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ORIGINAL RESEARCH

Prevalence and Risk Factors of Hypovitaminosis D in Nigerian Children with Sickle Cell Anaemia

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Abstract

Background: Vitamin D deficiency (VDD) has been linked to some acute and chronic bone disorders that commonly complicate sickle cell anaemia (SCA) in children. Some of these bone diseases include chronic pain, reduced bone density and fractures. Despite Nigeria having the highest number of children with SCA in the world, there is a paucity of data on vitamin D status and the associated risk factors in affected children.
Objective: To determine the prevalence and risk factors for hypovitaminosis D in children with sickle cell anaemia in steady-state.
Methods: A total of 174 children with sickle cell anaemia aged one to eighteen years were recruited at the Sickle Cell Foundation Centre, Lagos. Baseline sociodemographic, clinical, anthropometric and laboratory parameters (serum 25-hydroxyvitamin D, corrected serum calcium and alkaline phosphatase) were recorded.
Results: The prevalence of vitamin D insufficiency and deficiency were 12.6 % and 72.5% respectively. Children below six years of age were less likely to have hypovitaminosis D compared to the older age groups (p = 0.017). The mean serum corrected calcium was lowest in subjects with vitamin D deficiency (p >0.001). Age and hypocalcaemia are independent predictors of hypovitaminosis D.
Conclusion: There is a high prevalence of vitamin D deficiency among children with sickle cell anaemia. Children aged below six years and with those with hypocalcaemia had higher odds of hypovitaminosis D.

Keywords: Children, Hypocalcaemia, Hypovitaminosis D, Sickle cell anaemia, Vitamin D deficiency, Nigeria, 25(OH)D.

Introduction

Globally, Nigeria has the largest number of children with sickle cell anaemia (SCA) with over 150,000 infants born with the disease yearly. [1] Children with SCA are prone to several complications with acute and chronic bone diseases being the commonest. [2] The commonest acute clinical manifestation of SCA is a vaso-occlusive crisis (VOC), often involving the long bones. Similarly, chronic bone diseases such as

osteopenia, osteoporosis, avascular necrosis and vertebral bone deformities are common in persons with SCA. [2]

Both acute and chronic sickle bone diseases have been associated with hypovitaminosis D. [3,4] Children with SCA are at higher risk of vitamin D deficiency (VDD) compared with their unaffected counterparts. This vitamin D deficient state may be due to several factors which include inadequate nutritional intake, a high metabolic rate which increases nutritional demand, [5] poor gastrointestinal absorption of nutrients due to mucosal damage as well as an impaired synthesis of active vitamin D from kidney injuries. [5, 6]

Globally, the prevalence of VDD among children with SCD is as high as 33% to 88.5%. [5, 7] Despite having the highest burden of children with SCA, there are few published data on the burden of VDD in Nigeria, and only one has reported its associated risk factors among Nigerian children with SCA. [7,8] The knowledge of the burden of VDD among Nigerian children with SCA and its associated risk factors may inform and guide evidence-based guidelines on appropriate vitamin D supplementation in this at-risk population. Therefore, this study evaluated the prevalence of hypovitaminosis D, and its associated potential predictors (age, gender, socioeconomic classes, nutritional status, serum calcium and alkaline phosphatase), among Nigerian children with SCA.

Methods

Study Site

This study was conducted at the Sickle Cell Foundation Centre, Idi-Araba, Lagos, Nigeria - a private institution which partners with the government to provide comprehensive care for persons with Sickle Cell Disease (SCD). It also serves as a major reference diagnostic centre for patients with SCD attending various public and private hospitals in Lagos State and neighbouring states in Nigeria.

Study Population

The study population comprised children with SCA aged 1 to 18 years in steady-state.

