ORIGINAL ARTICLE

Vitamin D status and serum vitamin D binding protein levels in Nigerian children with nephrotic syndrome

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ABSTRACT

Introduction: Nephrotic syndrome is a chronic relapsing condition associated with urinary loss of albumin and other proteins such as vitamin D binding protein (DBP). We determined vitamin D status and serum DBP levels in children with nephrotic syndrome and compared them to healthy controls.

Methods: A cross-sectional study was performed over a six-month period in children less than 18 years of age. The children with nephrotic syndrome were categorised by disease status as either newly diagnosed, in remission, resistant to therapy, or in relapse. Vitamin D levels were regarded as sufficient if ≥75 nmol/L, insufficient if <75 nmol/L but ≥50 nmol/L, deficient if <50 nmol/L, and severely deficient if <25 nmol/L. Serum DBP was also measured.

Results: Fifty-five children with nephrotic syndrome and 24 controls were included in the study. There was no significant difference between the median ages of the cases (72.0 months, interquartile range (IQR) 48.0–120.0 months) and the controls (84.0 months, IQR 39.0–129.0 months). Severe vitamin D deficiency, deficiency and insufficient levels were documented in 54.5%, 41.8% and 3.6% of cases, respectively, significantly lower than the controls (P = 0.003). Vitamin D levels were higher in children with nephrotic syndrome in remission than in those who were not (30.3 ± 15.2 nmol/L vs 19.6 ± 11.0 nmol/L, P = 0.004). In the groups who were in remission, newly diagnosed, relapsing, and resistant, the median vitamin D levels were 30.3 nmol/L, 20.1 nmol/L, 19.2 nmol/L and 9.4 nmol/L, respectively (P = 0.031).

Conclusions: Hypovitaminosis D occurs frequently in Nigerian children with nephrotic syndrome as well as in apparently healthy controls. Routine supplementation of vitamin D should be considered in children with nephrotic syndrome irrespective of whether the disease is in remission or not, or whether it is steroid-sensitive or not.

Keywords: nephrotic syndrome; vitamin D deficiency; vitamin D binding protein.

INTRODUCTION

Nephrotic syndrome is a condition characterised by heavy proteinuria, hypoalbuminaemia, hypercholesterolaemia and generalised body swelling. It is the commonest chronic kidney disease in children in developing countries, including Nigeria [1-3].

Over ninety percent of 25-hydroxyvitamin D (25(OH)D) and 1,25 dihydroxy vitamin D are bound to vitamin D binding protein (DBP) and albumin. The increased glomerular permeability in nephrotic syndrome results in urinary loss of albumin and globulins such as DBP [4].

Urinary loss of vitamin D with these carrier proteins is one of the factors responsible for a high prevalence of vitamin D deficiency in persons with nephrotic syndrome [5]. The reduced intestinal absorption of calcium secondary to hypovitaminosis D may result in hypocalcaemia and reduced bone mineral density [6].

There have been previous reports of high prevalence of vitamin D deficiency in children with chronic kidney disease [7,8]. Despite the common occurrence of nephrotic syndrome in Nigeria, a tropical region whose

population is at risk of vitamin D deficiency, no data are available on the local prevalence of vitamin D deficiency in children with this condition. Our study aimed to determine the burden of vitamin D deficiency in Nigerian children with nephrotic syndrome, determine the relationship of vitamin D deficiency with DBP, and to identify biochemical and clinical correlates of hypovitaminosis D.

METHODS

A prospective comparative study was conducted at Lagos State University Teaching Hospital (LASUTH), Ikeja, Nigeria, between May and November 2018. The hospital is a major referral centre that receives patients from both public and private hospitals in Lagos state and its environs. The Nephrology Clinic is a well-established clinical unit and training centre.

Our cases were children with a documented diagnosis of nephrotic syndrome and without comorbidities. The controls were apparently healthy children who were having routine follow-up visits at least four weeks after resolution of an acute illness which had been managed at the outpatient department. All the controls had normal urinalysis and kidney function. The sample size was estimated using data from a previous study [9] on the difference in mean vitamin D levels between cases and controls.

Information on the participants was obtained from hospital records and from the caregivers. Socioeconomic class was determined using the Oyedeji classification, which is based on the educational level and occupation of the parents [10].

