

Status of Metabolic Bone Disease in Pediatric Steroid Resistant Nephrotic Syndrome: Study from North India

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Abstract

Background: Children with steroid resistant nephrotic syndrome (SRNS) are at a greater risk of metabolic bone disease due to biochemical derangements by corticosteroids and immunosuppressant therapy. The present study was undertaken to evaluate the calcium-vitamin D status in children with SRNS.

Method: A cross-sectional case control study was performed to investigate the calcium-vitamin D status in 50 patients of SRNS and 40 healthy controls. Serum levels of 25 hydroxy vitamin D [25(OH) D], calcium, phosphorus, alkaline phosphatase (ALP) and parathyroid hormone (PTH) were estimated. The SRNS patients were further divided into 3 groups according to their Up:Uc ratio: Group A) 16 patients in complete remission, Group B) 14 patients in partial remission and Group C) 20 patients in relapse.

Results: Vitamin D and calcium levels were significantly lower in the SRNS patients ($p < 0.0001$). Lower levels of vitamin D and calcium were found in the relapse phase ($p < 0.01$ and $p = 0.001$). PTH and ALP levels were higher ($p < 0.05$ and $p = 0.001$). Up:Uc ratio with vitamin D and calcium showed a significant negative correlation ($p < 0.01$ and $p < 0.05$) whereas a positive correlation was seen with PTH and ALP ($p < 0.05$ for both).

Conclusion: There is a clear diminution of serum 25 (OH) D in patients with SRNS which reverts rapidly to normal after cessation of proteinuria which may associate with severe nephrotoxicity. Prophylactic therapy with vitamin D should be routinely advocated in these patients.

Keywords: Steroid resistant nephrotic syndrome; Vitamin D; Children; Parathyroid hormone; Alkaline phosphatase; Metabolic bone disease

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Introduction

Idiopathic steroid resistant nephrotic syndrome (SRNS) of children can be defined as a child with nephrotic syndrome who fails to show complete remission of symptoms after 4 weeks of daily therapy with oral prednisolone at a dose of 2 mg/kg/day [1,2]. The incidence of this disease varies from 10-30% [3,4]. Steroid resistance can be grouped into primary resistance in which there is failure of complete remission after treatment during the first time of nephrotic syndrome presentation, whereas in secondary resistance the child initially responds well after which he shows a recurrence of symptoms and fails to completely respond to steroid treatment [5]. Children with SRNS are more prone to

complications due to unremitting course of the disease which tends to progress to end-stage renal disease (ESRD) and due to the side effects of immunosuppressive therapy [1]. Non renal complications are observed due to the marked urinary excretion of albumin and other intermediate sized plasma proteins, resulting in the altered metabolism of many plasma proteins, protein bound substances and certain cellular and tissue proteins. Vitamin D metabolites are relatively hydrophobic and circulate bound to proteins, primarily to the vitamin D-binding protein (VDBP) and to a lesser extent albumin. VDBP is a 59Kda protein that is filtered easily by the nephrotic glomeruli. It has a high affinity for the major circulating vitamin D metabolite 25-hydroxyvitamin D (25(OH) D) and a 10- to 100-fold lower affinity for the vitamin D

hormone 1, 25-dihydroxyvitamin D (1, 25(OH)₂ D) [6]. Suboptimal 25(OH) D concentration due to loss of vitamin D-binding protein may lead to secondary hyperparathyroidism and metabolic bone disease notably osteomalacia. Not only this but the long-term use of glucocorticoids may lead to reduced bone formation mainly by directly acting on the bone cells and also antagonising the actions of parathyroid hormone (PTH) and vitamin D. In a study by Freundlich et al. [7] in 8 children with nephrotic syndrome, hypocalcemia, modest hyperparathyroidism and low calcidiol levels were observed during relapse phase which normalised during remission. In another prospective study by Nielson [8] and his co-workers the prevalence of vitamin D deficiency was observed to be 93% in children with nephrotic syndrome. Children with SRNS have a protracted clinical course and may have long standing proteinuria. Although metabolic bone disease have been recognised in children with nephrotic syndrome, to the best of our knowledge no comprehensive study till date has been done in SRNS patients in a large cohort. Therefore the aim of the present study was to evaluate the calcium - vitamin D metabolism in children with SRNS.

