ORIGINAL ARTICLE

Evaluation of Vitamin D Status in Children with Nephrotic Syndrome in Remission in a Tertiary Care Hospital of North India

Ashima Badyal, Koushal Kumar*

Abstract

Childhood nephrotic syndrome (NS) is characterized by massive proteinuria, hypoalbuminemia, hyperlipidemia associated with peripheral edema. Children with NS lose 25-OH vitamin D binding protein in urine, and can have low blood levels of this metabolite. The present hospital-based study was carried out on a total of 50 children with nephrotic syndrome who are in remission, in the age group of 1-15 years of either gender, attending to our pediatric nephrology clinic. 46% had clear deficiency of vitamin D, while insufficiency was present in 28% and normal levels in only 26% of patients. There was significant difference ($p<0.05$) in 25-OH vitamin D levels between frequent relapsers (FR) as compared to infrequent relapsers (IR) and first episode of nephrotic syndrome. Hypocalcemia was present in 86% of patients, hypophosphatemia in 10% of patients, hyperphosphatemia in 50% and raised alkaline phosphatase in 36% of patients. Strong positive correlation is observed between serum calcium and vitamin D levels ($r=0.720$; $p<0.001$) and moderate negative correlation between phosphorous and vitamin D levels ($r= -0.577$; $p<0.001$, but insignificant relation between vitamin D and alkaline phosphate levels ($r= -0.248$; $p<0.082$). It is concluded that vitamin D deficiency is common among children with nephrotic syndrome even after remission of proteinuria. There exists a strong positive correlation between serum calcium and vitamin D levels.

Key Words

Nephrotic Syndrome, Vitamin D, Serum Calcium, Phosphorus, Alkaline Phosphate

Introduction

Childhood nephrotic syndrome (NS) has an incidence of 90-100 per million population of India. It is characterized by massive proteinuria, hypoalbuminemia, hyperlipidemia associated with peripheral edema (1). During nephrotic syndrome vitamin D binding globulin (DBG), which binds upto 98% of the 25-OH vitamin D and has low molecular weight, lower than that of albumin, is lost in urine causing low 25-OH vitamin D levels (2). If the magnitude of such losses of 25-OH vitamin D is marked and its duration is prolonged, a state of vitamin D deficiency may ensue and be responsible for the abnormalities of calcium homeostasis (3). Many of these patients may develop rickets and osteomalacia even with normal renal function (4). In view of all the above facts, the present study was undertaken to evaluate the levels of vitamin D, calcium, phosphate and alkaline phosphate in children with nephrotic syndrome in remission.

Material and Methods

This hospital-based study was carried out on 50 children with nephrotic syndrome in remission who attended our Paediatric Nephrology Clinic in the Department of Paediatric, GMC Jammu, in collaboration with Department of Biochemistry, GMC Jammu over a period of 6 months. The study has been approved by Institutional Ethical Committee of GMC, Jammu.

Inclusion criteria: Children with 1-15 years of age of either gender, with first episode of nephrotic syndrome, treated with twelve weeks of steroids therapy and in remission; and children with relapse of nephrotic

From the Department of Biochemistry and Paediatrics*, Govt. Medical College, Jammu, J&K- India
Correspondence to: Dr. Koushal Kumar, Lecturer Paediatrics, SMGS Hospital, Govt. Medical College, Jammu (J&K)

syndrome, treated with six weeks of steroids therapy and/or on immune-modulators/alternate immune-suppression (levamisole, cyclophosphamide, MMF, Tacrolimus) and in remission.

Children with nephrotic syndrome not in remission; children with age less than 1 year and more than 15 years; children with secondary causes of nephrotic syndrome and significant renal lesions; children suffering from chronic infections like tuberculosis, diabetes mellitus, cystic fibrosis; children suffering from mal-absorption (celiac diseases), moderate to severe protein energy malnutrition or protein losing enteropathy; children with deranged renal or liver functions; children on anti-epileptics; and children already on vitamin D supplementation, were excluded from the study.

Written informed consent was taken from parent/ guardian of each child before including them in study. Detailed history was taken, anthropometry and detailed clinical examination was done to rule out other causes. 6 ml of venous blood sample was aseptically collected as per the standard guidelines and protocol. After centrifugation, serum was allowed to separate and subsequently analyzed for BUN, creatinine, LFTs, calcium, phosphorus, alkaline phosphate and vitamin D levels. Vitamin D was estimated with Abbott Architect chemiluminescent-micro-particle-immunoassay (5).

