Original Article

Risk factors for low bone density in pediatric nephrotic syndrome

Corina Lisa, Madarina Julia, Pungky A Kusuma, Tonny Sadjimin

Abstract

Background Disturbances in bone mineral metabolism and side effects of corticosteroid treatment may cause decreased bone density in patients with nephrotic syndrome (NS).

Objectives To compare the prevalence of low bone mineral density (BMD) in children with and without NS and to assess the effect of corticosteroid treatment on bone density in NS patients.

Methods We conducted a retrospective, cohort study in children aged 5-18 years diagnosed with NS for more than 2 months prior to data collection, and in children without NS as a control. BMD was assessed on calcaneal bone with ultrasound bone densitometry. Serum calcium, albumin, creatinine and phosphate levels were also assessed.

Results The prevalence of low BMD was significantly higher in NS patients than non-NS subjects, 73.3% (22 in 30) vs. 33% (11 in 33), respectively. The prevalence ratio was 6.3 (95% CI 2.1 to 18.9). NS patients had lower serum calcium levels, with mean difference of -0.17 (95% CI -0.27 to -0.07 mMol/L), P=0.009, and lower serum albumin, with mean difference of -0.88 (95% CI -1.27 to -0.49 g/L); P<0.001, than non-NS subjects. After adjusting for other risk factors, we found NS to be an independent risk factor for low BMD. Steroid-resistant and steroid-dependent patients had lower BMD than steroid-sensitive subjects (P=0.02). There was also a significant correlation between the onset of corticosteroid treatment and BMD (r=0.3; P=0.02).

Conclusions NS patients had higher risk for low BMD compared to normal subjects. Response to steroid treatment influences the severity of impaired bone density. **[Paediatr Indones. 2011;51:61-5]**.

Keywords: hypocalcemia, bone density, corticosteroids, nephrotic syndrome

Children with NS are at risk for disturbances in bone mineral metabolism such as hypocalcemia, vitamin D deficiency and secondary hyperparathyroidism.[1-7] Corticosteroid therapy as a treatment protocol for NS may also worsen the process of bone formation, both directly or indirectly.[3,8-10]

Little research has been conducted on the incidence of bone density disorders and hypocalcemia in pediatric NS in Indonesia. A case-control study in the United States found 14 cases of hypocalcemia out of 15 NS patients, while an Egyptian study found 5 cases out of 30 patients.[1,11] It has been estimated that 52% of patients with glomerular disorders receiving long-term corticosteroid therapy show a decrease in bone mineral density (BMD).[12] Mittal et al. reported changes in bone histology in 30 patients with NS, 56.7% with osteomalacia and 10.0% with bone mineralization defects.[5] A study in India reported osteopenia in 61/100 subjects (61%) and osteoporosis in 22/100 (22%) subjects. A study in Iran (2006) of 37 subjects reported that 12% had osteopenia.[13] Patients who were steroid-resistant or steroid-dependent, or

From the Department of Child Health, Medical School, Gadjah Mada University /Sardjito Hospital, Yogyakarta, Indonesia

Reprints request to: Madarina Julia, Department of Child Health, Medical School, Gadjah Mada University / Sardjito Hospital, Jl. Kesehatan no. 1 Yogyakarta, Indonesia. Tel. 62-274-561616, Fax. 62-274-583745, E-mail: *madarinajulia@yahoo.com*

those with frequent relapses, were at even higher risk of bone mineralization abnormalites because of their exposure to higher total dose of corticosteroids.[13,14]

The aims of this study were to compare the prevalence of hypocalcemia and abnormal bone density (low BMD) in children with and without NS, and assess the influence of cumulative doses of corticosteroids on bone mineral density.

Methods

We conducted a retrospective cohort study in two stages: stage one involved assessing the prevalence of hypocalcemia and abnormal bone density conditions in children with nephrotic syndrome compared to children without nephrotic syndrome, and stage two involved assessing the influence of cumulative corticosteroid doses on bone density.

The study was conducted in December 2006. Subjects were children aged 5-18 years who were either inpatients or outpatients at the Sardjito Hospital. Subjects were divided into 2 groups, those with and without NS. Subjects with NS fulfilled the inclusion criteria: diagnosed with NS more than 2 months prior to data collection, had a fairly complete medical record and received corticosteroid treatment at Sardjito Hospital. Non-NS subjects, serving as a control group, consisted of patients without NS who were not suffering from chronic diseases associated with hypoalbuminemia, had no history of consumption of medication causing disorders of bone metabolism in the last one year (corticosteroids, methotrexate, non-steroidal anti-inflammatory drugs (NSAIDs), anticonvulsants, and rifampicin), and had no history of absorption disorders such as Crohn's disease, ulcerative colitis, or celiac disease.

