Vitamin D status is insufficient in the majority of children at diagnosis of nephrotic syndrome

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ABSTRACT

INTRODUCTION: Children with nephrotic syndrome (NS) are treated for at least 12 weeks with high doses of prednisolone, which may be harmful to the bones. Vitamin D deficiency is also harmful to the bones.

METHODS: This was a prospective study of consecutive children with first episode of NS at the time of their diagnosis before treatment with glucocorticoids. The following plasma levels were measured: 25-hydroxy-vitamin-D (25(OH)D), albumin, ionised calcium, phosphate, parathyroid hormone (PTH), alkaline phosphatase and creatinine. The glomerular filtration rate (GFR) was estimated from the Schwartz formula, and only patients with normal values were included.

RESULTS: A total of 14 children were examined, 13 (93%) had 25(OH)D deficiency including 12 (86%) with moderate or severe vitamin D deficiency. The plasma 25(OH)D was positively associated with plasma albumin ($p = 0.031$) and negatively with PTH ($p = 0.003$), phosphate ($p = 0.016$) and body mass index percentile ($p = 0.022$). PTH was negatively associated with albumin ($p = 0.019$) and the estimated GFR ($p = 0.007$), and positively associated with phosphate ($p = 0.008$), 24-h urine protein/m^2 ($p = 0.018$) and systolic blood pressure percentiles ($p = 0.048$).

CONCLUSION: The vitamin D status was insufficient in 93% of the patients. We suggest that vitamin D status in children with NS be measured routinely at the time of diagnosis so that an individual treatment strategy for vitamin D deficiency can be given. Further studies are needed to evaluate the effect of such treatment.

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Nephrotic syndrome (NS) in children is steroid-responsive in the vast majority of cases and the widely accepted treatment is high-dose prednisolone (60 mg/m^2/day, maximum 80 mg/day) for6 weeks followed by 6 weeks of alternate-day prednisolone (40 mg/m^2) [1, 2]. The risk of experiencing relapses is more than two out of three [1]. Relapses are treated with daily prednisolone and non-responsive cases with other kinds of immunosuppressive therapy [1, 2]. Totally, children with NS are treated for a long period with doses of prednisolone far exceeding 5 mg daily, which caused a reduction in bone mineral density and a rapid increase in the risk of fracture in a meta-analysis of adults [3]. For adults treated with a daily dose of prednisolone above 5 mg, early use of preventive measures against glucocorticoid-induced osteoporosis is recommended [3]. Steroids cause osteopenia and osteoporosis by inhibiting osteoblasts, stimulating bone resorption [3, 4] and contributing to a negative calcium balance [5].

Vitamin D deficiency is also harmful to the bones. Rickets and osteomalacia are seen on radiologic images showing cortical thinning of long bones, stress fractures and metaphyseal widening and fraying [6].

During NS, vitamin D-binding globulin (DBG), which binds up to 98% of the 25-hydroxy-vitamin D (25(OH)D) [7] and has a molecular weight lower than that of albumin [4], may be lost in the urine causing a low 25(OH)D [4, 8].

The Danish Paediatric Society recommends that children who are treated with prednisolone for NS receive 0.5 l milk and 10 microgram vitamin D a day prophylactically [1]. Furthermore, The Danish Paediatric Society recommends that children older than 12 months with severe vitamin D deficiency are treated with 125 micrograms of vitamin D daily for three months and then with 10-20 micrograms daily for another three months [9], which is in accordance with the Lawson Wilkins Paediatric Endocrine Society, which also states that paediatricians in general should adopt a low threshold for examining vitamin D status [6].

The aim of this study was to measure 25(OH)D at the time of diagnosis in the first episode of NS in order to study vitamin D deficiency in this special population. To our knowledge, there are no previous reports of such measurements.

METHODS

Patients and setting

Included in this prospective study were consecutive patients at the Department of Paediatrics, Hvidovre Hospital, Denmark, at the time of diagnosis of their first episode of NS, defined as hypoalbuminaemi (< 25 g/l), proteinuria (> 1 g/m^2/24 h) and oedema. The patients underwent measurements of 25(OH)D, parathyroid hormone, phosphate, alkaline phosphatase and ionised calcium. The study period ran from 1 January 2010 to 15 June 2014. Every patient participated once.

Excluded were patients treated with medicines

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ABBREVIATIONS

BP = blood pressure
BMI = body mass index
DBG = vitamin D binding globulin
GFR = glomerular filtration rate
NS = nephrotic syndrome
$(OH)_2D$ = dihydroxy-vitamin D
PTH = intact parathyroid hormone

known to affect the vitamin D metabolism, apart from vitamin D and calcium supplementation, in accordance with the Danish dietary reference intake for age and ethnicity [10], as well as patients with a reduced glomerular filtration rate (GFR), GFR < 90 ml/minute/1.73 m^2, as estimated by the Schwartz formula.

