ORIGINAL ARTICLE

Bioavailable Vitamin D Levels in Children With First Episode Nephrotic Syndrome: A Longitudinal Study

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ABSTRACT

Objective: To estimate the levels of serum bioavailable vitamin D in children presenting with first episode nephrotic syndrome (FENS) at diagnosis and after 4 weeks of standard steroid therapy while the child is in remission, and compare the same with age-sex matched healthy controls.

Methods: We included children aged 1 month to 12 years presenting as FENS and estimated the serum calcium, phosphorus, alkaline phosphatase, 25-hydroxy vitamin D [25(OH)D], parathormone, serum and urine vitamin D binding protein (VDBP) at diagnosis and after 4 weeks of standard steroid therapy while the child is in remission. We also included age-sex matched healthy controls for comparison. Bioavailable and free 25(OH)D were estimated at enrolment and at 4 weeks of therapy.

Results: The mean (SD) 25(OH)D level (ng/mL) in children with FENS was 11.3 (6.1) at diagnosis and 13.6 (6.2) at 4 weeks follow-up, while the observed value in healthy controls was 16 (7) ng/mL. The median (IQR) serum VDBP level in FENS at enrolment was 223.0 (144, 305.5) µg/mL. There was significant correlation between serum VDBP and serum albumin levels ($P = 0.04$). At 4 weeks (remission), the median (IQR) VDBP levels increased to 554.5 (383, 644.75) µg/mL ($P < 0.001$). The median (IQR) free 25(OH)D levels (pg/mL) in children with FENS was 1.07 (0.8, 1.6) at enrolment and 0.53 (0.37, 0.86) at 4 weeks follow-up. The median (IQR) bioavailable vitamin D in FENS during proteinuria was 0.58 (0.4, 0.83) ng/ml, much lower as compared to controls 0.97 (0.85, 1.08) ng/mL ($P < 0.001$). On follow-up at 4 weeks of remission the median (IQR) bioavailable vitamin D levels increased to 0.87 (0.59, 1.42) ng/mL ($P = 0.015$). There was a very strong positive correlation between free vitamin D and bioavailable vitamin D ($r = 0.9$, $P < 0.001$); a strong negative correlation between serum VDBP and bioavailable vitamin D ($r = - 0.69$, $P < 0.001$). There was a positive correlation between 25 (OH)D and bioavailable vitamin D ($r = 0.63$, $P < 0.001$). Serum VDBP and serum albumin showed statistically significant positive correlation ($r = 0.37$, $P < 0.05$).

Conclusion: Children with FENS are deficient in vitamin D. The free and bioavailable vitamin D levels are reduced in children with FENS during the proteinuric phase. Further studies to assess the association between bioavailable vitamin D and 25(OH)D with bone mineral density are needed in children with nephrotic syndrome to validate the utility of bioavailable vitamin D in clinical practice.

Keywords: *Bone health, Child, Kidney, Proteinuria*

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INTRODUCTION

Childhood nephrotic syndrome (NS) shows a prompt response to high dose corticosteroids in majority of the patients; however, the disease frequently has a relapsing course warranting repeated courses of glucocorticoids, thereby predisposing these children to steroid toxicity. At physiological doses, glucocorticoids have a role in osteoblastic differentiation but at higher doses they promote apoptosis of osteoblasts [2]. The standard dose of prednisolone for the management of relapsed NS is 60 mg/m^2/day, which far exceeds the daily dose of 5 mg which itself is considered to be a risk factor for steroid-induced osteoporosis in adults [1]. This indicates that bone health of children with steroid sensitive nephrotic syndrome (SSNS) should be monitored using serum vitamin D assay and estimation of bone mineral density (BMD). Urinary losses of low molecular weight proteins that are necessary for the transport of metals, hormones and drugs like thyroid binding globulin, cortisol binding protein, vitamin D binding protein (VDBP) in addition to albumin, is a major complication in patients with NS [3]. Low VDBP manifests as low ionized calcium levels and tetany. Estimation of bioavailable vitamin D which represents the fraction of vitamin D which is not bound to VDBP but is either bound to albumin or circulates in free form is

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important [4]. Although there is some evidence to indicate that bioavailable vitamin D levels are decreased in adults with nephrotic syndrome [3]. A recent study showed that there was not much difference in the levels of free 25-hydroxy vitamin D [25(OH)D] in children presenting as relapsed nephrotic syndrome or those in remission when compared with healthy controls [5]. There is a paucity of studies on bioavailable vitamin D levels in children with idiopathic FENS and therefore this study was planned to assess the levels of serum bioavailable vitamin D in children with FENS at diagnosis and after receiving 4 weeks of steroid therapy while in remission.

