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ORIGINAL RESEARCH

Prevalence and factors influencing sub-optimal serum levels of 25-hydroxyvitamin D among children with sickle cell anaemia in south-west Nigeria

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Abstract

Background: Sub-optimal levels of vitamin D worsen morbidity in sickle cell anaemia (SCA). Increased frequencies of pain episodes and haemolysis have been linked with the deficiency and or insufficiency of vitamin D among individuals with SCA. In Nigeria, the country with the highest SCA burden, data on the prevalence and risk factors for sub-optimal vitamin D in SCA is scanty.

Objectives: The objectives of this study were to determine the prevalence of depressed vitamin D and examine the influence of sociodemographic characteristics and anthropometric parameters on the serum levels of 25-hydroxyvitamin D (25-OHD) among Nigerian children with SCA.

Methods: In this cross-sectional comparative study, serum 25-OHD levels of 95 children with SCA and 75 age- and gender-matched haemoglobin AA control were quantified by high performance liquid chromatography over a six-month period.

Results: A higher proportion of children with SCA had sub-optimal 25-OHD compared with the controls: 12.6% vs. 2.7%, p = 0.013, 95% CI = 1.124.4. The mean serum 25-OHD of the children with SCA was also significantly lower (41.8 ± 9.8ng/mL vs. 45.2 ±8.1ng/mL, p = 0.017, 95% CI = 1.6 6.1). Serum 25-OHD was not related to age, sex, social class, presence of underweight, overweight/obesity and stunting in both bivariate and multivariate analyses.

Conclusion: The prevalence of low serum vitamin D was higher among SCA patients than the matched controls. The serum level was not influenced by their sociodemographic and nutritional status.

Key words: 25-hydroxyvitamin D, Children, Cholecalciferol, Nigerian, Sickle cell anaemia, Prevalence.

Introduction

Deficiency or insufficiency of vitamin D is a public health issue, not only because of its adverse effects on calcium and bone homeostasis, but also because of several other extra-skeletal tissue disturbances. These extra-skeletal tissue disturbances include chronic pain, cardiovascular diseases, nephropathy, asthma, depressed immunomodulatory and anti-inflammatory effects and subsequent predisposition to infections.[1] Individuals with sickle cell disease (SCD) are particularly prone to low serum levels of vitamin D. [1,2] Although the exact pathophysiologic mechanism is unknown, the characteristic low serum vitamin D is believed to be related to decreased appetite and poor dietary food intake, increased basal metabolic rate and associated higher nutritional demands, reduced physical activity, exposure to sunlight and concurrent renal impairment. [1,2]

The first report of vitamin D deficiency (VDD) in patients with SCD was made by Buisson *et al.* [2] in the United States in 2004. The researchers examined the serum vitamin D status among 65 homozygous Haemoglobin SS children aged 5 to 18 years and compared with 33 healthy African-American and 76 healthy non-African American children aged 7 to 10 years. The researchers concluded that children with haemoglobin SS had significantly lower serum vitamin D concentration than healthy children, and a higher proportion of those with haemoglobin SS (65%), compared with the healthy African-Americans (6%) and non-African Americans (0%), had VDD. More than a decade after this initial observation, the clinical relevance of these findings and the effects on SCD management are still subjects of exploration, especially in climes where SCD burden is highest. In Sub-Saharan Africa, including Nigeria, data on serum vitamin D status in SCD are scanty. The available report is based on the observations made among adults,[3] unlike in many parts of the temperate region of the world, where several data exist on the prevalence of VDD or insufficiency among both adults and children with SCD. [1,2,4,5]

Empirical evidence obtained from tropical Africa near the equator, Nigeria inclusive, suggest that serum 25-OHD is relatively higher compared with the Caucasians. [3] This observation is likely due to the fact that the serum level of 25-OHD is affected by the amount of skin exposure to ultraviolet radiation and some genetic variations in vitamin D receptors. In spite of the compelling evidence of low vitamin D among individuals with SCD, VDD remains under-diagnosed and under-treated among Nigerian children with the disease. Vitamin D supplementation, in addition to improving bone health and associated acute and chronic bone pain, may also help to reduce the rates and severity of infections, which are common among children with SCD. In the present study, the prevalence of sub-optimal serum vitamin D and the associated risk factors among children with SCD from south-west, Nigeria, were determined. It is also hypothesized that serum vitamin D level of children with SCD would be lower than the healthy age and sex-matched haemoglobin AA controls.

