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Evaluation of Vitamin D in Sickle Cell Anaemia Patients at Steady State

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Abstract

Background: Sickle cell anaemia is a major public health problem in sub-Saharan Africa with high morbidities like bony alterations and bone fragility especially in those with vitamin D deficiency. There is paucity of research data on bone biomarkers in patients with sickle cell anaemia especially in Northern Nigeria. The aim of this study was to evaluate vitamin D in adult sickle cell anaemia patients at steady state.

Materials and Methods: Seventy-seven patients with sickle cell anaemia and equal number of apparently healthy matched controls were recruited for the study. Various biochemical parameters of bone metabolism were measured. Data were analysed using IBM SPSS version 23.0.

Results: The mean age of SCA adult patients and controls were 22.86 ± 0.76 and 24.36 ± 1.00 years respectively (p = 0.106) years. Serum calcium and 25OHD were significantly lower in the patients, with 47% and 91% of them had a value below the normal range. PTH, ALP, and phosphate were significantly higher in the patients, with 73%,87% and 61% res pectively, having values above the normal range.

Conclusion: Sickle cell anaemia patients have hypocalcaemia tendency associated with supranormal PTH and ALP, and imply impaired intestinal absorption of calcium and vitamin D leading to disturbed calcium metabolism which might contribute to the skeletal changes seen in sickle cell anaemia.

Keywords: Sickle cell anaemia, steady state, vitamin D.

Introduction

Sickle Cell Anaemia (SCA) is the prototype and severe form of Sickle Cell Disease (SCD), with an autosomal recessive inheritance (1). Globally, sickle cell affects 100 million people and it is responsible for 50% of mortality in those with severe form of the disease (2). Steady state is a period free of crises extending from at least three weeks since the last clinical event and three months or more since the last blood transfusion, to at least one week before the start of new clinical event (3). Due to complications of this chronic inflammatory disease associated with chronic haemolysis, vaso-occlusion, and tissue hypoxia, bone metabolism is adversely affected (4). Painful vaso-occlusive crises or osteomyelitis are the acute clinical manifestations whereas osteonecrosis, osteoporosis and osteopenia can be seen in long term. Vitamin D and calcium are required for optimal bone health with 90% of required vitamin D synthesis coming from exposure to sunlight. Dark skinned individuals usually require 5-10 times the exposure to sunlight to produce the same amount of vitamin D in their skin as do pale skinned people (5,6). Skin pigment absorbs solar ultraviolet radiation and diminishes the cutaneous production of vitamin D. This might explain, in part the high incidence of vitamin D deficiency in individuals of African descent. Osteopenia and osteoporosis have a frequency of up to 82% of adults with SCA (7,8). However, osteoporosis and demineralization of bone in SCA seems to be multifactorial. Bone marrow expansion and cortical thinning, hypogonadism, hypothyroidism, hypoparathyroidism, low vitamin D levels, direct toxic effects of iron overload on osteoblast, calcium and zinc deficiencies and reduced physical activity as well as low body mass index (BMI) plays a role in the bone metabolism that may be connected to the hypermetabolic state in SCA. Chronic inflammatory processes, chronic anaemia, increased cardiac load, rapid erythropoiesis, increased protein turnover, and oxidative stress all contribute to the hypermetabolic condition (9). Vitamin D status in SCA patients in this study area is not known, this study was aimed to evaluate vitamin D levels and its predictor in patients with SCA.

Materials and methods

The study was granted ethical approval by the Health Research and Ethics Committee of Usmanu Danfodiyo University Teaching Hospital Sokoto (certificate number: UDUTH/HREC/2019/No. 835).

This was a cross-sectional descriptive study carried out on adult with SCA at steady state attending haematology outpatient clinic in Usmanu Danfodiyo University Teaching Hospital, Sokoto from August to December 2019. Seventy-seven 77 SCA patients of both sexes (36 males and 41 females) aged between 18 years and 45 years were included in the study, similarly, seventy-seven (44 males and 33 females) age and sex matched healthy adults making the control group which were either siblings or relatives of the patients as well as staffs

