

Study on Vitamin D Status in Children with Nephrotic Syndrome in Rajendra Institute of Medical Sciences

Dr Bhardwaj Narayan Chaudhary¹, Dr Vivek Kumar Sinha², Prof(Dr) Anil Kumar Chaudhary³

1- Senior Resident, Department of Pediatrics, RIMS, Ranchi

2- Junior Resident, Department of Pediatrics, RIMS, Ranchi

3- Prof & HOD, Department of Pediatrics, RIMS, Ranchi

Date of Submission: 15-06-2021

Date of Acceptance: 30-06-2021

I. Introduction

Nephrotic syndrome is the clinical manifestation of glomerular diseases associated with heavy (nephrotic- range) proteinuria >3.5g/24hr or a urine protein : creatinine ratio >2. The triad of clinical findings associated with nephrotic syndrome arising from the large urinary losses of protein are hypoalbuminaemia (≤ 2.5 g/dl), edema, and hyperlipidemia (cholesterol >200mg/dl). Nephrotic syndrome is a disease with primary glomerular abnormality with a relapsing course and usually responds to steroid treatment. It is because of alteration of permeability of the glomerular capillary wall, which results in inability to restrict the urinary loss of proteins. Patients with nephrotic Syndrome losses 25-hydroxyvitamin D in the urine and can have low blood levels of this metabolite. These patients may also have secondary hyperparathyroidism with normal renal function and display evidence of defective mineralization of bone and enhanced bone resorption. 25-Hydroxyvitamin D (25-OHD) circulates in blood, bound to Vitamin D binding protein. Of the possibility is that patient with nephrotic syndrome lose 25-Hydroxyvitamin D with protein in the urine. If the magnitude of such losses of 25-Hydroxyvitamin 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. Use of steroids on long term basis can cause osteoporosis and necrosis of the head of the femur and affects the bone mineral content (BMC) and bone mineral density (BMD) in children. Bone mineralization during childhood affects the BMC in the adult skeleton. Diminished bone mineralization has been reported in children with nephrotic syndrome by using conventional radiography, densitometry methods, biochemical bone markers and by bone histology. Many of these patients may develop osteoporosis even with normal renal function. This is of therapeutic significance because these children would merit prophylactic therapy with calcium and Vitamin D. In studies of pediatric patients with prevalent primary and secondary NS, 25-hydroxyvitamin D deficiency is present. Initially, these abnormalities were thought to be transient in nature and resolved with remission, but recent data suggests that 25(OH)Vit D deficiency persists despite achieving remission. Abnormal vitamin D metabolism in idiopathic NS is multifactorial, with contributions from losses of both vitamin D binding protein and 25(OH) Vitamin D in the urine. The urinary losses of vitamin d binding protein may be secondary to proteinuria, overwhelming the proximal tubule reabsorption via megalin and cubulin pathways. Deficiency in 25(OH)Vitamin D may lead to hypocalcaemia, hyperparathyroidism, and diminished bone mineral density/content. Vitamin D deficiency has also been associated with multiple systemic effects including elevated blood pressure, metabolic syndrome, cardiovascular disease, anemia, and impaired immune system regulation. Complicating the impact of vitamin D deficiency on the bones in children with NS is the repeated exposure to glucocorticoids. The initial treatment at diagnosis and with each relapse exceeds the 5mg/day of prednisolone shown to cause osteoporosis in adults. This had led to an increased interest in the impact of pediatric NS on the bone metabolism in developing children. Children with NS have been shown to have decreased bone mineralization, although the mechanism was not defined. Furthermore, adult survivors of steroid-sensitive minimal change disease show persistent bone abnormalities. These findings have led to an increased interest in different strategies and trails around supplementation of calcium and vitamin D in children with NS. There is a significant gap in the knowledge about 25(OH)Vitamin D levels in patients with incident NS and the effect of disease course on these levels following initial treatment. To date, there has not been a study evaluating Vitamin D deficiency in children with prospective observation beginning with the initial diagnosis of NS in Rajendra Institute of Medical Science, Ranchi. This research was conducted to examine and analyse the Vitamin D status in Nephrotic Syndrome patient to help in proper management of this patient.