Study design, duration and sample size

This study reports baseline cross-sectional data of a prospective, quasi-experimental trial on vitamin D treatment in children with SCA with hypovitaminosis D. The sample size was calculated based on the prevalence of hypovitaminosis D of 88.5% on 113 subjects reported by Abok, *et al.* [7] in Nigeria. The sample size obtained by calculation was 156 with an added 10% attrition rate. The total size was stratified *a priori* into three age groups: five years and below (n = 57), 6-10 years (n = 58) and 11-18 years (n = 59). The study was conducted between February and May 2018.

Sample Selection and technique

Children with SCA aged 1 to 18 years in steady-state were included in the study while children with tuberculosis, asthma, renal or hepatic diseases as well as those on steroids, anticonvulsant therapy, hydroxyurea or chronic blood transfusion were excluded. The eligible subjects were recruited consecutively until the desired sample size for each age bracket was completed.

Ethical considerations

The study was approved by the Health Research Ethics Committee of the Lagos State University Teaching Hospital, Ikeja, Nigeria (NHREC0/04/2008). Written informed consent was obtained from caregivers and verbal assent was obtained from subjects seven years and above.

Data collection

Data on demographic (age, sex), nutritional status and social characteristics (socioeconomic

status) were obtained from the parents and caregivers of each child with the aid of a researcher-designed questionnaire.

Definition

Steady-state was defined as the absence of any sickle cell crises in the four weeks preceding recruitment into the study and no history of blood transfusion in the preceding four months. [9] The socioeconomic statuses of the primary caregivers were classified into upper, middle and lower socioeconomic strata using a previously designed scale for Nigerian homes by Oyedeji. [10] The scale uses the level of education (scored from 1 to 5) and occupation (scored from 1 to 5) of each of the parents; the mean score for each family was rounded up to the nearest whole number to derive the socioeconomic index score. Scores of 1-2, 3 and 4-5 translated to upper, middle and lower socioeconomic levels, respectively.

Anthropometry

Bodyweight was measured to the nearest 0.1kg using a digital weighing scale (Seca 799 electronic column scale, Germany). Body length was measured in children below two years of age using an infantometer (Seca 210) while height was measured to the nearest 0.1cm using a stadiometer in older children. The Body-Mass Index (BMI) was calculated as weight/ height^2 (Kg/m^2). Anthropometric parameters (weight, height/length were converted to Z-scores using the *Anthro*® and *Anthoplus*® software (WHO, Version 3.2.2. and 1.0.4 respectively). The nutritional status of each child was classified, using the WHO growth reference for children and adolescents from 5 years to 19 years. [11] The WHO anthropometry soft-ware was used to generate the z-scores. [12] Therefore, the weight-for-age z-score (WAZ) less than -2 was classified as acute malnutrition while WAZ greater than +2 defined overweight. In subjects below ten years, height/length for age z score (HAZ) less than -2SD was classified as stunting while HAZ less than –3 SD was described as severe stunting. Normal HAZ was defined as ≥ –2 to +2 z score. In adolescents aged ten years and above, stunting was defined as HAZ lower than -2SD. In under-5 children, BMI Z scores were classified thus: < –2SD was thinness, <–3SD was severe thinness, –2SD to + 1SD was normal, values greater than + 1 SD suggested children at risk of overweight, > +2 SD as overweight, and > +3 SD as obese. BMI classification for subjects above five years of age were as follows: overweight: > +1 SD but ≤ +2 SD, obesity: > +2SD, thinness: < –2SD and severe thinness: < –3SD. [11]

Serum Calcium

Serum calcium was analysed using Arsenazo III reagent. To enable correction for the effect of albumin on calcium, serum albumin was also assayed using the bromocresol green method. The corrected serum calcium for albumin was calculated using the Payne's recommendation [Corrected calcium = measured calcium (mg/dL) + 0.8 (40- albumin (g/L)].[13] The normal range of corrected serum calcium was taken as values between 8.5 and 10.5mg/dl. [14] Therefore, hypocalcaemia and hypercalcaemia were defined as serum calcium below 8.5mg/dL and above 10.5mg/dL respectively. [14]

