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Plasma homocysteine, B vitamins and bone mineral density in osteoporosis: a possible risk for bone fracture

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Abstract

Background: Changes in plasma total homocysteine(tHcy) folic acid, vitamins B12 and B6 in individuals with osteoporosis are reported to impair collagen cross-linking and contribute to low bone mineral density (BMD). There is paucity of information on these associations in osteoporotic patients at risk of bone fractures in Nigeria. The study evaluated plasma tHcy, folic acid, vitamins B_{12} and B_6, in relation to BMD in individuals with osteoporosis.

Methods: Fifty osteoporotic patients age 57.05±1.9 years were selected and fifty non osteoporotic volunteer's age 54.8±0.9 years were included as controls. The osteoporotic group consisted of 11 males and 39 females (1:3.5) while the controls consisted of 13 males and 37 females (1:2.8) respectively. Bone mineral density, anthropometric indices plasma tHcy, folic acid, vitamins B_{12} and B_6, were determined using standard procedures.

Results: The results showed remarkably significant increase in plasma tHcy ($p<0.001$) (180%) compared with the control value. Striking significant decreases were observed in folic acid (62%), vitamins B12 (42%), B_6 (59%) and BMI $p<0.001$) compared with control values. Positive correlation was obtained between vitamin B_{12} and BMD ($r = 0.311$, $p<0.05$).

Conclusion: Significant increase in tHcy with corresponding decreases in folic acid, vitamins B_{12} and B_6 are related to decrease in BMD in osteoporotic patients. These changes could be important risk factors for bone fracture in osteoporotic Nigerians. Supplementation with the B vitamins may be beneficial to the patients.

Keywords: *Osteoporosis, Homocysteine, Folic acid, Vitamins B_{12}, B6 and Bone mineral density*

Résumé

Introduction: Les variations de l'homocystéine plasmatique totale (tHcy) de l'acide folique, des vitamines B_{12} et B_6 chez les personnes atteintes d'ostéoporose sont présentés à porter atteinte à réticulation du collagène et de contribuer à la faible densité minérale osseuse (DMO).Il y a peu d'informations sur ces associations chez les patients ostéoporotiques risque de fractures osseuses au Nigeria. L'étude a évalué plasma tHcy, acide folique, vitamines B_{12} et B_6, en ce qui concerne la DMO chez les personnes atteintes d'ostéoporose.

Méthodes: Cinquante patients ostéoporotiques' âge 57,05 ± 1,9 ans ont été sélectionnés et non cinquante bénévoles ostéoporotiques l'âge 54.8± 0,9 ans ont été inclus comme contrôles. Le groupe de ostéoporotique composée de 11 hommes et 39 femmes (1: 3,5) tandis que les commandes étaient composés de 13 hommes et 37 femmes (1: 2.8) respectivement. La densité osseuse minérale, les indices anthropométriques plasma homocystéine, l'acide folique, des vitamines B_{12} et B_6 ont été déterminés en utilisant des procédures standard.

Résultats: Les résultats ont montré une remarquable augmentation significative dans le plasma tHcy ($P<0,001$) (180 %) par rapport à la valeur de commande. Frappant diminutions significatives ont été observées en acide folique (62 %), les vitamines B_{12} (42 %), B_6 (59 %) et de BMI $P<0.001$) par rapport aux valeurs de contrôle. Une corrélation positive a été obtenue entre la vitamine B_{12} et la densité minérale osseuse (r 0,311, $P<0,05$).

Conclusion: Une augmentation significative de tHcy avec des réductionsconcordantes en acide folique, vitamines B_{12} et B_6 sont associés à diminuer la DMO chez les patients ostéoporotiques. Ces changements pourraient être d'importants facteurs de risque de fracture de l'OS ostéoporotiques aux Nigérians. Une supplémentation en vitamines B peut être bénéfique pour les patients.

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Introduction

The World Health Organization (WHO) study group in 1994 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." Osteoporosis was further demonstrated as a bone mineral density T- score of at least 2.5 standard deviations below a healthy, young white female. [1]. It is a global public health

Results

Results obtained are represented in tables 1-4.

