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Original Article

Calcium and vitamin D supplementation in children with frequently relapsing and steroid-dependent nephrotic syndrome

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Abstract

Background Children with frequently relapsing and steroid-dependent nephrotic syndrome (FRNS/SDNS) are at risk for osteoporosis due to impaired metabolism of calcium and vitamin D.

Objective To determine the effect of calcium and vitamin D supplementation on bone mineral density, serum ionized calcium levels and serum 25-hydroxy-vitamin D levels in children with FRNS and SDNS.

Methods A clinical trial with a before and after design was performed. Subjects were SDNS or FRNS pediatric patients ≥ 5 years of age. Subjects received 800 mg elemental calcium and 400 IU vitamin D supplementation for 8 weeks. Serum ionized calcium, serum 25-hydroxy-vitamin D [25(OH)D], and bone mineral density (BMD) were determined before and after the supplementation.

Results Of the 30 subjects, 28 completed the study. However, only 20 subjects underwent BMD determination before and after supplementation. Of the 28 subjects, 22 had hypocalcemia and 26 had low vitamin D levels. Osteopenia was found in 14/20 subjects and osteoporosis was in 2/20 subjects. After 8 weeks of supplementation, mean serum ionized calcium increased from low [1.15 mmol/L (SD 0.03)] to normal [1.18 mmol/L (SD 0.04)] ($P<0.001$) levels, but mean serum 25(OH)D only increased from vitamin D deficiency category [20 ng/mL (SD 7.7)] to vitamin D insufficiency category [25.5 ng/mL (7.7)] ($P=0.010$). Mean *z*-score BMD increased from -1.1 (SD 0.9) to -0.7 (SD 0.2) after supplementation ($P<0.001$).

Conclusion Calcium vitamin D supplementation effectively increased serum ionized calcium, serum 25(OH)D, and BMD in subjects with FRNS and SDNS. **[Paediatr Indones. 2012;52:16-21]**.

Keywords*: frequent relapse nephrotic syndrome, steroid dependent, supplementation, calcium, vitamin D, bone mineral density, osteoporosis*

Patients with nephrotic syndrome (NS), a glomerular disease, usually respond to steroid treatment, but may experience relapses. Of those who achieve remission, 60-70% will experience relapses and 50% of those with relapses will become frequent relapsers.[1,2] Steroid dependency will occur in 50% of frequent relapsers.[2] Treatment of FRNS and SDNS is challenging because children with these conditions must receive high-dose and long-term steroid therapy. Steroids are known to cause osteoporosis and affect bone mineral density (BMD) in NS patients.[3-6] During relapse, the condition is thought to worsen, due to frequent loss of vitamin D metabolites and calcium in the urine.[7,8] The disease itself and the treatment medication affects skeletal mineralization. Diminished bone mineralization has been reported in children with NS tested by densitometry methods.[5,9]

Calcium and vitamin D play an important role in maintaining bone health.[10,11] Studies on the role of calcium and vitamin D supplementation in NS

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patients are limited. However, longitudinal studies by Gulati *et al*.[12] showed that calcium and vitamin D supplementation may improve the BMD in NS patients. Randomized, controlled clinical trials by Bak *et al*.[13] concluded that calcium and vitamin D supplementation can reduce the incidence of low BMD in NS patients.

Although the deteriorative effect of steroid treatment on children's bones has been well known for years, no recommendation has been suggested for the prevention or treatment of diminished BMD in children with NS. There have been few Indonesian studies to date evaluating the role of calcium and vitamin D supplementation in FNRS and SDNS patients. This study was conducted to determine the role of calcium and vitamin D supplementation on BMD, serum ionized calcium levels, and serum 25(OH)D levels in children with FRNS and SDNS.

Methods

A clinical trial with a before and after design was performed in the Nephrology Outpatient Care Unit, Department of Child Health, Cipto Mangunkusumo Hospital, Jakarta from November 2010 until April 2011. Subjects were SDNS or FRNS children with normal renal function ≥ 5 years of age. Exclusion criteria were osteogenesis imperfecta, juvenile osteoporosis, history of calcium and vitamin D supplementation for the preceding 6 months, and previous bisphosphonate (anti-resorptive agent) therapy.

We consecutively recruited 30 subjects who fulfilled the study criteria, but only 28 completed the study. These subjects consisted of 16 FRNS patients and 12 SDNS patients. Only 20 subjects underwent BMD determination before and after supplementation. NS was defined by edema, massive proteinuria (> 40 mg/m^2/hr), hypoalbuminemia (<2.5 g/dl), and hyperlipidemia. Frequent relapse was defined as ≥ 2 relapses within the first 6 months after presentation or ≥ 4 relapses within any 12 month period. Steroid dependency was defined as two consecutive relapses while under steroid treatment or relapse at least 14 days after steroid withdrawal.