from the clinic who are willing to participate in the study. Patients were excluded if diagnosed with HbAS, or other form of genotypes apart from HbSS, were prescribed with calcium and vitamin D supplements, has acute complications or clinical crises within last three months, had other comorbid disease such as hepatitis, cancer, HIV, metabolic bone disease and endocrine dysfunction, refused to sign a written informed consent to par ticipate in the study. Diet and exposure to sunlight were the main sources of vitamin D. A questionnaire was used to collect data. It included demographic data, number of admissions for painful crises, number of blood transfusions, history of bone fracture, bone infection. Haemoglobin level and haemoglobin electrophoresis were obtained from the patient medical records. A volume of 5ml of venous blood were collected in plain vacutainer tubes, and the serum was separated and stored at -20^{0}C until the time of analysis. Serum calcium, phosphate and alkaline phosphatase (ALP) were measured using visible spectrophotometer (M© Jefferson Measuretech instrument Co, Ltd 722G, China-Shanghai), serum vitamin D and PTH were measured using commercially available ELISA kits (CTK Mono bind Inc., Lake Forest, CA, USA), and assays were performed and calculated according to the manufacturer's instructions.

The concentrations were expressed in ng/mL and pg/mL respectively, using Rayto microplate reader (Rayto life and Analy tical Sciences Co. Ltd RT-2100C, Guangmin New District, Shenzhen, PR China). The precision of the assay methods was determined by analysing a normal human sample commercially sourced. The intra-assay CVs of the methods used were 6.7%, 8.2% and 11.5% and the inter-assay CVs were 7.2%, 9.8% and 12.6% for PTH, and vitamin D respectively.

Statistical analysis

Statistical analysis was carried out using the statistical package for social sciences (SPSS Version 23) software. Variables were expressed as mean ± SEM. Comparisons of variables were performed using unpaired student t-test. Bivariate associations of the variables were assessed using Pearson's correlation coefficients and *p* values < 0.05 were considered statistically significant.

Results

This research work evaluated prevalence of vitamin D deficiency among 77 SCA patients at steady state attending haematology outpatient clinic UDUTH, Sokoto North-Western Nigeria. Table 1 shows the numbers and percentages of SCA patients with values either lower or higher than the normal range of the measured parameters. It is clear from the table that about 90.01% of the SCA patients have vitamin D levels below the lower normal range and 46.75% below the normal range of calcium respectively, whereas, 87.01%, 72.73% and 61.04% with values greater than the upper normal range for ALP, PTH and phosphate respectively.

Table 2 shows the frequency of crises (VOC)/ years in relation to analytes. Higher frequency of crises was found to be associated with low serum vitamin D and calcium and higher PTH, phosphate and ALP (P = 0.001, P = 0.05, P = 0.001, P = 0.05, P = 0.001, respectively)

Table 1: Percentage of Sickle cell anaemia patients with abnormal range of Vit D, PTH, Calcium, Phosphate and ALP

Analytes	Mean Value		
	Number of Cases (%)	(±SEM)	Range
25 OHD (< 20.00 ng/mL)	70/77 (90.01%)	11.0±0.64	23.155 - 1.91
PTH (> 65.00 pg/mL)	56/77 (72.73%)	92.83±0.15	725.15 - 24.97
Calcium (< 2.2 mmol/L)	36/77 (46.75%)	2.13±0.08	3.08 - 0.86
Phosphate (>1.45 mmol/L)	47/77 (61.04)	1.55±0.07	2.65 - 0.97
ALP (> 279 U/L)	67/77 (87.01%)	496.044±25.20	841.5 - 5.5

Data are expressed as mean ± SEM., as well as range values, 25OHD = 25 Hydroxy vitamin D, PTH = Parathyroid hormone, ALP = Alkaline phosphatase, using descriptive statistics.

Table 2: Frequency of crises (VOC) / Years in relation to serum concentrations of Vit D; PTH; Calcium; Phosphate and ALP

Analyte Values	FOC/YRs no >3	FOC/YRs no <3	*P*-value
25OHD (ng/mL)	2.758	11.24± 0.08	0.001
PTH (pg/mL)	725.145	91.12± 13.82	0.001
Calcium (mmol/L)	1.70	2.12± 0.06	0.05
Phosphate (mmol/L)	2.10	1.28± 0.07	0.05
ALP (U/L)	731.5	505.65± 25.68	0.001

Values are expressed as mean ± SEM: 0.05 was considered statistically significant, 25OHD = 25 hydroxyvitamin D; PTH = Parathyroid hormone; Phos = Phosphate; ALP = Alkaline phosphatase; VOC = Vaso-occlusive crises. Using Student's t- test.

Table 3 shows the effect of age on serum concentrations of analytes. The mean serum concentration of vitamin D, PTH and ALP increases with increasing age and resultant decrease in calcium and phosphate for cases with reverse effect in control, (P = 0.001, P = 0.001, P = 0.001, P = 0.05, P = 0.05 respectively).