The BMI and BMD ($p< 0.001$) were significantly reduced compared with the corresponding control values. There was remarkable significant increase in plasma tHcy when compared with the corresponding control value ($p<0.001$). Mean plasma folic acid, vitamins B_{12}, and B_6 were significantly reduced in the osteoporotic patients ($p<0.001$) compared with the corresponding control values (Table 1).

The mean plasma tHcy ($p<0.001$) was significantly increased when compared with the corresponding control males. Significant decreases were obtained in BMD, folic acid, vitamins B_{12} and B_6 ($p<0.001$) in osteoporotic females compared with the controls respectively (Table 3).

There was a significant correlation between BMD and plasma vitamin B_{12} ($r = 0.311$, $p<0.05$) only. All other parameters were not significantly correlated (Table 4).

Discussion

Osteoporosis is a global public health problem and reports [2, 3] have shown that the disease and its associated fractures are among the important causes of morbidity and mortality affecting millions of people worldwide.

The patients in this study as defined by the T-score value of the bone mineral density diagnostic for osteoporosis in accordance with the World Health Organization [1] criteria for osteoporosis, have primary densitometric osteoporosis. Seventy eight percent of the patients were women while twenty two percent were men. Forty four percent of the patients complained of back pain. All of them were urban dwellers. Obesity was not a characteristic

Table 3: Biophysical and Biochemical parameters of osteoporotic females and control females

Variables	Osteoporotic females (N=39)	Control females (N=37)	t-value	p-value
Age (yrs)	56.3±2.1	54.8±1.8	1.8	P>0.05
BMI (Kg/m²)	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
tHcy (μmol/L)	19.0±0.7	10.1±0.3	11.7	p<0.001
Folic acid (μmol/L)	85.7±4.2	140.8±4.3	-9.1	p<0.001
Vit B_{12}(μmol/L)	155.9±4.2	358.3±15.7	-12.1	p<0.001
Vit B_6(μmol/L)	44.4±2.1	77.3±1.8	-11.8	p<0.001

Values= Mean ± SEM
BMI=Body Mass Index BMD=Bone mineral density
tHcy= total homocysteine Vit B_{12}=Vitamin B_{12} Vit B_6=Vitamin B_6

Table 4: Pearson correlation coefficient of all parameters in osteoporotic patients

Variables	Age (years)	BMI (kg/m²)	BMD (T-score)	tHcy (μmol/L)	Vit B_{12} (pmol/L)	B_6 (nmol/L)	Vit B_{12} (pmol/L)
Age(years)							
BMI(kg/m²)							
0.312*							
BDM (T- score)							
tHcy(μmol/L)							
Folic acid (nmol/L)							
Vit B_{12} (pmol/L)			0.312*				
B_6 ((nmol/L)							

BMI=Body Mass Index BMD=Bone mineral density
tHcy = total homocysteine Vit B_{12}= Vitamin B_{12} Vit B_6= Vitamin B_6

when compared with the corresponding control male values. There were no significant differences in BMI and age (Table 2).

There was significant increase in mean plasma tHcy ($p<0.001$) compared with the control females. Significant decreases in mean BMI, plasma folic acid, vitamins B_{12} and B_6 ($p<0.001$) were obtained

Assay principle

The test is based on the competition between S-adenosyl-L-homocysteine (SAH) bound to the walls of micro-titre plate and the test sample. Protein bound homocysteine (Hcy) is reduced to free Hcy. The free homocysteine in the test sample is converted to SAH by SAH hydroxylase. The enzyme SAH hydrolase is specific for L-form of Hcy [16]. The peroxidase activity was measured spectrophotometrically. The absorbance is inversely related to the concentration of total homocysteine in the sample.

