Vitamin D Status of HIV Infected Patients in Kano, Nigeria

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Abstract
Background: *The use of highly active antiretroviral therapy (HAART) has considerably reduced the burden of HIV /AIDS in the past few years. However, the metabolic/endocrine complications associated with the disease and side effects arising from treatment with HAART remain as formidable challenges in patients' management.*
Aim: *The aim of this study was to evaluate vitamin D status of HIV/AIDS patients in our environment.*
Methods: *The study was descriptive cross sectional in design and recruited 300 participants, made up of 150 HIV sero-positive patients and 150 HIV sero-negative controls. The data collected included demographics, duration and type of antiretroviral therapy (ART) regimen, serum levels of vitamin D, Parathyroid hormone (PTH), Albumin, Calcium, Phosphate, CD4 count and viral load.*
Results: *The percentage of HIV positive patients with vitamin D deficiency, vitamin D insufficiency and those with optimal (adequate) vitamin D were 60%, 21% and 19% respectively. HIV positive patients on non-nucleoside reverse transcriptase inhibitors (NNRTI) regimen had higher prevalence (82.2%) of vitamin D deficiency than the patients on PI based regimen (17.8%).*
Conclusion: *This study showed that vitamin D deficiency and vitamin D insufficiency are common in patients with HIV/AIDS, most especially those on Non- Nucleosides reverse transcriptase inhibitors (NNRTI).*

Key words*: Vitamin D; Human immunodeficiency virus; highly active antiretroviral therapy*

Introduction

The Human immune deficiency Virus infection/ Acquired Immune Deficiency Syndrome (HIV/AIDS) is a global health problem that has affected over 78 million people and has caused over 35million deaths since it was first recognized in 1981, making the epidemic the most destructive in human history (UNAIDS, 2015). As at November 2015, about 36.7 million people were living with HIV globally (UNAIDS, 2015).

According to the 2016 joint United Nation Program Report on HIV/AIDS (UNAIDS), the number of HIV positive people receiving antiretroviral therapy (ART) was about 18.2 million (UNAIDS, 2016).Nigeria has the second highest HIV/AIDS population in the world with about 3.5 million people living with the disease as at 2016 with a prevalence rate of 3.1% (GAPPR, 2016). The current prevalence rate of HIV in Kano state is 1.3% and about 24% of adult HIV positive patients in Nigeria are on antiretroviral treatment (GAPPR, 2016).

Advances in treatment with anti-retroviral therapy significantly reduced morbidity and mortality resulting from the disease. However the use of Highly Active Antiretroviral Therapy (HAART) is accompanied by lots of side effects (GAPPR, 2016).Among the common metabolic complications associated with the use of HAART are: reduction in bone mineral density, serum lipid abnormalities, lactic academia and disorders of glucose metabolism and deficiency of some vitamins especially vitamin D (Childs *et al*, 2012).

Vitamin D deficiency among HIV infected patients is reported to be between 29% and 73% (Childs *et al*, 2012). Risk factors for vitamin D deficiency among HIV/AIDS patients include risk factors similar to those of the general population (Ene-Obong *et al*,2001; Maziya-Dixon *et al*, 2005; Glew *et al*, 2010; Kruger *et al*, 2011; Poopedi *et al*, 2011) and HIV-related risk factors' which include: duration of infection with the virus, CD4 cell count below 200cells/mm^3, current antiretroviral therapy and viral load (Childs *et al*, 2012).

Research report on vitamin D status of HIV patients in Nigeria is scarce to nearly non-existence and the metabolic complications associated with vitamin deficiency affect the quality of life of HIV/AIDS patients and may reduce compliance to treatment.

This study determined the prevalence of vitamin D deficiency/insufficiency among HIV/AIDS patient on therapy with a view to document its magnitude. The awareness of its magnitude would hopefully lead to improvement in the management strategies of patients with resultant reduction in morbidity, mortality and ultimately enhance quality of life of the patients.

Materials and Methods

This cross sectional descriptive study was conducted in a teaching hospital in northern Nigeria. The study recruited 150 HIV sero-positive patients and 150 HIV sero-negative apparently healthy individuals as controls. Pregnant women, Lactating mothers, patients with history of chronic alcohol abuse, mal-absorption, chronic kidney disease, chronic liver disease, and thyroid disorders were excluded from the study.