II. Aims And Objectives:

To study vitamin D status in children with nephrotic syndrome admitted or in OPD basis in Rajendra Institute of Medical Science ,Ranchi.

III. Materials And Methods:

The present study will be conducted in the Department of Paediatrics,Rajendra Institute of Medical Sciences, Ranchi.

All the cases registered in the study will be interrogated for detailed history, clinically examined thoroughly and investigated.

Study design: Hospital based prospective observational time bound study.

Sample size: Case: 50 nephrotic syndrome patient coming, indoor and outdoor basis in Department of Pediatrics, RIMS,Ranchi .

Study period: June 2020 to May 2021

Study tools: 5ml syringe,cotton swabs, clot vial ,tourniquet.

Inclusion Criteria:

- All cases of nephritic syndrome upto 17 years age admitted or in OPD basis in department of paediatrics RIMS, Ranchi.

Exclusion Criteria :

- Children who have taken Vitamin D supplementation in past six months.
- Children who do not have normal glomerular filtration rate.
- Children who are on anti epileptic therapy.
- Children with congenital nephrotic syndrome.

IV. Method Of Data Collection:

Children who are fulfilling the inclusion criteria coming to the hospital in outdoor and indoor basis with proper written consent is taken from parents of the children. Then detailed history regarding the present complaints, past history, number of relapses,family history and drug history was taken and recorded in a structured proforma. Detailed clinical examination including anthropometry was done at the time collection of samples. Height was measured using stadiometer, length was measured using infantometer. Weight was recorded using digital weighing scale. Systemic examination was done to rule out any comorbid conditions. After proper clinical examination 5ml venous blood was taken in a clot vial with aseptic methods and was sent for estimation of calcium (Ca), phosphorus (P), alkaline phosphatase and serum estimation of vitamin D. Samples for calcium, phosphorous, alkaline phosphatase and vitamin D levels were collected after the remission of the relapse or the 1st episode of nephrotic syndrome. Estimation of 25 OH Vitamin D levels was done by chemiluminescence immunoassay technology.

V. Results:

The present study consist of 50 cases of nephrotic syndrome, that were admitted 12 cases were excluded from the study. Out of 38 patients 16 cases were 1st episode of nephritic syndrome. Renal biopsy was done in 3 patients, of which 1 patient had minimal change disease, 1 patient had IgA nephropathy and 1 patient had focal segmental glomerulosclerosis. 12 out of 38 children had hypertension (Figure 1).

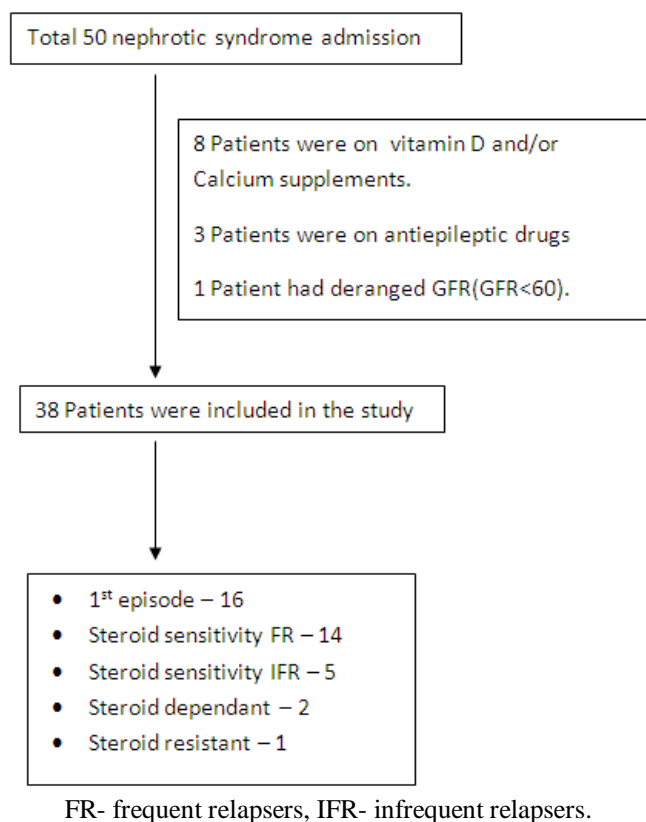


Figure 1: Inclusion and analytical sample flow.

