SHORT COMMUNICATION

Effects of Steroid Treatment on Bone Mineral Metabolism in Children with Glucocorticoid-sensitive Nephrotic Syndrome

C Koşan[1], G Ayar[2], Z Orbak[3]

ABSTRACT

Glucocorticoids have been used in nephrotic syndrome (NS) treatment for many years. In this study, we aimed to evaluate the effect of steroids on bone mineralization in children with glucocorticoid-sensitive nephrotic syndrome (GSNS).

Twenty children who were first diagnosed as GSNS received glucocorticoid therapy for four months. Before treatment, at the 4th and 12th week of initial therapy, bone mineral density (BMD) and levels of the markers for bone turnover were evaluated.

At the 4th and 12th week of treatment, mean serum calcium (Ca) and osteocalcin levels were found to be significantly lower than those at the beginning of the therapy. Mean serum total alkaline phosphatase (t-ALP), bone-specific alkaline phosphatase (b-ALP) and urine calcium creatinine ratio (Ca/Cr), urinary deoxypyridinoline levels were significantly increased in comparison to the beginning of therapy. There was no significant relationship between serum levels of phosphate and parathyroid hormone (PTH) at the beginning of treatment and at the 4th and 12th week of treatment. Mean value of BMD was significantly lower at the 4th and 12th week of treatment than that at the beginning of the therapy.

In conclusion, bone mineralization was negatively affected by steroid treatment in children with NS. These children should undergo regular BMD evaluation, and an appropriate therapeutic approach should be planned.

Keywords: Bone mineral density, children, glucocorticoid, nephrotic syndrome

Efectos del Tratamiento de Esteroides sobre el Metabolismo Mineral del Hueso en Niños con Síndrome Nefrótico Sensible a los Glucocorticoides

C Koşan[1], G Ayar[2], Z Orbak[3]

RESUMEN

Por muchos años se han venido usando glucocorticoides en el tratamiento del síndrome nefrótico (SN). Este estudio se encamina a evaluar el efecto de los esteroides sobre la mineralización ósea en niños con síndrome nefrótico sensible a los glucocorticoides (SNSG).

Veinte niños que fueron diagnosticados primeramente con SNSG, recibieron terapia con glucocorticoides durante cuatro meses. Antes del tratamiento, en las semanas 4 y 12 de la terapia inicial, se evaluaron la densidad mineral ósea (DMO) y los niveles de los marcadores del recambio óseo.

En el tratamiento de las semanas 4 y 12, se halló que el calcio (Ca) sérico promedio y los niveles de osteocalcina eran significativamente más bajos que los existentes a comienzos de la terapia. Los niveles de fosfatasa alcalina sérica total promedio, fosfatasa alcalina (t-ALP), fosfatasa alcalina especifica ósea media (b-ALP), la relación calcio/creatinina en la orina (Ca/Cr), y los niveles de

From: [1]Medical Faculty, Department of Nephrology, Ataturk University, Erzurum, Turkey, [2]Medical Faculty, Department of Paediatrics, Ataturk University, Erzurum, Turkey and [3]Medical Faculty, Department of Paediatric Endocrinology and Metabolism, Ataturk University, Erzurum, Turkey.

Correspondence: Dr Z Orbak, Ataturk University Medical Faculty, Department of Paediatrics, 25240, Erzurum, Turkey. E-mail: zerrinorbak@yahoo.com

deoxipiridinolina urinaria, aumentaron significativamente en comparación con los existentes al comienzo de la terapia. No hubo relación significativa alguna entre los niveles séricos de fosfato y hormona paratiroidea (PTH) ni al principio del tratamiento ni en las semanas 4 y 12 de tratamiento. El valor promedio de la DMO fue significativamente más bajo en las semanas 4 y 12 de tratamiento que al principio de la terapia. En conclusión, la mineralización del hueso fue afectada negativamente por el tratamiento con esteroides en los niños con SN. Estos niños deben tener una evaluación regular de DMO, para lo cual es necesario planear un enfoque terapéutico apropiado.

