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Research Article

Evaluation of Lipids and Lipoproteins in Relation with Bone Mineral Density in Patients at Risk of Bone Fractures

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Abstract

Osteoporosis, which contributes to morbidity and mortality, often coexists with cardiovascular disease, especially atherosclerosis. Changes in low-density lipoprotein choesterol (LDLC) and high-density lipoprotein cholesterol (HDLC) most relevant to cardiovascular disease (CVD), have been associated with reduced bone quality and increased fracture risk. HDLC, long understood as protective in CVD has recently been identified as a possible regulator of osteoblast cell differentiation. Lipid and lipoprotein oxidation products from a high fat diet have also been linked to loss of bone density in mice. Study on bone mineral density(BMD) in relationship with changes in plasma lipids in Nigerians at risk of bone fracture is scarce. This study was designed to evaluate BMD using the Dual Energy X-ray Absorptiometry (DXA) as well as lipids and lipoproteins in osteoporotic parients. One hundred subjects consisting of fifty osteoporotic patients aged 57±1.93 years with T-score below -2.5 (-2.5 to -7.5) and fifty controls aged 42.82±1.53 years with T- score of -0.6±0.4 were selected for this study. All biochemical and biophysical parameters were determined using standard procedures. Results showed significant increases in plasma total cholesterol(TC), LDLC ($p<0.001$) as well as TC/HDLC and LDLC/HDLC ($p<0.05$). There were remarkable significant decreases in plasma HDLC, BMI and BMD ($p<0.001$) respectively. There are compelling evidence from the results of this study that decreased BMD and increased plasma TC and LDLC as well as reduced HDLC are features of osteoporotic patients in Nigeria and this could lead to risks in bone fracture.

Keywords: osteoporosis , cholesterol, LDLC, HDLC, bone fracture; BMD

INTRODUCTION

World Health Organization (WHO) study group defined osteoporosis as "a systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fractures."

A decline in skeletal integrity may stem from adverse environmental conditions such as smoking, inactivity, or gastrointestinal inflammation and malabsorption. However, for a patient at risk for fragility fracture, strategic nutritional therapy has been thought to play a major impact in improving bone health. (Slemenda *et al.*,1996). Although estimates suggest 50 percent of the variance in peak bone mass as due to genetic factors (Kohlstadt,2006; Pocock *et al.*, 1987), it is also estimated that 30-50 percent of the genetic factors that influence bone strength can be affected by the environment in which bone is immersed (Parhami *et al.*,1999; Pocock *et al.*, 1987). The use of biomarkers and targeted nutritional intervention is a valuable, underutilized clinical tool.

Oxidative damage from free radicals is a major cause of degenerative diseases and contribute to osteoporosis (Parhami 2003), to the increase in osteoclastogenesis and subsequent bone loss. Reactive oxygen species (ROS), among the most damaging free radicals, are constantly produced during mitochondrial respiration. For instance, Grassi *et al.*, (2005) demonstrated in vivo that ROS are necessary for bone loss to occur in estrogen-deficient mice.

The biomarkers, low-density lipoprotein (LDL) and high-density lipoprotein (HDL), most relevant to cardiovascular disease (CVD), have also been suggested as indicators of reduced bone quality and increased fracture risk. HDL, long understood as protective in CVD, has been identified as a possible regulator of osteoblast cell differentiation (Moerman *et al.*, 2004).

Parhami *et al.*, (2003) demonstrated that products of lipid and lipoprotein oxidation from a high fat diet are the source of peroxisome proliferator-activated receptors gamma (PPARγ)-activating ligands and this has been linked to a loss of bone density in mice.(Yeung *et al.*, 2005; Parhami *et al.*, 2002). Causes of osteoporosis including obesity and diabetes are associated with bone marrow adiposity which greatly produces tumor necrosis factor-α (TNF-α) (Leslie *et al.*, 2012; Nielson *et al.*, 2012). One of the shared features is that

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osteoblasts and adipocytes differentiate from a common precursor cell in the bone marrow, the mesenchymal stem cell (Armstrong *et al.,* 2006). It is this commonality of osteoblast and adipocyte stem cells that links osteoporosis and obesity, two of the most common conditions. Bone marrow mesenchymal stem cells (mMSC) differentiate into either osteoblasts or adipocytes, depending on their environment.