Serum Alkaline Phosphatase

Serum alkaline phosphatase was assayed using the diethanolamine (DEA) method. [15] Normal ranges of serum alkaline phosphatase values according to age and sex are as follows:

a) Children below two years of age irrespective of gender: 150 to 420 U/L
b) Children between the ages of two and ten years irrespective of gender: 100 to 320 U/L
c) Female adolescents between 11 and 18 years: 150 to 420 U/L
d) Male adolescents between 11 and 18 years: 100 and 390 U/L. [16]

Serum Vitamin D Analysis

The automated random-access direct competitive three-step chemiluminescent binding assay which detects both 25(OH)D_2 and 25(OH)D_3 was used. The assay of 25(OH)D using 25-OH vitamin D ELISA kit has good accuracy and precision with a sensitivity of 94.2% and specificity of 98.8%. [17] The 25-OH Vitamin D ELISA Kit (OKEHO2569) was used for serum vitamin D analysis. [18] Vitamin D status of subjects was classified as sufficient (serum 25(OH)D of 75nmol/L or more), insufficient (serum 25(OH)D of 50 - < 75nmol/L) and deficient (serum 25(OH)D < 50 nmol/L) using the Endocrine Society Clinical Practice Guidelines. Hypovitaminosis D was defined as serum 25(OH)D below 75nmol/L. [19]

Data analysis and management

Data analysis was done using the Statistical Package for Social Sciences (SPSS) version 20.0. Test of normality was conducted using the Kolmogorov-Smirnov test. The demographics were presented as frequencies and percentages. Continuous variables were summarized as mean and standard deviation when normally distributed or as median and interquartile ranges if skewed. The comparison between categorical variables was done using Pearson's Chi-Square (or Fisher's exact test as necessary) while non-parametric tests such as the Mann Whitney and Kruskal-Wallis tests were used to compare the median values for two and three variables respectively. The relationship between 25(OH)D, the three age strata and serum alkaline phosphate levels were done using Kruskal-Wallis test. Potential factors that showed a significant bivariate relationship with vitamin D were entered into a binary logistic regression model to determine the clinical or biochemical parameters that are independently associated with hypovitaminosis D. The contribution of each variable was assessed with the adjusted odds ratio. A probability value less than 5% (0.05) was considered statistically significant at 95% confidence interval.

Results

Socio-demographic and nutritional characteristics

Table I shows the sociodemographic and nutritional characteristics of the subjects. The mean (±SD) age of the subjects was 8.5± 4.5 years with female to male ratio of 1:1.1.

Prevalence of hypovitaminosis D

The overall median (IQR) serum 25(OH)D was 40.3 nmol/l (30.6, 54.1nmol/l) ranging from 18.1 nmol/l to 145.5 nmol/l. One hundred and forty-eight (85.1%) children had serum 25(OH)D < 75 nmol/l: 126 (72.5%) were classified as "deficient" while 22 (12.6%) were classified as "insufficient". The remaining 26 (14.9%) had sufficient levels of serum vitamin D.

Factors associated with vitamin D deficiency

The median 25(OH)D levels were similar between males and females [41.6 nmol/l (31,5, 62.6) vs. 38.7 nmol/L (28.6, 50.1); p = 0.290]. There was a significant reduction in median levels of 25(OH)D as age increased. The median (IQR) level of 25(OH)D in children below 5 years of age was 56.6 nmol/l (27.1, 84.7). In children aged 6-10 years and 11-18 years, the median serum 25(OH)D were 46.2noml/l (26.5, 65.9) and 39.5 nmol/l (21.1, 57.9) respectively (Kruskal-Wallis test: p = 0.001) as shown in Table II.

The proportion of children with vitamin D deficiency increased with increase in age. Age and hypocalcaemia were significantly associated with vitamin D status as shown in Table III.