METHODS

We performed an observational study in children with idiopathic FENS aged 1 to 12 years and age- and sex-matched healthy controls, between January 2021 to June 2022, in the Division of Pediatric Nephrology at Lady Hardinge Medical College, a tertiary care teaching hospital in New Delhi, India. Prior approval from the institutional ethics committee was obtained.

The sample size was calculated based on a study from India where the mean (SD) bioavailable 25(OH)D levels (nmol/L) were observed as 1.59 (1.22) and 4.93 (4.15) in adults with FENS (cases) and controls, respectively [3]. Using alpha error of 5%, power of 90% and 95% confidence interval, the sample size was calculated to be 18 per group. Considering a dropout rate of 10%, the sample size was calculated as 20 per group. A sample size of 30 participants per group was committed based the recommendations of the scientific committee of the institute.

A written and informed consent from the caregivers and an assent, as applicable, were obtained before the enrolment of participants in this study. We excluded children with rickets and those with other underlying conditions predisposing to hypoalbuminemia (liver disease and severe acute malnutrition). We enrolled healthy age- and sex-matched population as controls. All patients were managed as per the guidelines of the Indian Society of Pediatric Nephrology [6]. At enrolment, a complete hemogram, kidney function tests, serum albumin, serum cholesterol, urine microscopy, and urine protein creatinine ratio were estimated. Additional investigations like serum calcium, phosphorus, alkaline phosphatase (ALP), 25(OH)D, serum parathormone (PTH), serum and urinary VDBP was performed, as needed. All patients were followed up after 4 weeks of steroid-induced remission when serum VBDP, serum albumin and 25(OH)D levels were measured. Free and bioavailable vitamin D were calculated using a formula adapted from the one suggested by Powe et al to calculate free and bioavailable testosterone (supporting free hormone hypothesis) [7]. Serum testosterone, sex hormone binding globulin and albumin and their respective binding constants were replaced with 25(OH)D, VDBP and albumin as summarized below:

Free 25(OH)D = $[-b + \sqrt{(b^2-4ac)}] / 2a$

a = Kdbp × Kalb × albumin + Kdbp

b = (Kdbp × DBP) – (Kdbp × 25(OH)D) + (Kalb × albumin) + 1

c = – [25(OH)D]

Kdbp = affinity constant between 25(OH)D and DBP

Kalb = affinity constant between 25(OH)D and albumin

Bioavailable 25(OH)D = (K_{alb} × albumin + 1) × [Free 25(OH)D]

Kdbp = 7×10^8 M^{-1}

Kalb = 6×10^5 M^{-1}

[*Concentrations of albumin, VDBP and 25(OH)D expressed in mol/L; Free and bioavailable 25(OH)D are converted to pg/mL and ng/mL, respectively*]

Statistical analysis: Data were analyzed using SPSS version 28. Categorical data were expressed as numbers (percentages) and were compared between the two groups using Chi square test or Kruskal Wallis test (if non-normal distribution of data). Continuous data were expressed as mean (SD) or median (IQR) and compared using Student's t-test or Wilcoxon rank test (if non-normal distribution of data). Correlation was done using Pearson correlation for normally distributed data and Spearman correlation for non-normally distributed data. $P < 0.05$ was considered to be statistically significant.

RESULTS

The baseline data of the study population is shown in **Table I**. Overall, the mean age of the study participants was 41.97 (28.93) months, with 34 boys and 26 girls. The baseline kidney function tests were normal, except for mild elevation in urea ($n = 6$). At baseline, the mean (SD) 25(OH)D levels were significantly lower in the case group compared to controls. After 4 weeks of therapy, the 25-(OH) D levels improved significantly but remained lower than the control group. Alongside, the PTH levels and urinary VDBP decreased significantly while the serum VDBP levels increased significantly at 4 weeks. The free 25(OH)D levels reduced significantly at 4 weeks, while the bioavailable 25(OH)D levels increased significantly at remission. The bioavailable 25(OH)D level in the control group was comparable to the level observed during

Table I Baseline Characteristics of Children with First Episode Nephrotic Syndrome and Healthy Controls

