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Original Article

Effect of calcium and vitamin D supplementation on serum calcium level in children with idiopathic nephrotic syndrome

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Abstract

Background Patients with idiopathic nephrotic syndrome (NS) may develop hypocalcemia caused by low levels of albumin and vitamin D-binding protein, which subsequently decreases calcium absorption in the intestine. Hypocalcemia may result in neuromuscular manifestations, such as Chvostek's and Trosseau's signs.

Objectives To evaluate the effect of calcium and vitamin D supplementation on hypocalcemia and its clinical manifestations in idiopathic NS children.

Methods A randomized, single-blind, controlled trial was performed in idiopathic NS patients aged 1−14 years. Subjects were divided into treatment and placebo groups. Subjects in the treatment group received 800 mg elemental calcium and 400 IU vitamin D supplementation, while they in control group received placebo syrup, both for 8 weeks. Serum calcium and manifestations of hypocalcemia were examined before and after supplementation.

Results Thirty subjects completed the study (15 in each group). Seventeen subjects experienced hypocalcemia. Chvostek's and Trosseau's signs were observed in 6 subjects in the treatment group and 2 subjects in the placebo group (P= 0.427). After 8 weeks of intervention, Chvostek's and Trosseau's signs disappeared in both groups, and calcium levels were significantly increased in both groups compared to the levels before intervention. However, there was no significant difference in serum calcium levels after 8 weeks between the treatment and placebo groups (P =0.707).

Conclusion Normalization of serum calcium levels and improved clinical manifestations of hypocalcemia occur both in NS patients who receive calcium and vitamin D supplementation and those who do not. **[Paediatr Indones. 2014;54:162-7.]**

Keywords: *idiopathic nephrotic syndrome, supplementation, calcium, vitamin D, hypocalcemia*

Calcium is an important element found in the body.[1] Approximately, 40% of calcium in the body binds to proteins, especially albumin and globulin.[1] Calcium absorption in the intestine depends on vitamin D level.[2] Patients with nephrotic syndrome (NS) may have hypocalcemia, due to hypoalbuminemia, loss of vitamin D-binding protein in the urine, and the use of steroid therapy.[3] Several studies have reported hypocalcemic states in children with NS.[4-7] Low levels of calcium may lead to neuromuscular, cardiovascular, mental, and bone formation disorders.[8,9] Tetany is characteristic of neuromuscular disorders due to hypocalcemia.[10] Symptoms may range from mild (perioral numbness, paresthesias, and muscle cramps) to severe (carpopedal spasm, laryngospasm, and focal or generalized seizures). The classic physical examination findings in patients with latent tetany include Trousseau's and Chvostek's signs.[10]

There have been limited studies on the effect of calcium and vitamin D supplementation on serum

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calcium levels and the clinical manifestations of hypocalcemia in NS patients. A study reports that NS patients who received calcium and vitamin D supplementation had significantly increased calcium ion levels and improved bone mineral density.[6] Another study in India shows a significant increase of serum calcium level, both in the group who were given calcium and vitamin D supplementation, and group who were not given the supplementation, in the first attack and infrequent relapse NS patients.[11]

We aimed to evaluate the effect of calcium and vitamin D supplementation on serum calcium levels and clinical manifestations of hypocalcemia in idiopathic NS patients.

Methods

This randomized, single-blind, controlled trial of parallel design with repeated measurements was performed from August 2012 to March 2013. Subjects were patients with idiopathic NS active disease, defined as patients having their first attack, infrequent relapsers, or frequent relapsers. Subjects had normal renal function as estimated by glomerular filtration rate (GFR) based on the Schwartz formula.[12] Children were aged 1−14 years and visited the Pediatrics Department of Dr. Hasan Sadikin Hospital, Bandung or Kota Bandung District Hospital. We excluded children with a history of calcium and vitamin D supplementation for 2 months prior to enrollment, severe malnutrition, or severe clinical manifestations of hypocalcemia, such as severe tetany, seizures, muscle spasms of the larnyx, and arrhythmias.

Nephrotic syndrome is defined by edema, massive proteinuria (>40 mg/m^2/hr or dipstick method ≥2+), hypoalbuminemia (<2.5 g/dL), and hyperlipidemia. Infrequent relapse was defined as <2 relapses within the first 6 months after presentation or <4 relapses within a 12-month period. Frequent relapse was considered to be ≥2 relapses within the first 6 months or ≥4 relapses within a 12 month period. Remission was defined as negative proteinuria for 3 consecutive days. Chvostek's sign was defined as twitching of the facial muscles produced by tapping on the facial nerve located 0.5 to 1 cm below the zygomatic process of the temporal bone, 2 cm anterior to the ear lobe, and on a line with the angle of the mandible. Trousseau's sign was defined as adduction of the thumb, flexion of the metacarpophalangeal joints, extension of the interphalangeal joints, and flexion of the wrist occurring after three minutes of inflation of a sphygmomanometer cuff above systolic blood pressure.