This study was approved by the Commission on Medical Research Ethics and Health, Medical School, Gadjah Mada University. Informed consent was obtained from all subjects' parents.

We calculated sample size based on the assumption that the proportion of events in the exposed group (P1) was 0.5 (50%) and that in the unexposed group (P2) was 0.15 (15%).[7,12] With type 1 error (α) set at 0.05, $Z\alpha = 1.96$ and strength (power) 80%, the minimum sample size was deemed to be 28 for each group. Children fulfilling criteria of either group were invited consecutively until the required number of subjects was met.

All subjects underwent blood sampling for measurement of serum albumin, calcium, phosphate and creatinine levels, as well as assessment of bone mineral density. Blood levels of the substances listed above were assessed using a calibrator kit manufactured by Vitros Chemistry in the Clinical Pathology laboratory of Sardjito Hospital. Anticoagulants were not added to the 3 ml blood specimens from each subject to avoid affecting the assay results.

Specimens were taken directly to the laboratory or stored at appropriate temperatures (room temperature, refrigerator or frozen) depending on the type of examination to be conducted, as serum calcium, phosphate and creatinine each have different temperature stabilities. Assay results were automatically shown by the calibrator that had been adjusted for quality parameters with specific reference values.

Bone mineral density on calcaneus was measured using Ubis Ultrasound densitometric 5000 series. Measurements were taken three times for each subject. Means of the three measurements were calculated and converted into z-scores. Consistency of the examinations was ensured by using only one examiner for all laboratory and bone mineral density measurements.

Comparisons were performed using either t test or chi-square test according to the type of the measurement. Logistic regressions were used to assess multiple risk factors for low bone mineral density. Pearson's correlation tests were used to assess the relationship between cumulative dose and onset of corticosteroid therapy to BMD.

Results

We recruited 30 subjects with NS and 33 subjects without NS. Baseline characteristics of the study subjects are shown in **Table 1**. Glomerular filtration rate (GFR), serum phosphate and creatinine levels were within normal limits and did not differ significantly between the two groups. There were significant differences in mean age, BMD, and serum calcium and albumin levels between the two groups.

The prevalence of hypocalcemia and low BMD

in the NS group was significantly higher than in the non-NS group (**Table 2**). Furthermore, children with low BMD had significantly lower levels of serum calcium and albumin (**Table 3**). Multiple logistic regressions were performed to assess the association between age, gender, hypocalcemia and hypoalbuminemia and low BMD (**Table 4**). The models showed that NS was a significant independent risk factor for low BMD.

Within the NS group, there were significant

Table 1. Characteristics of subjects

Characteristics n = 30	NS n = 33	Non-NS (95% CI)*	Mean difference
Mean age, months (SD)	110.9 (38.1)	145.4 (8.3)	-34.5 (-48.15 to -20.95)
Male gender, n	24	20	-
Mean serum calcium, mmol/L (SD)	2.34 (0.28)	2.51 (0.08)	-0.17 (-0.27 to -0.07)
Mean BMD (SD)	-2.80 (1.25)	-1.16 (1.27)	-1.63 (-2.27 to -1.01)
Mean serum albumin, g/dL (SD)	3.5 (1.1)	4.4 (0.21)	-0.88 (-1.27 to -0.49)
Mean GFR, mL/min per 1.73m2 (SD)	118.4 (20.7)	112.1 (19.1)	6.3 (-3.75 to 16.2)
Mean serum creatinine, mg/dL (SD)	0.70 (0.39)	0.76 (0.11)	0.06 (-0.07 to 0.21)
Mean serum phosphate, mmol/L (SD)	1.61 (0.32)	1.53 (0.16)	0.08 (-0.04 to 0.20)

BMD (

* t test except for gender: X2 test

Table 2. Prevalence of hypocalcemia and low BMD

	NS	Non-NS	Prevalence ratio (95% CI)	P
Prevalence of low BMD	22/30	11/33	6.3 (2.1 to 18.9)	<0.001
Prevalence of hypocalcaemia	6/30	0/33		0.009*

*Fisher's exact test

Table 3. Risk factors for low BMD in NS subjects

Characteristic	Low BMD (n=22)	Normal BMD (n=8)	Mean difference (95% CI)	P
Mean age, months (SD)	134.5 (25.4)	123.5 (36.8)	-10.8 (-26.7 to 5.1)	0.18
Male gender, n	20	6	-	0.37*
Mean serum calcium, mmol/L (SD)	2.15 (0.29)	2.35 (0.26)	-0.2 (-0.25 to -0.05)	0.005
Mean serum albumin, g/dL (SD)	3.5 (1.13)	3.7 (1.09)	-0.5 (-0.93 to -0.06)	0.025