Determination of B vitamins

Analysis of folic acid, vitamins B_{12} and B_6 were carried out using high performance liquid chromatography (HPLC) - waters 616/626

Principle: Separation by Liquid Chromatography is based on the distribution of solutes between a liquid mobile phase and a stationary phase. When particles of small diameter are used as stationary phase support, the technique is high performance liquid chromatography (HPLC). In HPLC, the stationary phase is composed of uniform, ultra-fine particles, which greatly increase its adsorptive area. The stationary phase is packed firmly into column. The resistance of flow in this column is high; therefore large pressures (500-5000 pounds per square inch) are required to deliver constant flow rates. Elute from the column is monitored using a variety of detectors such as ultra-violet or redox-potential electrode detectors. Commercial quality control samples were included in each assay for precision and accuracy.

Statistical analysis

All results were subjected to statistical analysis using SPSS version 14 (SPSS Inc, Chicago, USA) for windows. The result was expressed as mean ± SEM. Students't-test was used for statistical comparisons of means and the differences were regarded as significant at $p<0.05$. Pearson's correlation coefficient was used to assess relationship between biochemical and biophysical characteristics.

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)	57.0±1.9	54.8±0.9	1.8	P>0.05
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
tHcy (μmol/L)	18.8±0.5	10.2±0.2	14.1	p<0.001
Folic acid (μmol/L)	85.1±3.7	138.5±3.4	-10.5	p<0.001
Vit B_{12}(μmol/L)	155.3±6.0	363.0±12.6	-14.9	p<0.001
Vit B_6(nmol/L)	45.0±1.9	76.0±1.5	-12.9	p<0.001

Values= Mean ± SEM
BMI=Body Mass Index BMD=Bone mineral density
tHcy =Total Homocysteine Vit B_{12} =Vitamin B_{12} Vit B_6 =Vitamin B_6

Table 2: Biophysical and Biochemical indices of osteoporotic males and control males

Variables	Osteoporotic males N=11	Control males N=13	t-value	p-valu
Age (yrs)	55.8±4.6	53.1±2.6	1.1	P>0.05
BMI (Kg/m^2)	23.2±1.3	27.9±2.0	-1.9	P>0.05
BMD (T-Score)	-2.7±0.6	-0.7±0.1	-3.7	p<0.001
tHcy (μmol/L)	17.9±0.7	10.6±0.3	10.4	p<0.001
Folic acid (μmol/L)	82.9±8.3	132.1±4.8	-5.4	p<0.001
Vit B_{12}(μmol/L)	153.6±14.9	376.5±19.0	-9.0	p<0.001
Vit B_6(μmol/L)	47.3±3.8	72.1±2.8	-5.4	p<0.001

Values= Mean ± SEM
BMI=Body Mass Index
BMD=Bone mineral density tHcy= Total homocysteine
Vit B_{12} =Vitamin B_{12} Vitamin B_6 =Vitamin B_6

levels of plasma folic acid, vitamins B_{12} and B_6, [27]. Homocysteine thiolactone is highly reactive and conditions such as elevated plasma tHcy as reported in the osteoporotic group in the present study, favours its production. Homocysteine thiolactone may lead to protein functional damage by the inactivation of lysyl oxidase which is required for collagen cross linking and modification [28], invariably increased level of plasma tHcy could lead to increase in the risk of fracture through interference in collagen cross-linking. Thus suggesting that elevated plasma homocysteine may interfere with the development of the microarchitecture of bone, independent of the amount of minerals in the bone. This concept is corroborated by the absence of an association between total plasma homocysteine and BMD in the present study.

Conclusion

This study provides clear evidence that altered plasma tHcy, B vitamins and reduced BMD exist in osteoporotic Nigerian. These alterations could be risk factors for bone fracture in part. Dietary supplementation of folic acid, vitamins B_{12} and B_6 may be beneficial.

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feacture of the patients, they had BMI lower than the control subjects .

It is interesting to note that the present study revealed abnormaly raised mean plasma tHcy with corresponding decreased in BMD in all the osteoporotic patients. The mean BMD of the osteoporotic patients was higher than the control value with an increase of one hundred and eighty percent over the control value. There was gender variation in mean BMD value with male patients exhibiting lower value.