Parameters	*Group*		*P value*
	Case (n = 30)	*Controls (n = 30)*	
Age (mo)[a]	36 (24.75, 48)	36 (24, 47.5)	0.923
Age group[c]			
13-24 mo	8 (26.7)	9 (30)	0.869
25-72 mo	19 (63.3)	17 (56.7)	
73-143 mo	3 (10)	4 (13.3)	
Gender[c]			
Male	17 (56.7)	17 (56.7)	1.000
Female	13	13	
Family history of NS[c]	1 (3.3)	0	1.000
Hypertension[c]	11 (36.7)	0	< 0.001
Weight SDS[b]	-0.28 (0.98)	0.21 (0.79)	0.039
HAZ Score[a]	-0.94 (-1.64, 0.17)	-0.09 (-0.5, 0.51)	0.002
Short stature[c]	3 (10)	0	0.237
Clinical rickets[c]	0	0	1.000
Blood urea (mg/dL)[a]	27.5 (21.4, 35.5)	24 (18.3, 37.5)	0.255
Serum creatinine (mg/dL)[a]	0.3 (0.2, 0.3)	0.3 (0.3, 0.4)	0.238
Serum calcium (mg/dL)[a]	8.2 (7.8, 8.4)	9.75 (9.2, 10)	< 0.001
Ionized calcium (mg/dL)[a]	4.2 (3.9, 4.5)	4.6 (4.6, 4.8)	0.004
Serum phosphorus (mg/dL)[a]	5 (4.8, 5.6)	4.8 (4, 5)	0.005
Serum albumin (g/dL)[a]	1.2 (1, 1.5)	3.9 (3.7, 4)	< 0.001
Total cholesterol (mg/dL)[b]	463.3 (102.3)	124.1 (24.6)	< 0.001
Serum alkaline phosphatase (U/L)[a]	196.5 (164, 272)	122 (110.5, 175)	0.001
Parathormone (pg/mL)[a]	62.5 (38.2, 87.3)	52 (48, 65)	0.600
25-hydroxy vitamin D (ng/mL)[b]	11.3 (6.1)	16 (7)	0.007
VDBP (µg/mL)[a]	223.0 (144, 305.5)	578 (501.5, 627.2)	< 0.001
Free vitamin D (pg/mL)[a]	1.07 (0.8, 1.6)	0.62 (0.5, 0.7)	< 0.001
Bioavailable vitamin D (ng/mL)[a]	0.58 (0.40, 0.83)	0.97 (0.85, 1.08)	< 0.001
UP/UC[a]	6.3 (3.2, 7.9)	0.08 (0.04, 0.12)	< 0.001
Urine VDBP (µg/mL)[a]	30 (15, 170)	3 (0.72, 4.22)	< 0.001

Values expressed as [a]median (IQR), [b]mean (SD) or [c]n (%)
HAZ Height-for-age z score, NS Nephrotic syndrome, SDS Standard deviation score, UP:UC Urine protein to urine creatinine ratio, VDBP Vitamin D binding protein

remission. **Table II** shows the change in parameters after 4 weeks of therapy (during remission). There was a very strong positive correlation between free 25(OH)D and bioavailable vitamin D levels (r = 0.9, $P < 0.001$); a strong negative correlation between serum VDBP and bioavailable 25(OH)D (r = -0.69, $P < 0.001$). There was a positive correlation between total 25(OH)D and bioavailable vitamin D (r = 0.63, $P < 0.001$). A positive correlation was present between urine UP/UC and urine VDBP (r = 0.51, $P = 0.004$). The serum VDBP and serum albumin showed statistically significant positive correlation (r = 0.37, $P < 0.05$).

We treated 10 patients out of 30 patients with vitamin D therapy, but they continued to remain vitamin D deficient (<12 ng/ml) even at four weeks of therapy.