Methods

In this cross sectional, comparative study, children aged 4 to 11 years with homozygous SS haemoglobin who were in steady state and age and the sex-matched haemoglobin AA children were included. The diagnosis of Sickle cell anaemia (SCA) was made by haemoglobin electrophoresis. The children were consecutively recruited by convenience sampling at the Paediatric Haematology Clinic of the Wesley Guild Hospital unit of the Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria. The controls were age- and sex-matched, apparently healthy haemoglobin AA children, who accompanied their siblings to the Paediatric Haematology Clinic or those seen at the Children General Outpatient Clinic of the hospital, for school-entry medical tests. Excluded from the study were children with acute illness or crises in the preceding four weeks, blood transfusion in the preceding three months, children on chronic blood transfusion program, significant chronic illnesses (*e.g.* chronic kidney disease, cerebral palsy, Down syndrome). In addition, children receiving medications known to affect growth or nutritional status (such as growth hormone and glucocorticoid), use of vitamin D for any reason were not included.

The study was approved by the Ethics and Research Committee of the hospital (ERC/2016/02/14). All the parents/caregivers of the selected children gave informed consent and children older than 7 years gave assent before commencing the study.

Data on the socio-demographics (age, sex and socioeconomic status) were obtained from each subject through a pre-tested questionnaire. Socioeconomic status was determined using the occupation of the father and the highest academic qualification of the mother, according to the model of Olusanya *et al.* [6] The occupation of the father was scored 1 to 3 while the educational qualification of the mother was scored 0 to 2. The minimum and maximum total scores ranged from 1 to 5 respectively. The subjects with a total score of 1 or 2 were categorised as upper class (i.e. social class 1), total score of 3 as middle class (social class 2) and those with score of 4 or 5 as lower class (social class 3).

Growth status

Body weight was measured to the nearest 0.1 kg on a Scaletronix digital electronic scale (Scaletronix, White Plains, NY) while height was measured to the nearest 0.1 cm using a stadiometer (Holtain, Crymych, UK). All measurements were carried out by the same investigator. From the body weight and height, the body mass index (BMI) was calculated from the formula: weight (kg)/height^2 (m^2). The body weight and height were then transformed into Z-scores for height/age (HAZ), weight/age (WAZ),

weight/height (WHZ) and BMI (BMI-Z). The Z-scores were compared with the reference values provided on the WHO Child Growth Standards [7] for children younger than five years, and National Center for Health Statistics (NCHS)/Centers for Disease Control and Prevention 2000 reference standards [8] for children older than five years. Underweight was defined by BMI-Z less than -2 SD from the mean, overweight by BMI-Z > 2 SD but = +3 SD from the mean and obesity by BMI-Z > +3 SD. BMI-Z score between -2 SD and +2 SD from the mean for age and sex was regarded as normal. In addition, wasting and stunting were defined as WHZ and HAZ < -2 SD respectively.

Nutritional assessment

The assessment of the nutritional status of subjects was based on the Triceps Skinfold Thickness (TSF), mid upper arm circumference (MUAC), upper arm area (UAA), upper arm fat area (UAFA) and the upper arm muscle area (UAMA). Measures of MUAC were combined with the TSF thickness to calculate UAA, UAFA and UAMA, as shown in the following equations: [9] UAMA (cm^2) = (MUAC - πTSF)^2/4π; UAA (cm^2) = π/4 (MUAC/π)^2; UAFA (cm^2) = UAA - UAMA and Fat % = UAFA X 100/UAA, Where π is 3.1416.