Weight and height were measured using a Seca weighing scale and stadiometer, and body mass index calculated. Waist circumference was measured at the level of the umbilicus using a flexible inelastic tape measure and hip circumference (was taken as the widest part of the buttocks.

Blood pressure measurement was performed using a mercury sphygmomanometer with an appropriately sized cuff on the left arm. An average of three readings of sitting blood pressure was considered as the subject's blood pressure.

The cases were classified as either newly diagnosed, in relapse, resistant or in remission. "Newly diagnosed" refers to cases diagnosed less than six months prior to enrolment, "relapse" refers to cases with ≥2+ protein in the urine after having achieved remission on three consecutive days, "remission" is defined by negative or trace proteinuria for three consecutive days, and "resistant" are cases with persistent proteinuria despite eight weeks of daily steroid

therapy [11,12]. Steroid-sensitive nephrotic syndrome is defined as achieving remission with steroid therapy alone [11]. In line with the unit's protocol, all children with nephrotic syndrome are routinely given 400 IU of vitamin D daily during relapse and while on steroid therapy.

Venous blood samples for serum calcium, albumin, creatinine and 25(OH)D were taken from all participants. The 25(OH)D levels were measured by an experienced scientist using high-performance liquid chromatography in the LASUTH biochemical laboratory. Samples for 25(OH)D were stored at −20°C until the time of analysis. Normal serum corrected calcium was defined as 2.20–2.70 mmol/L. Serum creatinine was determined by the modified Jaffe reaction using an Erba XL 600 automated analyser with the assay standardised with the National Kidney Disease Education Program (NKDEP) laboratory working group protocol. Urine protein was analysed using a colorimetric method (pyrogallol red). Urine creatinine was analysed using the Jaffe reaction without deproteinisation. Glomerular filtration rate (GFR) for each participant was estimated using the Schwartz formula. Only children with estimated GFR greater than 60 mL/min/1.73 m^2 were recruited.

The vitamin D status was defined using the Endocrine Society Clinical Practice guidelines [13]. Participants with serum 25(OH)D <50 nmol/L were identified as being vitamin D deficient, with severe deficiency defined as serum 25(OH)D <25 nmol/L. Insufficiency was defined as 25(OH)D 50–75 nmol/L and sufficient as at least 75 nmol/L.

Data were analysed using the Statistical Package for Social Sciences software version 24 for Windows (SPSS Inc., Chicago, IL, USA). Normality of numerical data was assessed using the Kolmogorov–Smirnov test and then summarised using means and standard deviations or medians and interquartile ranges, as appropriate. Means were compared between groups using independent sample T tests. Pearson's correlation was used to assess relationships between laboratory results (serum creatinine, serum albumin, GFR, serum calcium, urine protein/creatinine ratio) and DBP and 25(OH)D. Categorical variables were described using frequency and percentages, and associations evaluated using chi-squared and Fisher's exact tests. P values less than 0.05 were considered significant.

Ethical approval for the study was granted by the Health Research and Ethics Committee of LASUTH (reference no. LREC.06/10/790).

RESULTS

Among the children who were recruited, five cases and six controls were excluded because their blood samples were haemolysed and not suitable for analysis. The number of

cases and controls included in the study was 55 and 24, respectively. Table 1 summarises their sociodemographic and clinical characteristics. There was no significant difference between the median age of the cases [72.0 months, interquartile range (IQR) 48.0–120.0 months] and the controls (84.0 months, IQR 39.0–129.0 months). The cases tended to be of lower social class. They also had higher waist-to-hip ratios than the controls (P = 0.008); other anthropometric parameters and blood pressures were similar. More males than females were included in each of the groups but this difference was not statistically significant.

Almost half of the cases (47%) were in remission, with 26% newly diagnosed, 25% in relapse and 1.9% considered resistant. A total of 51 cases (93%) were on steroid therapy. The median duration was 6.0 months (IQR 2–12 months), with a mean dose of 28.2 ± 20.2 mg/day. Overall, 39 of the cases (77%) and none of the controls were on vitamin D supplementation. The median duration of vitamin D supplement use among the cases was 10.0 (IQR 1.3–16.0) months.

Table 2 summarises the laboratory data of the participants. Serum albumin was lower in cases than controls, as were the vitamin D levels (24.3 versus 29.9 nmol/L, P = 0.024). There was no difference in the median DBP levels.