20 patients were males and 18 patients were females. Male to female ratio was 1.1:1. Age wise distribution of cases is shown in Table 1. Mean age of onset of nephrotic syndrome was 4.6 years and median age at study entry was 7 years.

Table 1: Age wise distribution of cases (n=38)

Age (years)	No. of patients	Percentage
1-3	8	21.05%
4-6	12	31.50%
7-9	6	15.70%
10-12	10	26.31%
13-15	2	5.26%

Table 2 shows distribution according to pattern of response to corticosteroid therapy. Among the patients of 1st episode, all were responsive to steroids, Among the relapsed cases, 5 patients were on immunomodulators, of which 3 patients were on levamisole, 1 patient on cyclophosphamide and cyclosporine and 1 patient on tacrolimus. Only one child out of 15 children of <5years of age had severe wasting according to WHO classification using weight for height /length standard deviation charts. None of the children had severe acute malnutrition. Three out of 19 children of >5years of age had severe wasting or wasting according to WHO classification using standard deviation charts of BMI for age.

Table 2: Distribution according to pattern of response of corticosteroid therapy (n=38).

Category	No. of patients	Percentage
Steroid Sensitivity	16	42.10%
	5	13.15%
	14	36.84%
Steroid dependant	2	5.26%
Steroid resistant	1	2.63%

Out of 38 children 6(15.7%) children had clinical features of hypocalcemia in form of muscle spasm and muscle cramps. None of the children had fracture. Out of 38 patients 18(47.36%) patients had insufficiency of vitamin

D levels and vitamin D levels were normal in 8(21.05%) patients. None of them had toxicity. Mean vitamin D levels was 12.6 and median vitamin D levels were 15ng/ml(Table 3).

Table 3: Vitamin D levels in nephrotic syndrome (n=38)

Vitamin D levels	No. of patients	Percentage
Deficiency (<12ng/ml)	18	47.36%
Insufficiency (12-20ng/ml)	12	31.57%
Sufficiency (20-100ng/ml)	8	21.05%
Toxicity (>100ng/ml)	0	0%

Vitamin D deficiency was present in 18(47.36%) and insufficiency was present in 12(31.57%) children. Normal levels were present in 8(21.05%) children. Mean levels of 25 OH vitamin D in males was 14.89 ng/ml and in females was 28.16ng/ml. There was no statistically significant difference of vitamin D levels between males and females. Levels of Vitamin D were compared among different age groups. Mean vitamin D levels in age group of 1-5years was 21.33ng/ml, in 6-10 years was 18.49ng/ml and in 11-15 years was 22.62ng/ml. ANOVA test was applied to compare between the groups and f value was 0.09313 and p value was 0.9113. There was no significant difference of vitamin D levels among the different age groups (Table 4).

Table 4: Age wise distribution of vitamin D levels (n=38)

Vitamin D levels	1-5 years	%	6-10 years	%	11-15 years	%
Deficiency	9	23.68	8	21.05	0	0
Insufficiency	6	15.78	4	10.52	2	5.26
Sufficiency	3	7.89	2	5.26	4	10.5
Total	18	47.36	14	36.84	6	15.78

Levels of vitamin D were compared among the patients with different number of relapses.

Total number of relapses were between 1 and 13. Mean vitamin D levels in patients with 1-5 relapses was 28.53ng/ml, in patients with 6-10 relapses was 12.69ng/ml and in patients with 11-15 relapses was 10.78ng/ml. ANOVA test was applied and there was statistically significant difference in levels of Vitamin D among different groups. Vitamin D levels were low in patients with more number of relapses (Table 5).

Levels of vitamin D were studied among patients with 1st episode, frequent relapsers, infrequent relapsers and SSNS/SRNS group.