Elevated triglycerides, reduced control of blood glucose, abnormally increase in bone marrow adiposity and a suppression of osteoblastic activity as well as the activation of the adipocyte-specific transcription factor PPARγ have been reported in aging mice. Magnetic resonance imaging reveals that postmenopausal women have twice the level of bone marrow fat as premenopausal women, and the lower the bone density the greater the saturated-fat content. (Adami *et al.*, 2004).

Parhami (Parhami *et al.*, 2002) has hypothesized that accumulation of bone marrow fat in an environment of increased oxidative stress increases lipoprotein oxidation. Although, this association is often dismissed as a consequence of aging, studies have shown that this relationship remains significant after age adjustment (Rosen and Bouxsein, 2006). The common finding of simultaneous vascular calcification and osteoporosis in individual patients suggests that local tissue factors govern regulation of biomineralization (Cui *et al.*, 2005). Bone and vascular tissue share several features at the molecular and cellular levels. Bone marrow contains endothelial cells, preosteoblasts, and monocyte-derived osteoclasts, all of which have counterparts in the artery wall.

Atherosclerosis and osteoporosis involve recruitment and differentiation of monocytic cells that differentiate into macrophage-foam cells in artery and osteoclasts in bone. However, in bone and bone osteoblasts, report has indicated that osteoblastic differentiation is inhibited by oxidized lipids and hyperlipidemia (Xu *et al*., 1995). Because the immature osteoblasts are located immediately adjacent to the subendothelial matrix of bone vessels, lipid accumulation in subendothelial matrix would be expected to inhibit differentiation of the bone-forming cells. In addition, oxidized lipids induce endothelial expression of monocyte chemotactic factors and M-CSF, a potent inducer of osteoclastic differentiation, oxidized lipids would be expected to promote bone resorption by recruitment and differentiation of osteoclast precursor cells. Consistent with this possibility is a report which showed that high-fat diet inhibits bone growth in chickens (Parhami *et al*., 2001). Study has also shown that mice placed on a high-fat diet developed not only atherosclerosis but also osteopenia (Parhami *et al*., 2001)

Study on the relationship of osteoporosis and bone mineral density as well as changes in lipid profile has not been studied in Nigerians with low bone mineral density. This study was designed to evaluate the relationship between bone mineral density, lipids and lipoproteins in osteoporotic patients.

MATERIALS AND METHODS

Fifty patients with osteoporosis attending the General Outpatient Clinic of the Family Medicine Department of the University College Hospital, Ibadan Nigeria and fifty apparently healthy volunteers were selected for this study. Written /oral Informed Consent was obtained from all participants before commencement of study. Ethical approval was obtained from the University of Ibadan /University College Hospital Ethical Committee.

All patients with BMD of T-Score above<1.0 (-0.9 to -4.5). were included in the study.

Patients on osteolek (bisphosphonates) or other antiresorptive drugs as well as patients on lipid lowering drug and other aliments that could affect the outcome of this study were excluded.

Demographic and medical history: An interview based questionnaire was used to collect demographic information and medical history from all subjects.

Anthropometric measurement: The weight of the participants without shoes was taken using Seca adult weighing scale. Height was also measured using a meter rule calibrated on a wall. Body mass index was calculated using Weight/Height^2 (kg/m^2).

Bone mineral density measurement

The Dual Energy X-ray Absorptiometry (DXA) scan supplied by the manufacturer (Osteosys, Poland) for scanning and analysis was used to obtain the BMD. Daily quality control was carried out to allow the densitometer to standardize and calibrate against a standard calibration block before measurement of the distal radius.

Blood sample collection

5ml of overnight fasting (10-12 hours) blood samples were collected by standard venipuncture without stasis and were dispensed into Dipotassium Ethylene Diamine Tetra acetic Acid (K2EDTA) bottles and placed on ice bag immediately and processed within 1 hour of collection. The blood samples were spun at 3500 rpm for 10 minutes using MSE centrifuge and the plasma was separated into clean plain containers and stored at -20°C until analyzed.

