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Original Article

Comparisons of bone mineral density in steroid dependent, frequent relapse, and infrequent relapse nephrotic syndrome children

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Abstract

Background Children with nephrotic syndrome, especially those with steroid dependent and frequent relapse are at greater risk of reduced bone mineral density (BMD).

Objective To determine bone mineral density (BMD) in steroid dependent and frequent relapse compared to infrequent relapse nephrotic syndrome.

Methods We conducted a cross-sectional study at the Child Health Department, Cipto Mangunkusumo Hospital, from August until November 2009. Subjects were 5 to 18 year-old children with steroid dependent nephrotic syndrome (SDNS), frequent relapse nephrotic syndrome (FRNS), or infrequent relapse nephrotic syndrome (IRNS). Ionized calcium level, vitamin 25(OH)D_3 level, and BMD were measured using dual energy x-ray absorptiometry (DEXA).

Results 11 SDNS and 11 FRNS children (group I) were compared with 22 IRNS children (Group II). Children of SDNS and FRNS had significantly longer duration of illness, more relapses, longer steroid therapy duration, and greater cumulative steroid dose compared to group II (IRNS). There were no differences between the two groups with regard to mean ionized calcium level and vitamin 25(OH)D_3 level. Children in group I had lower *z*-scores compared to group II, but the difference was not statistically significant [mean (SD) -1.182 (1.21) vs -0.795 (1.25), $p=0.305$]. Subgroup analysis showed that SDNS children had lower *z*-scores than FRNS [-1.791 (1.17) vs -0.573 (0.94), $p=0.019$] and IRNS [-1.791 (1.17) vs -0.795 (1.25), $p=0.026$].

Conclusion Children with SDNS have significantly lower BMD *z*-scores compared to those with FRNS and IRNS. **[Paediatr Indones. 2010;50:193-8]**.

Keywords *bone mineral density, steroid dependent nephrotic syndrome, frequent relapse, infrequent relapse.*

Almost 90% children with idiopathic nephrotic syndrome achieve remission when treated with initial standard treatment of International Study for Kidney Diseases in Children (ISKDC) which consists of 4-week full dose and 4-week alternate dose of prednisone. Those who achieve remission in the first 4 weeks, so called steroid sensitive nephrotic syndrome (SSNS), 60-70% will experience relapse and 50% of them become frequent relapse.[1,2] Iriani et al[3] in Jakarta, reported that 37% of 171 patients in 1985-2000 became frequent relapse and steroid dependent. Treatment of these groups are challenging due to high-dose and long-term corticosteroid therapy.[2]

More than 44.2% children with SSNS who receive long-term corticosteroid experience side effects of steroid during adulthood, mainly osteoporosis, short stature, overweight, and obesity.[4] Corticosteroid causes osteoporosis directly by increasing osteoclast activity and decreasing osteoblast activity; indirectly

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by decreasing calcium absorption, reduces adrenal and gonadal glucocorticoid productions, and inhibit collagen type I and tissue inhibitor of metalloproteinase gene expression.[5,6] In patients with nephrotic syndrome, especially those who are SDNS and FRNS, the condition is worse by frequent loss of vitamin D metabolites and calcium in the urine during relapse.[7,8] Children with nephrotic syndrome who receive high-dose and long term corticosteroid therapy are at greater risk of reduced bone mineral density (BMD). This study was conducted to compare bone mineral density in children with SNDS, FRNS, and IRNS.

Methods

A cross-sectional study was performed in Nephrology Outpatient Care, Child Health Department, Cipto Mangunkusumo Hospital Jakarta on August until November 2009. Subjects were 5-18 year-old children wirh SNDS, FRNS, and IRNS with normal renal function. We excluded patients who were steroid resistant, those who had never relapse, and those with underlying structural bone abnormalities (osteogenesis imperfecta, juvenile osteoporosis, prior calcium and vitamin D supplementation for 6 months, or previous anti-resorptive agent therapy).

