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Original Article

Severity of Vitamin D Deficiency in Children with Nephrotic Syndrome: A Study from Tertiary Care Center in Northern India

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ABSTRACT. In nephrotic syndrome (NS) due to podocytopathies, loss of vitamin D binding globulin along with albumin in urine leads to Vitamin D deficient state. We aimed to study the severity of vitamin D deficiency and its clinical correlation in children with NS. We performed a cross-sectional study at a tertiary care hospital in Northern India. Enrolment of children aged 1–18 years was done. Patient's detailed history, numbers of relapse, treatment details, and data regarding various immunomodulatory drugs treatment. Vitamin D level was estimated, and its status was further classified as deficiency <20 ng/mL and insufficiency 20–30 ng/mL as per Global Consensus Recommendations on evaluation, treatment, and prevention of vitamin D deficiency. Continuous variables were compared using tests such as unpaired *t*-test, Kruskal–Wallis test, and Wilcoxon rank sum test depending on the distribution of parameters. Categorical variables were compared using Chi-squared tests or Fisher's exact test. A total of 96 children with NS were screened, of which 77.1% had vitamin D deficiency. The mean serum vitamin D level was 14.393 ± 8.52 ng/mL. Among the 48 children of the first episode of NS 36 were deficient (36/48 = 75%). Whereas in the relapse category, 30 patients had infrequently relapsing NS (deficient 24/30 = 80%). Eleven children had frequently relapsing NS; among them, 10 were vitamin D deficient (10/11 = 90.9%), and there was a negative correlation between vitamin D level and duration of illness. Vitamin D deficiency is a common comorbidity in children with NS. Given the putative immunomodulatory property of vitamin D, this deficiency should be identified and treated routinely in all cases of NS.

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Introduction

Nephrotic syndrome (NS) remains the primary renal ailment of childhood. An annual incidence of NS in children in the Western world has been estimated to be 2–7/100,000 children, while it is

slightly higher in South Asian ethnicity.[1] Minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS) are the two most common histopathological findings on invasive biopsy. The main determinants of the disease prognosis are underlying histopathology and the response to corticosteroid treatment.[2]

Podocytes present in nephron play a critical role in the maintenance of glomerular filtration barrier and structural integrity and its injury and loss leads to proteinuria and progressive sclerosis.[3] Study done by Kim et al[3] indicates that podocytes express the receptors for interleukin-4 and interleukin-13, if activated by these cytokines, might disrupt glomerular permeability, contributing to proteinuria. Multiple immunologic and non-immunologic diseases of the kidney, like idiopathic MCD and FSGS (acquired podocytopathies), can be the cause of podocyte injury.[4] Another surrogate evidence of the immunologic basis of NS is the role of immunomodulators such as corticosteroids and calcineurin inhibitors, which are known to have a protective mechanism of podocyte against injury and loss.[5] The differentiation of precursor monocytes into more mature phagocytic macrophages is stimulated by the action of vitamin D on macrophages. Macrophages have their 1α-hydroxylase and to generate internal 1,25(OH)2D3 require sufficient ambient levels of 25-hydroxy-vitamin D (25[OH]D) substrate.[6]

During NS, vitamin D-binding globulin (carrier protein), which has a molecular weight lower than that of albumin, binds up to 98% of the 25(OH)D, and patients with active NS excrete vitamin D binding protein (VDBP) along with albumin in their urine.[7]

Vitamin D deficiency in NS can cause numerous skeletal manifestations like a significant loss of trabecular bone in axial skeletons such as the spine, hips, and ribs, which leads to further risk of developing fracture, hypocalcemia and long-term sequelae like rickets, osteomalacia. Hence, it can be prevented by early supplementation of vitamin D to inhibit defective mineralization of bone due to imbalance between calcium and phosphorus.[8,9]

As established in preceding sections, vitamin D has a unique position in the pathophysiology of NS where, on the one hand, there is putative loss, thus creating a vitamin D deficient state while, on the other hand, given the immunomodulatory properties of vitamin D, its derangement may alter the natural course of NS. This has prompted a multitude of studies.