Table 5: Vitamin D levels according to number of relapses among the relapsers. (n=19)

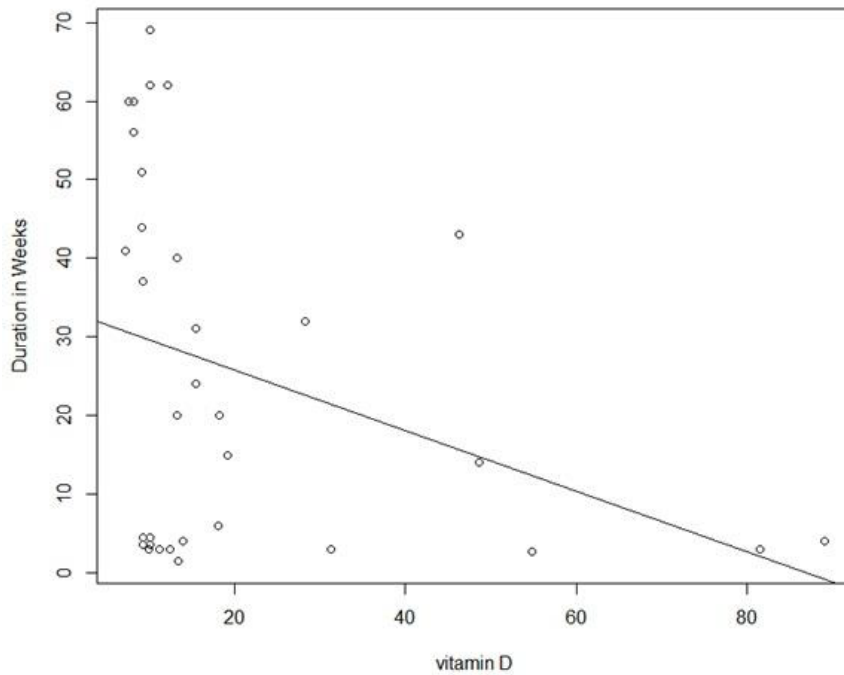
Vitamin D status	1 to 5 relapses	6 to 10 relapses	10 to 15 relapses
Insufficiency	2	1	2
Sufficiency	3	1	0
Toxicity	0	0	0
Total	5	9	5
Mean vitamin D levels			

Mean 25 OH vitamin D levels were low in Frequent relapsers (13.87ng/ml) as compared to SDNS/SRNS group (14.69ng/ml), infrequent relapsers (23.25ng/ml) and 1st episode of nephrotic syndrome (26.74ng/ml). There was statistically significant difference of vitamin D levels among the patients in these groups (Table 6).

Table 6: Association between FR, IR, 1st episode of nephrotic syndrome and SDNS/SRNS and vitamin D levels(n=38).

Vitamin D Status	FR(n=14)	1 st Episode(n=16)	IR(n=5)	SDNS/SRNS(n=3)
Deficiency	8(57.1%)	8(50%)	2(40%)	0(0%)
Insufficiency	4(28.5%)	3(18.75)	3(60%)	2(66.6%)
Sufficiency	2(14.28%)	5(31.25%)	0(0%)	1(33.33%)

Comparison was done between total cumulative duration of steroid treatment and levels of vitamin D. There was negative correlation between them. Patients with longer duration of steroid treatment had lower levels of vitamin D levels (Figure 2). Mean duration of steroid treatment in this study was 25.70 weeks.



Pearson correlation coefficient=-0.343.

