Original Article

Vitamin D level in nephrotic syndrome, Factors of impact?

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Abstract

Introduction
Nephrotic syndrome (NS) usually responds to steroid therapy; however, the majority of children relapse, resulting in prolonged and repeated courses of steroid therapy. Vitamin D is a vital component of bone metabolism and calcium homeostasis. Maintenance of adequate levels of vitamin D is recommended to maintain good bone health and other organ systems of NS patients. The study aimed to assess serum 25 (OH) D levels in children with NS, compare its level with healthy age and sex-matched controls and compare its level in different types of NS.

Patients and Methods
It was a cross-sectional study for measuring 25(OH) D levels in 60 patients with NS and 60 apparently healthy children, to be compared as controls. Nephrotic patients were classified as steroid sensitive (20 cases) steroid dependent (20cases), and steroid resistant (20 cases). Serum 25-hydroxyvitamin [25(OH) D] was measured using Enzyme Linked Immune Sorbent Assay (ELISA) technique.

Results

Serum 25(OH) D was significantly lower in NS patients than control group ($p<0.001$).Steroid resistant nephrotic syndrome (SRNS) patient group showed the highest drop than the other 2 groups ($p<0.001$). There was significant negative correlation between vitamin D level and number of relapses, 24 hours urinary proteins and serum cholesterol.

Conclusion
It is recommended that NS patients especially on long term steroid therapy should undergo regular follow up of vitamin D level and early prophylactic supplementation with calcium and vitamin D should be advised.

Keywords
Nephrotic syndrome, Vitamin D.

Running Title Nephrotic Syndrome, Vitamin D

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Introduction

Nephrotic syndrome (NS) is the most common renal disease of childhood with normal renal function. It usually responds to steroid therapy; however, the majority of children relapse, resulting in prolonged and repeated courses of steroid therapy[1].Previous studies have shown that bone mineral density is reduced relatively early in this disorder[2].Prednisone remains the preferred therapy in the children with steroid sensitive NS as long as there is no significant steroid toxicity. However, steroids are known to cause osteoporosis by inhibiting osteoblasts and increasing bone resorption [3].

Children with NS often display a number of calcium homeostasis disturbances causing abnormal bone histology, including hypocalcemia, reduced serum vitamin D metabolites, impaired intestinal absorption of calcium, and elevated levels of immunoreactive parathyroid hormone (PTH). There is also loss of a variety of plasma proteins and minerals in the urine as well as steroid therapy [4].

Vitamin D (which includes both D2 and D3) is not really a vitamin. It behaves like a hormone and carries out essential biological functions through endocrine, paracrine, and intracrine mechanisms [5].Maintenance of adequate levels of vitamin D is recommended, not only to maintain good bone health, but also to provide for its non-osseous functions [6].

Vitamin D plays a crucial role in a large number of physiological functions and its deficiency is associated with many acute and chronic illnesses including disorders of calcium metabolism, autoimmune diseases, some cancers, type 2 diabetes mellitus, infectious diseases and cardiovascular diseases [7]. Some studies have been reported that supplementation with calcium and vitamin D is beneficial in preventing bone loss. The treatment with a high dose of vitamin D3 may correct the abnormalities, which suggests vitamin D3 should be used in children with protracted active NS [8].

The aim of this study was to assess whether NS patients under standard therapy have vitamin D deficiency compared to controls and whether vitamin D status differs according to type of NS (steroid sensitive nephrotic syndrome, steroid dependent nephrotic syndrome and steroid resistant nephrotic syndrome) and therapy.

Patients and Methods

This study is a cross-sectional study conducted at Children Hospital of our university during the period from December 2015 to June 2016, included 60 patients with nephrotic syndrome and 60 apparently healthy children randomly chosen from children coming to general clinic in the children hospital as controls. Written consent to participate in the study was taken from their parents.

Nephrotic syndrome patients were stratified into 3 groups; steroid sensitive nephrotic syndrome (SSNS) [20 patients], steroid dependent nephrotic syndrome (SDNS) [20 patients] and steroid resistant nephrotic syndrome (SRNS) [20 patients]. SSNS is defined as occurrence of remission within six weeks of prednisone at dose of 60 mg/m^2/day, SRNS is defined as failure to develop remission after six weeks of prednisone at dose of 60 mg/m^2/day and SDNS is defined as acquire two consequent relapse during corticosteroid therapy withdrawal or after 14 days after treatment was completed [9].