Data for Indian settings, moreover, in sub-Himalayan region are not well known and there is a paucity of studies on this subject and previous studies estimating 25(OH)D levels in nephrotic remission stage have reported mixed results. Hence, this study was done to assess the severity of vitamin D deficiency and its clinical correlation in children with NS.

Materials and Methods

This cross-sectional hospital-based study was conducted among children of NS in the age group of 1–18 years at a tertiary care center of Northern India from January 2018 to June 2019.

Inclusion criteria

Any child between the age group 1 and 18 years diagnosed with NS visiting outpatient department, emergency, or admitted during the study time frame was enrolled in the study.

Exclusion criteria

Children with preexisting rickets, neoplastic diseases, chronic malabsorption, chronic liver disease, chronic kidney disease, congenital renal anomalies, receiving drugs other than glucocorticoids which can cause adverse skeletal effects (e.g., phenytoin, phenobarbitone, methotrexate).

Written assent from eligible children and informed consent from their parents were obtained prior to recruitment in the study. The Ethical Committee of the Institution approved this study. A chart was prepared to document date of diagnosis, dates of relapses, number of relapses, treatment details (Vitamin D supplements, immunomodulator drugs), immunization history, socioeconomic history, and steroid

toxicity. Cases were stratified according to age of onset, duration of NS, and current steroid therapy. Management of cases was done as per unit protocol.

Total circulating 25(OH)D was measured by using electrochemiluminescence immunoassay method in the Department of Biochemistry. Siemens ADVIA Centaur Immunoassay System was used for the quantitative determination of a total of 25(OH) vitamin D in human serum.

Global consensus recommendations on the evaluation, treatment, and prevention of vitamin D deficiency by Holick et al[10] was used to define vitamin D status as – "sufficiency: >30 ng/mL (>75 nmol/L); insufficiency: 20–30 ng/mL (50–75 nmol/L); deficiency: <20 ng/mL (>50 nmol/L)."

Statistical Analysis

Data were analyzed using IBM SPSS Statistics version 23 software (IBM Corp., Armonk, NY, USA). Categorical variables were expressed as proportions or percentages. Continuous variables were expressed as mean ± standard deviation (SD) or as medians with total and interquartile ranges based on the distribution of data. Shapiro–Wilk test was utilized to assess the normal distribution of data and compared using tests such as unpaired *t*-test, Kruskal–Wallis test, and Wilcoxon rank sum test depending on the distribution of parameters. Categorical variables were compared using Chi-squared tests or Fisher's exact test. The Spearman rank test was used to calculate the statistical significance with a double-sided *P*-value and a significance level <0.05, indicating statistical significance.

Results

Socio-demographic characteristics

A total of 96 children in the age group of 1–18 years (mean age: 6.75 ± 3.6 years) were included in the study. The sociodemographic profile is described in Table 1.

Disease and treatment characteristics

The mean age of onset of NS in this study was 4.94 ± 2.97 years and half of the participants enrolled in this study suffered from first episode of NS. Table 2 summarizes the disease and treat-ment characteristics of participants.

Distribution of serum Vitamin D level

The variable vitamin-D Level (ng/mL) was not normally distributed (Shapiro–Wilk test: *P* <0.001) (Figure 1). The median (interquartile range) of vitamin-D Level (ng/mL) was 13.10 (11.18). The vitamin-D Level (ng/mL) ranged from 2.4 to 50.3 ng/mL.

More than three-fourths of enrolled participants

Table 1. Socio-demographic profile of the children with nephrotic syndrome (*n* = 96).