Plasma Lipids and Lipoproteins Estimations

Plasma TC was determined using commercial kit according to the method of Allain et al, (1974), and HDLC was determined after precipitating out LDLC and VLDLC in sample using the method of Allain *et al*., (1974),

Triglyceride was determined using commercial kit based on enzymatic hydrolysis with lipases. (McGowan *et al*., 1982)

LDL cholesterol was calculated using Friedwald *et al*., 1972 formula,

TC - (TG/5+HDLC) =LDLC (mg/dl)

Accuracy and precision of all biochemical tests were monitored by including commercial quality control samples within each batch of test assay.

Statistical Analysis

All results were subjected to statistical analysis using SPSS version 14 (SPSS Inc, Chicago, Illinois) for windows. The results were expressed as mean plus standard error of mean.

Student t-test and analysis of variance (ANOVA) were used for statistical comparisons and the differences regarded as significant at $p<0.05$. Pearson's correlation coefficient was used to assess the relationship between parameters.

Table 1

Biophysical and Biochemical parameters of osteoporotic patients and controls

Variables	Osteoporotic patients N=50	Control subjects N=50	t-Value	p-Value
Age (yrs)	57±1.9	42.8±0.9	5.8	p<0.001
BMI (Kg/m^2)	25±0.9	30.8±0.9	-3.7	p<0.001
BMD (T-Score)	-2.0±0.4	-0.6±0.4	-3.3	p<0.001
TC (mg/dl)	234.0±5.5	142.4±4.4	13.1	p<0.001
TG (mg/dl)	86.2±6.2	64.9±3.4	3.0	p<0.05
HDLC (mg/dl)	41.1±2.0	55.8±2.4	4.7	p<0.001
LDLC (mg/dl)	161.5±5.5	88.3±5.1	9.9	p<0.001
TC /LDLC	1.5±0.3	2.2±0.4	-1.9	NS
TC /HDLC	0.2±0.0	0.3±0.2	-2.8	p< 0.05
LDLC/HDLC	3.3±0.2	2.5±0.2	2.5	p<0.05

TC= total cholesterol; HDLC =high density lipoprotein cholesterol; BMI =body mass index; BMD = bone mineral density; LDLC= low desity lipoprotein cholsterol; N= number

Table 2

Biophysical and Biochemical parameters of osteoporotic male and control males

Variables	Osteoporotic males N=11	Control males N=13	t-Value	p-Value
Age (yrs)	55.8±4.6	40.1±2.6	3.1	p<0.05
BMI (Kg/m^2)	23.2±1.3	27.9±2.0	-1.9	NS
BMD (T-Score)	-2.7±0.6	-0.7±0.1	-3.7	p<0.001
TC (mg/dl)	226.3±9.9	137.0±10.0	6.3	p<0.001
TG (mg/dl)	97.6±20.6	64.7±7.3	1.6	NS
HDLC (mg/dl)	40.7±4.6	57.1±4.5	2.5	p<0.05
LDLC (mg/dl)	149.7±10.4	82.9±11.5	4.2	p<0.001
TC /LDLC	1.6±0.1	2.4±0.6	-1.3	NS
TC /HDLC	0.3±0.1	0.3±0.1	-1.3	NS
LDLC/HDLC	2.8±0.3	2.5±0.5	0.5	NS

RESULTS

Table 1 shows the biophysical and biochemical parameters in all patients and controls. The patients were older than the control subjects (p<0.001). The BMI, BMD, plasma HDLC (p< 0.001) and HDLC /TC (p< 0.05) were significantly reduced when compared with the corresponding control values. There were remarkable significant higher plasma TC, and LDLC when compared with the corresponding control values (p<0.001) respectively. TG and LDLC/HDLC were also significantly higher (p<0.05) compared with the corresponding control values.

Table 2 shows the biophysical and biochemical parameters in osteoporotic male patients and control males. There was significant increase in age (p<0.05) as well as plasma TC and LDLC (p<0.001) when compared with the control males. On the other hand, there were significant decreases in BMD and HDLC (p<0.001) when compared with the control values. There were no significant differences in BMI, plasma TG, TC/LDLC, HDLC/TC, LDLC/HDLC ratios compared with the corresponding controls values.