Material and Methods

Study design and participants

The present case control study was conducted by the Department of Biochemistry and Pediatrics, Chacha Nehru Bal Chikitsalya Hospital (CNBC), New Delhi. The study included a total of 50 patients (30 males and 20 females) clinically classified as steroid resistant nephrotic syndrome in the age group of 1 to 12 yrs presenting to the outpatient department and indoor of CNBC along with 40 age matched controls. The study was approved by the scientific committee and informed consent was taken from all patients. All patients were in remission phase or with persistent proteinuria at the time of the study. Diagnosis of nephrotic syndrome was based on generalized edema, proteinuria (Up:Uc>2.0 mg/mg), hypoalbuminemia (serum albumin <2.5 g/dl) and hypercholesterolemia (serum cholesterol >200 mg/dl). Remission was defined as complete with urine albumin nil (or Up:Uc<0.2 mg/mg) or partial with urine albumin trace (or Up:Uc between 0.2-2.0 mg/mg) for 3 consecutive days in early morning specimens. Relapse was defined as urine albumin 3 + or 4+ (or Up:Uc>2.0 mg/mg) for 3 consecutive days in early morning specimens, having been in remission previously. Any patient with secondary nephrotic syndrome, hypertension, macroscopic hematuria or abnormal renal function was excluded from the study. Patients were treated with enalapril, prednisolone, cyclosporine and/or cyclophosphamide. Regrouping of the patients was done according to their urine protein creatinine ratio (Up:Uc). The subjects were divided into three groups A) remission/normal (Up:Uc<0.2 mg/mg) which included 16 patients, B) partial remission/sub-nephrotic (Up:Uc=0.2-2.0 mg/mg) which included 14 patients and C) relapse/nephrotic range (Up:Uc>2.0 mg/mg) which included 20 of the patients. The Inclusion criteria were (1) Age between 1 and 12 years (2) Idiopathic Nephrotic Syndrome (3) Normal serum creatinine. Exclusion criteria included patients with osteogenesis imperfecta, juvenile osteoporosis, history

of calcium and vitamin D supplementation for the preceding 6 months and previous bisphosphonate (anti-resorptive agent) therapy. Each participant underwent a detailed study of history recording followed by a detailed physical examination which included weight, height, blood pressure measurement, quantification of proteinuria, estimation of serum electrolytes, blood urea, serum creatinine, total cholesterol and serum albumin etc. 25 (OH) vitamin D was measured by an ELISA kit from DLD Diagnostika GMBH (Alderhost, Hamburg, Germany). PTH levels were estimated by chemiluminescence method on Access-2 Beckman Coulter analyser. Routine biochemical parameters were estimated on Olympus AU-400 analyser.

Statistical analysis

Statistical Package for Social Sciences (SPSS), version 16 (Chicago,IL,USA) was used for data analysis. All data was expressed as the mean ± standard deviation. The data between SRNS patients and controls was analysed by unpaired student's t-test. SRNS patients were grouped according to their Up:Uc ratio and were compared by 1 way analysis of variance (ANOVA). Correlation between Up:Uc ratio, PTH, Vitamin D and other analytes was done by Pearsons Correlation Coefficient. A p value of less than 0.05 was denoted as statistically significant.

Results

The present study included 50 children with SRNS, amongst which 30 (60%) had minimal change disease, 13 (26%) of the subjects had focal segmental glomerulosclerosis, 5 (10%) were found to have membranoproliferative glomerulonephritis and the remaining 2 (4%) patients had idiopathic membranous nephropathy. As shown in **Table 1**, 30% of the children (n=15) had hypocalcemia at the time of examination (calcium cut off value<8.5 mg/dL). On assessment of their vitamin D status 54% (n=27) of the SRNS children had frank vitamin D deficiency (Vitamin D<30 nmol/L) and the remaining 46% (n=23) were vitamin D insufficient (Vitamin D 30- 75 nmol/L).

Table 2 shows the comparison of demographic and biochemical parameters between the SRNS cases and control. No significant difference in gender or age was observed between the cases and control; however BMI of the SRNS patients was significantly lower than that of controls (p<0.05). Serum creatinine (p<0.01) and total cholesterol (p<0.001) were significantly higher in the SRNS cases than control; on the other hand serum albumin was significantly decreased amongst the cases (p<0.0001). 25(OH) Vitamin D levels (p<0.0001) and total calcium (p<0.001) were

Table 1 Clinical characteristics of the SRNS patient.

Clinical Manifestations	N (%)
Calcium Status	
Hypocalcemia (<8.5 mg/dL)	15 (30%)
Normal Level	35 (70%)
25 (OH) Vitamin D Status	
Vitamin D Deficient (<30 nmol/L)	27 (54%)
Vitamin D Insufficient (30-75 nmol/L)	23 (46%)
Vitamin D Sufficient (>75 nmol/L)	0 (0%)

significantly decreased in the SRNS cases whereas PTH was significantly increased ($p < 0.01$). No significant difference was observed in blood urea, phosphorus and ALP in between cases and control.

