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Original Research Article

Study of prevalence of vitamin D deficiency in nephrotic syndrome

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ABSTRACT

Background: Patients with nephrotic syndrome (NS) lose 25-hydroxyvitamin D in the urine and can have low blood levels of this metabolite. Corticosteroid therapy on long term basis can cause osteoporosis and affects the bone mineral content (BMC) and bone mineral density (BMD) in children. Hence this study was undertaken to study the prevalence of Vitamin D deficiency in children with Nephrotic syndrome.
Methods: It is a time bound prospective hospital based observational study done at Department of Paediatrics, KIMS Hospital, Hubli. A detailed history and clinical examination including anthropometry was taken for cases of Nephrotic syndrome admitted to the hospital. 5ml venous blood was collected and sent for estimation of calcium (Ca), phosphorus (P), Alkaline phosphatase (ALP) and 25(OH) Vitamin D levels.
Results: Mean age of onset of nephrotic syndrome was 4.6 years and median age at study entry was 7 years. Male to female ratio was 1.4:1. Vitamin D deficiency was present in 16(47.05%) children and insufficiency was present in 11(32.35%) children with nephrotic syndrome. Wasting was present in 11.76% and stunting was present in 50% of the children with nephrotic syndrome. There was no statistically significant difference of vitamin D levels with respect to sex and age group. Frequent relapsers had low levels of vitamin D levels as compared to 1st episode and infrequent relapsers. There was moderately significant positive correlation between serum calcium and vitamin D levels and negative correlation between phosphate levels and vitamin D levels.
Conclusions: Vitamin D deficiency is common in children with nephrotic syndrome even after the remission of proteinuria.

Keywords: Bone mineral content, Bone mineral density, Nephrotic syndrome, Vitamin D

INTRODUCTION

Nephrotic syndrome is a disease with primary glomerular abnormality with a relapsing course and usually responds to steroid treatment.[1,2] It is because of alteration of permeability of the glomerular capillary wall, which results in inability to restrict the urinary loss of proteins. Nephrotic syndrome is characterized by massive proteinuria, hypoalbuminemia, hyperlipidemia associated with peripheral edema.[1] Patients with Nephrotic syndrome (NS) lose 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.[3]

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.[2,4,5]

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.[3]

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.[6] Hence this study was undertaken to study the prevalence of Vitamin D deficiency in children with Nephrotic syndrome admitted to Pediatric ward, Karnataka institute of Medical Sciences, Hubli.

METHODS

This was a prospective observational hospital based time bound study.

Inclusion criteria

- All cases of Nephrotic syndrome up to 18 years of age admitted to Department of Paediatrics, KIMS Hospital, Hubli, between 1st December 2015 and 30th November 2016 were included in the study.

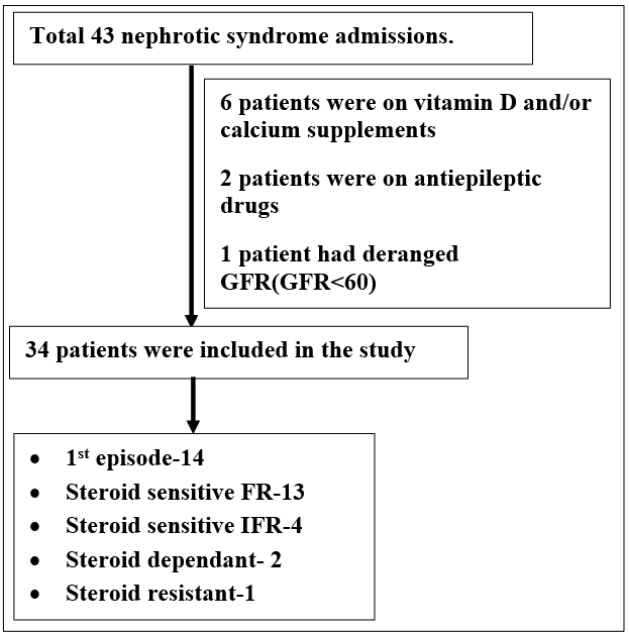
Exclusion criteria

- Children who have taken Vitamin D supplementation in past six months, not have normal GFR, on antiepileptics, with congenital nephrotic syndrome were excluded from the study.

After admission to the hospital, detailed history regarding the present complaints, past history, number of relapses family history and drug history was taken and recorded in a structured proforma. A written informed consent was taken from the parents. Detailed clinical examination including anthropometry was done at the time of collection of samples. Height was measured using stadiometer, length was measured using infantometer. Weight of the patient was recorded using digital weighing scale. Systemic examination was done to rule out other comorbid conditions. 5ml venous blood was collected in plain bottle, centrifuged and serum was sent for estimation of calcium (Ca), phosphorus (P), alkaline phosphatase (ALP) and serum for estimation of vitamin D was collected and stored at -20 centigrade. 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 (Advia Centaur, Seimens).

RESULTS

The present study consist of 43 cases of nephrotic syndrome, that were admitted from 1st December 2015 to 30th November 2016, out of which 34 cases were included in the study and 9 cases were excluded from the study. Out of total 34 patients 14 cases were 1st episode of nephrotic 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. 11 (32.35%) out of 34 children had hypertension (Figure 1).