DISCUSSION

The mean (SD) 25(OH)D levels in our study at enrolment were 11.3 (6.1) ng/mL which falls under the definition of deficiency of vitamin D according to the Indian Academy of Pediatrics (IAP) guidelines [8]. At 4 weeks, the mean (SD) vitamin D levels were 13.6 (6.2) ng/mL, suggestive of vitamin D insufficiency. Cetin et al reported mean (SD) serum vitamin D levels during remission in children with

Table II Change of Various Parameters at First Episode and After 4 Weeks of Follow-up

Parameters	*First episode*	*Follow up*	*P value*
Weight (kg) [a]	13 (11.2, 16.8)	11.65 (9.82, 15)	< 0.001
MAP (mmHg) [a]	72 (68.2, 73)	69 (67.3, 71.8)	< 0.001
Hypertension [c]	11 (36.7)	2 (6.7)	0.003
Elevated blood pressure[c]	5 (16.7)	5 (16.7)	
Stage 1 hypertension[c]	4 (13.3)	2 (6.7)	
Stage 2 hypertension[c]	2 (6.7)	0	
Hemoglobin (g/dL)[a]	11.85 (10.4, 12.5)	10.8 (9.9, 11.5)	< 0.001
Hematocrit[b]	34.88 (3.6)	31.53 (3.7)	< 0.001
Urea (mg/dL)[a]	27.5 (21.4, 35.5)	31 (24.3, 40.8)	0.365
Creatinine (mg/dL)[a]	0.3 (0.2, 0.3)	0.26 (0.2, 0.3)	0.391
Serum albumin (g/dL)[a]	1.2 (1, 1.5)	3.8 (3.5, 4)	< 0.001
Serum calcium (mg/dL)[a]	8.2 (7.8, 8.4)	10 (9.1, 10.2)	0.335
Ionized calcium (mg/dL)[a]	4.2 (3.9, 4.5)	4.4 (4.1, 4.6)	0.193
Serum phosphorus (mg/dL)[a]	5 (4.8, 5.6)	4.5 (4.3, 5)	0.012
Alkaline phosphatase (U/L) [a]	196.5 (164, 272)	147 (115.3, 159)	0.004
Total cholesterol (mg/dL) [b]	463 (102.3)	155.4 (33.4)	< 0.001
Parathormone (pg/mL)[a]	62.5 (38.24, 87.33)	45.5 (35, 58.8)	0.024
25(OH)D (ng/mL) [b]	11.3 (6.1)	13.6 (6.2)	0.033
VDBP (µg/mL) [a]	223 (144.0, 305.5)	554.5 (383, 644.8)	< 0.001
Free vitamin D (pg/mL) [a]	1.07 (0.8, 1.6)	0.53 (0.37, 0.86)	< 0.001
Bioavailable vitamin D (ng/mL) [a]	0.58 (0.40, 0.83)	0.87 (0.59, 1.42)	0.015
UP/UC[a]	6.33 (3.2, 7.9)	0.1 (0.09, 0.15)	< 0.001
Urinary VDBP (µg/mL) [a]	30 (15, 170)	2.8 (1.12, 5.42)	< 0.001

Values expressed as [a]median (IQR), [b]mean (SD) or [c]n (%)
MAP Mean arterial pressure, VDBP Vitamin D binding protein, UP:UC Urine protein to urine creatinine ratio, 25(OH)D 25-Hydroxy vitamin D

infrequently relapsing nephrotic syndrome after a mean steroid intake of 1 year as 16.4 (9.09) ng/mL [9]. Lower levels of ionized calcium, high PTH and high ALP observed in a setting of vitamin D deficiency suggested biochemical abnormalities related to abnormal bone mineral metabolism and ill effects of bone health. They demonstrated that even 12 weeks of glucocorticoid therapy is a risk factor for osteoporosis and that the risk of osteoporosis was not dependent on the cumulative steroid dose. Since, the management of FENS consist of 12 weeks of glucocorticoid therapy, we recommend that serum vitamin D levels be tested at baseline, and those found to be deficient should be offered supplements to prevent the adverse effects on bone health. Choudhary et al emphasized on routine calcium and vitamin D supplementation, irrespective of vitamin D status, for osteoprotection in children with nephrotic syndrome [10]. We also found that healthy controls in our population had vitamin D insufficiency and while the children with FENS showed improved vitamin D status following remission at 4 weeks, they continued to have insufficient vitamin D status. It is noteworthy that although India is a tropical country with good sunlight, our healthy children are vitamin D insufficient.

Surve et al assessed the influence of VDBP levels on vitamin D status of under-five healthy children and showed that physiologic variations in PTH and VDBP are to be considered in children before deciding treatment strategies. PTH and VDBP were considered as non-modifiable risk factors for vitamin D deficiency in children, especially those under the age of 5 years. The study also emphasised on the free hormone hypothesis and the importance of measuring free and bioavailable vitamin D, rather than total 25(OH)D, to prevent under- or overestimation of vitamin D status in children [11]. The median (IQR) serum VDBP level in our children with FENS during proteinuria was 223.0 (144.0, 305.5) µg/mL

WHAT THIS STUDY ADDS?