History of illness was obtained from each subject, including disease onset, duration of illness, number of total relapses, steroid therapy duration, and cumulative steroid dose. Urea and creatinine levels were also tested. Subjects received 800 mg elemental calcium and 400 IU vitamin D supplementation daily for 8 weeks. Serum ionized calcium, serum 25(OH)D, and BMD were determined before and after supplementation. Serum ionized calcium was measured by Ciba-Corning Model 288 blood gas analyzer® (normal range 1.17-1.29 mmol/L) and serum 25(OH)D_3 by Elecsys Vitamin D3 (25-OH) 2010 cobas e411®, using electrochemiluminescence immunoassay (ECLIA).

BMD of the lumbar spine (L_1-L_4) was measured by dual energy x-ray absorptiometry (DEXA) Lunar GE Medical Systems®, and the patients' data were expressed as *z*-scores (number of SDs from the mean values of healthy Asian children matched for sex and chronological age). A lumbar spine BMD *z*-score of between -1.0 and -2.0 was classified as osteopenia, while a *z*-score of less than -2.0 was classified as osteoporosis.

Data was analyzed using SPSS for windows version 17.0 program, with $P<0.05$ considered as significant. Study approval was obtained from the Ethics Committee, Faculty of Medicinem, University of Indonesia.

Results

We recruited 28 subjects for our study, consisting of 16 FRNS and 12 SDNS patients. There were 21 boys and 7 girls (3:1) aged 5-14.7 years. Gender, age, age at onset of illness, body weight, body height, nutritional status based on body mass index (BMI), and blood pressure between the two groups were comparable. Subjects with SDNS had greater cumulative steroid dose compared to that of FRNS subjects. All subjects had normal renal function (**Table 1**).

Overall, 22/28 subjects were hypocalcemic (serum ionized calcium level <1.17 mmol/L). Twenty-six subjects had low serum vitamin D levels, with 10 subjects classified to be in a state of insufficiency (serum 25(OH)D at 20 - 30 ng/mL) and the other 16 subjects to be in a state of deficiency (serum 25(OH) D at < 20 ng/mL). There were no differences in

Table 1. Subjects' characteristics

Characteristic	FRNS (n = 16)	SDNS (n = 12)
Gender		
Male, n	11	10
Female, n	5	2
Median age, years (range)	8.3 (5.1-14.5)	6.8 (5-13.7)
Median onset, years (range)	3 (1.8-8.6)	2.6 (1-7.8)
Median duration of illness months (range)	64 (30-131)	35.5 (11-138)
Mean total no. of relapses (SD)	12.2 (6)	11 (4.5)
Median steroid therapy duration, months (range)	34.5 (18-89)	25 (9-62)
Mean cumulative steroid dose, mg (SD)	14,943 (7,137)	20,780 (27,937)
Mean body weight, kg (SD)	30 (8.8)	23.8 (10.4)
Mean body height, cm (SD)	125 (13.6)	115 (15.3)
Median BMI, kg/m2 (range)	19.8 (13.8-23.5)	17.1 (13.1-21.5)
Nutritional status (based on BMI)		
Undernourished, n	0	1
Well-nourished, n	9	8
Overweight, n	1	2
Obese, n	6	1
Blood pressure		
Mean systolic, mmHg (SD)	104 (11)	107 (12)
Mean diastolic, mmHg (SD)	72 (10)	72 (7)
Median urea, mg/dL (range)	17 (12-34)	16.5 (9-26)
Mean creatinine, mg/dL (SD)	0.43 (0.12)	0.37 (0.1)

SD: standard deviation

calcium status or vitamin D status between the two groups (**Table 2**).

Of the 20 subjects who underwent bone densitometry, 14/20 had osteopenia (z-score BMD between –1.0 and –2.0) and 2/20 had osteoporosis (z-score BMD < -2.0) (**Table 3**). **Table 4** shows the mean serum ionized calcium levels, mean serum 25(OH)D levels and BMD before and after 8 weeks of supplementation. Mean serum ionized calcium increased from low [1.15 mmol/L (SD 0.03)] to normal [1.18 mmol/L (SD 0.04)] (P <0.001). However, mean serum 25(OH)D only increased from the category of vitamin D deficiency [20 ng/mL (SD 7.7)] to insufficiency [25.5 ng/mL (SD 7.7)] (P=0.01), possibly indicating a suboptimal duration of supplementation. Mean z-score BMD increased from the category of osteopenia [-1.1 (SD 0.9)] to normal [-0.7 (SD 0.2)] after supplementation (P<0.001).