FR- frequent relapsers, IFR- infrequent relapsers.

Figure 1: Inclusion and analytical sample flow.

20 patients were males and 14 patients were females. Male to female ratio was 1.4:1. 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=34).

Age (years)	No. of patients	Percentage
1-3	7	20.59
4-6	10	29.41
7-9	6	17.65
10-12	9	26.47
13-15	2	5.88

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 to corticosteroid therapy (n=34).

Category		No. of patients	Percentage
Steroid sensitive	1st episode	14	41.17
	Infrequent relapsers	4	11.76
	Frequent relapsers	13	38.24
Steroid dependant		2	5.8
Steroid resistant		1	2.94

Out of 34 children 4(11.76%) children had clinical features of hypocalcemia in form of muscle spasm and muscle cramps. None of the children had fracture. Out of 34 patients 16(47.05%) patients had deficiency of vitamin D levels and 11(32.35%) patients had insufficiency of vitamin D levels and vitamin D levels were normal in 7(20.59%) patients. None of them had toxicity. Mean vitamin D levels was 20.35029 and median vitamin D levels were 12.28ng/ml (Table 3).

Table 3: Vitamin D levels in nephrotic syndrome (n=34).

Vitamin D levels	No.of patients	Percentage
Deficiency (<12ng/ml)	16	47.05
Insufficiency (12-20 ng/ml)	11	32.35
Sufficiency (20-100 ng/ml)	7	20.58
Toxicity (>100 ng/ml)	0	0.00

Vitamin D deficiency was present in 12(60%) males and 4(28.57%) females and insufficiency was present in 6(30%) males and 5(35.71%) females. Normal levels were present in 2(10%) males and 5(35.71%) females. 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=34).

Vitamin D levels	1-5 years	%	6-10 years	%	11-15 years	%
Deficiency	7	46.66	9	64.28	0	0.00
Insufficiency	5	33.33	3	21.42	3	60.00
Sufficiency	3	20.00	2	14.28	2	40.00
Total	15	100.00	14	100.00	5	100.00

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=17).

Vitamin D status	1 to 5 relapses	6 to10 relapses	10 to15 relapses
Deficiency	0	8	2
Insufficiency	2	1	1
Sufficiency	2	1	0
Toxicity	0	0	0
Total	4	10	3
Mean vitamin D levels	28.53ng/ml	12.69ng/ml	10.78ng/ml

f value=8.1629, *p*-value=0.002602.

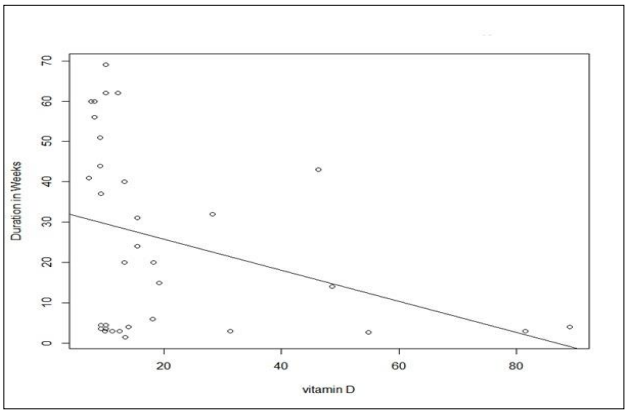
Mean 25 OH vitamin D levels were low in Frequent relapsers (13.87ng/ml) as compared to SDNS/SRNS group (14.69ng/ml), infrequent relapsers (23.25ng/ml) and 1st episode of nephrotic syndrome (26.74ng/ml). There was statistically significant difference of vitamin D levels among the patients in these groups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

Table 6: Association between FR, IR, 1st episode of nephrotic syndrome and SDNS/SRNS and vitamin D levels (n=34).

Vitamin D status	FR (n=13)	1st episode (n=14)	IR (n=4)	SDRN/ SRNS (n=3)
Deficiency	6 (42.85%)	9 (69.23%)	1 (25%)	0 (0%)
Insufficiency	4 (28.57%)	2 (15.38%)	2 (50%)	3 (100%)
Sufficiency	4 (28.57%)	2 (15.38%)	1 (25%)	0 (0%)
Mean vitamin D levels	13.87ng/ml	26.74ng/ml	23.25ng/ml	14.69ng/ml

f value=1.2872, *p*-value=0.02919.

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.

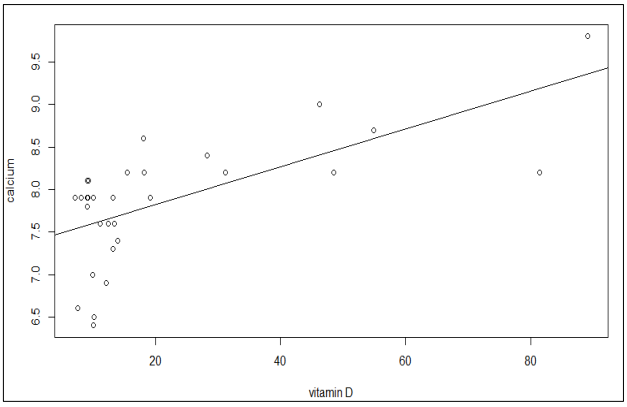
Figure 2: Correlation between cumulative duration of steroid treatment and vitamin D levels.