Ethical approval to carry out this study was obtained from the Ethical Research Committee of the hospital and informed written consent was obtained from all subjects after verbal explanation on the aim and objectives of the study in a language well understood by them.

A pre-tested questionnaire was administered to obtain the bio-data and biophysical parameters of all subjects that consented to participate in the study.

Subsequently, five (5) milliliters of blood was collected from each of the subjects into a gel/clot activator bottle. The blood samples were then spun immediately in a centrifuge to obtain sera which were then frozen at -20^oC until analysis. Serum vitamin D is stable at this temperature for 24 weeks (Lewis and Elder PA, 2008). Analyses of the samples were done in batches until completion.

Quantitative measurement of vitamin D in the form of 25(OH)D was done using chemiluminescence immunoassay technique on Elecsys 2010 immunology analyser (Hart *et al*., 2006).

Vitamin D levels> 75nmol/l were considered adequate/optimal, levels between 50-75nmol/L were considered insufficient, while levels <50 nmol/L represent deficiency (Holick, 2007).

PTH was also measured using chemiluminescence immunoassay technique on Elecsys 2010 immunology analyser (Hermsen *et al*.,2002).

Serum calcium, phosphate and albumin were measured manually using O- Cresolphthalein complexone method, Phosphomolybdate complex method and Bromocresol green (BCG) method respectively (Robertson and Marshall, 1979; Doumas *et al*, 1971;Tietz ,2008).

All data generated were processed and analyzed using a computer based Statistical Package for the Social Sciences (SPSS) version 16.0. Mean and Standard Deviation (SD) were used to summarize quantitative data while proportions and percentages were used for qualitative data.

Statistical analysis to compare qualitative variables was done using chi square test, while analysis of quantitative variables was done using student t-test. Nonparametric data was analyzed using nonparametric statistics (Kruskal-Wallis test). A confidence interval of 95% was used and a P value of <0.05 was considered significant.

Results

The socio-demographic characteristic of the study subjects is shown in Table1. The age distribution of the study subjects revealed that the highest number of subjects in both study groups were between 30-39 years of age. The control group were slightly older and had mean age of 40.3±9.5 years while the HIV positive patients had mean age of 38.0± 12.8 years. However, the observed difference in the mean age between the patients and controls was not statistically significant (P = 0.082).

The sex distribution of the study subjects showed a female preponderance with male : female ratio of 1:2. Majority of the study subjects were married.

Table 2 shows the mean concentration of serum Vitamin D, Calcium, Phosphate, Albumin, PTH, CD4 count and viral load of the study subjects.

The mean serum levels of vitamin D, calcium, phosphate and albumin were lower in HIV positive patients compared to the controls while the mean serum level of PTH was higher in the HIV patients than the controls. However, the differences observed in the mean levels of vitamin D, phosphate, albumin and PTH between the HIV patients and the controls were statistically significant while the difference in the mean calcium concentration between the two groups was not statistically significant.

The HIV sero-positive patients were found to have deficient (60%), insufficient (21%) and adequate (19%) vitamin D levels respectively while 48%, 39% and13% of the control group had deficient, insufficient and adequate vitamin D levels respectively.

A higher number of the HIV patients with vitamin D deficiency were on NNRTI based HAART regimen (82.2%) compared to those on PI based regimen (17.8%)(Table 3). All the HIV positive patients with vitamin D insufficiency (100%) were on NNRTI based regimen. A significant positive correlation was demonstrated between HAART and vitamin D (P=0.009). Adequate serum levels of vitamin D was found in 28 (18.7%) HIV patients(Table 3).

The distribution pattern of the CD4 count among the HIV positive patients showed that five (5) patients had CD4 count less than 200 cell/µL, seventy-seven (77) patients had their count between 200-499 cells/µL while sixty- eight (68) patients had CD4 count greater than 500 cells/µL. And 65% of the HIV patients had viral load of <50 copies/ml while 35% had >50 copies/ml. However, no significant relationship between CD4 cell count and vitamin D deficiency was observed in our study but a negative relationship was found between Vitamin D deficiency and viral load.

Discussion

This study was conducted between October, 2015 and April, 2016. The age group with the highest number of HIV positive patients was 30-39 years. This suggests that HIV infection was highest among young individuals in the study area. This observation is in agreement with United Nations progress report on Nigeria (Leah and Ayiwulu, 2010).