Table I: Socio-demographic and nutritional characteristics of subjects

Parameters	*Frequency*	*Percentages*
Age groups (Years)		
1 to 5	57	32.8
6 to 10	58	33.3
11 to 18	59	33.9
Gender		
Male	91	52.3
Female	83	47.7
Social class		
Upper	53	30.5
Middle	38	21.8
Lower	83	47.7
Nutritional classification		
***Weight–for–Length/Height (n = 57)**		
Normal	50	87.7
Wasting	7	12.3
****Weight–for –age (n = 115)**		
Normal	101	87.8
Underweight	14	12.2
Length/Height of age (n = 174)		
Normal	139	79.8
Stunting	24	13.8
Severe stunting	11	6.32
BMI–for–age (n = 174)		
Thinness	30	17.2
Normal	137	78.7
ǂAt risk/Obese	7	4.1

***Weight for height among subjects ≤ 5 years with Length/Height within 65-120cm.[17]**
****Among subject ≤10 years**
ǂAt risk of overweight in under 5s and overweight in subjects above 5 years

Association between Vitamin D categories, Serum Calcium and Alkaline Phosphatase

Table IV shows the mean serum calcium and median alkaline phosphatase levels according to vitamin D status. The mean corrected serum calcium was lowest in children with vitamin D deficiency but highest in the children with vitamin D sufficiency (p <0.0001). Post-hoc analysis showed that the "sufficient" group had higher mean serum calcium than the "insufficient" group (p = 0.025) or the "deficient" group (p <0.001). There was no significant difference in the mean serum calcium levels between the "insufficient" and "deficient" groups (p = 0.975). Concerning serum alkaline

phosphatase, no significant relationship was established with vitamin D status.

Age and serum calcium values were entered into the logistic regression model for predictors of hypovitaminosis D. As shown in Table V, both factors were retained in the model as having an independent association with hypovitaminosis D.

Table II: Relationship of median serum 25(OH)D levels to gender and age groups

Age Groups (Years)	*Gender*	*Median Serum 25(OH)D (Interquartile range)*	*Males vs. Females (p-value)*
1 to 5	Overall	56.6 (27.1, 84.7)	
	Male	55.9 (22.3, 84.6)	0.557
	Female	57.5 (27.5, 87.3)	
6 to 10	Overall	46.2 (26.5, 65.9)	
	Male	49.5 (29.4, 69.9)	0.185
		42.8 (23.5, 62.1)	
11 to 18	Overall	39.5 (21.1, 57.9)	
	Male	42.9 (20.6, 55.3)	0.438
	Female	36.4 (22.9, 49.9)	

Comparison of the median 25(OH)D across the three age groups (H = 15.089, p = 0.001)

Discussion

The prevalence rates of vitamin D deficiency and insufficiency in this cohort were 72.5% and 12.6%, respectively. Age and serum calcium levels were observed to be independent predictors of vitamin D deficiency. The high prevalence rate of vitamin D deficiency among children with SCA in the present study is in agreement with previous reports. The high prevalence rates may be explained by several factors such as reduced appetite that contributes to micronutrient deficiency; high nutritional demands as a result of increased resting metabolic rate repeated intestinal gut infarction resulting in reduced intestinal absorption of micronutrients such as vitamin D; and reduced production of 1,25 dihydroxyvitamin D from the kidneys due to functional and structural disruption of the renal system from repeated microinfarction. [5,20,21]

An earlier Nigerian study reported a similarly high prevalence rate of 88.5% of hypovitaminosis D among children with SCA. [7] The prevalence rate of vitamin D deficiency in the present cohort was close to 63.2% earlier reported from Turkey. [22] However, it is comparatively lower than 96.4% reported by Jackson and co-researchers, [23] from Washington, USA, using the same cut-off values, presumably due to the inclusion of subjects with co-morbidities such as asthma (42.5%) - a disease condition associated with increased risk of vitamin D deficiency.