Nephrotic syndrome was defined by edema, massive proteinuria (>40 mg/m^2/h), hypoalbuminemia (<2,5 g/dL), and hyperlipidemia. Frequent relapse was defined as ≥2 relapses within the first 6 months of presentation or ≥4 relapses within any 12 month period. Steroid dependency was defined as two consecutive relapses under steroid treatment or relapses at least 14 days after steroid withdrawal, while infrequent relapse was defined as <2 relapses within the first 6 months of presentation or <4 relapses within any 12 months period. Remission was defined as urinary protein excretion below 4 mg/m^2/h or protein/creatinine ratio below 0,2 mg/mg for 3 consecutive days. A relapse was considered to be the reccurence of massive proteinuria in patients who were on remission.[1,9]

We took a detailed history of characteristics of each subject including onset of disease, duration of illness, total number of relapses, steroid therapy duration, cumulative steroid dose, relapse free interval, prednisone free interval, ureum and creatinine level. Serum ionized calcium, vitamin 25(OH)D_3, and urinary protein/creatinine ratio were analyzed using the standard methods. Serum ionized calcium was measured with Ciba-Corning Model 288 blood gas analyzer® (normal range 1.17-1.29 mmol/L). Vitamin 25(OH)D_3 was measured with Elecsys Vitamin D3 (25-OH) 2010 cobas e411®, using ECLIA (*electrochemiluminescence immunoassay*) method, intraassay coefficient variation was 4.0% and interassay coefficient variation was 5.7%.

Bone mineral density (BMD) of the lumbar spine (L_1-L_4) was measured with dual energy x-ray absorptiometry (DEXA) Lunar GE Medical Systems®, and the patient's data values were expressed as the *z*-score (number of SDs from the mean values of healthy Asian children matched for sex and chronological age). A lumbar spine BMD *z*-score between -1.0 and -2.0 was classified as osteopenia, while a *z*-score of less than -2.0 was classified as osteoporosis. Written informed consent was received from the parents before the study, and the study protocol was approved by the Research Ethics Committee, Medical School, University of Indonesia. Data was analyzed using SPSS for windows version 17.0 program, with $P<0.05$ considered as statistically significant.

Results

We consecutively recruited 44 subjects who fulfilled the study criteria, consisting of 22 SDNS and FRNS children (11 subjects each) defined as group I and 22 IRNS children (group II), either in relapse or remission. There were 30 boys and 14 girls (2:1) aged 5-17.3 years. Gender, age, onset of illness, body weight, body height, and nutritional status based on body mass index (BMI) between two groups were comparable. Children in group I (SDNS and FRNS) had significantly longer duration of illness, more total number of relapses, longer steroid therapy duration, and greater cumulative steroid dose compared to group II (IRNS) (**Table 1**).

Overall, 29 (66%) subjects had hypocalcemia (ionized calcium level <1,17 mmol/L) and 35 (79%) subjects had low levels of vitamin D, 16 (36%) in insufficiency state (25(OH)D_3 levels between 20 and 30 ng/mL), and 19 (43%) subjects were in deficiency

Table 1. Subject and disease characteristics

Parameter	SDNS/FRNS (n = 22)	IRNS (n = 22)	P
Gender: Boy/girl	13/9	17/5	0.195 Φ
Age, median (range) yr	8.7 (5.6-15)	6.6 (5-17.3)	0.067#
Onset, median (range) yr	3 (1.5-11.3)	4.5 (1.2-16)	0.301#
Duration of illness, median (range) yr]	57 (12-131)	15.5 (3.6-96)	<0.001#
Number of total relapse, mean (SD)	9.4 (5.4)	3 (2.5)	0.000*
Steroid therapy duration, median (range) mo	26 (4.5-28)	4 (2-38)	<0.001#
Cumulative steroid dose, mean (SD) mg	13,476 (8,879)	4,649 (4,142)	<0.001*
Body weight, mean (SD) kg	30 (9.2)	27 (12.7)	0.341*
Body height, mean (SD) cm	126 (12.6)	120 (19.2)	0.211*
BMI, median (range) [kg/m^2]	18.5 (15-25.7)	16.8 (14.5-22.6)	0.162#
Nutritional status, n			
Well nourished	17	17	0.816
Overweight	3	1	
Obesity	2	4	
Ureum, median (range) mg/dL	19 (8-39)	22 (7-105)	0.025#
Creatinine, mean (SD) mg/dL	0.49 (0.19)	0.56 (0.24)	0.336*

SD: Standard Deviation, Φ *Chi Square,** Independent t Test, # Mann Whitney Test

Table 2. Calcium status, vitamin D level, and degree of proteinuria

Parameter	FRNS/SDNS (n = 22)	IRNS (n = 22)	Σ	P
Calcium status				0.340Φ
Normal	6	9	15	
Hypocalcemia	16	13	29	
Vitamin D status				0.987Ψ
Normal	3	6	9	
Insufficiency	10	6	16	
Deficiency	9	10	19	
Degree of proteinuria				0.605 Ψ
Normal	15	15	30	
Moderate	0	3	3	
Nephrosis	7	4	11	

Φ*Chi Square Test* Ψ Kolmogorov-Smirnov Test

state (levels <20 ng/mL). Thirty subjects were on remission and 14 subjects were on relapse. There were no differences with regard to calcium or vitamin D status, and degree of proteinuria between the two groups (**Table 2**).