The overall sex distribution of study subjects revealed female gender preponderance with a ratio of 2:1. This also holds true for the HIV positive patients. This observation suggests that HIV was commoner among females than males in the study area. The result is in keeping with the findings of a study conducted in Awka (South-East Nigeria) in which female preponderance of 68.9% was reported (Nwozor and Nwankwo, 2013). Another study conducted in South Africa among HIV patients also reported a female preponderance

(Pettifor et *al*, 2005).However, the study by Illiyasu and colleagues in Kano reported a male preponderance of 54.6% (Iliyasu *et al*, 2004).

Majority of the HIV sero positive patients were married. This observation is in agreement with the report of a study carried out in Nassarawa-Eggon, Nigeria (Leah and Ayiwulu, 2010). The high number of married HIV positive patients may be due to increased level of awareness of HIV infection, readily available centres and personnel for voluntary counselling and testing (VCT) services and reduced levels of stigmatization in the society (Leah and Ayiwulu, 2010).

This study found a lower vitamin D level among HIV positive patients when compared to the HIV negative controls. An assessment of the HIV positive group also showed that HIV patients on non-nucleoside reverse transcriptase inhibitors (NNRTI'S) had higher tendency of developing vitamin D deficiency compared to patients on protease inhibitors (PI).

In a study carried out among young Israeli women, 65% of those of Ethiopian origin had Vitamin D deficiency (Shahar *et al*, 2012). A similar study in United States of America by Women's Interagency HIV group also observed 60% Vitamin D deficiency among HIV positive participants (Adeyemi *et al*, 2011). However, prevalence of Vitamin D deficiency in a study on Brazilian HIV sero positive participants was found to be 40.65%%(Conrado *et al*, 2011). Also, in a study of an Iranian population, prevalence of vitamin D deficiency was 86.7% among adult HIV infected patients (Etminani-Esfahani *et al*, 2011). Lower levels of vitamin D were detected in symptomatic HIV patients when compared to asymptomatic ones in another study conducted in United States (Adeyemi *et al*, 2011).

The various degrees of vitamin D deficiency reported in HIV populations may be due to variations in methods of serum vitamin D measurement and the diagnostic cut-off points used to define vitamin D deficiency in those studies. Other contributing factors may include co-infections, younger age, smoking, body mass index, race, CD4 cell count, presence of severe diseases and metabolic syndrome, female sex, active intravenous drug abuse, advanced stage of HIV, NNRTI use, viral load and albumin levels (Wasserman and Rubin, 2010).

Several researches have evaluated the impact of antiretroviral drugs on vitamin D metabolism. Both protease inhibitors (PIs) and non-nucleosides reverse transcriptase inhibitors (NNRTIs) have been associated with vitamin D metabolic pathways (Ellfolk *et al*, 2009). The PIs have been shown to inhibit 1α and 25 α- hydroxylation of vitamin D in hepatocytes and monocyte cultures (Ellfolk *et al*, 2009). Reduced conversion of 25-OH vitamin D to its active metabolite may explain the increased 25-OH vitamin D in subjects with low 1, 25 $(OH)_2D$. NNRTI's have been found to increase 25-OH vitamin D catabolism, through the induction of CYP24 and reduced transcription of CYP2R1, a 25-hydroxylase (Ellfolk *et al*, 2009).

Though this study observed a lower calcium concentration among HIV sero positive patients compared to the sero-negative controls, this difference is not statistically significant ($p>0.05$). Other studies on serum calcium levels amongst HIV positive patients also demonstrated no significant correlation between HIV/AIDS and serum calcium levels. It is likely that after HIV/AIDS had caused a reduction in levels of vitamin D and calcium, the body compensates by mobilising calcium from body stores (bone). This further depletes body stores and affects mineralization of the bones (Favus, 2006).

An assessment of serum phosphate level among HIV patients in this study showed a significantly lower level of phosphate compared to the sero-negative controls. This is in agreement with similar study conducted previously (Fernandez-Fernandez *et al*, 2011).The phosphaturic effect of PTH on the kidneys may be responsible for the low level of phosphate observed in this study. In this circumstance, PTH increases the tubular secretion of phosphate into the urine thereby worsening the deficiency state.