Clinical evaluation

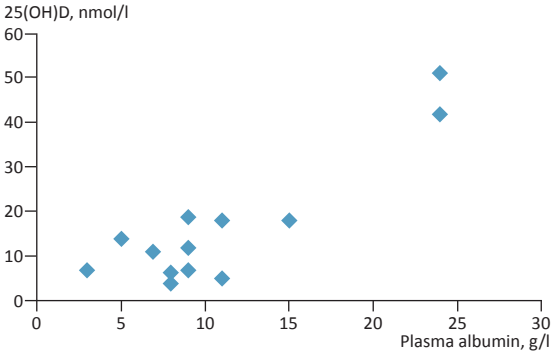
At the time of diagnosis of NS, weight and height were measured with a Wireless Column Scale (Secagm bh & co., Germany) and a Harpenden Stadiometer (Holtain Limited, Kymych Dyfed, Britain), respectively. Body surface was estimated from the Du Bois´ nomogram. Body mass index (BMI) was calculated and evaluated and the values compared to national reference data [11]. The blood pressure (BP) was measured at the time of diagnosis in accordance with the recommendations from the American Academy of Paediatrics, and the levels of the systolic BP were transformed into BP percentiles [12].

Paraclinical methods

All paraclinical components were analysed at the Department of Clinical Biochemistry, Hvidovre Hospital. Until July 2013, the total plasma concentration of 25(OH)D was measured in ten patients by Liaison (DiaSorin) and thereafter in four patients by Cobas e601 (Roche Diagnostics). The results of 25(OH)D measurements in these four patients were 23, 8, < 8 and 5 nmol/l, respectively. The values were therefore not transformed as the two different methods give similar results in the low concentration area. The results of 25(OH)D measurement were in two cases < 10 nmol/l and in one case < 8 nmol/l, which were transformed into 7 nmol/l and 5 nmol/l, respectively, in the analyses and in **Figure 1**. Vitamin D deficiency was defined as 25(OH)D < 50 nmol/l and further categorised as mild (25-50 nmol/l), moderate (10-25 nmol/l) or severe (< 10 nmol/l) [6, 9].

FIGURE 1

Concentrations of 25-hydroxy-vitamin-D (25(OH)D) and plasma albumin in children with first episode of nephrotic syndrome at the time of diagnosis.



The intact parathyroid hormone (PTH) was assayed on the Roche immunoassay platform by Cobas e601 (Roche Diagnostics). Plasma concentrations of albumin, creatinine, phosphate and alkaline phosphatase were measured by photometric analysis by Cobas c501 (Roche Diagnostics), and the 24-h urine protein was measured by turbidemetric analysis by Cobas c501 (Roche Diagnostics). Ionised calcium was measured by ion selective electrodes (Konelab), and the estimated GFR was calculated by the Schwartz formula.

Statistical analyses

Numerical outcomes are given in medians and ranges. The Spearman rank test was used to calculate the statistical significance with a double-sided p-value and a significance level below 0.05 indicating statistical significance. The associations were analysed using the IBM SPSS Statistics 19 software.

Trial registration: not relevant.

RESULTS

Results are shown in **Table 1**. Included were 14 children, eight of whom were boys. The median age was 3.4 years (range: 0.9-11.6 years). With regard to associated diagnoses, two children had Schönlein-Henochs Purpura and two had asthma, one of whom was treated with 200 microgram budesonide twice daily. At the time of diagnosis, five children received daily vitamin tablets with ten microgram vitamin D. Ethnically, eight children were Caucasian (57%), four Pakistani, one Filipino and one Turkish. The time of diagnosis occurred during the spring for seven children, during the summer for two children, during the autumn for one child and during the winter for four children.

Vitamin D deficiency was found in 93% (13/14) of the patients; 86% (12/14) had moderate or severe vitamin D deficiency. The patient with normal vitamin D had a 25(OH)D of 51 nmol/l. The median P-albumin was 9 g/l (range: 3-24 g/l). The median 24-h urine protein was 3.8 g/m^2/24-h (range: 1.2-7.1 g/m^2/24 h). The PTH was elevated in three patients, and alkaline phosphatase was

TABLE 1

Data on 14 consecutive children with first episode of nephrotic syndrome at time of diagnosis.