BMD (

* t test except for gender: X^2 test

Table 4. Multiple logistic regression models for risk of low BMD

Variable	Adjusted OR (95% CI)	P	Adjusted OR (95% CI)	P	Adjusted OR (95% CI)	P	Adjusted OR (95% CI)	P
Nephrotic syndrome*	8.2 (1.9 to 34.2)	0.004	7.3 (1.7 to 31.1)	0.007	7.7 (1.8 to 32.6)	0.005	7.16 (1.7 to 30.2)	0.007
Hypocalcemia †	-		2.2 (0.21 to 22.4)	0.5	-		5.4×10^8 (0; uncountable)	1
Hypoalbuminemia#	-		-		1.7 (0.16 to 18.8)	0,6	0 (0; uncountable)	1
Gender (male)	0.9 (0.25 to 2.9)	0.8	1.2 (0.35 to 4.3)	0.8	1.18 (0.35 to 4.05)	0.8	1.1 (0.34 to 3.9)	0.8
Age(months)	0.99 (0.9 to 1.0)	0.5	0.99 (0.9 to 1.01)	0.5	0.99 (0.9 to 1.01)	0.5	0.99 (0.9 to 1,1)	0.5

m=male
* with nephrotic syndrome vs. without nephrotic syndrome
† hypocalcemia vs. normal
# hypoalbuminemia vs. normal

differences in BMD (P=0.02, One-way ANOVA) among the three groups of patients based on steroid responsiveness. Post-hoc tests showed significant differences between the steroid-sensitive and steroid-resistant groups (P=0.02), and between the steroid-sensitive and steroid-dependent groups (P=0.01). However, no significant difference was observed between the steroid-resistant and steroid-dependent groups (P=0.72).

T-test revealed no significant differences in the mean cumulative dose of corticosteroid (mg / kg) between children with low BMD compared to those with normal BMD. There was also no correlation between cumulative dose of corticosteroids with BMD (r= 0.2 and P= 0.26, Pearson correlation). However, there was a significant but weak correlation between the onset of corticosteroid treatment with BMD (r= 0.3 and P= 0.02, Pearson correlation).

Discussion

We observed lower mean blood calcium levels in subjects with NS than in non-NS subjects. The prevalence of hypocalcemia in children with NS was 6/30 children (20%), significantly higher than that in children without NS (0/33), (P= 0.009, Fischer exact test). Children with NS have proteinuria, which can lead to hypoalbuminemia and vitamin D deficiency, both important factors in calcium metabolism. Similar findings were also observed in several other studies. [1,2,5,15]

The lower than expected prevalence of hypocalcemia in the NS group might be related to calcium and vitamin D supplementation during the active phase of disease. Supplementation of calcium and vitamin D has been shown to increase serum calcium levels in children with NS. [16-18]

We observed 73.3% of NS subjects had low BMD, compared to only 33.3% of those without NS. Increased prevalence of bone density abnormalities were reported by Gulati et al., who found osteopenia in 61% and osteoporosis in 22% of cases out of 100 NS subjects. [3] In addition, Basitrania et al. reported 12% of 37 NS cases had osteoporosis. [13]

Multiple logistic regression analysis showed no correlation in age, hypocalcemia or hypoalbuminemia to low BMD. However, NS was observed to be an independent risk factor for low BMD. In contrast to our results, Gulati et al. reported predictive factors for abnormal bone density in NS patients to be higher age at onset, low calcium intake, and cumulative dose of corticosteroids. [3]

Although we did not observe an association between cumulative doses of corticosteroids and BMD (r=0.2 and P=0.26), we saw a correlation between the age of onset of corticosteroid treatment and BMD (r=0.3 and P=0.02), i.e. the younger the age corticosteroid was given, the lower the BMD. It seemed that low bone density in children with NS was unlikely to be due to a single factor, neither hypocalcemia nor corticosteroid per se, but may be related to the accumulation of several factors. For example, hypocalcemia and its secondary hyperparathyroidism, vitamin D deficiency, child's age and phase of growth at onset of exposure to corticosteroid treatment, and cumulative dose of corticosteroids may contribute to low BMD. [4,6,8,12,14]

Basitrania et al. showed that lower BMD in NS patients was associated with higher cumulative doses of corticosteroids. These higher doses were observed in patients who were steroid-dependent, with an earlier onset of treatment and longer duration of disease. [13] However, Shouman et al. and Mishra et al. showed that higher cumulative doses of corticosteroids were not associated with lower bone density in NS patients. [11,19] Esbjorner et al. also concluded that duration of corticosteroid therapy and cumulative dose of steroids were not associated with bone density abnormalities. [20]

We observed that children with NS had a higher risk for hypocalcaemia and low BMD, although we could not single out the most important factor associated with low BMD except for the presence of NS. In spite of evidence that supplementation with calcium and vitamin D might reduce the risk for hypocalcemia, this would not necessarily reduce the risk for low BMD. [16-18]

This was the first study assessing the risk for low BMD in NS patients at Dr. Sardjito Hospital, Yogyakarta. Although most information was collected directly obtained primary data, the information on cumulative doses of corticosteroids were secondary data obtained from medical records. In addition, some information on factors affecting BMD was not collected because of difficulty in obtaining details from the medical records,

e.g. episodes of hypoalbuminemia and hypocalcaemia, or use of corticosteroids before referral.