Table 2. Calcium and vitamin D status before supplementation

Criteria	FRNS (n = 16)	SDNS (n = 12)	P
Calcium status			0.159$^{\Phi}$
Hypocalcemia	13	9	
Normal	3	3	
Vitamin D status			0.719$^{\Psi}$
Normal	2	0	
Insufficiency	5	5	
Deficiency	9	7	

$^{\Phi}$ Chi-square test $^{\Psi}$ Kolmogorov-Smirnov test

Table 3. Bone mineral density in FRNS and SDNS patients before supplementation

Criteria	FRNS (n = 10)	SDNS (n = 10)	P
Bone mineral density			0.975$^{\Psi}$
Normal	2	2	
Osteopenia	7	7	
Osteoporosis	1	1	

$^{\Psi}$ Kolmogorov-Smirnov test

Discussion

Disorders of mineral metabolism may occur in NS patients with normal kidney function, including hypocalcemia and hypovitaminosis D.[14] Hypocalcemia in NS patients may be due to loss of calcium in the urine, decreased calcium absorption in the intestine, and steroidal side effects.[8,15] As a consequence, BMD may decrease, leading to the risk of fracture.[14]

Table 4. Serum ionized calcium, serum 25(OH)D and BMD before and after supplementation in FRNS and SDNS groups

Parameter by group	Before supplementation	After supplementation	P
FRNS (n=16)			
Mean ionized calcium, mmol/L (SD)	1.15 (0.02)	1.19 (0.04)	0.042*
Mean 25(OH)D, ng/mL (SD)	20.8 (8.5)	27.4 (6.8)	0.021*
SDNS (n=12)			
Mean ionized calcium, mmol/L (SD)	1.15 (0.03)	1.18 (0.04)	<0.001^
Mean 25(OH)D, ng/mL (SD)	18.9 (6.7)	22.8 (8.4)	0.217*
FRNS and SDNS (n=28)			
Mean ionized calcium, mmol/L (SD)	1.15 (0.03)	1.18 (0.04)	<0.001*
Mean 25(OH)D, ng/mL (SD)	20 (7.7)	25.5 (7.7)	0.010*
FRNS (n=10)			
Mean *z-score* BMD L1-4 (SD)	-1.2 (1)	-0.7 (1.3)	0.325*
SDNS (n=10)			
Mean *z-score* BMD L1-4 (SD)	-1 (0.7)	-0.8 (0.9)	0.018^
FRNS and SDNS (n=20)			
Mean *z-score* BMD L1-4 (SD)	-1.1 (0.9)	-0.7 (0.2)	0.003^

*Paired t-test; ^Wilcoxon test

Clinical manifestations of hypocalcemia vary from asymptomatic to convulsions, depending on the duration, severity, and rapidity of the hypocalcemia. Diagnosis should not depend on the ionized calcium level at one random examination.[16] Signs and symptoms of hypocalcemia usually occur if the ionized calcium level is < 1.1 mmol/L and rapid intravenous correction is indicated if the level is < 0.8 mmol/L.[17]

In our study, 22/28 subjects were hypocalcemic (serum ionized calcium < 1.17 mmol/L), but no subjects had < 1.1 mmol/L serum ionized calcium. Furthermore, all subjects with hypocalcemia were asymptomatic. Similar to our results, Nurmalia *et al.*[18] reported the proportion of hypocalcemia in FRNS and SDNS subjects to be 16/22. We observed that the pre-supplementation mean serum ionized calcium was 1.15 mmol/L (SD 0.03), similar to the results of Nurmalia *et al.*[18] [1.14 ng/mL (SD 0.04)].

Serum 25(OH)D tends to decrease in NS patients due to the loss of vitamin D-binding protein in urine. Vitamin D plays an important role for maintenance of calcium and phosphorus metabolism, involved in controlling interactions between the intestine, bone, and kidney. The active form of vitamin D ($1,25(OH)_2D$) has several functions: stimulating the absorption of calcium and phosphorus in the intestine, calcium mobilization from bone, and stimulating calcium reabsorption at the distal renal tubules (parathormone-dependent).[19] Without vitamin D, only 10 to 15% of dietary calcium is absorbed.[20]

We found that 26/28 subjects had low levels of vitamin D, with 10 subjects in a vitamin D insufficiency state and 16 subjects in a deficiency state. Nurmalia *et al.*[18] reported the proportion of vitamin D insufficiency to be 10/22 and vitamin D deficiency to be 9/22. Our subjects had mean serum 25(OH)D level of 20 ng/mL (SD 7.7), while Nurmalia *et al.*[18] reported a similar mean serum 25(OH)D of 21.32 ng/mL (SD 6.65).