Children in group I had lower *z*-scores of BMD L_1-L_4 compared to group II, but the difference was not statistically significant. Five out of 22 subjects and 2/22 subjects in group I and II, respectively, had osteoporosis, but none experienced fracture (**Table 3**).

There were also no differences between the two groups in terms of the mean ionized calcium level and mean vitamin 25(OH)D_3 level (**Table 4**). Furthermore, subgroup analysis which divided group I to SDNS and FRNS subgroups, showed that SDNS children had lower *z*-scores of BMD L_1-L_4 than FRNS [-1.791 (1.17) vs -0.573 (0.94), P=0.019] and IRNS [-1.791 (1.17) vs -0.795 (1.25), P=0.026]. Cumulative steroid dose in subgroup SDNS but there were no differences between the two groups with regard to the total number of relapses, steroid therapy duration, relapse free interval, prednisone free interval, ionized calcium and vitamin 25(OH)D_3 level. Subgroup analysis of FRNS compared to IRNS showed that *z*-scores of BMD L_1-L_4 between the two groups were not different (**Table 4**).

On univariate analysis, we observed that there were weak inverse correlation between onset of

Table 3. Bone mineral density in nephrotic syndrome patients

Parameter	SDNS/FRNS (n = 22)	IRNS (n = 22)	P
z-score BMD L_1-$_4$, mean (SD)	-1.182 (1.21)	-0.795 (1.25)	0.305*
z-score whole body BMD, mean (SD)	-0.786 (0.83)	-0.573 (0.82)	0.396*
Osteopenia (-2.0<*z-score*<-1.0)	4	10	0.052Φ
Osteoporosis (*z-score*<- 2.0)	5	2	0.412Ω

*Independent t Test , Φ *Chi Square Test*, Ω Fisher's test

Table 4. Comparison of bone mineral density in SDNS, FRNS subgroups, and IRNS group

Parameter	SDNS (n=11)	FRNS (n=11)	IRNS (n=22)	P
Number of total relapse, mean (SD)	9.9 (5)	9 (6)	3 (2.5)	<0.001π
Steroid therapy duration, median (range) mo	36 (45-128)	25 (11-122)	4 (2-38)	<0.001Ω
Cumulative steroid dose, mean (SD) mg	16,563 (10,921)	10,389 (5,031)	4,649 (4,142)	<0.001π
Relapse free interval median (range) wk	6 (0-28)	28 (0-88)	8 (0-48)	0.530Ω
Prednison free interval, median (range) wk	0 (0-10)	0 (0-14)	3 (0-80)	0.081Ω
Ionized calcium, mean (SD)mMol/L	1.14 (0.04)	1.14 (0.05)	1.16 (0.03)	0.254π
25(OH)D_3 level, mean (SD) ng/L	19.91 (6.53)	22.73 (8.30)	24.59 (13.69)	0.764Ω
z-score BMD L_1-$_4$, mean (SD)	-1.791 (1.17)	-0.573 (0.94)	-0.795 (1.25)	0.036π

π One-way Anova test Ω Kruskal-Wallis Test

Table 5. Correlation of age, onset of illness, duration of illness, total relapses, steroid therapy duration, cumulative steroid dose, body mass index, ionized calcium level, and vitamin D level with low BMD L_{1-4} z-score

Variable	r (correlation coefficient) θ	P θ
Age	-0.228	0.137
Onset of illness	-0.484	0.001*
Duration of illness	0.206	0.179
Total relapses	0.337	0.025*
Steroid therapy duration	0.410	0.006*
Cumulative steroid dose	0.403	0.007*
BMI (Body Mass Index)	0.165	0.285
Ionized Calcium	0.101	0.515
Vitamin D level	0.371	0.013*

θSpearman's test, * *p*<0.05 statistically significant

illness (P=0,001) and weak positive correlation between total number of relapses (P=0,025), steroid therapy duration (P=0,006), cumulative steroid dose (P=0,007), and low level of vitamin D (P=0,013) with low z-scores of BMD L_1-L_4 (less than -1.0). Other parameters; age, duration of illness, BMI, and ionized calcium level were not correlated with low z-scores of BMD L_1-L_4 (**Table 5**).

