levels of estrogen, cortisol, calcium and alkaline phosphatase activity in the serum of HIV infected women. This is beneficial. Thus the measurement of the serum levels of estrogen, cortisol, calcium and alkaline phosphatase activity can be used in line with CD4 count for the assessment of osteoporosis and bone associated disorder in HIV infection.

#### 4. CONCLUSION

Conclusively, it is evident that HIV infection and osteoporosis produce alteration both in bone mineral metabolism and hormonal levels as revealed by changes in levels of different hormonal studies, and also characteristic changes in calcium and alkaline phosphatase activity. This study reveals that premenopausal and postmenopausal women with HIV infection have reduced estrogen and higher levels

of cortisol and alkaline phosphatase activity when compared with premenopausal and postmenopausal Control female subjects. The progressive alteration of these biochemical and hormonal parameters is an indication that their measurement may be useful tools in diagnosis, treatment, monitoring and management of osteoporosis as well as other bone associated disorders in HIV infected patients.

## CONSENT

All author(s) hereby declare that written informed consent was obtained from all the patients who participated in this study.

## ETHICAL APPROVAL

All author(s) hereby declare that all experiment and procedure have been examined and approved by the appropriate board of ethics committee of Nnamdi Azikiwe University Teaching Hospital Nnewi, South East Nigeria, and research have therefore been performed in accordance with the standards laid down in the 1964 Declaration of Helsinki.

## REFERENCES

- [1]. Brian K, Alldredge KK, Young M, Lloyd Y, Wayne A, Kradjan B, *et al. Applied therapeutics: the clinical use of drugs*. 2009; 47: 101–113.
- [2]. Sweet MG, Sweet JM, Jeremiah MP, Galazka SS. Diagnosis and treatment of osteoporosis. *Am Fam Physician* 2009; 79: 193-200.
- [3]. Bruera D, Luna N, David DO, Bergoglio LM, Zamudio J. Decreased bone mineral density in HIV-infected patients is independent of antiretroviral therapy. *AIDS* 2003; 17: 1917-1923.
- [4]. Brown TT, Qaqish RB. Antiretroviral therapy and the prevalence of osteopenia and osteoporosis: a meta-analytic review. *AIDS* 2006b; 20: 2165-2174.
- [5]. Sinnesael M, Boonen S, Claessens F, Gielen E, Vanderschueren D. Testosterone and the male skeleton: a dual mode of action. *J. Osteop* 2011; 240-328.
- [6]. Vega D, Maalouf NM, Sakhaee K. CLINICAL Review #: the role of receptor activator of nuclear factor-kappaB (RANK)/RANK ligand/osteoprotegerin: clinical implications. *J Clin Endocrinol Metab* 2007; 92: 4514-4521.
- [7]. Scott WN. *Anatomy & Physiology Made Incredibly Easy*. Lippincott Williams & Wilkins 2011; Philadelphia PA.
- [8]. Brummett BH, Kuhn CM, Boyle SH, Babyak MA, Siegler IC, *et al.* Cortisol responses to emotional stress in men: association with a functional polymorphism in the 5HTR2C gene. *Biol Psychol* 2012; 89: 94–98.
- [9]. Sarkar BC, Chauhan UP. A new method for determining micro quantities of calcium in biological materials. *Anal. Biochem*, 1967; 20: 155-166.
- [10]. McComb RB, Bowers GN, Posen S. Alkaline phosphatase. *New York: Plenum Press* 1979; 268–269.
- [11]. Tsang BK, Armstrong DT, Whitfield JF. Steroid biosynthesis by human ovarian follicular cells in vitro. *J Clin Endocrinol Metab* 1980; 51: 1407-1411.

- 283 [12]. Engvall E, Perlman P. Enzyme-linked immunosorbent assay (ELISA). Quantitative  
284 assay of immunoglobulin G. *Immunochemistry* 1971; 8 (9): 871-874.  
285
- 286 [13]. Hakulinen MA, Saarakkala S Dual energy X-ray laser measurement of calcaneal mineral  
287 density. *Physics in med Bio* 2003; 48 (12): 1742-1752.  
288
- 289 [14]. Guthrie JR, Dennerstein L. Risk factors for osteoporosis. A review. *Medscape Women's*  
290 *Health* 2000; 5 (4): 1-2.  
291
- 292 [15]. Zaidi M, Blair HC, Monga BC, Abe E, Haung CLH. Osteoclastogenesis, bone  
293 resorption and osteoclast based therapeutics. *J Bones Mineral Research* 2007; 18 (4):  
294 599-609.  
295
- 296 [16]. Yin MT, Zang CA, McMahon DJ. Higher rates of bone loss in postmenopausal HIV-  
297 Infected women: a longitudinal study. *J clin endocrinol metab* 2012; 97 (2): 554-562.  
298
- 299 [17]. Waugh, E.J., Lam, M.A., Hawker, G.A., McGowan, J., Papaioannou, A., Cheung,  
300 A.M., et al. Risk factors for low bone mass in healthy 40–60 year old women: a  
301 systematic review of the literature. *Osteoporosis international: a journal established*  
302 *as result of cooperation between the European Foundation for Osteoporosis and the*  
303 *National Osteoporosis Foundation of the USA* 2009; 20 (1): 1–21.  
304
- 305 [18]. Berk LS, Tan SA, Berk D. Cortisol and Catecholamine stress hormone decrease is  
306 associated with the behavior of perceptual anticipation of mirthful laughter". *The*  
307 *FASEB J* 2008; 22 (1): 946-111.  
308
- 309 [19]. Soldatos G, Sztal-Mazer S, Woolley I, Stockigt J. Exogenous glucocorticoid excess as a  
310 result of retonavir-fluticasone interaction. *Intern Med J* 2005; 35 (1) : 67-68  
311
- 312 [20]. Bolland MJ, Grey AB, Horne AM. Annual zoledronate increases bone density in  
313 HAART- treated HIV-infected men: a randomized controlled trial. *J clin Endocrinol*  
314 *metab* 2007; 92 (4): 1283-1288.  
315
- 316 [21]. Wester VL, Staufenbiel SM, Veldhorst MA, Visser JA, Manenschijn L et al. Long-  
317 term cortisol levels measured in scalp hair of obese patients. *Obesity* 2014; 56 (2):  
318 234-240.  
319
- 320 [22]. Heaney RP. Calcium, dairy products and osteoporosis. *J Am College Nutrit* 2000; 19  
321 (2): 838-998.
- 322 [23]. Nordin BE. Calcium and Osteoporosis. *Nutrition* 1997; 13 (7-8): 664-686.
- 323 [24]. Hellman P, Albert J, Gudhend M. Impaired parathyroid hormone release in HIV  
324 infection. *AIDS Research Hum Retroviruses* 1994; 10: 391-392.  
325
- 326 [25]. Dhanwal DK. Thyroid disorders and bone mineral metabolism *Indian J Endocrinol*  
327 *Metab* 2011 Jul; 15(Suppl2): S107–S112.  
328
- 329 [26]. Gibellini D, De Crignis E, Ponti C. HIV-1 triggers apoptosis in primary osteoblast and  
330 HOBT cells through TNF- $\alpha$  activation. *J Med Virol* 2008; 80: 1507-1514.