- Bioavailable vitamin D is lower in children with first episode nephrotic syndrome compared to healthy controls.

which is comparable to the mean (SD) value of 210.0 (137.40) µg/mL observed in another study in Indian adults with idiopathic NS [3]. The lower levels of VDBP are attributed to the urinary proteinuria beyond the compensatory capacity of liver. At 4 weeks follow-up, the VDBP levels in cases were comparable to the values observed in healthy controls, possibly due to a compensatory increase in VDBP synthesis by the liver and genetic variation in gene coding for VDBP.

Assessment of VDBP is of paramount importance in children with steroid resistant NS, frequently relapsing NS and steroid dependant NS, as these children continue to have proteinuria for prolonged periods. VDBP levels must be assessed with extra caution as it might be affected by the presence of sepsis, as suggested by Waldron et al that VDBP acts a negative acute phase reactant [12]. We did not find any difference in VDBP levels in children with or without infection at the onset of disease. In our study serum VDBP and serum albumin showed statistically significant positive correlation and for every one unit decrease in albumin, VDBP decreased by 117.9 units. This is similar to the study by Aggarwal et al showing similar correlation ($r = 0.264$, $P = 0.008$) [3].

Importance of measuring free and bioavailable vitamin D levels, rather than total 25(OH)D needs to be stressed to prevent under- or overestimation of vitamin D status in children, so that vitamin D may be supplemented if required. According to free hormone hypothesis, it is the free form of the hormone which is active rather than the total 25(OH)D. Therefore, we calculated the free 25(OH)D levels based on the serum albumin, VDBP and total 25(OH)D levels. As the serum albumin level increased during remission, the free form of vitamin D decreased. Hence, it would be ideal to measure free vitamin D levels during FENS and relapse to know the exact vitamin D status in children with FENS. The fraction of 25(OH)D which is loosely bound to albumin is also available for action at target sites, therefore bioavailable vitamin D levels should be measured in children with NS. We also observed that the mean bioavailable vitamin D increased and the VDBP decreased with an increase in age. The bioavailable vitamin D levels of healthy controls were comparable to children with FENS at 4 weeks of remission which suggests that bioavailable vitamin D is especially useful to study vitamin D status in children during proteinuria. In our study bioavailable vitamin D showed a significant correlation with serum albumin, VDBP and 25(OH)D.

Denburg et al showed lower levels of free and bioavailable vitamin D in pediatric chronic kidney disease (CKD) secondary to glomerular disorders having nephrotic range proteinuria compared to other etiologies of CKD, supporting the evidence that bioavailable vitamin D reflects the vitamin D status better in children with proteinuria [4]. The mean bioavailable vitamin D levels in children with CKD secondary to focal segmental glomerulosclerosis (FSGS) was 0.8 ng/mL and those secondary to congenital anomalies of the kidney and urinary tract (CAKUT) was 3.4 ng/mL. The median bioavailable vitamin D levels observed in children with FENS was of 0.58 ng/mL in our study.

The strength of our study includes a follow-up evaluation of patients at 4 weeks once they had attained remission which showed near normalization of bioavailable vitamin D levels. Additionally, our study provides normative values of bioavailable vitamin D levels in healthy Indian children which were lacking. Limitations include that we did not assess the bone mineral density in our study population, a small sample size and a short duration of follow-up

We recommend that a simple intervention like supplementing maintenance doses of vitamin D3 and calcium in children with NS, especially steroid dependent and steroid resistant NS to improve their bone health [8, 13]. This supplementation is especially recommended in Indian children where there is a high prevalence of vitamin D insufficiency.

We conclude that children with FENS are deficient in vitamin D and therefore there is a need to ascertain the free and bioavailable vitamin D levels especially during proteinuria. Further studies showing correlation of bioavailable vitamin D and 25(OH)D with bone mineral density and parathormone are required in children to validate the clinical application of measuring bioavailable vitamin D in children with SSNS and SRNS.

Ethics clearance: Institute Ethics Committee (IEC) No. LHMC/IEC/2020/PG thesis/100, dated Dec 28, 2020.

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