Patient Group included: nephrotic patients on steroid therapy. Their age ranged from 3 to 16 years old. nephrotic patients associated with other causes of vitamin D deficiency as liver cell disease, obesity, malabsorption, chronic bacterial infection, autoimmune disease, chronic illness, cancer and also patients on drugs that interfere with vitamin D as anticonvulsant drugs, anti-tuberculous drugs, statins and thiazide diuretics were excluded from the study.

All patients were subjected to history taking including; age, gender, age of onset of nephrotic syndrome, current dose and response to steroids ,immunosuppressive drugs, vitamin D and calcium supplementation (if any) and number of relapses and clinical examination including; weight and its percentile, vital signs (including blood pressure),presence of edema and cushinoid features, presence of infection (cellulitis, chest infection, etc.) and any other complications (e.g. thrombosis or renal impairment).

Routine laboratory investigations were recorded including; complete blood count [using automated hematology coulter (Cell Dyne 3700, Abbott laboratories; North Chicago, USA)], serum albumin, total calcium and serum creatinine (measured by standard methods on multi-channel auto-analyzer; Hitachi, Japan), total cholesterol (using Beckman CX5 synchron automated machine) and 24 hours urinary proteins. Renal biopsy results (if done) were recorded. Measurement of 25(OH) D level in serum using Enzyme-linked immune-sorbent assay (ELISA). The kit was supplied by Bioassay Technology Laboratory, China.

Statistical Analysis

Data was collected, checked, revised and inserted into the computer program. Data was analyzed by SPSS statistical package version 17. Excel computer program was used to tabulate the results, and represent it graphically. Quantitative variables were expressed as mean and standard error. Qualitative variables were expressed as count and percent.

One Way ANOVA was used to declare the significant difference between groups at $p<0.05$. Duncan multiple comparison test at $p<0.05$ was used to declare the significant between each two groups. Chi square test used to declare the significant difference in the distribution between groups at $p<0.05$[10].

Results

There was a significant correlation between sex and nephrotic syndrome. Nephrotic syndrome is more common in males (P value=0.018).**(**Table 1) shows demographic data and laboratory investigations of all nephrotic patients.**(**Table 2) shows demographic data of the subgroups of nephrotic patients. There was a statistically significant shorter duration of illness in SSNS in comparison to the other 2 groups (P value=0.025) and also a statistically significant lower number of relapses in SSNS in comparison to the other 2 groups (p-value <0.001). **(**Table 3) shows weight percentiles and steroid treatment among subgroups of nephrotic syndrome. It revealed no significant difference between the 3 subgroups of nephrotic patients regarding weight percentiles but a statistically significant difference was present in steroid treatment pattern between the 3 subgroups (p-value=0.040).

The most common used immunosuppressive drug was cyclosporine, used by 10 patients (40%) while the least drug was mycophenolate mofetil, used by 1 patient (4%). Cyclophosphamide was used by 9 patients (36%) and azathioprine was used by 5 patients (20%). **(**Table 4) shows comparison between the subgroups of nephrotic patients regarding laboratory investigations. There was a statistically significant increase in 24 hours urinary proteins in SRNS group in comparison to the other 2 groups (p-value < 0.001).Renal biopsy was done for 21 nephrotic patients. Fourteen patients (23.3%) showed minimal change nephrotic syndrome (MCNS), 3 patients (5%) showed mesangio-proliferative glomerulonephritis (MPGN) and 4 patients (6.7%) showed focal segmental glomerulosclerosis (FSGS) while 39 patients (65%) did not undergo biopsy.

Figure (1) show comparison between cases and controls regarding vitamin D level which shows a statistically significant decrease in vitamin D level of nephrotic cases in comparison to controls (p-value <0.001). Figure (2) compare vitamin D level between the 3 subgroups of nephrotic syndrome. Mean serum vitamin D level was 13.5 ±4.74, 9.8±2.21and 5.64 ±1.6 ng/dl in SSNS, SDNS and SRNS, respectively. There was a statistically significant lower level of vitamin D level in SRNS group in comparison to the other 2 groups of nephrotic syndrome (p-value < 0.001). Table (5) shows significant negative correlation between vitamin D level and number of relapses, 24 hours urinary proteins and cholesterol with p-value 0.03, <0.001 and 0.008, respectively.