Patient no.	Sex	Age, yrs	Ethnicity	Plasma albumin, g/l	Urine protein, g/m²/24 h	Plasma 25(OH) D, nmol/l	Plasma PTH, pmol/l	Plasma Ca^{2+}, mmol/l	Plasma phosphate, mmol/l	Estimated GFR, ml/min/1.73 m²	Vitamin D supplement
1	F	2.8	Caucasian	9 ↓	2.7 ↑	< 10 ↓	–	–	–	217	Yes
2	M	2.6	Pakistani	3 ↓	3.3 ↑	< 10 ↓	–	1.19	1.98	165	Yes
3	M	2.8	Filipino	15 ↓	2.5 ↑	18 ↓	4.2	1.17	1.26	121	–
4	M	6.9	Caucasian	9 ↓	1.2 ↑	12 ↓	3.8	1.26	–	126	–
5	M	5	Caucasian	9 ↓	3.7 ↑	12 ↓	4.3	1.33	1.56	151	Yes
6	F	8	Caucasian	24 ↓	1.2 ↑	51	2.7	1.32	1.44	171	–
7	F	2.6	Caucasian	7 ↓	4.4 ↑	11 ↓	5.2	1.22	1.59	104	–
8	M	11.6	Caucasian	9 ↓	5.2 ↑	19 ↓	4.3	1.28	1.75	128	–
9	M	3.8	Pakistani	5 ↓	3 ↑	14 ↓	5.3	1.24	1.59	116	–
10	M	10.2	Caucasian	24 ↓	3.8 ↑	42 ↓	4	1.24	1.18	129	–
11	M	2	Caucasian	11 ↓	6.2 ↑	23 ↓	4.7	1.3	1.53	175	Yes
12	F	3	Pakistani	8 ↓	5 ↑	8 ↓	7.5 ↑	1.19	1.67	103	–
13	F	6.3	Pakistani	8 ↓	4.1 ↑	5 ↓	7.5 ↑	1.26	1.65	94	–
14	F	0.9	Turkish	11 ↓	7.1 ↑	< 8 ↓	7.2 ↑	1.33	1.81	106	Yes

F = female; GFR = glomerular filtration rate; M = male.
↓ = decreased. ↑ = increased.

elevated in one patient. The phosphate and ionised calcium were normal in all patients. The median estimated GFR was 128 ml/min/1.73 m² (range: 94-217 ml/min/1.73 m²).

The 25(OH)D associated positively with plasma albumin (p = 0.031), Figure 1, and negatively with PTH (p = 0.003), phosphate (p = 0.016) and BMI percentile (p = 0.022). The PTH associated negatively with albumin (p = 0.019) and estimated GFR (p=0.007), and positively with phosphate (p = 0.008), 24-h urine protein/m² (p = 0.018) and systolic BP percentiles (p = 0.048). Systolic BP percentiles were negatively associated with estimated GFR (p = 0.030).

Furthermore, creatinine associated positively with age (p = 0.002), weight (p = 0.019), height (p = 0.003) and body surface (p = 0.005), and associated negatively with estimated GFR (p = 0.026).

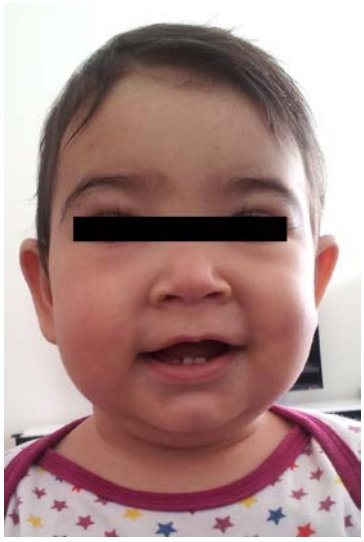
DISCUSSION

At the time of diagnosis, 93% of the present children with NS had vitamin D deficiency, and the 25(OH)D was positively associated with plasma albumin. No data were influenced by treatment with glucocorticoids, since the measurements were done before initiation of treatment and since it was the patients' first episode of NS. Previously, this positive association between 25(OH)D and albumin was reported in 16 children with NS, of whom six were treated with prednisolone [13].

The 25(OH)D was negatively associated with PTH, but a secondary hyperparathyroidism was present as a sign of severe vitamin D deficiency only in 21% of the cases. The regulating role of PTH may also be seen in the association between PTH and albumin, 24-h urine protein/m², estimated GRF, phosphate and systolic BP percentiles, respectively. To our knowledge, these associations have not previously been reported in children with a first episode of NS at the time of diagnosis.

The associations in the present study indicate the following sequence: A nephrotic patient looses albumin and DBG in the urine and gets a low vitamin D status. The gastrointestinal calcium uptake is reduced; serum calcium tends to decrease and PTH and phosphor increase. A high PTH level increases conversion of 25(OH)D into $1,25(OH)_2D$. When the 25(OH)D is low, plasma calcium is maintained by increased osteoclastic activity, which reduces the mineral content of the bones. This sequence further accelerates when treatment with glucocorticoid is initiated, which also leads to an increased loss of calcium in the urine [14].