Palabras claves: Densidad mineral ósea, niños, glucocorticoides, síndrome nefrótico

West Indian Med J 2012; 61 (6): 628

INTRODUCTION

Steroids are known to cause osteoporosis and affect the bone mineral content (BMC) and bone mineral density (BMD) in children (1, 2). It has been shown in adults that nephrotic syndrome (NS) itself, even with normal renal function, is associated with alterations in bone and mineral metabolism (1). Glucocorticoids cause rapid, dose-dependent bone loss and an increased risk of fracture in adults. During childhood and adolescence, skeletal modelling results in sex- and maturation-specific increases in bone density. Children may be especially vulnerable to the effects of glucocorticoids on bone formation and peak bone mass (3). Therefore, we selected glucocorticoid-sensitive nephrotic syndrome (GSNS) without substantial systemic inflammatory involvement to examine the effects of glucocorticoids on the growing skeleton and investigated alterations in BMD and in markers for bone turnover over a short period of time before and after high-dose glucocorticoid administration.

SUBJECTS AND METHODS

Twenty children (seven girls and 13 boys) who were first diagnosed as GSNS were included in the study. During treatment, all patients were steroid responders and none of them had any relapse.

Their mean age was 6.85 ± 3.25 years. Medical history, physical examination, purified protein derivative test, complete blood count, serum urea, creatinine, cholesterol, triglycerides, total protein, albumin, electrolytes, serum C_3 (complement), C_4 and hepatitis B surface antigen, urinalysis, urine protein excretion rate and abdominal ultrasound were carried out at the time of admission. All patients had massive proteinuria (> 40 mg/m^2 per hour), hypoalbuminaemia (< 2.5 mg/dL), hypercholesterolaemia and oedema. Nephrotic patients between one and seven years of age without macroscopic haematuria, hypertension, low serum C_3 levels and renal failure were not included in the study. All children were white and prepubertal. Prior to the nephrotic syndrome, the patients were normally growing children free of chronic disease. No patient had previously taken glucocorticoids or any medications affecting calcium and bone metabolism such as diuretics and vitamin D metabolites.

Bone mineral density and levels of the markers for bone turnover including serum calcium (Ca), phosphate (P), total alkaline phosphatase (t-ALP), bone-specific alkaline phosphatase (b-ALP), parathyroid hormone (PTH), osteocalcin (OC) and urinary calcium creatinine ratio (Ca/Cr), urinary deoxypyridinoline (DPyr) were measured. Serum calcium levels were corrected for hypoalbuminaemia according to the standard formula (if serum albumin level was less than 2.5 gr/dL, corrected Ca = measured Ca level (mg/dL) + 0.8 x [4-albumin(gr/dL)]. The new onset patients were treated with 2 mg/kg per day prednisolone (maximum 60 mg) in three divided doses for four weeks, followed by a single morning dose of 2 mg/kg on alternate days for an additional four weeks. Afterward, a single morning dose of 1 mg/kg on alternate days was used during the third 4-week and then tapered. Intravenous methylprednisolone was not used, neither was supplementation with calcium and vitamin D. Parameters of bone metabolism were re-evaluated at the 4th and 12th week of initial therapy.

Venous blood samples and urine samples were obtained in the morning following an overnight fasting. Serum t-ALP and b-ALP, Ca and P, urine Ca, creatinine and cholesterol, triglycerides, total protein and albumin were estimated by an autoanalyser. Osteocalcin levels were evaluated by "IMMULITE otoanalizör". Serum PTH and DPyr levels were measured by chemiluminescence enzyme immunoassay.

Bone mineral density was measured by dual-energy X-ray absorptiometry (DEXA; Hologic QDR 4500 Elite densitometer). Results for vertebrae L-1 through L-4 were averaged to obtain the patient's total vertebral bone mass.