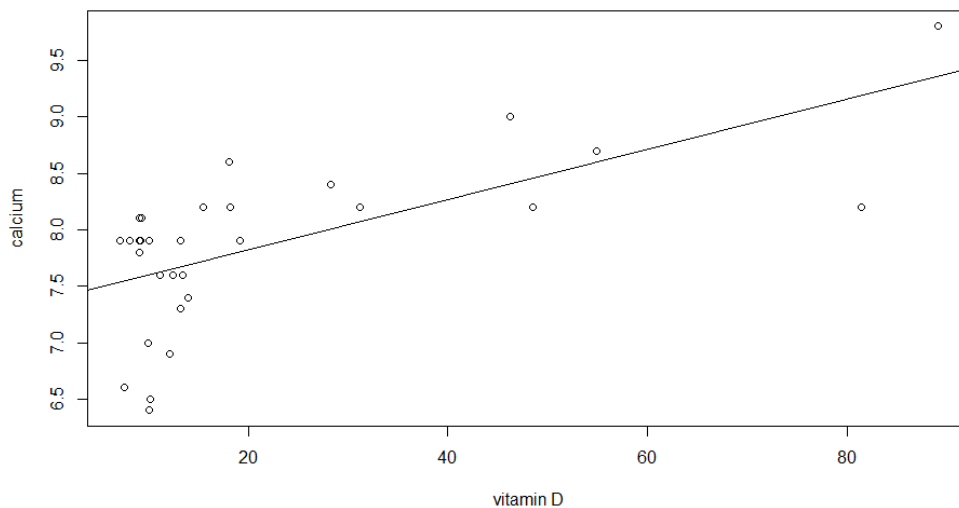
Figure 2: Correlation between cumulative duration of steroid treatment and vitamin D levels.

Out of 38 patients hypocalcemia was found in 33 (86.84%) patients, and none of the patients had hypercalcemia. Calcium levels were normal in 5 (13.15%) patients (Table 7).

Table 7: Comparison between Vitamin D and Calcium levels(n=38).

Vitamin D levels	Hypocalcaemia	Normal Calcium levels
Deficiency	18	0
Insufficiency	10	2
Sufficiency	5	3
TOTAL	33	5

None of the patients with normal Calcium levels had Vitamin D deficiency. Vitamin D and Calcium has a moderate correlation (Figure 3).



Pearson's correlation coefficient=0.6203.

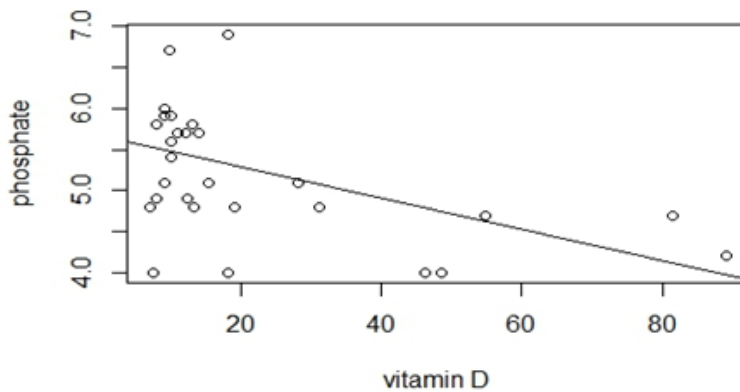
Figure 3: Correlation between calcium and vitamin D

Table 8: Comparison between vitamin D and phosphorous levels (n=38).

Vitamin D levels	Hypophosphatemia	Normal	Hyperphosphetemia	Total
Deficiency	2	6	10	18
Insufficiency	2	5	5	12
Sufficiency	4	4	0	8
Total	8	15	15	38

Hypophosphatemia was present in 8(21.05%) of the patients and 15(39.47%) patients had normal phosphate levels and 15(39.47%) patients had hyperphosphatemia (Table 8).

There was moderate negative correlation between phosphorous and Vitamin D levels (Figure 4).



Pearson’s correlation coefficient=-0.5149.

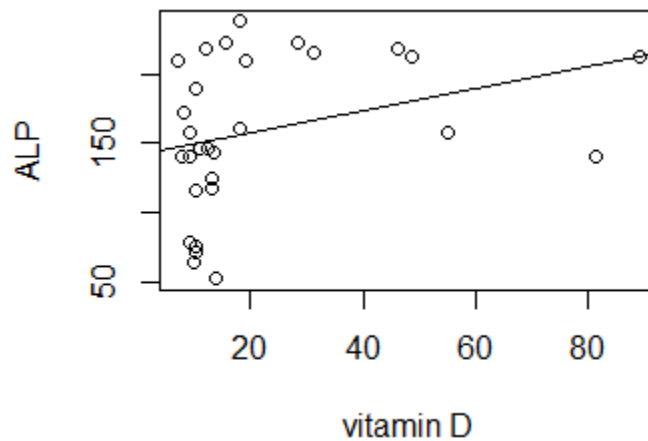
Figure 4: Correlation between phosphate and vitamin D levels.