Table 3 shows the biophysical and biochemical parameters in osteoporotic females and control females. There were significant increases in age, plasma TC, LDLC (p<0.001) as well as TG and LDLC/HDLC (p<0.05). On the other hand, there were significant decreases in BMI and plasma HDLC (p<0.001) as well as BMD and TC/HDLC ratio (p<0.05). There was no significant difference in TC/LDLC ratio.

Comparison of same parameters between male and female patients showed no significant changes.

Table 4 shows Pearson's Correlation coefficient (r) of all parameters in osteoporotic patients. There was a significant correlation between age and TC (r=0.349, p<0.05); LDLC (r=0.416, p<.01); and LDLC/HDLC (r=0.328, p<0.05) were obtained. Inverse correlation between age and TC/LDLC (r= -0.293, P<0.05) and TC/ HDLC (r= -0.332, P<0.05). TC was significantly correlated with LDLC(r= 0.856, p<0.01) and inversely correlated with TC/LDLC (r=-0.332, p<0.05) TC/HDLC (r= -0.305, p<0.05) respectively. There was significant correlation between TG and TC/LDLC (r= 0.289, p<0.05). Similarly HDLC showed a significant correlation with TC/LDLC(r= 0.669, p<0.05) and TC/HDLC (r= 0.835, p<0.01) with a corresponding inverse correlation with LDLC/HDLC (r= -0.831, p<0.01).

LDLC showed correlation with LDLC/HDLC (r= 0.614, p<0.01) HDLC (r= -0.679, P<0.01). TC/LDLC was significantly correlated with TC/HDLC (r= 0.845, p<0.01) and inversely correlated with LDLC/HDLC (r= -0.781, p<0.01). BMD and BMI were not correlated with any of the parameters.

DISCUSSION

The patients were clinically diagnosed as having primary densitometric osteoporosis as defined by the T-score value of the bone mineral density diagnostic for osteoporosis in accordance with the World Health Organization (1994) criteria for osteoporosis. Seventy eight percent (78%) of the patients were women while twenty two percent (22%) were men, an indication stuggesting in part that women are possibly more prone to osteoporosis.

Table 3:

Biophysical and Biochemical parameters of osteoporotic female and control females

Variables	Osteoporotic females N=39	Control females N=37	t-value	p-value
Age (yrs)	57.3±2.1	43.8±1.8	4.8	p<0.001
BMI (Kg/m^2)	26.8±1.1	31.8±1.0	-3.4	p<0.001
BMD (T-Score)	-1.8±0.5	-0.6±0.1	-2.3	p<0.05
TG (mg/dl)	82.9±5.5	65.0±3.8	2.7	p<0.05
HDLC (mg/dl)	41.3 ±2.2	55.4±2.8	3.9	p<0.001
LDLC (mg/dl)	161.8±6.3	90.2±5.6	8.8	p<0.001
TC/LDLC	1.5±0.1	2.1±0.4	-1.5	NS
TC/HDLC	0.2±0.1	0.3±0.1	-2.4	p<0.05
LDLC/HDLC	3.4±0.2	2.5±0.2	2.5	p<0.05
TC (mg/dl)	236.2±6.5	144.3±4.8	11.3	p<0.001

TC= total cholesterol; HDLC =high density lipoprotein cholesterol; BMI =body mass index; BMD = bone mineral density; LDLC= low desity lipoprotein cholsterol; N= number