Table 3: Effect of age on serum concentrations of vitamin D; PTH; Calcium; Phosphate; and ALP levels for Cases and Controls

Age group/ year	25OHD (ng/mL)	PTH (pg/mL)	Calcium (mmol/L)	Phos (mmol/L)	ALP (U/L)
CASES					
18 - 27	10.92±008.	79.49±14.87	2.13±0.05	1.64±0.08	504.49±20.42
28 - 37	11.78±0.05	139.30±15.02	2.06 ±0.07	1.61±0.03	536.25±19.87
38 - 47	11.86±0.07	282.39±12.67	2.00±0.04	1.08±0.05	580.56±22.54
CONTROLS					
18 - 27	15.02±0.05	18.55±13.45	2.53±0.06	1.22±0.02	400.18±23.45
28 - 37	11.79±0.06	17.65±12.87	2.69±0.07	1.25±0.07	385.68±20.25
38 - 45	9.02±0.04	7.84±17.40	2.59±0.05	1.18±0.04	310.69±19.58
P - VALUE	0.001	0.001	0.05	0.05	0.001

Values are expressed as mean ± SEM: 0.05 was considered statistically significant, 2 5OHD = 25 hydroxyvitamin D; PTH = Parathyroid hormone; Phos = Phosphate; ALP = Alkaline phosphatase; Using one-way ANOVA. P values were statistically significant if <0.05 for both cases and controls, in all p value are less than 0.05. Data expressed as Mean ± SEM.

Table 4 shows the effect of Body mass index BMI on serum concentrations of analytes for both cases and controls. Increase in BMI is associated with increase in serum concentration of vitamin D and PTH for both cases and controls with resultant decrease of calcium, phosphate and ALP activity, (P = 0.001, P = 0.001, P = 0.05, P = 0.05, P = 0.001, respectively).

Table 4: Effect of body mass index on serum concentration of Vit D; PTH; Calcium; Phosphate; and ALP levels for Cases and Controls

BMI (Kg/m^2)	25OHD (ng/mL)	PTH (pg/mL)	Calcium (mmol/L)	Phos (mmol/L)	ALP (U/L)(mmol)
CASES					
< 18.5	10.19±0.05	74.55±13.89	2.18±0.07	1.62±0.03	519.70±21.90
18.5 - 24.9	11.62±0.07	112.71±15.06	2.67±0.04	1.59±0.05	518.42±20.60
25 - 29.9	15.88±0.04	237.16±13.43	1.79±0.06	1.57±0.08	316.25±22.40
CONTROLS					
18.5 - 24.9	12.49±0.08	16.37±12.06	2.63±0.08	1.23±0.05	430.53±19.00
25 - 29.9	13.82±0.06	17.42±14.90	2.56±0.05	1.21±0.08	338.55±22.50
P- VALUE	0.001	0.001	0.05	0.05	0.001

Values are expressed as mean ± SEM: 0.05 was considered statistically significant, 25 OHD = hydroxyvitamin D; PTH = Parathyroid hormone; Phos = Phosphate; ALP = Alkaline phosphatase BMI = body mass index. Using one-way ANOVA. P values were statistically significant for both cases and controls.

Discussion

The results presented show the concentrations of some of the biochemical parameters related to bone metabolism in SCA. The reference ranges of our laboratory (Calcium; 2.2-2.55 mmol/L, PTH; 15.00-65.00 pg/mL, 25OHD; less than < 20ng/mL as defi ciency, Phosphate; 0.87-1.45 mmol/L, ALP; 98-279 U/L). However, some of the studied SCA patients had values outside the limits of these normal ranges.

This study evaluated the prevalence of vitamin D deficiency in 77 patients with SCA. The prevalence of low serum level of vitamin D less than < 20ng/mL was 90.91%. These findings were consistent with previous reports, in which the prevalence of 93% were observed (10). Similarly, a prevalence of 84% were observed in a study conducted by (11). Furthermore, a prevalence of 92% and 70.9% of vitamin D deficiency less than 20ng/mL were observed respectively by (12). Also agrees with 75% prevalence in a study by (13). The observed prevalence estimates in SCA population of vitamin D deficiency range from 56.4% to 96.4%, (12), seem to be serious health problem around the world, not only because it affects bone metabolism (12), but also has been shown to affect the prognosis of many diseases such as CVD, nephropathy, and chronic pain (14). This vicious cycle caused by all these complications reduces both quality of life and survival of SCA (15). Furthermore, a high prevalence rate range of 65% - 100% was reported (16,17). However, a lower prevalence of vitamin D deficiency less than 20ng/mL of 63.1% were observed (18), as well as 52.47% (19). Low serum level of vitamin D in SCA could be attributed to high concentration of melanin in the skin, decreased appetite and low food intake, increased basal metabolic rate and higher nutritional demands to sustain normal physiologic functioning (20). These changes might affect the rate of the hydroxylation step of vitamin D which may lead to decreased concentration of 25OHD in the blood (20).