At the time of evaluation 16 patients were found to be in complete remission, 14 patients in partial remission and 20 were in relapse phase. Total cholesterol was found to be significantly increased ($p < 0.01$) in the SRNS patients in the relapse phase whereas serum albumin was significantly decreased ($p = 0.000$). 25(OH) Vitamin D and total calcium were lowest in the relapse phase ($p < 0.01$ and $p = 0.001$ respectively), whereas PTH levels were significantly elevated ($p < 0.05$) in the nephrotic phase. No difference in levels of phosphorus was observed in the 3 phases whereas ALP levels showed an increase in the relapse phase ($p = 0.001$) as seen in **Table 3**. In **Table 4**, Up:Uc ratio was correlated with the various

biochemical parameters in the SRNS patients. It showed a significant positive correlation of total cholesterol, PTH and ALP with Up:Uc ratio as $p < 0.001$, $p < 0.05$, $p < 0.05$ respectively. On the other hand albumin, 25-(OH) Vitamin D and calcium showed a significant negative correlation with Up:Uc ratio ($p < 0.0001$, $p < 0.01$, $p < 0.05$ respectively).

Discussion

In our study, 30% of the SRNS children ($n = 15$) were found to have hypocalcaemia. Vitamin D deficiency (Vitamin D < 30 nmol/L) was observed in 54% of our cases ($n = 27$) and insufficiency (Vitamin D = 30-75 nmol/L) was seen in 46% cases ($n = 23$). Similar findings were observed by Malluche et al. [9] in a series of 6 nephrotic syndrome patients in which hypocalcemia was observed amongst 5 of the patients and vitamin D deficiency in all the subjects. Studies by Goldstein et al. [10], Schmidt-Gayk et al. [11], and Barragry et al. [12] reported that patients with nephrotic syndrome had very low levels of 25-hydroxy vitamin D most probably because of loss of this metabolite in urine. Goldstein et al. [10] found that patients with nephrotic syndrome and normal renal function had low blood concentrations of ionized calcium and modest elevations in the blood level of parathyroid hormone (PTH). He suggested that a state of vitamin D deficiency exists in these patients and may underlie the derangements in calcium homeostasis. These were in accordance with our findings, where the mean vitamin D and total calcium levels in the SRNS cases was significantly lower than the controls. PTH level also showed a modest significant elevation in the cases in comparison to controls. Comparison was done amongst the remission, partial remission and relapse groups where vitamin D and total calcium was found to be lowest in the relapse phase whereas PTH and ALP levels were highest in the relapse phase. Phosphorus levels showed no significant change in the three groups. Our findings were in accordance with Freundlich [7] and his co-workers who observed hypocalcemia, modest hyperparathyroidism and low calcidiol levels in a case series of 8 children with nephrotic syndrome. According to him all biochemical changes in nephrotic syndrome are potentially reversible and tend to normalize during the remission period. Our data also supported these findings. Vitamin D plays a pivotal role in the maintenance of calcium and phosphorus metabolism and is actively involved in controlling interactions between the intestine, bone and kidney. Without vitamin D only 10-15% of the dietary calcium is absorbed [13]. In our study all subjects had low levels of vitamin D with 27 subjects suffering from frank deficiency and the remaining 23 had insufficiency.

Children with SRNS are prone to metabolic bone disease because of biochemical derangements as a result of renal disease as well as therapy due to corticosteroids as well as immunosuppressant drugs. Steroids reduce bone formation through decreasing osteoblast number and stimulation and decrease synthesis of matrix constituents. They also increase bone resorption through decreasing serum calcium, osteoprotegerin and adrenal androgens and increase PTH levels [14]. However there are controversial reports regarding bone mineral alteration during

Table 2 Distribution of adherence to treatment of patients ($n = 196$).

Characteristics	SRNS Cases ($n = 50$)	Control ($n = 40$)	p-value
Gender (M/F)	30 (60%)/20 (40%)	20 (50%)/20 (50%)	$p > 0.05$
Age (Years)	7.27 ± 3.31	7.04 ± 3.20	$p > 0.05$
BMI (Kg/m ²)	20.1 ± 1.85	21.0 ± 1.76	$p < 0.05$
Blood Urea	22.9 ± 11.10	19.32 ± 16.56	$p > 0.05$
Serum Creatinine	0.67 ± 0.38	0.45 ± 0.22	$p < 0.01$
Total Cholesterol	230.41 ± 121.61	151.38 ± 30.62	$p < 0.001$
Serum Albumin	3.20 ± 0.98	4.23 ± 0.53	$p < 0.0001$
25 (OH) D	31.3 ± 17.9	61.26 ± 30.35	$p < 0.0001$
PTH	47.9 ± 36.5	31.05 ± 15.23	$p < 0.01$
Total Ca	9.02 ± 1.37	9.98 ± 0.93	$p < 0.001$
Phosphorus	4.50 ± 0.88	4.54 ± 1.46	$p > 0.05$
ALP	209.97 ± 110.65	231.33 ± 115.42	$p > 0.05$

Table 3 Biochemical parameters in the SRNS Cases according to their Up:Uc ratio.