Table 1 Demographic data and laboratory investigations of nephrotic patients

	Mean± SD	**Median**	**Minimum**	**Maximum**
Age	8.54 ± 3.32	9.00	3.00	14.00
Duration of illness (year)	3.74 ± 2.99	3.00	.08	10.00
Age of 1st attack (year)	5.30 ± 2.45	4.00	3.00	11.00
Number of relapses	2.52 ± 2.50	2.00	.00	8.00
Serum creatinine(mg/dL)	0.47 ± 0.20	0.45	0.10	0.90
Serum albumin (mg/dL)	3.55 ± 0.87	3.70	1.10	5.50
24 hrs urinary proteins (g/24h)	1.27 ± 1.86	.23	.02	8.50
Serum cholesterol (mg/dL)	267.06 ± 131.90	231.00	109.00	650.00
Serum calcium (mg/dL)	9.31 ± 0.86	9.00	7.40	11.70
Vitamin D level	16.69 ± 13.64	10.05	4.20	53.40

SD: Standard deviation

Table 2 Comparison between the subgroups of nephrotic patients regarding demographic data

	SSNS		**SDNS**		**SRNS**		**P-value**
	Mean ± SD	**Median**	**Mean ± SD**	**Median**	**Mean ± SD**	**Median**	
Age (year)	7.75 ± 3.01	7.00	9.68 ± 2.77	10.00	9.73 ± 3.34	10.00	0.077
Duration of illness (year)	2.50 ± 2.51	2.00	4.65 ± 2.91	4.00	4.11 ± 3.22	4.00	0.025
Age of 1st attack (year)	5.28 ± 2.62	4.00	5.03 ± 2.22	4.00	5.62 ± 2.60	4.00	0.634
Number of relapses	0.50 ± 0.69	0.00	4.00 ± 2.03	4.00	3.10 ± 2.85	3.00	< 0.001

SSNS: Steroid sensitive nephrotic syndrome, **SDNS:** Steroid dependent nephrotic syndrome
SRNS: Steroid-resistant nephrotic syndrome **SD:** Standard deviation

Table 3 Comparison between the subgroups of nephrotic patients regarding weight percentiles & steroid treatment

		Subgroups of nephrotic						P value
		SSNS		SDNS		SRNS		
		Count	%	Count	%	Count	%	
Weight percentiles	≤25th percentile	7	35.0%	6	30.0%	7	35.0%	0.624
	50th-90th percentile	11	55.0%	12	60.0%	8	40.0%	
	>90th percentile	2	10.0%	2	10.0%	5	25.0%	
Steroid treatment pattern	No treatment	6	30.0%	2	10.0%	0	0.0%	0.040
	Every day	3	15.0%	2	10.0%	6	30.0%	
	Alternate day	11	55.0%	16	80.0%	14	70.0%	

SSNS: Steroid sensitive nephrotic syndrome, **SDNS:** Steroid dependent nephrotic syndrome
SRNS: Steroid-resistant nephrotic syndrome

Table 4 Comparison between the subgroups of nephrotic patients regarding laboratory investigations

	SSNS		SDNS		SRNS		P-value
	Mean ± SD	Median	Mean ± SD	Median	Mean ±SD	Median	
Serum creatinine (mg/dL)	0.50 ± 0.18	0.50	0.40 ± 0.17	0.40	0.52 ± .24	0.50	0.134
Serum Albumin (mg/dL)	3.82 ± 0.74	3.95	3.55 ± 0.55	3.60	3.18 ± 1.11	2.90	0.086
24 hours urinary proteins (g/24h)	0.36 ± 0.77	0.09	1.02 ± 1.91	0.30	2.55 ± 2.00	2.60	< 0.001
Serum Cholesterol (mg/dL)	231.40 ± 113.36	194.00	244.95 ± 76.25	238.00	334.80 ± 171.62	290.00	0.059
Total Calcium (mg/mL)	9.34 ± 0.70	9.40	9.36 ± 0.63	9.00	9.22 ± 1.20	9.00	0.453