MUAC was measured on a freely hanging left upper arm midway between the acromion and the olecranon process using a flexible but non stretchable tape to the nearest 0.1cm in all the subjects. TSF was measured using the Harpenden Skinfold Callipers. The skin was pinched between index finger and the thumb half-way down the back of the arm. This was then gently gripped by the callipers to measure the skin fold thickness in millimeters. The mean of two measurements was recorded for each subject.

Serum 25-Hydroxyvitamin D level

Serum 25-OHD levels were quantified using an automated Waters 616/626S Transducer Pump high performance liquid chromatography (HPLC) machine at a wavelength of 198nm. The quantification was obtained using the linear regression equation previously generated from the calibration curve. Serum levels of 25-hydroxyvitamin D = 30ng/mL were considered sufficient, 20 29.9 ng/mL as insufficient, values < 20ng/mL as vitamin D deficient and values < 10 ng/mL as severely deficient.[4]

Statistical Analysis

Statistical tests were performed with SPSS version 17.0. Independent sample t-test and Mann-Whitney U test were used to compare the mean/median serum vitamin D levels and other continuous data as applicable. Possible associations between categorical variables were tested using the Pearson Chi-Square or Fisher's Exact tests. The influence of sociodemographic and nutritional variables on serum vitamin D status was further tested using the binary logistic regression analysis. The statistical significance level for the alpha error was taken as $P < 0.05$ and 95% confidence interval exclusion of unity.

Results

A total of 170 children, comprising 95 with homozygous Hb SS (cases) and 75 with haemoglobin AA (controls) were studied. The overall mean age of the subjects was 7.1 ± 2.5 years with the male: female ratio of 1.3:1. The baseline sociodemographic characteristics of the patients and the controls were similar. (Table I).

A higher proportion of children with SCA compared with the controls were underweight (29.5% vs. 16.0%, p = 0.04) and stunted (12.6% vs. 0%, p = 0.004), with corresponding significantly lower median BMI-Z scores (-0.96 vs. -0.39, p = 0.004) and HA-Z scores (-0.29 vs. 0.22, p = 0.001). In addition, the patients had lower MUAC, UAMA, UAA and UAFA compared with the controls (p = 0.001). (Table II).

Sociodemographic variables and serum 25-OHD

Gender, age and social class did not significantly influence the level of serum 25-OHD in both patients and controls.

Gender: Among the patients, the mean serum 25-OHD levels of the males and females were similar (42.0 ± 9.4ng/mL and 41.6 ± 10.3ng/mL respectively; p = 0.857). The mean serum 25-OHD levels of the males and females were also comparable among the controls (46.4 ± 7.4ng/mL vs. 43.3 ± 9.0ng/mL, p = 0.104). The gender distribution between the groups with sub-optimal and normal vitamin D levels was similar, p = 0.330 (Table III).

There was no statistically significant correlation between age and the serum 25-OHD levels of the patients and controls (r = -0.02, p = 0.849 and r = 0.14, p = 0.222 respectively). The mean ages of the groups with normal and sub-optimal serum vitamin D levels were similar: 7.5 ± 2.5 years vs. 6.6 ± 2.4 years, p = 0.253 (Table III).

Table I: Sociodemographic characteristics of the patients and the controls

Characteristics	*HbSS* (N = 95)	*HbAA* (N = 75)	*P-values*
Gender Male/Female	52/ 43	45/ 30	0.535
Age			
Mean age ± SD (Years)	7.35 ± 2.47	6.80 ± 2.57	0.160
<5 years	19 (20.0)	20 (26.7)	
5 10 years	62 (65.3)	45 (60.0)	0.590
>10 years	14 (14.7)	10 (13.3)	
Socioeconomic class			
Class 1	15 (15.8)	11 (14.7)	
Class 2	54 (56.8)	43 (57.3)	0.979
Class 3	26 (27.4)	21 (28.0)	