Two subjects had osteoporosis but none experienced fractures. Children with NS, especially those who are frequent relapsers or steroid-dependent, are exposed to the risk of reduced BMD, and thus have a greater risk of fracture or inadequate peak bone mass attainment.[16] These conditions may occur due to biochemical and metabolic disturbances caused by loss of protein and minerals in urine during the nephrotic stage.[7,8]

In addition to calcium and vitamin D levels, side effects of steroid therapy are another important factor affecting bone health. Steroids are the mainstay of therapy for NS patients. It is uncertain when the most rapid bone loss occurs, but it is thought that the highest decrease occurs in the first 6-12 months of steroid therapy. Several studies showed that the extent of the decrease of bone mass correlated with the dose and duration of therapy. The greater dose and the longer duration of steroid therapy, the greater the bone loss. Therefore, patients with NS, particularly FRNS and SDNS, have a higher risk of

impaired BMD.[15,21] Risk factors for reduced BMD are cumulative steroid dose >500 mg, steroid exposure >12 months, prednisone dose >7.5 mg/day, and ≥4 courses of high-dose prednisone.[22]

We found osteopenia in 14/20 subjects and osteoporosis in 2/20 subjects. Nurmalia *et al.*[18] reported osteopenia in 4/22 subjects and osteoporosis in 5/22 subjects. In a study on 100 subjects with FRNS, SDNS, infrequent NS (IFNS), and steroid-resistant NS (SRNS), Gulati *et al.*[12] found that 61% had osteopenia and 22% had osteoporosis. In contrast Leonard *et al.*[22], Mishra *et al.*[23], and Shouman *et al.*,[24] found that children with NS had BMDs not significantly different from healthy children. Our subjects had mean BMD z-score of -1.1 (SD 0.9), which was included in the osteopenia category, similar to results of Nurmalia *et al.*[18] Compliance in our study was good in both groups (82.2 % and 80.1%).

Two previous studies examined the role of calcium and vitamin D supplementation in NS patients. In a study by Gulati *et al.*,[12] 88 subjects with FRNS, SDNS, IFNS, and SRNS received fixed doses (500 mg calcium carbonate equivalent to 200 mg elemental calcium and 200 IU vitamin D) per day for 6-12 months. They reported that the mean spinal BMD values were significantly better on follow-up (0.607±0.013 g/cm2) as compared to baseline values (0.561±0.010 g/cm2) (P<0.0001).[12] Bak *et al.*[13] examined the role of calcium and vitamin D supplementation in IFNS and new NS patients. They were given 1000 mg elemental calcium and 400 IU vitamin D for 8 weeks. BMD was significantly decreased in both the treatment and non-treatment groups, but the percentage of BMD decrease was found to be significantly lower in the treatment group than in the non-treatment group (4.6±2.1% vs. 13.0±4.0%, respectively; P<0.001).

Side effects due to the use of calcium and vitamin D supplementation have been rarely reported. The maximum safe dose of vitamin D is 2000 IU/day,[25] while that of elemental calcium is 2500 mg/day.[26] Vitamin D intoxication is defined as the occurrence of hypercalcemia (>2.65 mmol/L) and increased level of 25(OH)D (>150 ng/mL).[27] Hypercalcemia causes symptoms such as anorexia, nausea, vomiting, polyuria, weakness, constipation, abdominal pain, headache, arrhythmia, and bradycardia.[28] No side effects were reported by our subjects after 8 weeks of calcium and vitamin D supplementation.

A limitation of this study was the design used (before and after treatment) because we had no control subjects who did not receive supplementation. Another limitation was the lack of analysis of dietary intake of calcium and vitamin D. Compliance rate was also obtained by history-taking only and thus may result in inaccurate information.

In conclusion, we observed that supplementation with 800 mg elemental calcium and 400 IU vitamin D daily for 8 weeks, effectively increased serum ionized calcium levels, serum 25(OH)D levels, and BMD in NS patients. Since 25(OH)D levels were not adequately increased, further randomized, controlled trials with longer treatment duration and more subjects are needed. No side effects were associated with the supplementation.

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