All subjects were treated for NS in accordance with the consensus treatment of idiopathic NS in children issued by the Nephrology Coordination Task Force of the Indonesian Pediatric Society.[13] We allocated subjects into groups using a permuted-block randomization method. The treatment group received 800 mg elemental calcium and 400 IU vitamin D supplementation and the control group received a placebo in the form of a syrup, both daily for 8 weeks. Serum calcium and clinical manifestations of hypocalcemia were examined at the initiation and completion of the study. Creatinine levels were measured at the onset of the study. Both creatinine and serum calcium were measured by *Modular P800* (normal range for creatinine was 0.7−1.2 mg/dL and for serum calcium was 8.4−11.0 mg/dL). Clinical manifestations of hypocalcemia was defined by Chvostek's and Trousseau's signs.

Two-way ANOVA test was used to analyze the changes in serum calcium levels at different points of time, and the influence of treatment mode. McNemar's test was used to compare the clinical manifestations of hypocalcemia. Data was analyzed using the Statistical Package for Social Sciences software (SPSS Inc., Chicago, IL, USA) version 17. A P value of less than 0.05 was considered to be statistically significant. This study was approved by the Ethics Committee of the Faculty of Medicine, University of Padjadjaran.

Results

Thirty subjects (21 boys and 9 girls) aged 1−14 years enrolled and completed this study. Descriptive characteristics of all subjects are presented in **Table 1**. Age, gender, onset of illness, duration of illness, number of relapses, duration of steroid use, nutritional status, creatinine levels, serum calcium levels, serum calcium status, and type of nephrotic syndrome were similar between the two groups. Mean age at onset of illness was 75.9 months in the treatment group and 73.6 months in the placebo group. Mean duration of steroid

use in the treatment and placebo groups were 358.73 days and 216.07 days, respectively.

At the beginning of the study, 17 out of 30 subjects experienced hypocalcemia. At the end of the study, the serum calcium levels increased in both the treatment [8.09 (1.43) to 9.16 (0.74) mg/dL] and placebo [8.3 (0.97) to 9.16 (0.8) mg/dL] groups **(Table 2)**.

Serum calcium levels were significantly higher in all subjects after 8 weeks than at the onset of the study (Factor-A; P=0.001). However, there were no significant differences in serum calcium levels between the calcium and vitamin D supplementation group and the placebo group after 8 weeks (Factor-B; P=0.707) **(Table 2)**.

At the onset of the study, there were 6/15 in the treatment group and 2/15 in the placebo group experienced either Chvostek's or Trousseau's signs (P= 0.427), and all such patients had serum calcium levels ≤7.4 mg/dL. At the end of the study, none of

Table 1. Subjects' characteristics

Characteristics	Treatment group (n=15)	Placebo group (n=15)
Age, months		
Mean (SD)	87.8 (35.7)	81 (43.18)
Range	29–148	27–156
Age at onset of illness, months		
Mean (SD)	75.9 (30.3)	73.6 (41.2)
Range	29–148	19–144
Gender, n		
Male	9	12
Female	6	3
Duration of illness, days		
Mean (SD)	360.27 (667.14)	224.4 (369.82)
Range	7–2,160	3–1,440
Number of relapses		
Mean (SD)	1 (1.41)	0.8 (1.01)
Range	0–4	0–3
Duration of steroid therapy, days		
Mean (SD)	358.73 (668.02)	216.07 (372.49)
Range	0–2,160	0–1,440
Nutritional status, n		
Well-nourished	13	15
Overweight	2	-
Mean creatinine levels (SD), mg/dL	0.4 (0.12)	0.38 (0.17)
Mean serum calcium levels (SD), mg/dL		
First attack	7.48 (1.44)	7.81 (0.56)
Infrequent relapsers	9.40 (1.00)	9.03 (0.66)
Frequent relapsers	8.92 (0.96)	8.72 (1.41)
Serum calcium status, n		
Hypocalcemia	7	10
Normal	8	5
Type of nephrotic syndrome, n		
First attack	9	8
Infrequent relapse	1	3
Frequent relapse	5	4

Table 2. Serum calcium levels at onset and 8 weeks of study in both groups

Serum Ca^{2+} level / Factor-B (Treatment)	A_1 Mean serum Ca^{2+} at study onset (SD), mg/dL	A_2 Mean serum Ca^{2+} at 8 weeks (SD), mg/dL
B_1 (Treatment group)	8.09 (1.43)	9.16 (0.74)
B_2 (Placebo group)	8.30 (0.97)	9.16 (0.8)

Note: Two-way ANOVA: Factor-A: P=0.001; Factor-B: P=0.707

the patients had either sign **(Table 3).** No side effects of calcium and vitamin D supplementation were found. Remission of NS occurred in 13 subjects of the treatment group and 12 subjects of the placebo group (P=1.00) **(Table 4).**

achieve remission with proteinuria resolution, serum calcium levels usually return to normal.[15]

There were no significant differences in serum calcium levels (P=0.707) between the supplementation and placebo groups after 8 weeks of treatment.