Out of 34 patients hypocalcemia was found in 30(88.23%) patients, and none of the patients had hypercalcemia. Calcium levels were normal in 4 (11.76%) patients (Table 7).

Table 7: Comparison between vitamin D and calcium (n=34).

Vitamin D levels	Hypocalcaemia	Normal Ca levels
Deficiency	16	0
Insufficiency	10	1
Sufficiency	4	3

None of the patients with normal calcium levels had Vitamin D deficiency. There was moderate correlation between calcium and Vitamin D levels (Figure 3).



Pearson's correlation coefficient=0.6203.

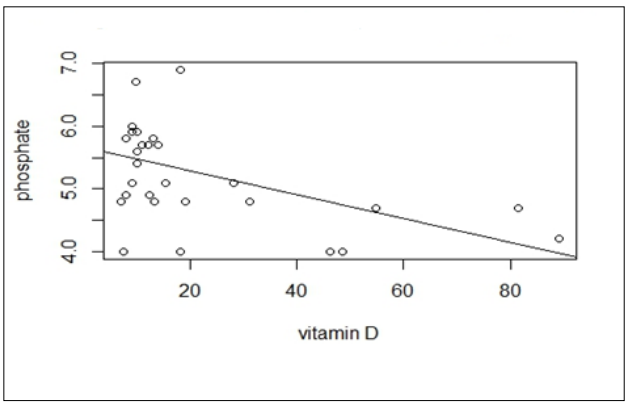
Figure 3: Correlation between calcium and vitamin D levels.

Table 8: Comparison between vitamin D and phosphorous levels (n=34).

Vitamin D levels	Hypophosphatemia	Normal	Hyperphosphatemia	Total
Deficiency	1	4	11	16
Insufficiency	1	5	5	11
Sufficiency	3	4	0	7
Total	5	13	16	34

Hypophosphatemia was present in 5(14.70%) of the patients and 13(38.24%) patients had normal phosphate levels and 16(47.06%) patients had hyperphosphatemia (Table 8).

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



Pearson's correlation coefficient=-0.5149.

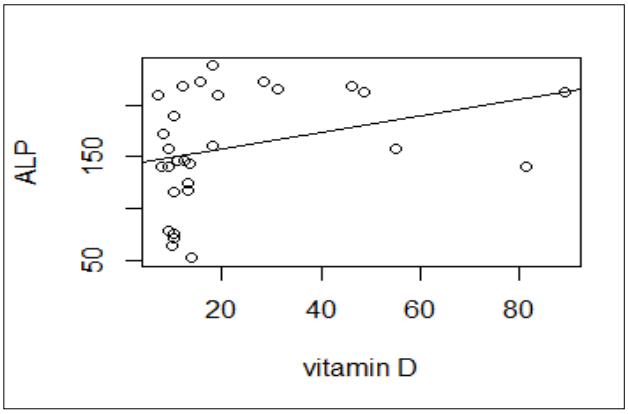
Figure 4: Correlation between phosphate and vitamin D levels.

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	5	11
Insufficiency	6	5
Sufficiency	6	1
Total	17	17

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.

DISCUSSION

This was a hospital based cross sectional descriptive time bound study conducted between 1st December 2015 and 30th November 2016. 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 1.4:1. In this study only 4 (11.76%) 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 17 (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 BMI.[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 16(47.05%) children and insufficiency was present in 11(32.35%) 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 27(79.41%) children had Vitamin D levels of <20ng/ml. Median levels of Vitamin D levels in this study were 12.28ng/ml.

There was no significant difference in 25 OH Vitamin D levels among males and females in a study conducted by Weng et al, whereas levels of 25 OH Vitamin D were higher in males in a study conducted by Aggarwal et al level.[7,13] In present study Mean levels of 25 OH Vitamin D in males was 14.89ng/ml and in females was 28.16ng/ml. In a study conducted by Banerjee et al there was a negative correlation between 25 OH Vitamin D levels and age.[14] In present study there was no such correlation with the age.

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, with mean levels of 25 OH vitamin D levels being 23.25ng/ml and 13.87ng/ml respectively. In this study there was a negative correlation between the total cumulative 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.

CONCLUSION

Vitamin D deficiency is common in children with nephrotic syndrome even after the remission of proteinuria with mean vitamin D levels of 20.35ng/ml. 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 (13.87ng/ml) as compared to infrequent relapsers (23.25ng/ml) and 1st episode of nephrotic syndrome (26.74ng/ml). This could be explained due to repeated exposure to corticosteroids in frequent relapsers. Hypocalcaemia was seen in 88.23% of the patients, with mean calcium levels of (7.83mg/dl) and there was positive correlation between calcium and Vitamin D levels. Hyperphosphatemia was present in 47.06% of patients with mean phosphate levels of 5.28mg/dl which was in normal range. 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.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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