Parameters	Complete remission / Normal (Up:Uc < 0.2) (N=16)	Partial remission / Sub-nephrotic (Up:Uc = 0.2-2.0) (N=14)	Relapse / Nephrotic (Up:Uc > 2.0) (N=20)	p value
Total Cholesterol	158.78 ± 29.5	222.43 ± 150.2	293.31 ± 116.18	$p < 0.01$
Albumin	4.03 ± 0.53	3.30 ± 0.88	2.46 ± 0.74	$p = 0.000$
25 (OH) D	41.6 ± 15.9	31.4 ± 17.5	22.9 ± 16.0	$p < 0.01$
PTH	37.3 ± 15.4	23.6 ± 10.9	58.5 ± 53.9	$p < 0.05$
Calcium	9.9 ± 1.0	9.0 ± 1.6	8.3 ± 0.94	$p = 0.001$
Phosphorus	4.71 ± 0.76	4.53 ± 1.06	4.30 ± 0.83	$p > 0.05$
Alkaline Phosphatase	170.8 ± 69.2	198.9 ± 98.25	268.6 ± 40.5	$p = 0.001$

Table 4 Correlation of Up:Uc ratio with biochemical parameters in SRNS cases.

Parameters	Correlation with Up:Uc ratio	
Albumin	$r = -0.654$	$p < 0.0001$
Total Cholesterol	$r = 0.465$	$p < 0.001$
25 (OH) D	$r = -0.406$	$p < 0.01$
PTH	$r = 0.296$	$p < 0.05$
Calcium	$r = -0.538$	$p < 0.05$
Phosphorus	$r = -0.090$	$p > 0.05$
Alkaline Phosphatase	$r = 0.290$	$p < 0.05$

steroid and immunosuppressant therapy. Bak et al. [15] reported that bone mineral density (BMD) decreased at a rate of $13.0 \pm 4\%$ in a steroid treated group whereas in another study [16] no such effects were observed on the BMD. However several studies suggest that glucocorticoids are not solely responsible for this syndrome and that cyclosporine A (CsA) may also play a role. Cyclosporine A affects the immune system by inhibiting IL-2 production and release and T-lymphocyte activation. The immune system is said to control the bone mineral metabolism at a local level and cyclosporine (CsA) might affect bone [17]. In a study by Basiratnia et al. [18] in Iraq seven NS patients received CsA together with steroid; the changes in their bone were not different with those of the steroid treated group. Although the role of CsA is still controversial, some reports are in favor [19] and others are against the bone demineralization effect of CsA [20]. However studies have shown higher remission rates in children with SRNS when steroid therapy was combined with CsA therapy [21-23]. According to Tune et al. [24], however CsA nephrotoxicity is more severe in SRNS than in steroid dependent NS regardless of the biopsy pattern. Children with NS are vulnerable to the effects of glucocorticoids on bone formation as they grow up. In addition SRNS children may develop prolonged proteinuria and immunosuppressive agents used in treatment may be associated with severe nephrotoxicity. Apart from this

other factors may also contribute to increased bone resorption such as nutritional deficiency, hypoproteinemia, immobilization, and proinflammatory cytokines excessively produced in active inflammation, which triggers excessive osteoclastic activity [25]. Replacement therapy with vitamin D and calcium should therefore be routinely advocated to minimize the deleterious effects of deficiency on body homeostasis. Though our study had the limitations of a small sample size, no follow up of vitamin D status in SRNS subjects and lack of determination of bone mineral density in SRNS subjects, we were still able to establish a relationship between calcium-vitamin D status and proteinuria.

Conclusion

To conclude the normal range of 25-(OH) D levels found in our study agree well with that reported by other groups. There is a clear diminution of serum 25-(OH) D in patients with SRNS which reverts rapidly to normal after cessation of proteinuria. Further studies are required on a larger sample size to ascertain our findings. The role of prophylactic therapy in these patients needs to be evaluated further.

Conflict of Interest

The author(s) confirm that this article has no conflict of interest.

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