Alkaline phosphatase was elevated in 17(50%) patients (Table 9).

Table 9: Comparison between vitamin D and alkaline phosphatase levels.

Vitamin D levels	Raised ALP	Normal ALP
Deficiency	10	8
Insufficiency	5	7
Sufficiency	4	4
Total	19	19

There was a weak correlation between alkaline phosphatase and Vitamin D levels (Figure 5).



Pearson’s correlation coefficient=0.3352.

Figure 5: Correlation between alkaline phosphatase levels and vitamin D.

VI. Discussion

This was a hospital based cross sectional descriptive time bound study conducted between 1st June 2020 and 30stMay 2021. In present study authors have measured calcium, phosphate, Alkaline phosphatase and Vitamin D levels 2 weeks after the patient attained remission, that is in absence of proteinuria. All patients had normal renal parameters Children with nephrotic syndrome receive steroids multiple times and for longer duration. Changes in levels of calcium and Vitamin D metabolites in patients with nephrotic syndrome are considered to be following urinary losses of these metabolites or their carrier proteins or secondary to corticosteroid therapy, especially in long term therapeutic courses.⁶ In this study mean age of onset of nephrotic syndrome was 4.6 years and median age at study entry is 7 years. Male to female ratio was 1.1:1. In this study only 4 (10.52%) children had wasting according to the WHO classification by using standard deviation chart for either weight for height/length or BMI for age. Stunting was present in 19 (50%) children of study population as per WHO classification using Standard Deviation charts of height/length for age. None of the children had severe acute malnutrition. This was similar to a study conducted by Weng et al in which SSNS patients had lower height and higher BML.⁷ Vitamin D is an important hormone required for bone metabolism and it involves regulation of calcium and phosphorous balance for bone mineralization and remodelling. Deficiency of Vitamin D in growing age causes rickets. Increased prevalence vitamin D deficiency in children with nephrotic syndrome has led to increased interest in the impact of nephrotic syndrome and its treatment on bone development in the children. According to the study conducted by Leonard et al, a daily dose of steroid as low as 5mg/day can contribute to osteoporosis in adults.⁸ This is of concern in children with nephrotic syndrome because childhood is a period of high bone turnover, with very high rates of bone formation required to maintain adequate mineralization of the rapidly growing skeleton and steroid treatment protocol in nephrotic syndrome excess the dose at which adult osteoporosis risk can increase. The cause of this bone loss due to corticosteroids is multifactorial. There can be direct inhibition of the bone forming osteoblast and an effect on overall calcium balance. In children with steroid sensitive nephrotic syndrome (SSNS), repeated episodes of proteinuria may be the most likely cause of low levels of 25 OH vitamin D as children with SSNS usually have multiple number of relapses.⁷ In present study median number of relapse of nephrotic syndrome was 6.5. It is known that children with active nephrotic syndrome are prone to the development of 25 OH Vitamin D deficiency. Vitamin D deficiency(<20ng/ml) in nephrotic syndrome was first studied by Freundlich et al in 1985 in 16 children with active nephrotic syndrome, in which all children were found to have deficiency of 25 OH Vitamin D.⁹ This group extended these findings and revealed that children in relapse had a mean 25 OH Vitamin D levels of 9ng/ml and during remission these levels improved to a mean of 30ng/ml. Huang et al reported similar findings of normalized 25 OH Vitamin D levels in remission in 25 children with nephrotic syndrome.¹⁰ These findings suggested that 25 OH Vitamin D deficiency in this population may be transient. Since then there are many studies suggesting that 25 OH Vitamin D levels may not normalize completely when children with nephrotic syndrome attain remission. Low levels of 25 OH Vitamin D were also found in patients with nephrotic syndrome in studies conducted by Schmidt-Gayk et al and Offerman et al.^{11,12} In 2005 Weng et al measured, levels of 25 OH Vitamin D levels in children with nephrotic syndrome in remission and it was found that 68% of children had vitamin D levels <20ng/ml and 90% children had <30ng/ml.⁷ In a study conducted by Aggarwal et al in 2016, deficiency of 25 OH Vitamin D was found in 74% of children with nephrotic syndrome and insufficiency was found in 26% of children.¹³ In present study Vitamin D deficiency was present in 18(47.36%) children and insufficiency was present in 12(31.57%) children with nephrotic syndrome. This difference in the findings is because of the new classification of the Vitamin D levels which was proposed in the year 2016 by IAP. In other studies levels of <20ng/ml was taken as deficiency whereas in present study <12ng/ml is taken as deficiency. Total of 30(78.94%) children had Vitamin D levels of <20ng/ml. In a study conducted by Weng et al number of relapses did not affect the concentration of 25 OH Vitamin whereas in a study conducted by Biyikli et al there was a significant difference in 25 OH Vitamin D levels between frequent relapsers and infrequent relapse, which was similar in present study.¹⁵ There was a significant difference in 25OH vitamin D levels between the patients of 1st episode of nephrotic syndrome and frequent relapsers. In this study there was a negative correlation between the totalcumulative duration of steroid exposure and Vitamin D levels, i.e. children with longer duration of treatment with steroids like frequent relapsers and steroid dependant nephrotic syndrome had lower levels of 25(OH) vitamin D. No study has described association between total cumulative duration of steroid therapy and levels of vitamin D. In a study conducted by Bak et al serum calcium levels were low in active nephrotic syndrome and increased after remission, which was related to improvement of the hypoalbuminemia, and when adjusted to hypoalbuminemia serum calcium levels were found to be normal.² Goldstein et al conducted a study among 12 patients with nephrotic syndrome out of which 11(91.66%) had hypocalcaemia.³ In present study also similar results (88.23%) were found. Whereas in a study conducted by Banerjee et al in 2013 serum calcium and phosphate levels were normal in all children with nephrotic syndrome.¹⁴ In a study conducted by Mittal et al also levels of ionized calcium and iPTH were normal in patients with nephrotic syndrome with normal renal function but mean total serum calcium as well as 24 hour