Elevated plasma total homocysteine obtained in the osteoporotic subjects did not correlate with any of the measured parameters; an indication that suggests that increased plasma tHcy could be an independent risk factor for osteoporosis. An earlier report [11] showed that plasma tHcy >15μmol/L can give rise to 2.5 fold increase risk of bone fracture. It could be speculated from the results of the present study that these patients are more at risk of bone fracture with tHcy of 18μmol/L. Another observation is that the male subjects showed a higher mean plasma tHcy level than the females. Earlier studies [9,15] have consistently shown that plasma tHcy is higher in the male than female, previous studies [17,18] from our laboratory have consistently showed higher mean plasma tHcy value in male.

It is noteworthy that the patients have markedly decreased plasma vitamins B_6, B_{12} and folic acid, well known cofactors in homocysteine metabolism. In this environment, the greater proportion of the population is low income earners. and the affordable diet is mainly carbohydate based and low in vitamin nutrients [19]. This could be the underlying cause of the low plasma B vitamins level. It is possible that these changes are contributory factors to the predisposition to osteoporosis. It can be inferred from the available evidence in this study that dietary supplementation of the B vitamins may play major roles in part, in ameliorating osteoporosis in Nigerian-Africans.

The present findings are consistent with the result of a study [20] that examined the relationship among homocysteine, B-vitamins status and BMD in older Americans in whom decreased level of plasma folic acid was obtained. Several observational studies suggest that poor dietary intakes and low plasma concentrations of B vitamins are associated with decreased BMD, greater bone loss, and higher risk of osteoporotic fracture [20, 21].

Plasma tHcy levels are influenced by dietary intake of folic acid, vitamins B_{12} and B_6 as demonstrated by previous study (20). In another study, Cagnacci [22] suggested a direct effect of folic acid on bone tissue and a positive association between folic acid level and BMD was reported in post-menopausal women. The osteoporotic patients in this study had significantly lower BMD with a corresponding decreased plasma folic acid. It could not be speculated from the present study whether the low BMD with corresponding decreased plasma folic acid are mediated through elevated plasma homocysteine. The decreased plasma vitamin B_{12} with corresponding decreased BMD in the patients further suggests that decreased plasma vitamin B_{12} could have contributory effect on bone fracture. This is congruent with earlier findings [12,23] which demonstrated that vitamin B_{12} was independently associated with BMD. Several cross sectional studies including a previous study by the younger Framingham offspring cohort study group [24, 25], found a direct association between vitamin B_{12} and BMD. Vitamin B_6 was also lower in the osteoporotic patients independent of BMD in the patients. Mclean *et al*, [23] demonstrated that decreased vitamin B_6 was associated with greater bone loss and that adjustment for BMD attenuated the association with fracture indicating that insufficient plasma vitamin B_6 may contribute to greater bone fragility through a reduction in bone mass. Our result is congruent with a recent investigation of men and women in the Rotterdam study in which increased dietary intake of vitamin B_6 was directly related to improved BMD and inversely related to risk of osteoporotic fracture [13].

Evidently, elevated plasma tHcy level obtained in the osteoporotic group in the present study could be linked to decreased levels of folic acid, vitamins B_{12} and B_6, all of which are involved in the metabolism of homocysteine during remethylation and tans-sulfuration processes. The mechanism linking elevated plasma tHcy level with BMD is equivocal. A proposed hypothesis posits that the mechanism underlying the association between the homocysteine level and risk of osteoporotic fracture may be attributed to interference by homocysteine in collagen cross-linking [26]. Since collagen cross-links are important for the stability and strength of the collagen network, interference with the formation of cross-links results in an altered bone matrix which then leads to increase bone fragility [26]. The mechanism by which elevated homocysteine interferes with collagen cross-linking could be as a result of protein homocysteinylation giving rise to the formation of homocysteine thiolactone when the normal remethylation or trans-sulphuration pathways are altered due to inadequate

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observée; alors, les antioxydants ont montré une corrélation négative avec LH et FSH tandis qu'une corrélation positive avec l'estradiol et de progestérone. *Conclusion:* La présente étude a révélé que la phase peri-menopausale et la phase post-ménopause normale sont associés à la tension d'oxydative. Donc il peut être à l'avantage quand les deux phases sont dirigées dans le taux de déficit hormonal si l'antioxydant est un complément.