We conclude that NS patients have a higher risk for low BMD compared to normal subjects. Response to steroids influenced the severity of impaired bone density, but cumulative dose of corticosteroids was not associated with low BMD. Since we did not observe specific risk factors for low BMD in NS patients, further studies may be aimed at assessing the detailed mechanisms of low BMD in patients with NS.

Acknowledgments

References

1. Freundlich M, Bourgoignie D, Zilleruelo JJ, Jacob G, Cantenbury A, Strauss JM. Bone modulating factors in nephrotic children with normal glomerular filtration rate. Pediatrics. 1985;76:280-5.

2. Weng FL, Shults J, Herskovitz RM, Zemel BS, Leonard MB. Vitamin D insufficiency in steroid sensitive nephrotic syndrome in remission. Pediatr Nephrol. 2005;20:56-63.

3. Gulati S, Godbole M, Sing U, Gulati K, Srivastava A. Are children with idiopathic nephrotic syndrome at risk for metabolic bone disease? Am J Kidney Dis. 2003;41:1163-9.

4. Grymonprez A, Proesmans W, Van Dyckl M, Jans I, Goos G, Bouillon R. Vitamin D metabolites in childhood nephrotic syndrome. Pediatr Nephrol. 1995;9:278-81.

5. Mittal SK, Dash SC, Tiwari SC, Agarwal SK. Bone histology in patients with nephrotic syndrome and normal renal function. Kidney Int. 1999;5:1912-9.

6. DeLuca HF. Vitamin D endocrinology. Ann Intern Med. 1976;85:367-77.

7. Lyman D. Undiagnosed vitamin D deficiency in the hospitalized patient. Am Fam Physician. 2005;71:299-304.

8. Prummel MF, Wiersinga WM, Lips P, Sanders GT, Sauerwein HP. The course of biochemical parameters of bone turnover during treatment with corticosteroids. J Clin Endocrinol Metab. 1991;72:382-6.

9. Biyikli NK, Emre S, Sirin A, Bilge I. Biochemical bone markers in nephrotic children. Pediatr Nephrol. 2004;15:35-7.

10. Freundlich M, Jofe M, Goodman WG, Salusky IB. Bone histology in steroid-treated children with non-azotemic nephrotic syndrome. Pediatr Nephrol. 2004;19:400-7.

11. Shouman MG, Abdallah N, Meguid AE, Salama EEE, El Ghoroury E, Abou-Ismail LA, et al. Bone mineral density markers in children with steroid sensitive idiopathic nephrotic syndrome. Int J Acad Res. 2010;2:150-5.

12. DeDeus BR, Ferreira ZA, Kirstajn GM, Heilberg IP. Osteopenia in patients with glomerular disease requiring long-term corticosteroid therapy. Nephron Clin Pract. 2003;94:c69-74.

13. Basitrania M, Fallahzadeh MH, Derakhshan A, Hosseini-Al-Hashemi G. Bone mineral density in children with relapsing nephrotic syndrome. Iran J Med Sci. 2006;31:82-6.

14. Mushtaq T, Ahmed SF. The impact of corticosteroids on growth and bone health. Arch Dis Child. 2002;87:93-6.

15. Freundlich M, Bourgoignie JJ, Zilleruelo G. Calcium and vitamin D metabolism in children with nephrotic syndrome. J Pediatr. 1986;108:383-7.

16. Bagga, A. Revised guidelines for management steroid sensitive nephrotic syndrome. Indian J Nephrol. 2008;18:31-9.

17. Bak M, Serdaroglu E, Guclu R. Prophylactic calcium and vitamin D treatments in steroid-treated children with nephrotic syndrome. Pediatr Nephrol. 2006;21:350-4.

18. Gulati S, Sharma RK, Gulati K, Singh U, Srivastava A. Longitudinal follow-up of bone mineral density in children with nephrotic syndrome and the role of calcium and vitamin D supplements. Nephrol Dial Transplant. 2005;20:1598–603.

19. Mishra OP, Meena SK, Singh SK, Prasad R, Mishra RN. Bone mineral density in children with steroid-sensitive nephrotic syndrome. Indian J Pediatr. 2009;76:1237-9.

20. Esbjorner E, Arvidsson B, Jones IL, Palmer M. Bone mineral content and collagen metabolites in children receiving steroid treatment for nephrotic syndrome. Acta Pediatr. 2001;90:1127-30.