urine calcium was low.¹⁶ In a study conducted by Mehta et al in showed that Serum Calcium levels were significantly lower in frequent relapsers and steroid dependant nephrotic syndrome compared to 1st episode of nephrotic syndrome and infrequent relapsers.¹⁷ This was similar in present study. Goldstein also demonstrated that infusion of parathyroid extracts into patients with nephrotic syndrome elicited a markedly reduced calcaemic response, suggesting that these patients have a skeletal resistance to the calcaemic action of PTH. Several factors may contribute to this abnormality like hyperphosphatemia, uremia and vitamin D deficiency.³ In a study conducted by Gulati et al it was found that children administered repeated (and hence higher) doses of steroids, i.e. frequent relapsers and steroid dependant nephrotic syndrome patients were more often symptomatic, had significantly lower calcium and higher Alkaline Phosphatase levels.⁶ In present study also children with frequent relapses had lower calcium levels. In a study conducted by Mittal et al there was no correlation between serum calcium and 25 OH vitamin D levels, which was not similar in present study.¹⁶ There was moderately significant positive correlation between serum calcium and vitamin D levels, and moderately negative correlation between Serum Phosphate and Vitamin D levels. However, there was no significant correlation between alkaline phosphatase and vitamin D levels in present study. This can be possible as alkaline phosphatase levels are affected by other factors also like liver disease.

VII. Conclusion

Vitamin D deficiency is common in children with nephrotic syndrome even after the remission of proteinuria. There was no significant difference of Vitamin D levels in different age groups and sex. Levels of vitamin D were lower among the patients with higher number of relapses as compared to patient with less number of relapses. Frequent relapsers had lower mean value of Vitamin D levels as compared to infrequent relapsers and 1st episode of nephrotic syndrome. This could be explained due to repeated exposure to corticosteroids in frequent relapsers. Hypocalcaemia was seen in 86.84% of the patients, and there was positive correlation between calcium and Vitamin D levels. Hyperphosphatemia was present in 39.47% of patients. Alkaline Phosphatase was increased in 50% of the patients, but mean levels (156IU/L) were almost normal. This could be due to Alkaline Phosphatase levels may be affected by other factors. Four children had clinical features of hypocalcaemia in form of muscle spasm and muscle cramps. In conclusion, patients with increased duration and number of relapses have more Vitamin D deficiency, hence would benefit from supplements.