From this study, the value of calcium was closer to the lower limit of the normal range, the prevalence of low serum calcium was 46.75%, this finding was consistent with previous report, in which the prevalence of 50% was observed (21), but does not agree with the prevalence of 14% observed by (22, 23). Reduction in serum calcium levels in SCA patients might be attributed to the impairment of the digestive and absorptive function (24), perhaps because the patients are usually confined indoor and are less exposed to sunlight than their normal counterparts therefore, they have low vitamin D store (25OHD), and subsequently, skeletal abnormalities (25). Some studies were able to show high level of phosphate in SCA patients while others failed to do so, from this study 61% of the cases were found to have high level of phosphate this is in accordance with 50% increase in phosphate level in a study by (23), but contrary to 14% increase in phosphate level reported by (26). High phosphate levels may be attributed to the altered renal handling of phosphate in SCA patients, which is associated with increased clearance of sodium due to suggested resistance to the phosphaturic effect of fibroblast growth factor 23 (27). It is well known that modest increase in serum phosphate levels significantly affect red-cell metabolism, increase red-cell metabolism, increase 2, 3- diphosphoglycerate DPG levels, and caused decreased affinity of oxygen for haemoglobin, i.e., a rightward shift of the haemoglobin-oxygen dissociation curve (28). Such changes could have significant effects on these patients since a rightward shift in haemoglobin dissociation curve will be associated with decreased oxygen saturation and an increased sickling of red cells, as well as shortening of the delay time for haemoglobin polymerization (28).

Similarly, some studies were able to show high level of PTH while others have failed to show and, in some studies, remain unchanged. From this study 73% of cases were found to have high level of PTH and this agreed with 71.6% in a study conducted by (13) but contrary to 18.4% in a study reported by (11), as well as 31% by (24,29). High levels of PTH in SCA may be due to physiological adjustment in response to a hypocalcaemia tendency (13), elevated levels of PTH will also stimulate the hydroxylation of 25OHD in the kidney to form 1-25, $(OH)_2D$ (30), which in turn stimulates intestinal absorption of vitamin D and calcium. The net result of these processes is to maintain normal serum calcium. If, however, the gastrointestinal absorption of calcium remains insufficient, physiological adjustments would be required to maintain normal serum calcium, which will be achieved mainly by the increased bone resorption, and in consequence, the bone structure will suffer (30). Moreover, significant reduction in PTH concentration could be attributable to iron overload from repeated blood transfusion and subsequent endocrine failure (31).

While some studies have shown increased levels of ALP in SCA patients with reduced vitamin D, others have failed to show it (18,19). From this study serum ALP was significantly high 87.01% and this is in agreement with 74% in a study conducted by (32). Association of ALP a marker for bone turn over with low vitamin D status in SCA patients is however not clear (18,19). However, correlation between higher mean ALP and low mean serum vitamin D denotes severity of bone damage and is helpful guide of progress in the management of bone pains in SCA (33). Bone ALP is the principal enzyme fraction that is often increases during Sickle cell crises and also suggested that there is correlation between severity of crises, serum ALP activities, and isoenzyme patterns (32), similarly, elevation of ALP activities in SCA patients could be detected even when the participants are asymptomatic.

Conclusion

There is high prevalence of vitamin D deficiency in SCA patients in Sokoto, suggesting that serum vitamin D may play an important role in bone metabolism through its association with parathyroid hormone, alkaline phosphatase, and phosphate, and may provide additional information for pathogenesis of bone loss in this disease. The present data warrant further investigation to determine whether serum calcium/phosphate and PTH values normalize in SCA patients with vitamin D repletion in a prospective way, which will be an important and cost-effective intervention in this group of patients. Bone metabolic biomarkers should be included in the periodic investigations, tools for screening and monitoring as well as prediction of complications in management of SCA.

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Competing interest

The authors declare that they have no competing interests**.**

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