Table 3. Clinical manifestations of hypocalcemia at the onset and at 8 weeks of study

Parameter	Treatment group (n=15)	Placebo group (n=15)	P value
Neuromuscular signs (Trosseau's or Chvostek's)			
At study onset, n			
(+)	6	2	
(-)	9	13	0.427*
After 8 weeks, n			
(+)	-	-	
(-)	15	15	

* McNemar's test

Table 4. Outcome disease in both groups after 8 weeks of study

Parameter	Treatment group (n=15)	Placebo group (n=15)	P value
Remission, n	13	12	1.00*
No remission, n	2	3	

* Fisher's exact test

Discussion

Calcium levels in nephrotic syndrome patients may decrease due to several mechanisms: hypoalbuminemia, decreased calcium absorption in the intestine due to loss of vitamin D-binding protein in the urine, and the use of steroid therapy.[3] Two studies reported that low albumin levels in NS patients were asssociated with low levels of ionized and serum calcium.[4, 7] A study reported low levels of 25 hydroxy-vitamin D (25 (OH)D) in children with frequent relapse and steroid-dependent NS.[6] We found that 17/30 subjects experienced hypocalcemia at the onset of the study (**Table 1**). Similarly, other studies reported that hypocalcemia is found at a high incidence among NS patients.[4, 6, 7]

There was a significant increase in serum calcium levels after 8 weeks in both groups. Therefore, we cannot conclude that calcium and vitamin D supplementation affected the alteration in serum calcium levels. One explanation may be that remission usually occurs in 95% of idiopathic NS patients with minimal change disease (MCD) after an 8-week course of steroid therapy.[14] Also, in patients with idiopathic NS who

The lack of difference in serum calcium levels between the two groups maybe because we only assessed serum calcium level at 8 weeks of study. At this end point, the majority of patients had achieved remission. As such, we should assess the serum calcium level at least twice during the study in order to further discern an effect of calcium and vitamin D supplementation. In idiopathic NS patients, serum calcium level is low, in accordance with the decrease in abumin level, but returns to normal after remission is achieved which usually occurs after 8 weeks.[14,15] Steroid therapy can also affect serum calcium levels in NS patients.[16] Steroids may inhibit calcium absorption by lowering the expression of a specific protein transporter in the duodenum.[17] A study in NS patients showed that after the 4th and 12th weeks of steroid treatment, serum calcium levels were significantly lower than those at the initiation of therapy.[16]

Hypocalcemia can lead to clinical manifestations related to neuromuscular, cardiovascular, and mental disorders, as well as disturbances in bone formation.[8, 9] Tetany is a common clinical manifestation associated with neuromuscular disorders.[10] Trousseau's and Chvostek's signs are the physical findings

in patients with latent tetany.[10] At the onset of our study, we found that 8/30 subjects had Trousseau's and Chvostek's signs, and there was no statistical difference between both groups. At 8 weeks of study, there were no Chovstek's or Trosseau's signs in either group. All subjects who experienced Trousseau's and Chvostek's signs at the beginning of the study had serum calcium levels $\geq$7.4 mg/dL at 8 weeks of study. Since clinical manifestations of tetany usually occur if serum calcium level falls between 7.0 to 7.5 mg/dL, [10] this may explain our findings. In the future, we suggest assessing the improvement of clinical manifestations of hypocalcemia at least twice during the study to observe if more rapid clinical improvement occurred due to calcium and vitamin D supplementation.

There were 25/30 subjects with complete remission of NS. Nephrotic syndrome with minimal change disease (MCD) shows good response to steroid therapy, as more than 70% patients achieve remission.[18]

Side effects that may occur due to calcium and vitamin D supplementation include anorexia, weight loss, weakness, fatigue, disorientation, vomiting, dehydration, polyuria,

constipation, fever, chills, abdominal pain, and renal dysfunction.[19] No side effects were reported during this study.

A limitation of our study is the lack of dietary analysis to assess dietary intake of calcium and vitamin D. Also, the assessments on serum calcium levels and improvement of hypocalcemia clinical manifestations were performed only at 8 weeks of study, and should have been done at least twice to assess for an earlier effect of calcium and vitamin D supplementation.

In conclusion, normalization of serum calcium levels and improved clinical manifestations of hypocalcemia occur both in NS patients who receive calcium and vitamin D supplementation and those who do not. Good management of NS patients leads to improved serum calcium levels, with or without supplementation.

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