Table 4:
Correlation coefficients of all parameters in osteoporotic patients

Variables	Age (yrs)	BMI (Kg/m^2	BMD (T-Score)	TC (mg/dl)	TG (mg/dl)	HDLC (mg/dl)	LDLC (mg/dl)	TC/LDLC	TC /HDLC	LDLC/HDLC
Age (yrs)				.349^{x}			.416^{xx}	-293^{x}	-.332^{x}	
BMI (Kg/m^2)										
BMD (T Score)										
TC (mg/dl)	.349*			.817$^{x\,x}$			-.856^{x}	-.332^{x}	-.305^{x}	
TG (mg/dl)								. 289^{x}		
HDLC (mg/dl)								.669$^{x\,x}$	.835$^{x\,x}$	-.831$^{x\,x}$
LDLC (mg/dl)	.416^{xx}			.856$^{x\,x}$				-.753^{x*}	-.679$^{x\,x}$	.614$^{x\,x}$
TC/LDLC	-293^{x}			-.332^{x}	289^{x}	.669$^{x\,x}$	-.753$^{x\,x}$		.845$^{x\,x}$	-.781$^{x\,x}$
TC /HDLC	-.332^{x}			-.305^{x}		.835$^{x\,x}$	-.679$^{x\,x}$	.845$^{x\,x}$		-.916$^{x\,x}$
LDLC/HDLC	.328^{x}					831$^{x\,x}$	614^{xx}	-.781$^{x\,x}$	-.916$^{x\,x}$	

* p< 0.05 ; ** p< 0.01 ; TC= total cholesterol; HDLC =high density lipoprotein cholesterol; BMI =body mass index
BMD = bone mineral density; LDLC= low desity lipoprotein cholsterol; N= number

Forty four percent (44%) of the patients complained of back pain. All participants were urban dwellers. Patients claimed not to have smoked cigarette nor consumed alcohol. The mean BMI of the osteoporotic patients was essentially lower than control subjects. Changes in lipid and lipoprotein oxidation in the pathophysiology of osteoporosis has attracted interest recently (Parhami, 2003; Young and McEneny, 2001) Markedly increased plasma TC, LDLC and their ratios were obtained in all patients with osteoporosis in this study. These increases could have important clinical implications due to the likely significant comorbidity profile, an indication suggesting an increased risk for bone fracture and coronary artery disease. These increases in plsama TC and LDLC corresponded with decreased BMD in the patients. In earlier studies (Rajamannan, 2008; Brownbill and Ilich, 2006) no correlation was reported between TC, LDLC and BMD, an observation similar to the result of the present study. However, few studies (Solomon *et al*., 2005; Zabaglia *et al*., 1998) demonstrated that increased TC and LDLC were inversely related with BMD in both pre and post-menopausal women. In another study a positive relationship between BMD and TG levels was reported. Cui *et al.*, (2005) reported that elevated level of TG had a significant correlation with BMD value at the trochanter site in post-menopausal women. Their finding is incongruent with the result of the present study which showed no association between TG and BMD. There are conflicting repeorts on the relationship between plasma HDLC and BMD, While available evidence (Von der Recke *et al.*, 1999) suggested an inverse relationship; the present result showed no association between HDLC and BMD a finding also similar to an earlier observation (Graham *et al*., 2010).

The increased LDLC and reduced HDLC obtained in this study suggest a possible early CVD risk in osteoporotic patients. Dyslipidimia couple with decreased BMD are notable indicators of risk of bone fragility. An earlier study has indicated that increased plasma TG suppresses osteoblastic activity(26). Evidence from a study has also indicated that osteoblast and adipocytes share a common progenitor from stromal cells in bone marrow and this has led to the postulation that a relationship between hyperlipidemia and BMD could be the missing link between osteoporosis and atherosclerosis (Rosen and Bouxsein, 2006). It may therefore be proper to speculate from the results of this study that osteoporotic patients could be more prone to bone fracture in part as a result of reduced BMD coupled with increased plasma TC and LDLC. These changes could potentiate early CVD event. Previous evidence showed that osteoporotic patients are at increased risk of acute cardiovascular events independent of age and cardiovascular risk profile and the increase in risk is proportional to the severity of osteoporosis at the time of diagnosis (Young McEneny, 2001). Infact, evidence from earlier study also indicated that for every 1 mg decreased in plasma HDLC, the risk of developing CVD is 2.5 times higher (Goldbourt *et al*., 1997).

CONCLUSION

There are compelling evidence from the results of this study that decreased BMD and increased plasma TC and LDLC as well as decreased HDLC are features of osteoporotic patients in Nigeria and these changes could lead to risks of bone fracture. Further study is warranted on larger sample size.

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