Parameters	Mean±SD/*n* (%)
Age (years)	6.75±3.60
Age	
1–5 years	45 (46.9)
6–10 years	39 (40.6)
11–18 years	12 (12.5)
Gender	
Male	70 (72.9)
Female	26 (27.1)
Residence	
Hilly area	18 (18.8)
Non-hilly area	78 (81.2)
Socio-economic status	
Upper middle	14 (14.6)
Lower middle	34 (35.4)
Upper lower	46 (47.9)
Lower	2 (2.1)

Table 2. Disease and treatment characteristics in children with nephrotic syndrome (n = 96).

Parameters	Mean±SD/n (%)
Age of onset (years)	4.94±2.97
Age of onset	
1–5 years	63 (65.6)
6–10 years	30 (31.2)
11–18 years	3 (3.1)
Duration of illness (weeks)	91.15±131.44
Type of nephrotic syndrome	
First episode	48 (50.0)
Infrequent relapse	30 (31.2)
Frequent relapse	11 (11.5)
Steroid resistance	3 (3.1)
Steroid dependent	4 (4.2)
Disease status	
Active	91 (94.8)
In remission	5 (5.2)
Total number of relapses	2.00±3.57
Treatment	
Steroids	81 (84.4)
Steroids+levimasole	6 (6.2)
Steroids+tacrolimus	2 (2.1)
Steroids+tacrolimus+cyclosporine	1 (1.0)
Steroids+mycophenolatemofetil	1 (1.0)
Off steroids	5 (5.2)

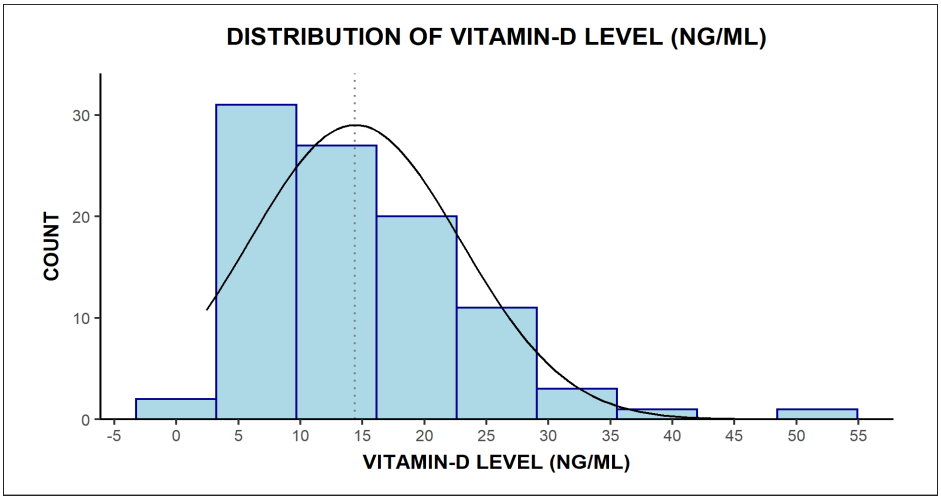

Figure 1. Histogram depicting the distribution of Vitamin D level (ng/mL).

Table 3. Comparison of Vitamin D level (ng/mL) (n = 96) in children with active disease versus those in remission.

Vitamin-D level (ng/mL)	Disease status		Wilcoxon test	
	Active	In remission	*W*	*P*
Median (IQR)	13 (10.85)	23.9 (9.2)	116.000	0.067
Range	2.4–50.3	9.31–28.6		

(77.1%) were vitamin D deficient, while vitamin D insufficiency was seen in a further 18.8% of participants.

Comparison of Vitamin D levels: Disease activity

Median vitamin D level in children with active NS was compared with those in remission using the Wilcoxon test. There was no significant difference between the groups in terms of vitamin-D Level (ng/mL) (W = 116.000, P = 0.067) (Table 3).