In Denmark, vitamin D deficiency due to lack of sunlight is common [15], but the natural seasonal changes in 25(OH)D in Denmark did not affect the results, since the patients had vitamin D deficiency measured throughout the year. However, worldwide low levels of vitamin D status may be more important in NS than previously thought. In a study from Istanbul, 25(OH)D was also decreased in 20 children with NS and a normal estimated GFR. It was the first attack for four children and a relapse for 16 children. The 25(OH)D was significantly lower in the patients than in the healthy control subjects both before and after the high-dose glucocorticoid treatment [14]. Furthermore, in a study from South Florida, 25(OH)D was low in all 16 children and the bone mineral content was less than 90% of normal in the tested pa-



A ten-month-old girl at time of diagnosis of nephrotic syndrome. We suggest also measurement of the vitamin D status at the time of diagnosis of nephrotic syndrome, so that individual treatment strategy of a vitamin D deficiency may be given.
Photo: *Susanne Østergaard*, Hvidovre Hospital. The parents have accepted that the picture is published as part of the paper.

tients at the time of diagnosis of NS; however, six patients received glucocorticoids at the time of examination. All had a normal estimated GRF [13].

Besides lack of sunlight, pigmentation of the skin, diet and BMI are important factors affecting the vitamin D status, the latter because of the fat-soluble characteristics of the vitamin. The children in this study had a median BMI percentile of 80 at the time of diagnosis, and Vitamin D was associated with BMI percentile. Treatment with prednisolone will initially lead to loss of oedema and secondly to weight gain caused by fat accumulation [1]. This fat accumulation may decrease 25(OH)D further [6].

Supplementary vitamin D may quickly improve the 25(OH)D status in patients with NS. In adults with NS, vitamin D was normalised 48 h after treatment with 200 microgram of vitamin D daily [16], and in another study it was normalised within 1 month in eight of nine patients after treatment with 25 microgram vitamin D daily [17]. It was recently reported that among adult patients with non-dialysis chronic renal failure and albuminuria, treatment with vitamin D decreased the albumin in the urine [18]. Furthermore, vitamin D modulates the B- and T-lymphocyte function [6].

Based on the present study, we suggest that children with a first episode of NS may benefit from measurement of vitamin D status at the time of their diagnosis, so an individual strategy for treatment with vitamin D may be provided in order to avoid the potential harmful effects of vitamin D deficiency. Altered bone and vitamin D status were previously thought to occur only in children with long-standing, massive proteinuria, like those with Finnish-type congenital NS or treatment-resistant NS [19]. In 2005, however, it was reported that children with steroid-sensitive NS, which constitutes the vast majority of childhood idiopathic NS [1], had a lower 25(OH)D than healthy children. These patients were in remission for at least 14 days prior to the date of the study visit. It was suggested that children with remitted steroid-sensitive NS may benefit from routine measurement of 25(OH)D [19]. Recently, it was suggested to test 25(OH)D repeatedly to guide the dose-adjustment of vitamin D in children with glucocorticoid treated glomerulopathies [20]. The suggestion was based on a study of 55 children with glomerulopathies and normal estimated GFR, where the majority were treated with oral glucocorticoid and/or other immunosuppressive medications at the time of analysis. It appeared that 75% had a 25(OH)D below 50 nmol/l and 38% had decreased spinal bone mineral density despite the fact that a significant number of the children were treated with calcium and/or vitamin D preparations to avoid these conditions [20]. There are several strengths to this study: its prospective, consecutive design; the fact that patients were not influenced by the treatment with glucocorticoids at the time of measurement as it was the first episode of NS and all patients had a normal GFR. Furthermore, our suggestion that vitamin D should be measured at the time of diagnosis of NS is in accordance with the general guideline on measurement of vitamin D status when in doubt [6] and it does not cause further blood samplings. Our suggestion is also in line with previous suggestions for measurement of vitamin D in children with remitted steroid-sensitive NS [19] and with glucocorticoid-treated glomerulopathies [20].

A limitation of our study is that $1,25(OH)_2D$ was not measured. It has been reported that $1,25(OH)_2D$ was normal in 12 of 14 children with NS even though 25(OH)D was reduced or low and the bone mineral density was reduced [13]. However, the 25(OH)D had more effect on bone mineralisation than $1,25(OH)_2D$ in both uremic and nutritional osteomalacia [13]. Finally, we included a limited number of patients, representing only a Danish town area.

Further studies are important to demonstrate if treatment for vitamin D deficiency affects the bone mineral density and maybe also reduces the amount of albumin in the urine in children with NS.

CONCLUSION

We found that 93% of the children in a Danish population with a first episode of NS had vitamin D deficiency at the time of their diagnosis. Based on our results and the literature, we suggest that children may benefit from routine measurement of their vitamin D status at the time of diagnosis of NS for the first time or at relapse, so an individual strategy for treatment with vitamin D can be given in order to avoid the potentially harmful effects of vitamin D deficiency.

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