SSNS: Steroid sensitive nephrotic syndrome, **SDNS:** Steroid dependent nephrotic syndrome
SRNS: Steroid-resistant nephrotic syndrome **SD:** Standard deviation

Table 5 Correlation between vitamin D level and clinical & laboratory parameters of nephrotic patients

		Vitamin D level
Age (year)	**r-**	- 0.148
	p- value	0.252
Duration of illness (year)	**r-**	- 0.156
	p- value	0.227
Age of 1st attack(year)	**r-**	- 0.030
	p- value	0.818
Number of relapses	**r-**	- 0.276
	p- value	0.030
Weight (kg)	**r-**	- 0.116
	p- value	0.371
Systolic blood pressure(mmHg)	**r-**	0.096
	p- value	0.456
Diastolic blood pressure(mmHg)	**r-**	- 0.111
	p- value	0.390
Serum creatinine(mg/dL)	**r-**	- 0.180
	p- value	0.163
Serum albumin (mg/dL)	**r-**	0.190
	p- value	0.138
24 hrs urinary proteins(g/24h)	**r-**	- 0.457
	p- value	<0.001
Serum cholesterol(mg/dL)	**r-**	- 0.332
	p- value	0.008
Serum calcium(mg/dL)	**r-**	0.217
	p- value	0.090

r- = Correlation Coefficient

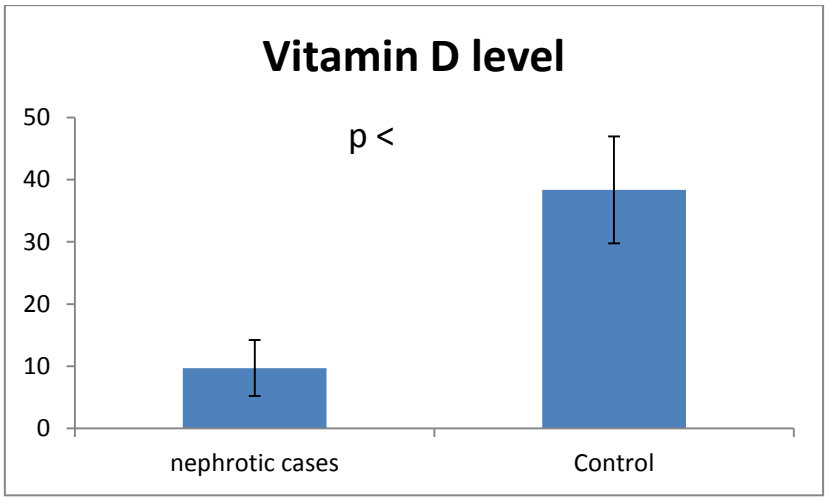

Figure 1 Comparison between nephrotic cases and controls regarding vitamin D level

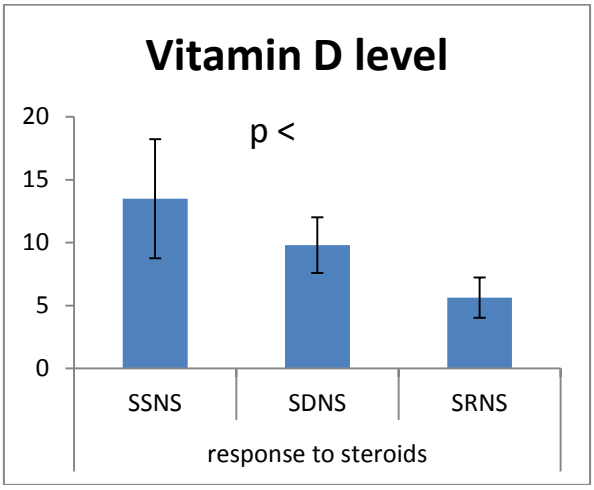

Figure 2 Comparison between the 3 subgroups of nephrotic syndrome regarding vitamin D level

Discussion

Low levels of 25-hydroxycholecalciferol [25 (OH) D] have been documented in NS patients during relapse due to the loss of both 25 (OH) D and its binding protein in urine at this time. However, since most NS relapses are short lasting, these low levels do not reflect the steady state of body stores **[11]**.Vitamin D-binding globulin (DBG), which binds up to 98% of the 25 hydroxy-vitamin D [25(OH) D] and has a molecular weight lower than that of albumin may be lost in the urine causing a low 25 (OH) D **[12].**