All the cases had hypovitaminosis D, with 55% having severe deficiency, 42% deficient and 4% insufficient. Among the controls, 17% were severely deficient, 4% insufficient and most (79%) deficient for vitamin D. A higher proportion of the cases had severe deficiency, deficiency or insufficiency compared to the controls (P = 0.003).

The vitamin D levels in children in remission were higher than those not in remission (30.3 ± 15.2 nmol/L versus 19.6 ± 11.0 nmol/L, P = 0.004). There was no difference in the DBP level between those in remission and those not in remission.

Table 1. Sociodemographic and clinical characteristics of participants.

	Cases (n = 55)	Controls (n = 24)	P value
Age in months	72.0 (48.0–120.0)	84.0 (39.0–129.0)	0.765
Sex			
Male	38 (69%)	18 (75%)	0.595
Female	17 (31%)	6 (25%)	
Socioeconomic class			
Upper	28 (51%)	24 (100%)	0.362
Middle	22 (40%)	0 (0%)	
Lower	5 (9%)	0 (0%)	
Ethnicity			
Yoruba	41 (75%)	22 (93%)	0.362
Ibo	12 (22%)	2 (8%)	
Other	2 (4%)	0 (0%)	
Anthropometric measures			
Weight (kg)	25.1 ± 10.7	26.8 ± 11.6	0.517
Height (cm)	119.2 ± 22.5	124.9 ± 26.0	0.321
BMI (kg/m^2)	17.1 ± 2.6	16.5 ± 2.2	0.386
WC (cm)	59.6 ± 8.3	59.3 ± 9.4	0.897
HC (cm)	64.4 ± 10.5	69.7 ± 24.6	0.214
Waist/hip ratio	0.9 ± 0.1	0.9 ± 0.1	0.008
Systolic BP (mmHg)	88.0 ± 17.4	89.0 ± 12.0	0.799
Diastolic BP (mmHg)	53.6 ± 15.0	51.2 ± 16.3	0.524
Nephrotic syndrome status			
Newly diagnosed	14 (26%)		
Remission	25 (47%)		
Relapsed	13 (25%)		
Resistant	1 (2%)		

Abbreviations: BMI, body mass index; WC, waist circumference; HC, hip circumference; BP, blood pressure.

Table 2. Biochemical parameters of the participants.

	Cases (n = 55)	Controls (n = 24)	P value
Serum creatinine (mg/dL)	0.6 (0.4–0.8)	0.5 (0.5–0.7)	0.952
Serum albumin (mg/dL)	1.64 (1.4–2.1)	4.35 (4.1–4.6)	<0.001
GFR (mL/min/1.73 m^2)	88.8 (75.4–137.4)	104.0 (85.1–118.9)	0.640
DBP (nmol/L)	55.5 (45.0–72.0)	56.0 (52.1–74.2)	0.321
Serum 25(OH)D (nmol/L)	24.3 (14.9–35.3)	29.9 (27.1–34.5)	0.024
Serum calcium (mmol/L)	2.4 (2.3–2.5)	2.2 (2.1–2.5)	0.217
Urine protein/creatinine ratio (g/g)	6.33 ± 0.91		
Vitamin D status			
Severe deficiency	30 (55%)	4 (17%)	0.003
Deficiency	23 (42%)	19 (79%)	
Insufficiency	2 (4%)	1 (4%)	
Sufficiency	0 (0%)	0 (0%)	

Abbreviations: GFR, glomerular filtration rate; DBP, vitamin D binding protein; 25(OH)D, 25-hydroxyvitamin D.

Table 3. Correlation of clinical and laboratory parameters with serum levels of vitamin D and vitamin D binding protein.

	DBP		Vitamin D	
	r	P	r	P
Age	0.256	0.059	−0.150	0.274
Weight	0.229	0.093	−0.116	0.397
Height	0.271	0.046	−0.157	0.252
BMI	0.012	0.931	0.048	0.727
Systolic BP	−0.147	0.289	−0.039	0.777
Diastolic BP	0.000	0.998	−0.007	0.960
Serum albumin	−0.040	0.780	−0.071	0.622
GFR	−0.032	0.819	−0.119	0.395
Urine albumin/creatinine ratio	−0.080	0.627	0.120	0.468
Serum creatinine	0.044	0.752	−0.020	0.887

Abbreviations: DBP, vitamin D binding protein; BMI, body mass index; BP, blood pressure; GFR, glomerular filtration rate.