References

- [1]. Nelson W, Kliegman R. Nelson Textbook of Pediatrics. Philadelphia, PA: Elsevier; 2016.
- [2]. Bak M, Serdaroglu E, Guclu R. Prophylactic calcium and vitamin D treatments in steroid-treated children with Nephrotic syndrome. *Pediatr Nephrol.* 2006;21:350-4.
- [3]. Goldstein DA, Haldimann B, Sherman D, Norman AW, Massry SG. Vitamin D metabolites and calcium metabolism in patients with nephrotic syndrome and normal renal function. *J Clin Endocrinol Metabol.* 1981;52(1):116-21.
- [4]. Kinra S, Rath B, kabi B. Indirect quantification of lipid peroxidation in steroid responsive nephrotic syndrome. *Arch Dis Child.* 2000;82:76-8.
- [5]. Elsevier Clinical Advisory Board, Clinical Update: Nephrotic Syndrome. 2012.
- [6]. Gulati S, Sharma RK, Gulati K, Singh U, Srivastava A. Longitudinal follow-up of bone mineral density in children with Nephrotic syndrome and the role of calcium and vitamin D supplements. *Nephrology, dialysis, transplantation : Official publication of the European Dialysis and Transplant Association - European Renal Association.* 2005;20(8):1598-603.
- [7]. Weng FL, Shults J, Herskovitz RM, Zemel BS, Leonard MB. Vitamin D insufficiency in steroid- sensitive nephrotic syndrome in remission. *Pediatr Nephrol.* 2005;20:56-63.
- [8]. Leonard MB, Feldman HI, Shults J, Zemel BS, Foster BJ, Stallings VA. Long-term, high-dose glucocorticoids and bone mineral content in childhood glucocorticoid sensitive nephrotic syndrome. *N Engl J Med.* 2004;351:868-75.
- [9]. Freundlich M, Bourgoignie JJ, Zilleruelo G, Jacob AI, Canterbury JM, Strauss J. Bone modulating factors in Nephrotic children with normal glomerular filtration rate. *Pediatr.* 1985;76(2):280-5.
- [10]. Huang JP, Bai KM, Wang BL. Vitamin D and calcium metabolism in children with nephrotic syndrome of normal renal function. *Chin Med J.* 1992;105:828-32.
- [11]. Schmidt-Gayk H, Grawunder C, Tschöpe W, Schmitt W, Ritz E, Pietsch V, Andrassy K, Bouillon R. 25-hydroxy-vitamin-D in nephrotic syndrome. *Lancet.* 1977;310(8029):105-8.
- [12]. Offermann G, Von Herrath D, Schaefer KV. Serum 25-hydroxycholecalciferol in uremia. *Nephron.* 1974;13(4):269-77.
- [13]. Aggarwal A, Yadav AK, Ramachandran R, Kumar V, Kumar V, Sachdeva N, et al. Bioavailable vitamin D levels are reduced and correlate with bone mineral density and markers of mineral metabolism in adults with nephrotic syndrome. *Nephrol.* 2016;21(6):483-9.
- [14]. Banerjee S, Basu S, Sengupta J. Vitamin D in nephrotic syndrome remission: a case-control study. *Pediatr Nephrol.* 2013;28(10):1983-9.
- [15]. Biyikli NK, Emre S, Sirin A, Bilge I. Biochemical bone markers in nephrotic children. *Pediatr Nephrol.* 2004;19(8):869-73.
- [16]. Mittal SK, Dash SC, Tiwari SC, Agarwal SK, Saxena S, Fishbane S. Bone histology in patients with nephrotic syndrome and normal renal function. *Kidney Int.* 1999;55(5):1912-9.
- [17]. Mehta P, Nanda S. Comparison of calcium metabolism in different subgroups of nephrotic syndrome in children. *Indian J Child Health.* 2016;3(3):216-9.