Vitamin D deficiency was taken as serum 25(OH)D level <20 ng/ml which was further divided into:

- Insufficiency: serum 25(OH)D level 12-20 ng/ml
- Deficiency: serum 25(OH)D level <12 ng/ml

Serum calcium, phosphorous and alkaline phosphate were measured in fully autoanalyser, Siemens clinical chemistry system (6).

Statistical analysis was carried out by using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp. Released 2017. Armonk, NY, USA). Categorial variables were shown in number and percentage (%) and continuous variables as mean ± SD. Comparison between continuous variables was made using independent sample *t*-test. Linear relationship between two variables was determined using Pearson correlation. $p \leq 0.05$ was considered to be statistically significant.

Results

Present study consisted of 50 cases of nephrotic syndrome in remission who attended our Paediatric Nephrology OPD, Department of Paediatrics, GMC Jammu, over a period of 6 months. Out of 50 patients, 29 were males, while 21 were females. Male to female ratio came out to be 1.38:1. Mean age of onset of syndrome was 4.9 years.

14 patients were of first episode of nephrotic syndrome, 20 steroid sensitive (SSNS) frequent relapsers (FR), 6 steroid sensitive (SSNS) infrequent relapsers (IR), 8 steroid dependent (SDNS) and 2 were steroid resistant (SRNS) (*Table 1*). Among the patients of 1st episode, all were found to be responsive to steroids. Among the relapsed cases, 10/26 patients were on immune-modulators/alternate immune-suppression (levamisole, cyclophosphamide). Among the 8-steroid dependent (SDNS), 5 patients were on MMF and the one on Tacrolimus. 2 patients of SRNS were on tacrolimus. Renal biopsy performed on the 3 patients who were on Tacrolimus, showed MCNS in 2 patients and FSGS in 1

Table 1: Association Between 1st Episode of Nephrotic Syndrome, FR, IR, SDNS/SRNS and Vitamin D Levels (n=50)

Vitamin D Status	1st Episode (n=14)	FR (n=20)	IR (n=6)	SDNS/SRNS (n=10)
Deficiency (<12 ng/ml)	3	15	2	3
Insufficiency (12-20 ng/ml)	5	2	1	6
Sufficiency (20-100 ng/ml)	6	3	3	1
Mean Levels	24.81±12.36 ng/ml	13.61±11.38 ng/ml	25.13±13.37ng/ml	15.30±4.62 ng/ml

FR: Frequent Relapsers, IR: Infrequent Relapsers, SDNS: Steroid Dependent, SRNS: Steroid Resistant

Table 2: Association Between Vitamin D and Calcium Levels(n=50)

Vitamin D Status	Hypocalcaemia	Normal Ca Levels	Pearson Correlation
Deficiency/ Insufficiency	37	0	.720
Normal	6	7	$p < .001$

patient.

13 of the total 50 cases had hypertension as per recent updated guidelines on hypertension by AAP (7). None of the children had severe clinical wasting or severe malnutrition affecting vitamin D levels. None of the children had clinical features of hypocalcemia. Also, none of the children had fracture.

46% of patients had deficiency of vitamin D levels. vitamin D insufficiency was present in 28% patients, while normal levels of vitamin D were present in only 26% patients. There was significant difference (p<0.05) in 25-OH vitamin D levels between frequent relapsers (FR) and infrequent relapsers (IR) (*Table 1*). The association between vitamin D and calcium levels was shown in *Table 2*. Hypocalcemia was present in 86 % of patients but none of the patients with normal calcium levels had deficiency of vitamin D. There is a strong positive correlation (r=0.720) between calcium and vitamin D levels and is statistically significant (p<0.001). Hypophosphatemia was present in 10% of patients, hyperphosphatemia in 50%, whereas normal level in 40% of patients showing moderate negative correlation between phosphorous and vitamin D levels (r= -0.577; p <0.001) *(Table 3)*. There seems to be a relation between vitamin D and alkaline phosphate levels also but not statistically significant (r= -0.248; p=0.082) *(Table 4)*.

Table 3: Association Between Vitamin D and Phosphorous Levels (n=50)

Vitamin D Status	Hypophosphatemia (<3.5 mg/dL)	Normal (3.5-6.5 mg/dL)	Hyperphosphatemia (>6.5 mg/dL)	Pearson Correlation
Deficiency/ Insufficiency	3	10	24	-.577
Normal	2	10	1	p < .001

Table 4: Association Between Vitamin D and Alkaline Phosphate Levels(n=50)

Vitamin D Status	Raised ALP	Normal ALP	Pearson Correlation
Deficiency/ Insufficiency	14	23	-.248
Normal	4	9	p < .082

Discussion

Children with nephrotic syndrome are treated with steroids for a longer duration. Changes in levels of calcium and vitamin D are considered to be due to urinary losses of these metabolites or their carrier proteins. These losses get exaggerated during relapse period. Such children may have multiple numbers of relapses.