Table 3 shows the relationship between clinical and laboratory parameters with 25(OH)D and DBP. Height was weakly correlated with DBP.

DISCUSSION

We have demonstrated a high prevalence of 25(OH)D deficiency in Nigerian children with nephrotic syndrome, with levels of vitamin D lower than in controls. This is similar to the findings in Egyptian children reported by Marzouk et al. [14]. In contrast, in India, Banerjee et al. [15] reported no differences between cases and controls. Contrary to our expectations, we found that our apparently healthy controls were also vitamin D deficient.

All the children with nephrotic syndrome in our study had 25(OH)D deficiency. In Miami, Freundlich et al. found levels <20 ng/mL (50 nmol/L) in all their participants [16] and in a multicentre North American study, Selewki et al. demonstrated that 25(OH)D deficiency was universal at diagnosis in all children with nephrotic syndrome and persisted at follow-up in a substantial number of patients [17]. In contrast, Som et al. [18] reported 25(OH)D deficiency in 66% of children with nephrotic syndrome. This variance may partly be explained by the differences in cut-off values used. While we used values from the Endocrine Society Clinical Practice guidelines, the Som et al. study used the American Academy of Paediatrics guidelines. The higher cuff-off values from the Endocrine Society Clinical Practice guidelines gives room for early intervention. Another reason may

be that less than half of our participants were in remission, compared to about 60% in the Som et al. study.

The majority of our patients with nephrotic syndrome had severe deficiency, comparable to findings reported in Indian children by Illalu et al. [19]. An earlier study [18] found lower rates of severe deficiency. Close to one quarter of the patients in our study were in relapse, when urinary loss of DBP is higher.

We found higher 25(OH)D levels in children who were in remission compared to those who were not. Similar to our findings, Freundlich et al. [20] reported that children in relapse had a mean 25(OH)D level of 9 ng/mL, and during remission a mean of 30 ng/mL. Little protein is lost during remission and thus there are higher levels of DBP in the bloodstream in that case.

Our children in remission recorded the highest vitamin D levels, followed by the newly diagnosed cases and then those in relapse. Cases with resistant nephrotic syndrome had the lowest levels of vitamin D. This is similar to the findings of Yousefichaijan et al., that vitamin D levels were lower in patients with steroid-dependent and steroid-resistant nephrotic syndrome than in patients with steroid-sensitive disease [21]. Banerjee et al. found that children with nephrotic syndrome had low levels of vitamin D for 12 weeks after relapsing, with a rise in levels as remission progressed [15].

The majority of our controls recorded hypovitaminosis, which has also been documented by earlier studies [9,22-24]. The controls were from higher socioeconomic classes, and likely experienced less outdoor activity and exposure to sunlight at home and at school.

Population-based reference ranges for 25(OH)D concentrations vary with diet, geography and exposure to sunlight; hence, health-based reference limits may be more appropriate [9]. There is a higher prevalence of vitamin D deficiency amongst African Americans than in white Americans; this could be due to lower synthesis of vitamin D which, in part, is genetically determined [25]. A study in healthy European populations revealed deficiencies in several countries including Denmark, France, Finland, England and Greece [26]. Vitamin D deficiency is associated with dark skin, insufficient sun exposure – because of excessive use of sunscreen with high SPF (sun protection factor), staying indoors for much of the day, wearing clothes covering most of the skin, living in northern latitudes during wintertime – obesity, chronic liver diseases, chronic intestinal diseases as well as chronic kidney diseases, and the use of drugs such as anti-epileptic medication and glucocorticoids [27,28].

We did not observe a correlation between 25(OH)D and serum DBP levels. A previous study did report a significant positive correlation, however, in keeping with the role of the carrier protein [29]. There was a negative correlation between serum DBP and proteinuria and a strong positive correlation between urinary DBP and urinary albumin [29].

Our study has some limitations. Dietary histories were not obtained so the possible influence of diet on vitamin D levels was not ascertained.

In conclusion, we recommend the active treatment of children with vitamin D deficiency with supplemental vitamin D, especially in patients with nephrotic syndrome. Our study has revealed that vitamin D deficiency is prevalent in our setting and that there is a need for the screening of healthy children and for the fortification of foods with vitamin D.

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