Written informed consent was received from parents, and the study protocol was approved by the ethics committee. Statistical analysis was conducted with the SPSS/PC version 15 programme. Results were compared by Student's paired *t*-test and Pearson's correlation.

RESULTS

Remission was achieved in all patients within four weeks of prednisolone treatment, and no relapse was observed during the study period. None of the patients developed pathological fractures. The follow-up calcium levels were significantly

lower compared with baseline value, especially at the 4th week (Table). While serum total and b-ALP levels, urine DPyr and Ca/Cr ratio progressively increased, serum OC levels decreased. Serum PTH and P levels did not change.

Table: Characteristics of patients with NS at the baseline and at the 4th and 12th week after glucocorticoid treatment

	Baseline mean ± SD	At 4th week mean ± SD	At 12th week mean ± SD	Baseline – 4th week *p*-value	Baseline – 12th week *p*-value
BUN (mg/dL)	17.35 ± 11.86	20.20 ± 13.66	16.6 ± 8.66	> 0.05	> 0.05
Creatinine (mg/dL)	0.62 ± 0.21	0.66 ± 0.22	0.63 ± 0.16	> 0.05	> 0.05
Total protein (g/dL)	4.06 ± 0.30	6.14 ± 0.88	6.71 ± 0.67	< 0.001	< 0.001
Albumin (mg/dL)	1.49 ± 0.29	3.47 ± 0.90	4.22 ± 0.70	< 0.001	< 0.001
Triglyceride (mg/dL)	497. 6 ± 175.8	271.3 ± 176.1	160.6 ± 52.6	< 0.001	< 0.001
Cholesterol (mg/dL)	475.4 ± 150.4	280.6 ± 132.8	198.6 ± 57.98	< 0.001	< 0.001
Proteinuria (mg/m^2/hr)	257.1 ± 257.9	14.90 ± 16.17	6.12 ± 7.8	< 0.001	< 0.001
Ca (mg/dL)	9.93 ± 0.44	8.98 ± 0.71	9.43 ± 0.73	< 0.001	< 0.01
P (mg/dL)	4.89 ± 0.85	4.36 ± 0.51	4.89 ± 0.92	> 0.05	> 0.05
T-ALP (U/L)	124.70 ± 59.27	147.65 ± 67.21	239.95 ± 189.35	< 0.05	< 0.01
B-ALP (U/L)	102.90 ± 56.35	124.45 ± 63.18	208.9 ± 166.34	< 0.05	< 0.01
PTH (pg/mL)	52.97 ± 39.38	57.01 ± 28.07	56.93 ± 26.63	> 0.05	> 0.05
OC (ng/mL)	13.19 ± 14.56	7.15 ± 8.17	3.6 ± 3.8	< 0.05	< 0.01
DPyr (nM DPyr/mM cre)	28.79 ± 22.79	33.65 ± 21.31	44.78 ± 27.68	< 0.05	< 0.001
Ca/Cr	0.07 ± 0.06	0.12 ± 0.13	0.22 ± 0.14	< 0.01	< 0.001
BMD (gr/cm^2)	-1.49 ± 1.44	-1.77 ± 1.46	-2.25 ± 1.5	< 0.001	< 0.001

BUN: blood urea nitrogen; Ca: adjusted serum calcium for hypoalbuminaemia; P: phosphate; T-ALP: total alkaline phosphatase; B-ALP: bone-specific alkaline phosphatase; PTH: parathyroid hormone; OC: osteocalcin; DPyr: urine deoxypyridinoline; Ca/Cre: calcium/creatinine ratio; BMD: bone mineral density

The cumulative doses of prednisolone were 1.5 ± 0.57 gr and 2.6 ± 0.98 gr at the 4th and 12th week of initial therapy, respectively. Bone mineralization decreased with the cumulative doses of prednisolone during the treatment.

Correlations between bone mineral density and bone turnover markers were insignificant at admission, the 4th and 12th weeks.