Vitamin D status of the patients and the controls

The mean serum 25-OHD of the patients (41.8 ± 9.8ng/mL) was significantly lower than the 45.2 ± 8.1ng/mL obtained among the controls (p = 0.017, 95% CI = 1.6 6.1). Among the patients, only 2 (2.1%) had VDD and 10 (10.5%) had vitamin D insufficiency whereas none of the controls had VDD, while 2 (2.7%) of them had vitamin D insufficiency. Overall, a higher proportion of children with SCA (12; 12.6%) compared with only 2 (2.7%) of the controls, had sub-optimal serum levels of 25-OHD (deficiency or insufficiency) (p = 0.013, 95% CI = 1.1 - 24.4).

Table II: Comparison of the growth and nutritional status of the children with sickle cell anaemia and the controls

Characteristics	*HbSS (N = 95)*	*HbAA (N = 75)*	*P-value*
Underweight	28 (29.5)	12 (16.0)	0.040
Normal BMI for age	63 (66.3)	59 (78.7)	0.076
Overweight	3 (3.2)	4 (5.3)	0.749
Obesity	1 (1.1)	0 (0)	1.000
Stunting	12 (12.6)	0 (0)	0.004
Normal height for age	75 (78.9)	61 (81.3)	0.699
Tall for age	8 (8.4)	14 (18.7)	0.048
MUAC (cm)	15.01 ±2.22	16.45 ± 2.29	<0.001*
TSF (mm)	16.53 ±1.44	16.59 ± 1.59	0.802*
UAMA (cm^2)	8.01 ± 3.68	10.41 ± 4.24	<0.001*
UAA (cm^2)	18.27 ±5.66	21.90 ± 6.38	<0.001*
UAFA (cm^2)	10.26 ±2.20	11.49 ± 2.44	0.001*
Fat %	57.67 ±6.33	53.68 ± 5.79	<0.001*
BMI-Z score	-1.04± 1.35	-0.27 ± 1.27	0.004**
HA-Z score	-0.52 ±1.33	0.56 ± 1.62	0.001**

KEY: * analysed by independent sample t-test; ** Analysed by Mann-Whitney U test. MUAC- Mid upper Arm Circumference; TSF- Tricep Skinfold Thickness, UAMA-Upper Arm Muscle Area; UAA Upper Arm Area; UAFA Upper Arm Fat Area; BMI

Social class: The distribution of the groups by socioeconomic class was similar: p = 0.980 (Table III).

Nutritional status and serum 25-OHD

The presence of underweight did not significantly influence vitamin D status. The proportion of the children with SCA with sub-optimal vitamin D levels who were underweight (25.0%; 3/12)] was similar to proportion among those with normal serum vitamin D levels (30.1%; 25/83); p = 0.722. The presence of overweight/ obesity, also did not have any significant relationship with the vitamin D status (p = 0.994).

The mean BMI-Z score of the 12 children with SCA and sub-optimal vitamin D (-1.0 ± 1.2) was similar to -1.0 ± 1.3 of the 83 with normal serum vitamin D levels (p = 0.827). The mean HA-Z scores of the two groups of SCA children were also similar; -0.1 ± 1.0 for those with sub-optimal vitamin D level, compared with -0.3 ± 1.3 for those with normal vitamin D level (p = 0.666). There was no significant correlation between either BMI-Z score or HA-Z score and serum vitamin D levels; r = -0.16, p = 0.119 and r = 0.13, p = 0.205 respectively. The mean TSF, MUAC, UAMA, UAA, UAFA and fat % between the two groups were not statistically different; p = 0.053, 0.327, 0.143, 0.208, 0.138 and 0.095 respectively. Using binary logistic regression, none of the analysed variables (age, gender, social class, presence of underweight, overweight/ obesity, stunting or fat percentage) predicted the presence of sub-optimal vitamin D status among children with SCA. (Table IV).