Comparison of Vitamin D levels: Type of nephrotic syndrome

Vitamin D Level (ng/mL) was not normally distributed in the five subgroups of the variable type of NS (Figure 2). *Post hoc* pairwise tests for the Kruskal–Wallis test performed using the Dunn Test method with Sidak correction were used to make group comparisons (Table 4).

Comparison of vitamin D levels: Type of nephrotic syndrome

Serum vitamin D levels were compared in

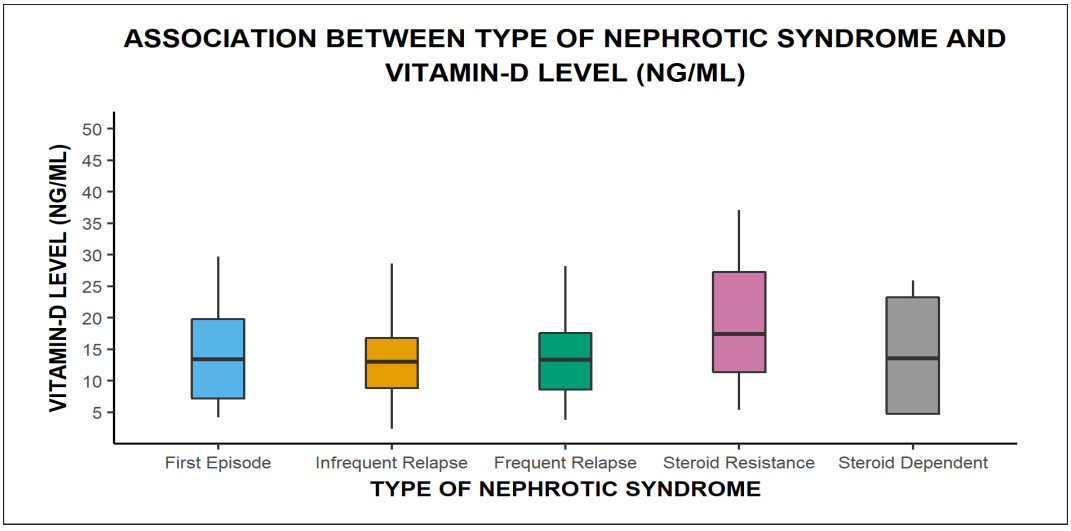

Figure 2. The Box-and-Whisker plot below depicts the distribution of Vitamin-D level (ng/mL) in the 5 groups (n = 96).

Table 4. Comparison of Vitamin-D level (ng/mL) (n = 96) in children with different types of nephrotic syndrome.

Vitamin-D level (ng/mL)	Type of nephrotic syndrome					Kruskal–Wallis test	
	First episode	Infrequent relapse	Frequent relapse	Steroid resistance	Steroid dependent	χ^2	*P*
Mean±SD	14.34±8.65	14.12±8.14	13.87±6.82	19.96±16.01	14.42±11.32	0.398	0.983
Median (IQR)	13.4 (12.61)	13 (7.91)	13.36 (8.98)	17.4 (15.86)	13.6 (18.53)		
Range	4.2–50.3	2.4–32.35	3.8–28.2	5.38–37.1	4.59–25.9		

Table 5. Comparison of vitamin-D level (ng/mL) (n = 96) in different treatment subgroups.

VitaminD level (ng/mL)	Treatment						Kruskal–Wallis test	
	Steroids (n=81)	Steroids+ levimasole (n=6)	Steroids+ tacrolimus (n=2)	Steroids+ Tacrolimus+ Cyclosporine (n=1)	Steroids +MMF (n=1)	Off steroids (n=5)	χ^2	P
Mean±SD	13.71±8.08	17.90±9.81	22.28±20.96	12.50±NA	4.80±NA	20.44±7.82	7.101	0.213
Median (IQR)	13 (10.06)	20.7 (14.8)	22.28 (14.82)	12.5 (0)	4.8 (0)	23.9 (9.2)		
Range	2.4–50.3	4.59–28.2	7.46–37.1	12.5–12.5	4.8–4.8	9.31–28.6		

different treatment subgroups. Five children who had achieved remission and were off steroids or any immunomodulator were also included. Kruskal–Wallis test was used to make group comparisons. There was no significant difference between the groups in terms of vitamin D Level (ng/mL) (χ^2 = 7.101, P = 0.213) (Table 5).