The study showed male predominance among nephrotic group (61.7% of the cases) (P=0.018). This was also reported (65%maleincidence) by Banerjee et al **[12].** Rahi et al., **[13]** who also reported male predominance. In our study male patients represented 16 patients (80%) of SSNS, 16 patients (80%) of SDNS and 10 patients (50%) of SRNS. Ghobrial et al., **[14]** found that 85% of the SSNS group, 75% of the SDNS group and 90% of the SRNS group were males. Hammad et al., **[15]** found that 65% of SRNS were males and Shah et al. **[16]** found that 66.7% of their SRNS patients were males. There was no significant difference between the subgroups of nephrotic patients regarding sex (p-value = 0.057). In agreement with our study, in retrospective cross-sectional study done by Situmorang et al., **[17]**which was conducted on 90 patients with nephrotic syndrome, there was no significant difference between sex of both groups (p=0.098).

In our study, age of nephrotic patients ranged from 3 years to 14 years with median of 9 years and mean of 8.54 ± 3.32. Banerjee et al., **[12]** studied 40 patients with NS, their age ranged from 4 years to 10 years with median 6.25 years. In our study, mean age showed no statistically significant difference between groups of nephrotic patients (p=0.077). In agreement with our study, Ghobrial et al., **[14]** found no significant difference between groups of nephrotic patients regarding age (P= 0.42), while Yousefichaijan et al., 2016 **[18]** observed a significant difference between groups of nephrotic syndrome being older in SRNS group (P=0.001). This difference between studies may be due to different number of patients included in each study and different ethnic groups. Another cross-sectional study done by Bennett et al., **[19]** which included children with SRNS (n = 24), SSNS (n = 28), and normal controls (n = 5), showed significant difference between groups regarding age being older in SRNS group (P =0.001).

Age at first attack in this study ranged from 3 to 11 years with median of 9 years and mean of 5.30 ± 2.45. On the contrary, Echeverri et al., **[20]** reported a lower age at first attack which ranged from 7 months to 16 years, with median of 25 months. This difference between studies may be due to different ethnic group. This study showed no statistically significant difference between groups of NS regarding age of first attack (P=0.634). On the contrary, Ghobrial et al., **[14]** found statistically significant lower age of onset of NS in SDNS group as compared to the other 2 groups (P=0.03).Weight of included patients showed no significant difference between the 3 subgroups (P= 0.624), which coincides with the same finding of Ghobrial et al., 2013 **[14].**

The study showed a statistically significant lower duration of illness in SSNS in comparison to the other 2 groups (P=0.025). On the contrary, Ghobrial et al., **[14]** found no significant difference between the 3 groups regarding duration of illness (P=0.89). There was significant higher number of relapses in SDNS group (P<0.001) compared to the other 2 groups. Similarly, Alt et al., **[21]** reported significantly higher relapse rate in SDNS (P=0.026).

In our study, 24 hours urinary proteins ranged from 0.02 to 8.5 g/24 hours with mean value of 1.27±1.86 g/24 hours. A descriptive study included 30 patients with nephrotic syndrome found that 24 hrs urinary proteins ranged from 2.33 to 5.2 g/24 hrs with the mean value of 3.28±2 g/24 hrs **[22]**.In our study there was significant higher level of 24 hours urinary proteins in SRNS compared to the other 2 groups (P<0.001). In agreement with our study, Ghobrial et al., **[14]** found a significant difference between the 3 groups in the 24-hour urinary protein levels being highest in the SRNS group (p-value = 0.001).

Regarding serum cholesterol in our patients, it ranged between 109 and 650 mg/dL with mean 267.06 ±131.9 mg/dL. A cross-sectional study in which 30 normal individuals and 30 patients with nephrotic syndrome was evaluated by Chandra and Kishore, **[23]**, regarding serum lipid profiles. They showed that the level of total cholesterol was significantly higher in the nephrotic patients. In our study, there was no statistically significant difference between subgroups of

nephrotic patients regarding serum cholesterol (p-value =0.059). On the contrary, Yousefichaijan et al. [18] observed that high range of cholesterol was observed in SRNS group more than other groups (FRNS, SDNS and SSNS) (p-value =0.001). Another study was done by Ghobrial et al., [14] reported that there was significant difference between the three groups in serum cholesterol being higher in the SRNS group (p-value <0.001). This is may be due to poor control of SRNS or frequent use of immunosuppressive drugs.