Table III: The relationship between sociodemographic and nutritional parameters and vitamin D status of children with SCA

Sociodemographic and nutritional parameters	*Sub-optimal vitamin D* **(N = 12)**	*Sufficient vitamin D* **(N = 83)**	*P- values*
Mean age	6.6 ± 2.4	7.5 ± 2.5	0.253
Gender (Male/ Female)	5/ 7	47/ 36	0.330
Upper social class (I)	2 (16.7)	13 (15.7)	1.000
Middle social class (II)	7 (58.3)	47 (56.6)	0.912
Lower social class (III)	3 (25.0)	23 (27.7)	0.845
Underweight	3 (25.0)	25 (30.1)	0.980
Normal BMI for age/sex	9 (75.0)	54 (65.1)	0.723
Overweight/ obesity	0 (0)	4 (4.8)	0.994
Stunting	0 (0)	12 (14.5)	0.345
Normal height for age	11 (91.7)	64 (77.1)	0.723
Tall for age	1 (8.3)	7 (8.4)	0.991
MUAC (cm)	14.3 ± 1.1	15.1 ± 2.3	0.053
TSF (mm)	16.9 ±1.4	16.5 ± 1.5	0.327
UAMA (cm^2)	6.6 ± 1.9	8.2 ± 3.8	0.143
UAA (cm^2)	16.3 ± 2.5	18.6 ± 5.9	0.208
UAFA (cm^2)	9.8 ± 0.9	10.3 ± 2.3	0.138
Fat %	60.5 ± 5.6	57.3 ± 6.3	0.095
BMI-Z score	-1.0 ± 1.2	-1.0 ± 1.3	0.827
HA-Z score	-0.1 ±1.0	-0.3 ± 1.3	0.666

KEY: BMI Body Mass Index; MUAC Mid Upper Arm Circumference; TSF Tricep Skinfold Thickness; UAMA Upper Arm Muscle Area; UAA Upper Arm Area; UAFA Upper Arn Fat Area; HAZ Height for age Z score.

Discussion

There are very few reports describing the relationship between SCD and serum vitamin D status in Nigeria unlike the multitude of reports from many developed countries. To the best of the author's knowledge, the index study is the first comparative report on vitamin D deficiency and or insufficiency among Nigerian paediatric population with sickle cell anaemia and their healthy haemoglobin AA counterparts.

It was observed that none of the controls had vitamin D deficiency while only 2.7% had insufficiency, and the mean serum 25-OHD was 45.2ng/mL. This is consistent with the report by Pfitzner *et al* [10] about two decades ago in a randomized cluster sample of 218 non-SCD children in Jos, Nigeria in which none of the study participants had vitamin D deficiency. The average value of 45.2ng/mL recorded in the index study was higher than 30ng/mL earlier observed by Oginni *et al.* [11] among apparently healthy, non-rachitic, non-SCD individuals from the same locality as the present study. In that previous study, children with haemoglobin AS were not excluded, unlike the present one in which the controls were only those with haemoglobin AA. These observations support the fact that Nigeria, which lies on latitude 10° north of the equator and longitude 8° east of the Greenwich Meridian, with abundant sunlight has a relatively low prevalence of vitamin D deficiency. [3, 10]

In the present study, 12.6% of children with SCA were either vitamin D deficient (2.1%) or insufficient (10.5%). These children with SCA also had significantly lower mean serum 25-OHD compared with the controls from the same geographical region. The lower serum 25-OHD in SCD has been attributed to a combination of reduced intake of vitamin D-rich foods, reduced exposure to sunlight because of longer indoor lifestyle, increased utilization of vitamin D for bone remodelling and higher body metabolism. [12]

Table IV: Binary logistic regression analysis on predictive variables for sub-optimal vitamin D status in children with sickle cell anaemia.