Correlation between duration of illness and vitamin-D level

Spearman correlation was used to examine the association between the duration of illness and vitamin D level. There was a weak negative correlation between the duration of illness (weeks) and vitamin D level (ng/mL), and this corre-lation was not statistically significant (rho = −0.05, P = 0.627) (Figure 3).

The scatterplot in Figure 3 depicts the correlation between duration of illness (weeks) and vitamin-D Level (ng/mL). Individual points represent individual cases. The blue trendline represents the general trend of correlation between the two variables. The shaded grey area represents the 95% confidence interval of this trendline.

Discussion

There has been a well-established fact that children with active NS are predisposed to develop of 25(OH)D deficiency. In this study, 77.1% of children with NS had deficient serum 25(OH)D concentrations and 18.8 % had insufficient serum 25(OH)D concentrations. Aggarwal et al[11] from India in 2016 showed that 74% of children diagnosed with NS were deficient of 25(OH)D, and 26% of children

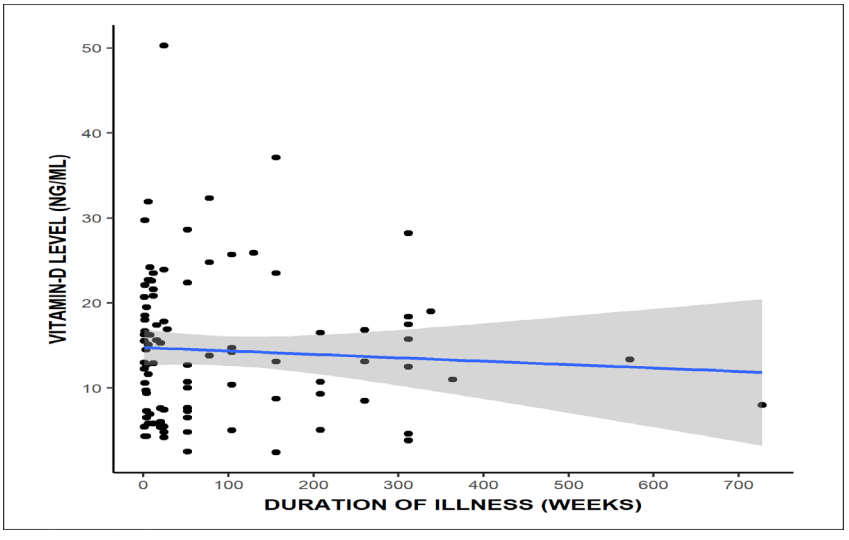

Figure 3. Correlation between duration of illness (weeks) and vitamin-D level (ng/mL) (n = 96).

were insufficient of 25(OH)D, which is almost similar to this study. Grymonprez et al[12] noticed that serum 25(OH)D was below 7 ng/mL in 10 out of 14 nephrotic children and in the low normal range in the remaining four patients. The average serum 25(OH)D levels were lower in the nephrotic patients than in the controls.

The results of this observational study demonstrated that 25(OH)D deficiency is prevalent both at active disease state and in remission. However, the mean levels of vitamin D in the active disease group and in the remission group are 14.06 ng/mL and 20.44 ng/mL, respectively. Similar findings were noted in a study done by Illalu et al.[13] But in another study by Banerjee et al,[9] it was found that serum 25(OH)D levels were lower within 3 months of relapse, but the levels were similar between the controls and patients with longer remission periods, suggesting that serum 25(OH)D levels were correlated with changes in the disease stage. In this study, the difference noted may be due to the persistence effect of long-term high-dose steroid therapy in an active disease period. Repeat testing of vitamin D level after 2–3 months of achieving remission could give a conclusion in this matter.