The result of this study demonstrated a significantly higher level of PTH among HIV patients than the controls. This finding coupled with relatively lower level of calcium further demonstrates that, the initial low level of calcium was responsible for the stimulation of PTH secretion which then caused bone resorption and tubular reabsorption of calcium thereby restoring serum calcium close to the reference range. This observation was also reported by Childs *et al*, (2012) who detected low serum vitamin D levels and increased serum PTH concentrations following anti-retroviral drug therapy owing to increased bone turnover and fractures (Obum-Nnadi *et al*, 2013). Paul *et al* also found a significantly higher mean concentration of PTH (65±26 pg/ml) in HIV positive patients receiving HAART compared to HIV negative controls (43±23, $p<0.001$)(Tafazoli and Khalili,2013).

The HIV positive patients in this study had lower serum albumin levels than the HIV negative controls. This finding is not unexpected given the wide range of nutritional disorders that can occur in HIV/AIDS patients. It is well known that patients with HIV/AIDS have low nutritional indices (Olayiwola *et al*, 2014). The relatively lower level of albumin may also contribute to the observed low serum calcium levels seen in the HIV positive patients studied. A study conducted in Zimbabwe among HIV patients on HAART showed similar pattern of serum albumin as observed in this study (Denise *et al*, 2013).

Conclusion

This study has shown that vitamin D deficiency and insufficiency are common among both HIV sero- positive patients on HAART and the HIV sero negative controls. HIV Patients on non-nucleosides reverse transcriptase inhibitors have sub-optimal vitamin D levels compared to those on Protease inhibitors.

Recommendations

Based on the high prevalence of vitamin D deficiency and insufficiency found in this study, the followings are recommended:

1. Baseline vitamin D measurement should be routinely done in all newly diagnosed HIV positive patients in order to detect patients with subnormal/deficiency levels.
2. All HAART eligible patients should have vitamin D levels known before the commencement of HAART.
3. Vitamin D supplementation should be given to all patients with subnormal/deficiency levels.
4. Routine measurement of Vitamin D should be encouraged among healthy individuals in our community.

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Table 1: Socio-demographic characteristics of the study subjects

Variable	Patients		Controls	
	N	%	N	%
Age Group (years)				
20-29	19	12.7	41	27.3
30-39	53	35.3	48	32.0
40-49	45	30.0	24	16.0
50-59	32	21.3	24	16.0
60+	1	0.7	13	8.7
Total	150	100.0	150	100.0
Gender				
Male	45	30.0	52	35.0
Female	105	70.0	98	65.0
Total	150	100.0	150	100.0
Marital Status				
Single	9	39.1	14	60.9
Married	86	41.0	124	59.0
Divorced	30	88.2	4	11.8
Widowed	25	75.8	8	24.2
Total	150	100.0	150	100.0

Table 2: Serum Vitamin D, Calcium, Phosphate, Albumin, PTH, CD4 Count and Viral Load (mean ± SD) of study subjects

Parameter	Subjects	Controls	p value
Vitamin D (nmol/L)	44.8±30.3	51.4±17.4	0.021*
Calcium (mmol/L)	2.3±0.2	2.4±0.2	0.057
Phosphate (mmol/L)	1.1±0.2	1.3±0.3	<0.001*
Albumin (g/L)	38.9±5.1	42.2±4.9	<0.001*
PTH (pmol/L)	50.3±35.9	37.7±18.0	<0.001*
CD4 Count (cells/µL)	480.9±211.4		
Viral load (copies/ml)	2601.1±10348		

Table 3: Vitamin D, Calcium, Phosphate, Albumin, PTH status of HIV patients on HAART

Parameters		HAART Regimen		p value~
		NNRTI	PI	
Vitamin D level				
Deficient	n	74	16	0.009*
	%	82.2	17.8	
Insufficient	n	32	0	
	%	100.00	0.00	
Adequate	n	22	6	
	%	78.6	21.4	
Calcium level				
Low	n	36	5	0.825
	%	28.1	23.8	
Normal	n	91	16	
	%	71.1	76.2	
High	n	1	0	
	%	0.8	0	
Phosphate level				
Low	n	16	3	0.796
	%	12.5	14.3	
Normal	n	111	18	
	%	86.7	85.7	
High	n	1	0	
	%	0.8	0.0	
Albumin level				
Low	n	1	0	0.368
	%	0.8	0.0	
Normal	n	126	20	
	%	98.4	95.2	
High	n	1	1	
	%	0.8	4.8	
PTH level				
Low	n	0	0	0.894
	%	0.0	0.0	
Normal	n	121	20	
	%	94.5	95.2	
High	n	7	1	
	%	5.5	4.8	

*=statistically significant, ~=Fisher's p value, PI= Protease Inhibitors