Also, the prevalence rate of vitamin D deficiency herein reported is higher than 50.4% found in a study of children with SCA in Spain. [24] This difference could have resulted from the fact that nearly half of the children in the Spanish study were already on vitamin D supplementation, thus reducing the chances of deficiency.

Table III: Relationship of vitamin D Status to Study Parameters

	Vitamin D Sufficiency	*Hypovitaminosis D*	*OR (95% CI)*	*p-value*
Age group (Years)				
1-5	14 (24.6)	43 (75.4)	Ref	
≥ 6	12 (10.3)	105 (89.7)	2.85(1.22-6.66)	**0.017***
Gender				
Male	18 (19.8)	73 (80.2)	Ref	
Female	8 (9.6)	75 (90.4)	2.31(0.95-5.65)	0.193
Social class				
Upper	8 (15.1)	45 (89.9)	Ref	
Middle	6 (15.8)	32 (84.2)	0.90 (0.30-3.00)	0.493
Lower	12 (14.4)	71 (85.5)	1.05 (0.40-2.77)	0.325
Calcium level				
Low (<8.5mg/dl)	3 (4.1)	70 (95.9)	Ref	
Normal/High (≥ 8.5mg/dl)	21 (24.1)	56 (75.9)	0.11 (0.03-0.4)	**<0.001***
ALP level				
Low/Normal	16 (14.5)	94 (85.5)	Ref	
High	8 (16.0)	42 (84.0)	0.89 (0.35-2.25)	0.314
***Weight–for–length/height (n = 57)**				
Wasting	2 (28.5)	5 (71.5)	Ref	
Normal	12 (24.0)	38 (76.0)	1.27 (0.22-7.39)	0.415
****Weight–for–age (n = 115)**	3 (21.4)	11 (78.6)	Ref	
Underweight	19 (18.8)	82 (81.2)	1.18 (0.30-4.64)	0.814
Normal				
Length/Height-for-age (n = 174)				
Stunting/Severe stunting	4 (11.4)	31 (88.6)	Ref	
Normal	22 (15.8)	117 (84.2)	0.69 (0.22-2.14)	0.194
BMI-for-Age (n = 174)				
Wasting	3 (10.0)	5 (90.0)	Ref	
Normal	20 (14.6)	117 (85.4)	1.1 (0.54-2.21)	0.591
Overweight/Obese	3 (42.8)	4 (57.2)	1.32 (0.4-2.4	0.293

***Weight for height among subjects ≤ 5 years with Length/Height within 65-120cm**

****Among subject ≤10 years**

ALP = Alkaline phosphatase; BMI = Body mass index

Table IV: Mean serum calcium and alkaline phosphatase according to Vitamin D status

	Vitamin D Sufficient (n = 26)	*Vitamin D Insufficient (n = 22)*	*Vitamin D Deficient (n = 126)*	*F*	*p-value*
Calcium(mg/dl)	9.82±1.1	8.77±1.3	8.60±1.2	10.031	**<0.001***
ALP (IU/L) (IQR)	310.6 (252.1, 392.4)	318.8 (265.7, 382.9)	283.4 (241.2, 347.4)	1.556	0.214⁺

***Derived from ANOVA**

⁺Derived from Kruskal-Wallis test

ALP = Alkaline phosphatase; IQR = Interquartile range

Table V: **Logistic regression model to determine independent predictors of hypovitaminosis D**

Parameters	*Odds Ratio*	*95% Confidence Interval*		*p-value*
		Lower	**Upper**	
Age (≥ 6 years)	0.448	0.247	0.812	0.008*
Hypocalcaemia	8.256	2.305	29.576	0.001*

CI - Confidence intervals; B - Intercept; SEC - Socio-economic class; ALP - Alkaline phosphatase