Vitamin D deficiency was prevalent in all five groups, i.e.,75.0% of first-episode NS children, 80.0% of infrequent relapsing NS, 90.9% of frequent relapsing NS (FRNS) , 66.7% of the steroid resistance group and 50.0% of the participants of steroid-dependent had a deficiency of vitamin-D Level. In contrast, Yousefichaijan et al[14] found that Vitamin D level was lower in patients with steroid dependent NS (SDNS) and steroid resistant nephrotic syndrome (SRNS) than in patients with steroid susceptible NS. Therefore, vitamin D levels can be used as an indicator in the prognosis of NS in patients. Another study by Dasitania et al[15] showed low level of vitamin D in children with FRNS and SDNS. However, in our study, vitamin D level was comparable between the first episode, FRNS and SDNS. It could be due to different cohort population were selected in previous studies and moreover low baseline levels of vitamin D in this study population. A study from India which was done by Sharma et al[16] in 2018, where 30% of the SRNS children (n = 15) were found to have hypocalcemia, vitamin D deficiency was observed in 54% of cases (n = 27) and insufficiency was seen in 46% cases (n = 23).

There was a weak negative correlation between the duration of illness (weeks) and vitamin D level (ng/mL). The children who were frequent relapsing (FRNS) or delayed in achieving remission (SRNS) had more loss of VDBP[7] with urine and needed to take corticosteroid for longer duration had more deficiency of vitamin D. It is supported by observation noted by Jesmin et al[17] that bone mineral density Z-score and duration of disease, are in an inverse relationship.

As there was no statistically significant difference in terms of vitamin D level between six treatment subgroups, i.e., children receiving only steroids, steroid + levamisole, steroids along with other immunomodulator drugs (cyclosporine, tacrolimus, mycophenolate mofetil) and the mean (SD) level of vitamin D in children not receiving any steroid, i.e. Off steroid group was 20.44 (7.82) ng/mL (range 9.31–28.6 ng/mL) it supports that mechanism of vitamin D deficiency in NS is not only due to steroid therapy but also it could be due to loss of VDBP.[7]

The mean (SD) level of vitamin D in the corticosteroid treated group was 13.71 (8.08) ng/mL. The exact mechanism of corticosteroids by which it causes vitamin D deficiency is not completely understood, although in 2000, Akeno et al[18] demonstrated that dexamethasone increased renal expression of vitamin D-24-hydroxylase, which caused degradation of active metabolites of vitamin D metabolites such as 25(OH)D and $1,25(OH)_2D$. Some other studies also support that steroids may enhance inactivation of 25(OH)D by upregulating 24-hydroxylase activity.[19,20]

This study has made an attempt to document the prevalence of vitamin D deficiency in a cohort of children suffering from NS in northern India, especially the sub-Himalayan region. However, we acknowledge certain limi-

tations inherent in this study. Given the high prevalence of vitamin D deficiency in the general population, it would have been meaningful to have a control for comparison to ascertain if the extent of vitamin D deficiency is significantly different in children with NS. However, due to logistic reasons and high cost of estimating vitamin D levels in the control group was not included as per the initial study plan. The sample size was not adequately powered; therefore may be no statistically significant difference was found between the group who received steroids and the groups that received different immuno-modulators.

Conclusions

There is a high prevalence of vitamin D deficiency and insufficiency in children with NS, which worsens with an increase in the duration of illness. Children assessed in the remission phase were also showed deficient and insufficient vitamin D status. Based on these findings, the study concludes that most of the children with NS will be benefited from routine measurement of their vitamin D status at the time of diagnosis. Given the immuno-modulatory properties of vitamin D and the findings in this study as well as similar studies, interventional clinical trials exploring the influence of vitamin D on the natural course of NS are needed.

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Conflict of interest: None declared.

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