DISCUSSION

Children with NS are prone to metabolic bone disease because of both biochemical derangements caused by the renal disease and corticosteroid therapy. There are controversial reports related to bone mineral alteration during steroid therapy. Bak *et al* (4) reported that BMD decreased at a rate of 13.0 ± 4.0% in a steroid treated group. On the other hand, no reduced BMD was found in another study (5). Also Leonard *et al* (3) reported that the subjects with GSNS had received an average of 23 000 mg of glucocorticoids over a 4-year interval and spine BMC did not differ significantly. These data suggested that intermittent treatment with high-dose glucocorticoids during growth was not associated with bone deficits in GSNS (6). Steroid-induced osteoporosis is observed within the first 6−12 months after the start of therapy and the risk of bone fracture is doubled in patients treated with steroids at more than the equivalent of 7.5 mg/day (6). Reduced total BMD has been found in steroid-treated children with NS as well as a reduction with the cumulative steroid dose in this present study where relatively high doses of prednisolone were used. The cause of decreased BMD may be explained by the higher glucocorticoid doses we used. There was not enough information on the decreasing time of BMD after prednisolone in children. The data also showed that decrease in BMD began as early as four weeks.

Nephrotic syndrome often occurs as a part of a systemic illness with an inflammatory state. The increased osteoclastic bone resorption accompanying inflammation is thought to be mediated by TNFα and other cytokines rather than by the RANKL, RANK, OPG pathway (7). On the other hand, it was reported that both 25(OH) vitamin D and calcitriol are transported by vitamin D binding globulin and albumin, which are depleted by heavy proteinuria-albumin losses. These changes contribute to secondary hyperparathyroidism and increased bone turnover (7, 8). In our study, serum PTH levels did not significantly change during the treatment period. These data suggest that 25(OH) vitamin D levels were not significantly decreased although 25(OH) vitamin D levels were not measured.

A reduced BMD is common, particularly in the first several months of treatment, when prednisolone doses exceed 5 mg/day. Glucocorticoid use is associated with reduced osteoblastic bone formation, increased apoptosis of both osteocytes and osteoblasts and increased ratio of RANKL to OPG that favours osteoblastic bone resorption (7). Glucocorticoids also indirectly affect bone. It causes reduced intestinal calcium absorption and increased urinary calcium losses (7). In the literature search, Bak *et al* (4) found normal calcium levels when adjusted for hypoalbuminaemia. We found that adjusted serum calcium levels for hypoalbuminaemia significantly decreased at the 4th week

compared with baseline value and then they increased at the 12th week; whereas Ca/Cr ratio progressively increased although serum Ca levels increased between 4−12 weeks. These data suggest that urinary calcium losses continue during glucocorticoid treatment. In addition, corticosteroid treatment significantly increased urinary calcium losses without leading to secondary hyperthyroidism.

While serum total and b-ALP, urine DPyr progressively increased, serum OC levels decreased in the present study. Osteocalcin, a non-collagenous protein of bone matrix, is known to be a good marker of bone formation and a sensitive indicator of the inhibitory effects of steroids. Bone-specific alkaline phosphatase, which is one of the isoenzymes, is produced by osteoblast, and a good marker of bone formation (9). Bone-specific alkaline phosphatase levels of our patients were higher than in healthy children, and increased during therapy. This condition may be related to increased bone turnover and improvement of massive proteinuria. However, there is some controversy. Why do serum b-ALP levels increase while serum OC levels decrease? Biyikli *et al* (10) found that both serum OC and ALP levels significantly decreased after the completion of steroid treatment. On the other hand, Lems *et al* (11) reported that OC levels decreased transiently but ALP levels remained unchanged. The cause of this may be multifactorial. It may be a consequence of reduction of osteoblast differentiation and an inhibition of the synthesis of osteoid by these cells. We hypothesize that b-ALP and OC could represent different aspects and different phases of osteoblast function. Thus, a case with inherited vitamin K deficiency was reported; this child had low osteocalcin level and high b-ALP levels and osteocalcin level increased while b-ALP level decreased after treatment (12). Corticosteroids also increase bone resorption; DPyr reflects the activity of osteoclast and it is often used as a marker of bone resorption.