The current study observed that the prevalence rate of vitamin D deficiency increased with increasing age. This is similar to findings documented by Basametur, *et al.* [25] in a cohort study of children in the UK. This is in keeping with the observation that epidermal concentration of 7-dehydrocholesterol, a cholesterol precursor of vitamin D decreases with age. [26] Similar to some earlier findings, [27,28] there was no relationship between sex and vitamin D deficiency in the current study. In contrast, however, Sridhar, *et al.* [29] and Jain Ram [30] reported a significantly higher prevalence rate of hypovitaminosis D in females. The predominance of hypovitaminosis D in females has been attributed to the relatively higher adiposity in females relative to males; increased adiposity impairs 25-hydroxylation, 1-α hydroxylation and bioavailability of vitamin D. [31] The absence of gender difference in the proportion of overweight/obese subjects in the current study may thus explain the non-association of hypovitaminosis D with gender. Also, the higher tendency of males to be more physically active than females and thus, to have a higher exposure to outdoor sunlight has been proposed as a likely reason for the relatively lesser prevalence rate of hypovitaminosis D in males. [32] Variation in season is not likely a reason for the high prevalence rate of hypovitaminosis D in the current study because the study was conducted solely in the dry season. However, the subjects' level of physical activity or exposure to sunlight was not assessed in the present study.

The present study observed that stunting was not significantly associated with vitamin D deficiency. However, all children with severe stunting had vitamin D deficiency. This finding is consistent with other reports. [33] Since stunting is a form of chronic malnutrition, children who are stunted are likely to have concomitant micro-nutrient deficiency including hypovitaminosis D. Conversely, hypovitaminosis D impairs linear growth and subsequently results in stunting. Vitamin D deficiency was not associated with underweight in the present study. This may be surprising because underweight children are at risk of associated micronutrient deficiencies (including hypovitaminosis D) compared to their normal-weight counterparts. It is plausible that other variables not within the scope of the present study may have confounded the potential influence of underweight. One such factor is the duration of the nutritional deficiency, which would have been impossible to ascertain within the scope of the present study.

In contrast to the findings from a previous study, [34] the present study did not observe an association between hypovitaminosis D and socio-economic status. This differs from the reports from developed countries where food fortification is highly practised. Lack of intake of micronutrient and availability of vitamin D in dietary sources even to those that can afford it in fortified foods are likely reasons for the low prevalence of vitamin D deficiency in the higher social groups in previous reports. Surprisingly, none of the participants was on any vitamin D supplement for at least six months before the

commencement of this present study. The practice of food fortification in Nigeria is rather poor at present, so even middle- or high-income earners may still be consuming low vitamin diet.

The mean serum calcium level of children with vitamin D deficiency was significantly lower than that of children with sufficient vitamin D in the present cohort. This is possibly due to poor intestinal absorption of calcium as a result of hypovitaminosis D, a micronutrient required for calcium absorption in the gut. No relationship was observed between hypovitaminosis D and serum alkaline phosphatase in the present study. This finding is similar to a previously documented report by Shareen, *et al.* [35] further buttressing the limitation of alkaline phosphatase as a screening marker for hypovitaminosis D.

The present study is limited by its single-centre nature, convenience sampling, by the cross-sectional design and the absence of a control group. Parameters such as serum parathyroid hormone and vitamin D binding protein levels that could affect serum vitamin D levels were also not assessed due to cost implication. Also, it is difficult to establish any causal relationship in the present study. Therefore, larger, multi-centre and controlled studies with randomly sampled affected and healthy Nigerian children are still required to provide better estimates of the burden and factors associated with VDD in children with SCA.

Conclusion

There is a high prevalence of vitamin D deficiency in Nigerian children with SCA. Children aged above five years and those with hypocalcaemia are more likely to have vitamin D deficiency. Routine vitamin D supplementation may be recommended in children with SCA, especially those between one and five years of age.

Authors' Contributions: AMO conceived and designed the study. AMO and DAO participated in data acquisition while AMO, UPO and ABA did the data analysis and interpretation. All the authors participated in manuscript drafting and review. All the authors approved the final version of the manuscript.
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