Introduction

Reactive Oxygen Species (ROS), collectively described as Oxygen Free Radicals (OFR) as well as Reactive Nitrogen Species (RNS), are products of normal cellular metabolism[1]. ROS and RNS are well recognized for playing dual roles as both deleterious and beneficial species, since they can be either harmful or beneficial to living systems[1]. The beneficial effects of ROS happen at low/moderate concentrations and involve physiological roles in cellular responses to noxia, as in defence against infectious agents and in the function of a number of cellular signaling systems[2]. Whereas, the harmful of ROS in biological systems happen when there is an overproduction of ROS/RNS on one side and a deficiency of enzymatic and non-enzymatic antioxidants on the other. Subsequently, Oxidative Stress (OS) results from metabolic reactions in which there is a disturbance in the equilibrium status of ROS and prooxidant/ antioxidant reactions in living organisms [2]. Previous study shows that OS influences the entire reproductive phases of women and even thereafter (i.e. menopause). It has also been suggested that the age-related decline in fertility is modulated by OS [3]. The pathological effects are exerted by various mechanisms including lipid damage, inhibition of protein synthesis, and depletion of Adenosine triphosphate (ATP)[4].

Perimenopause refers to the time before menopause till a year following the actual menopause. It is heralded by menopausal transition, a period when the endocrine, biological and clinical features of approaching menopause begins [5]. The perimenopausal period usually commence with irregular menses associated with shorter menstrual cycle length and onsets of vasomotor symptoms [5]. The most-noteworthy characteristic of the perimenopause is significant hormonal variability [5]. The median age of onset has been reported as between 45.5 and 47.5 years; the average duration is 4 years [6]. The World Health Organization has defined the menopause as the permanent cessation of menstruation resulting from loss of ovarian follicular activity [7]. Epidemiological study revealed that the average age for the final menstrual period is 48.4 years [8]. The biology underlying the transition to menopause includes central neuroendocrine changes as well as changes within the ovary, the most striking of which is a profound decline in follicle numbers. Grouped data show that mean changes in hormone levels become significant around the final menstrual period, with a decrease in Inhibin B (INH-B) concentration in early perimenopausal women being the most important and significant initial endocrine event at that time [5].

Menopause is an important landmark in the life of a woman [9]. It is defined as a physiological event in which there is at least twelve consecutive months of amenorrhoea caused by depletion of ovarian function [10].This results in various somatic, vasomotor, sexual and psychological symptoms that impair the overall quality of life of women [10-12]. Menopause has become an important subject of study because of the global increase in life expectancy resulting from better nutrition and improved health care delivery [13]. The introduction of governmental and other stakeholders' interventions targeted at achieving the Millennium Development Goals is also expected to increase the population of postmenopausal women. In 1990, the world population of postmenopausal women was reported to be 476 million, with 40% living in the industrialized world [14]. It is estimated that this figure will increase to 1200 million with 76% of these women in the developing countries by the year 2030 [14]. With the increasing average length of the postmenopausal life span, it has become imperative for healthcare providers to focus more attention on the health of this group of women to ensure that they enjoy this twilight years of their life optimally [13]. Estrogen has been shown to possess direct free radical scavenging properties at high concentrations, to enhance the activity of some naturally occurring antioxidant enzymes (particularly glutathione peroxidase), and to function as a chain-breaking antioxidant *in vivo* [15-17]. The human red blood cell (RBC) has an effective mechanism to prevent and neutralize oxidative stress (OS) induced damage. This is accomplished by a set of antioxidant enzymes such as GSH-Px, CAT, and SOD [18]. The antioxidant enzyme SOD widely distributed in all cells is present in high amounts in erythrocytes; the antioxidant enzyme plays an important role in scavenging the superoxide radical. SOD protects cells against superoxide radical by dismutation of the highly reactive superoxide anion to oxygen and to a less reactive oxygen species, hydrogen peroxide