Regarding serum calcium in our patients there was no significant difference between the subgroups of nephrotic syndrome (p-value =0.543). Another cross-sectional study was carried out by Mehta and Nanda, [24]on 30 children diagnosed as nephrotic syndrome divided into 3 groups (SSNS, FR/SDNS and patients in remission) found that serum calcium levels were significantly lower in patients in SDNS as compared to SSNS and patients in remission (p-value <0.01).

In our study, serum creatinine and serum albumin showed no significant difference between subgroups of nephrotic syndrome (p-value = 0.134 and 0.086, respectively). Similar findings were presented by Situmorang et al., [17] who found that difference in serum creatinine and serum albumin were statistically not significant between infrequent and frequent relapsing groups. On the contrary, Ghobrial et al., [14] reported that there was statistically significant higher serum creatinine level (p = 0.05) and statistically lower serum albumin (P<0.001) in SRNS compared to the other two groups.

In our study, vitamin D deficiency was found in 96.7% (58/60) of the nephrotic patients and there was significant lower 25 hydroxycholecalciferol [25(OH)D] level in nephrotic cases in comparison to controls (P <0.001). The same finding was reported in a prospective study conducted by Nielsen et al., [25] that was done on 14 nephrotic patients at Denmark and showed vitamin D deficiency in 93% (13/14) of the patients. On the contrary, a cross-sectional case-control study was performed by Banerjee et al., [12] to investigate 25 hydroxycholecalciferol [25(OH) D] level in 40 patients with NS in remission and 40 healthy controls. The level of 25(OH) D was not statistically different between the NS group and the control group (p-value =0.447). This variation between studies may be due to the different number of patients included in each study or included patients might be in remission on sampling. .Ethnic factor may be also responsible.

This study showed a statistically significant low vitamin D level in SRNS group in comparison to the other 2 groups of nephrotic syndrome (p-value <0.001).The same finding was reported in a cross-sectional study done by Weng et al., [26] who measured 25(OH) D in children with SRNS, SSNS and healthy controls and showed marked vitamin D deficiency in SRNS than SSNS and controls. A more recent cross-sectional study by Bennett et al., [19] attributed the more pronounced vitamin D deficiency in SRNS to the increased loss of urinary vitamin D binding protein (uVDBP) in comparison with SSNS ,as it showed that uVDBP was significantly higher (P<0.001) in patients with SRNS than in both patients with SSNS and controls.

In our study, there was a significant negative correlation between vitamin D level and number of relapses (p-value = 0.03). Similar finding was observed by Banerjee et al., [12]who investigated 25 hydroxycholecalciferol [25(OH)D] status in 40 patients with NS in remission and 40 healthy controls, in which vitamin D level was correlated with number of relapses (p-value=0.012). In our study there was a significant negative correlation between vitamin D level and 24 hours urinary proteins (p-value<0.001). In agreement of our study, a prospective study done by Nielsen et al., 2015 [25]showed that there was significant negative correlation between vitamin D level and 24 hours urinary proteins (p-value = 0.018).

Limitation in this study

The relatively small number of patients included in the study and lack of follow up of the patients.

Conclusion

Nephrotic syndrome patients especially on long term steroid therapy, high rate of relapses and massive proteinuria, should undergo regular assessment of vitamin D level. Early prophylactic supplementation with calcium and vitamin D is recommended with regular follow up of its level as increase in the dose is recommended according to patient needs.

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Statements

Ethics approval and consent to participate

This study protocol and consents were approved and deemed sufficient by the ethical scientific committee of the Cairo University hospital and was conducted in accordance with the University bylaws for human research. It conforms to the provisions of the Declaration of Helsinki in 2000. Informed written consent was obtained in every case from their legal guardians.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Conflict of interest

The authors declare no conflict of interest relevant to this article.

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