It is long recognized fact that children with nephrotic syndrome are prone to the development of 25-OH vitamin D deficiencies. Freundlich *et al.* (8) began to explore 25-OH vitamin D deficiencies in 1985 in 16 children with active nephrotic syndrome showing levels less than 20 ng/ml in all children. This group later in cohort of 58 children of nephrotic syndrome followed during relapses and remission, revealed mean 25-OH vitamin D levels of 9 ng/ml during relapse and 30 ng/ml during remission (9). Some of the authors also reported normalized 25-OH vitamin D levels during remission with prevalent nephrotic syndrome suggesting that 25-OH vitamin D deficiency may be transient. However, a recent study by Banerjee *et al.* (10) suggested that 25-OH vitamin D levels may not normalize when children go into remission. Weng *et al.* (11) in 2005 showed that over 90% of children had 25-OH vitamin D levels less than 30 ng/ml and 68% had levels less than 20 ng/ml during remission.

In a study conducted by Yousefichaijan *et al.* (12) on a total of 218 children, vitamin D level was deficient (<10 ng/mL) in 79% of SRNS, 83% of SDNS, and 17% of SSNS group (p value 0.0001), insufficient (10-30 ng/mL) in 81% of SRNS, 73% of SDNS, and 9% of SSNS group (p value = 0.0003), and sufficient (30-150 ng/mL) in 91% of SSNS, 17% of SDNS, and 7% of SRNS group (p value=0.002). In another study conducted by Cetin *at el.* (13), the serum levels of 25-(OH)D were lower than 30 ng/mL in 93.8% SSNS patients [deficiency: 76.7% patients, insufficiency: 23.3% patients]. However, serum 25-(OH)D levels were similar between the IFRNS and FRNS patients (16.4 ± 9.09 ng/mL vs. 15.9 ± 7.61 ng/mL, p>0.05) in their study. Thus, our findings are consistent with previous studies demonstrating 25-OH vitamin D deficiencies in nephrotic syndrome cases in remission to be 74% (37/50).

There was significant difference (p<0.05) in 25-OH vitamin D levels between the patients of first episode of nephrotic syndrome (24.81 ± 12.36 ng/ml) and IFR (25.13 ± 13.37ng/ml) when compared with frequent relapsers (13.61 ± 11.38 ng/ml) which is quite similar to the study

conducted by Bikyli *et al.* (14). In our study vitamin D level was lower in patients with SDNS and SRNS (8/10) than in patients with SSNS (29/40) which is similar to the study conducted by Yousefichaijan *et al.* (12) in 2018, therefore vitamin D levels can be used as an indicator for prognosis of nephrotic syndrome patients.

In our study, hypocalcaemia was present in 86% of children. Similar results were reported by Septarini *et al.* (15) who conducted a study among 28 children with frequently relapsing and steroid-dependent nephrotic syndrome and found that 22 out of 28 (78.57%) had hypocalcaemia. Besides, our study also showed hyperphosphatemia in 50% of children with moderate negative correlation between phosphorus and vitamin D and higher alkaline phosphate levels, however, no significant relation was found between vitamin D levels and alkaline phosphate. Similar findings were reported by Illalu *et al.* (16) who also demonstrated hyperphosphatemia in 47.06% (16/34) of the children with nephrotic syndrome and moderate negative correlation between phosphorus and vitamin D levels.

Nelson *et al.* (1) suggested routine measurement of vitamin D status in nephrotic syndrome, could be a strategy for treating individuals if suffering from vitamin D deficiency, however more studies are required to determine the role of such treatment.

Conclusion

It is concluded that vitamin D deficiency is common among children with nephrotic syndrome even after remission. Levels of vitamin D further found to be low among frequent relapsers (FR) as compared to infrequent relapsers and first episode of nephrotic syndrome. There exists a strong positive correlation between serum calcium and vitamin D levels and moderate negative correlation between phosphorous and vitamin D levels, but insignificant relation between vitamin D and alkaline phosphate levels. However, longitudinal studies are required to confirm these findings and evaluate effects of vitamin D on bones, particularly in frequent relapsers and steroid dependent nephrotic syndrome.

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