All biochemical changes with steroid treatment in NS are potentially reversible. Freundlich *et al* (13) found that biochemical abnormalities related to NS normalized during remission like in this report. Sierra *et al* (14) reported that the biochemical bone markers and BMD normalized after the total serum protein returned to control values in NS rats. Our data did not support this experimental result.

Children may be vulnerable to the effects of glucocorticoids on bone formation including peak bone mass while they grow up. Reduced bone mineral density may be as a consequence of inhibition of the bone-forming osteoblast and of activation of bone resorption but also of inhibition of intestinal absorption of calcium and its reabsorption by the renal tubule.

In conclusion, data from this study may point to a higher bone turnover and thus a reduced BMD in patients treated with high doses of steroids. These children should undergo regular BMD evaluation, and appropriate therapeutic approach should be planned.

REFERENCES

1. Olgaard K, Storm T, van Wovern N, Daugaard H, Egfjord M, Lewin E et al. Glucocorticoid induced osteoporosis in lumbar spine, forearm and mandible of nephrotic patients: a double blind study on the high dose long term effects of prednisolone versus deflazocort. Calcif Tissue Int 1992; **50:** 490−7.
2. Yildirim ZK, Buyukavci M, Eren S, Orbak Z, Sahin A, Karakelleoglu C. Late-side effects of high-dose steroid therapy on skeletal system in children with idiopathic thrombocytopenic purpura. J Pediatr Hematol Oncol 2008; **30:** 749−53.
3. Leonard MB, Feldman HI, Shults J, Zemel BS, Foster BJ, Stallings VA. Long-term, high-dose glucocorticoids and bone mineral content in childhood glucocorticoid-sensitive nephrotic syndrome. N Engl J Med 2004; **351:** 868−75.
4. 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.
5. Esbjörner E, Arvidsson B, Jones IL, Palmer M. Bone mineral content and collagen metabolites in children receiving steroid treatment for nephrotic syndrome. Acta Paediatr 2001; **90:** 1127−30.
6. Eastell R. Management of corticosteroid-induced osteoporosis. UK Consensus Group Meeting on Osteoporosis. J Intern Med 1995; **237:** 439−47.
7. Elder GJ. Nephrotic syndrome: don't forget the bones! Nephrology 2008; **13:** 43−4.
8. van Hoof HJ, de Sevaux RG, van Baelen H, Swinkels LM, Klipping C, Ross HA et al. Relationship between free and total 1,25-dihydroxyvitamin D in contributions of modified binding. Eur J Endocrinol 2001; **144:** 391−6.
9. Yang L, Grey V. Pediatric reference intervals for bone markers. Clin Biochem 2006; **39:** 561−8.
10. Biyikli NK, Emre S, Sirin A, Bilge I. Biochemical bone markers in nephrotic children. Pediatr Nephrol 2004; **19:** 869−73.
11. Lems WF, Gerrits MI, Jacobs JW, van Vugt RM, van Rijn HJ, Bijlsma JW. Changes in (markers of) bone metabolism during high dose corticosteroid pulse treatment in patients with rheumatoid arthritis. Ann Rheum 1996; **55:** 288−93.
12. Orbak Z, Selimoglu A, Doneray H. Inherited vitamin K deficiency: case report and review of literature. Yonsei Med J 2003; **44:** 923−7.
13. Freundlich M, Bourgoignie JJ, Zilleruelo G, Abitbol C, Canterbury JM, Strauss J. Calcium and vitamin D metabolism in children with nephrotic syndrome. J Pediatr 1986; **108:** 383−7.
14. Sierra RI, Specker BL, Jimenez F, Cruz C, Pedraza-Chaverri J. Biochemical bone markers, bone mineral content, and bone mineral density in rats with experimental nephrotic syndrome. Ren Fail 1997; **19:** 409−24.