Sociodemographic and nutritional parameters	*P*-values	95% CI	
		Lower	Upper
Age	0.598	0.964	1.021
Gender	0.831	0.489	1.778
Lower social class	0.078	0.320	1.344
Underweight	0.756	0.163	3.728
Overweight/Obesity	1.000	0.000	0.000
Stunting	0.534	0.158	35.361
Fat percentage	0.057	0.706	0.997

Globally, the proportion of SCD children with vitamin D deficiency/insufficiency varies depending on the definition of deficiency, location (latitude) and invariably, sun exposure, season of the year and race. [1,4] Defining deficiency as vitamin D level less than 20ng/ mL, the prevalence of vitamin D deficiency in SCD ranges from 56.4% to 96.4% in the temperate regions with little exposure to sunlight. [1] However, if deficiency is defined as serum level of = 10ng/mL, the prevalence could be as low as 33%.[13] Jackson *et al.* [14] reported from the United States that, 64% of the 139 children with haemoglobin SS had deficiency of vitamin D while Buisson *et al.* [2] reported a prevalence of 65%.

Findings from the other continents also revealed varying prevalences of vitamin D deficiency among individuals with SCD. In Madrid, Spain, Garrido *et al.*,[15] using definition of < 20ng/ mL, reported 56.4% prevalence rate of vitamin D deficiency among 78 children with SCD up to the age of 16 years. The children recruited into that study included those with haemoglobin SS, haemoglobin SC (HbSC), sickle beta thalassaemia-zero ($HbS\beta^0$) and sickle beta thalassaemia-plus ($HbS\beta^+$). Ozen *et al* [16] reported the prevalence rate of vitamin D deficiency among Turkish children with SCD as 63.1% using definition of <20ng/mL. Among the 99 Saudi children with HbSS, Mohammed *et al* [17] using a definition of <10ng/mL, found that 12% of the subjects were vitamin D deficient compared with 2% of the healthy controls from the same locality. The effects of climate and latitude on vitamin D status among people with SCD was explained in a comparative study of 18 children with SCA and age-, sex- and ethnicity-matched healthy children control in the Island of Curacao within the kindgom of Netherland by van der Dijs *et al*.[18] The study showed that none of the subjects had vitamin D deficiency using the definition of <10ng/mL. Increased exposure to sunlight was thought to account for the finding.

In the present study, none of the variables tested had significant relationship with the serum vitamin D levels. The climate/ season of the year, exposure to sunlight, type of food intake and genetic factors, rather than the age, gender, presence of underweight, BMI-Z score or HAZ score were thought to exert a more significant influence on serum vitamin D levels. [2, 3] The serum vitamin D levels had been observed to be higher in the seasons with higher temperatures, spring and summer. [19] However, the influence of age on serum vitamin D levels from previous studies is inconsistent. While Garrido *et al.* [15] observed lower serum vitamin D among children older than 5 years, Lal *et al* [20] reported an inverse correlation with age.

The present study was limited by our inability to quantify the amount of vitamin D present in the food taken by the subjects. Although attempts were made at doing this, the non-availability of a clear and integrated local software for nutrition data system for research (NDSR) which is needed to estimate dietary vitamin D from 24-hour dietary recall, hampered these efforts. The estimation could have helped to correlate dietary intake of vitamin D with the serum levels of 25-OHD and also determine the proportion of children with adequate daily intake (200IU/day). Previous reports on the relationship between vitamin D intake and serum 25-OHD are inconsistent. Peters *et al* reported no significant relationship among Brazilian adolescents. [21] However, van der Gaag and Brekhoff in their study of Netherland children during winter months reported a weak significantly positive correlation between dietary intake and serum levels of vitamin D. [22]

Conclusion

Nigerian children with SCA had sub-optimal levels of serum vitamin D than their age-, sex- and socioeconomically-matched healthy controls. In addition, the serum 25-OHD levels were not related to

sociodemographic characteristics or anthropometric parameters of the children studied. However, the prevalence of sub-optimal vitamin D levels among children with SCA was lower than recorded in most reports from the temperate regions of the world. Since vitamin D deficiency and insufficiency can be readily treated and the associated impacts corrected; regular serum vitamin D screening should be done among SCD patients. The role of serum vitamin D as a biomarker of SCD severity and its contribution to vaso-occlusive painful crises should be explored further.

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