Discussion

In this study, 29 (66%) subjects had hypocalcemia but only 1 subject experienced carpopedal spasm while the rest were asymptomatic. Clinical manifestations of hypocalcemia depend on the duration, severity, and rapidity of hypocalcemia; not only on the ionized calcium level at random examination.[10] Signs usually occur if the level is less than 1,1 mmol/L.[11]

Almost 99% of calcium is stored in the bones while 1% could be found in the blood, muscle, and the fluid between cells.[12] About 40% of the total blood Ca is bound to plasma proteins, primarily albumin. The remaining 60% includes ionized Ca (50%) plus phosphate and citrate complexes. The ionized or free Ca should be determined since this is the physiologically active form of Ca[13], but the etiology of hypocalcemia in nephrotic syndrome are multifactorial, not only simply related to hypoalbuminemia, but also abnormal blood levels of vitamin D metabolite and reduced intestinal calcium absorption.[7,8]

Serum 25(OH)D_3 decreases in NS patients because of the loss of vitamin D-binding protein in urine.[8,14] The active form of vitamin D (1,25$(OH)_2D_3$) stimulates the absorption of calcium and phosphor in the intestine, calcium mobilization from the bone, and

stimulates calcium reabsorption at distal renal tubulus (parathormon dependent).[15] Without vitamin D, only 10 to 15% of dietary calcium is absorbed.[14]

This study showed that 35 (79%) subjects had low levels of vitamin D, 16 were in vitamin D insufficiency state and 19 were in deficiency state. As we thought, up to now there are no other studies regarding the comparison of $25(OH)D_3$ level in such groups. This study highlighted the need of supplementation of vitamin D with or without calcium in nephrotic syndrome, especially those who are steroid dependent. The consensus issued by Nephrology Coordination Task Force, Indonesian Pediatric Society, recommends that children who receive long-term steroid therapy (more than 3 months) should be given calcium supplementation (250-500 mg/day) and vitamin D (125-250 IU).[1]

In this study, seven subjects had osteoporosis but none experienced any fractures. Children with nephrotic syndrome, especially those who have frequent relapses or steroid dependent, are exposed to the risk of reduced bone mineral density (BMD) and thus had a greater risk for developing fracture or inadequate peak bone mass attainment.[16]

Another important factor affecting bone health is the side effect of steroids, with the expected highest bone loss occuring in the first 6-12 months of steroid therapy. Several studies showed that the greater the dose and the longer duration of steroid therapy, the greater the bone loss. Maximum safe dose of steroid therapy remains controversial.[5,6] Risk factors of reduced bone mineral density are cumulative steroid dose >500 mg, steroid exposure >12 months, prednisone dose $>7,5$ mg/day, and ≥ 4 course of high-dose prednisone.[17,18]

Comparing BMD values, we observed that children in group I (SDNS and FRNS) had lower mean z-scores of BMD L_1-L_4 compared to group II (IRNS), but the difference was not statistically significant so we proceed to perform subgroup analysis which showed that SDNS children had lower z-scores of BMD L_1-L_4 than FRNS. It is likely that the difference of z-scores between SNDS and FRNS are caused by greater cumulative steroids since there were no differences regarding to the total number of relapses, steroid therapy duration, relapse free interval, prednisone free interval, ionized calcium and vitamin $25(OH)D_3$ level.

Subgroup analysis between FRNS compared to IRNS showed that z-scores of BMD L_1-L_4 were not different. Although FRNS subgroup had higher number of relapses, longer steroid therapy, and greater cumulative steroid, this occurrence might be caused by the longer relapse free interval of FRNS subgroup. As Kano et al[19] stated, the decrease of lumbal BMD after 16 weeks short-term prednisolone therapy were transient and would normalize by 16 weeks after the cessation of therapy. So, this might explain the results, since the relaps free interval median of FRNS group were 28 weeks while median of IRNS group were only 8 weeks.

Gulati, et al[16] in 2003 reported that bone mineral density in FRNS, SDNS and steroid resistant were lower than IRNS. The study was continued to assess the longitudinal changes in bone mineral density after calcium and vitamin D supplementation for 6-12 months. The results concluded that calcium and vitamin D supplementation were associated with reduced loss of BMD in steroid therapy.[20]

On univariate analysis, we observed that the BMD values were influenced by onset of illness, total number of relapse, steroid therapy duration, cumulative steroid dose,and vitamin D level, but not by age, duration of illness, and ionized calcium level. The limitations of this study are we did not asses calcium and vitamin D intake, weight bearing physical activity which could act as confounding factors. Also the evaluation of bone mineral density was cross-sectionally performed so the changes during relapse or remission are not avalaible.

We conclude that patients with SDNS who receive greater cumulative steroid dose are likely to have lower BMD value compared to FRNS and IRNS, and this group possibly requires vitamin D supplementation with or without calcium.

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