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Original Article

Parathyroid Hormone, Osteocalcin, and Bone Mineral Density in Children with Steroid-Dependent and Frequently-Relapsing Nephrotic Syndrome

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ABSTRACT

Background: A long-term corticosteroid treatment is often required for children with nephrotic syndrome, which may affect their bone metabolism.

Objectives: The aim of this work was to study bone mineral density (BMD) and some laboratory markers of bone metabolism (serum osteocalcin, parathyroid hormone, and serum alkaline phosphatase) in a group of nephrotic children who received multiple courses of prednisone.

Methods: We measured serum calcium, phosphorus, alkaline phosphatase (AP), osteocalcin (OC) and parathyroid hormone (PTH) in 30 children aged 3.8 to 12 years with steroid-dependent and frequently-relapsing nephrotic syndrome (21 males and 9 females), and in 15 age and sex matched controls. Measurement of bone mineral density (BMD) was performed for patients and controls using quantitative computed tomography (QCT).

Results: Nephrotic children had significantly lower serum levels of alkaline phosphatase (189.9 ± 15.4 IU/l), and osteocalcin (1.7 ± 1.5 ng/ml) compared with controls (226.6 ± 85.5 IU/l and 4.8 ± 1.1 ng/ml, respectively), $p \leq 0.05$ for each. Also, BMD was significantly lower in patients (118.2 ± 26.5 gm/cm^3) compared with controls (150.3 ± 33.5 gm/cm^3), $p \leq 0.05$. A significantly positive correlation was found between BMD and serum osteocalcin level (R: 0.447, p: < 0.05), and a significant negative correlation was found between BMD and the total dose of prednisone (R: - 0.546, p: < 0.01).

Conclusions: Repeated courses of prednisone used for treatment of children with steroid-dependent and frequently-relapsing nephrotic syndrome lead to decreased bone mineral density with decreased serum levels of osteocalcin and alkaline phosphatase, while serum PTH remains normal in the absence of hypocalcemia.

INTRODUCTION

In childhood the most frequent type of idiopathic nephrotic syndrome is the steroid responsive nephrotic syndrome. The most widely used derivative in the conventional treatment is prednisone. The aim of the treatment is to induce remission, to prevent relapses and to avoid side effects[1,2]. The standard regimen used worldwide consists of an initial treatment with continuous prednisone followed by alternate-day treatment[1]. In a number of children the relapsing course of the disease may potentiate the possibility of side effects due to prolonged therapy such as Cushingoid appearance, hypertension, cataracts, osteoporosis, and growth retardation[2].

Glucocorticoids have long been associated with osteoporosis and their effects on bone and mineral metabolism have been

extensively reviewed[3,4]. Pharmacologic doses of glucocorticoids are associated with decreased gastrointestinal calcium absorption and increased urinary calcium excretion, resulting in a negative calcium balance. Glucocorticoids are also associated with increased parathyroid hormone (PTH) secretion and decreases in gonadotropins, growth hormone, and adrenocorticotropic hormone (ACTH) release. Glucocorticoids directly inhibit bone formation by decreasing osteoblast differentiation and by inhibition of type 1 collagen synthesis. Glucocorticoids also stimulate bone resorption by directly enhancing osteoclast activity, as well as indirectly via increased PTH production and decreased gonadotropins[5].

Classical x-ray has been traditionally used for bone imaging. However, it is not sensitive enough to detect early changes in bone structure, because pathology becomes evident when 30-40% of bone mineral is already lost[6]. Quantitative computed tomography (QCT) is a more recent addition to the field of bone mineral analysis. It can identify the absolute mineral content of a specific volume of bone. It offers non-invasive determination of vertebral bone mineral content with exact three-dimensional localization of the measurement site, and separation of cortical from trabecular bone[7,8].

AIM OF THE WORK

The aim of this work was to study bone mineral density (BMD) and PTH in a group of children with steroid-dependent and frequently-relapsing nephrotic syndrome who received multiple courses of prednisone, in addition to serum osteocalcin and serum alkaline phosphatase (as markers of bone formation).

PATIENTS AND METHODS

This study was carried out at Mansoura Urology and Nephrology Center and included 30 children (21 males and 9 females) with steroid-dependent and frequently-relapsing nephrotic syndrome, their ages ranged from 3.8 to 12 years (7.85 ± 3.24 yrs). Patients were diagnosed according to the criteria submitted by the International Study of Kidney Diseases in Children[9].

Fifteen apparently healthy, children were included as a control group, they were selected from the outpatient clinic complaining from minor surgical problems, they were 9 males and 6 females, and their ages ranged from 4 to 14 years (8.80 ± 3.36 yrs).

All patients were subjected to careful history taking and thorough clinical examination, and the medical charts were reviewed to determine age, sex, disease duration, presence of edema, hypertension, pallor, growth retardation, bone deformities, muscle weakness, type of treatment, its duration, and the dates and numbers of relapses. The overall amounts of prednisone and the average daily prednisone dose that each patient received during therapy were calculated.

None of our selected patients had impaired renal function or other conditions unrelated to nephrotic syndrome that could affect bone health, and non of them received other immunosuppressive drugs or vitamin D preparations. Proteinuria and edema were documented to be absent at the time of the study visit.

Estimation of parathormone (PTH) level in the serum was performed by multivalent radioimmunoassay using commercially available ELISA kits (R & D system, Minneapolis, MN, USA).

Serum osteocalcin (OC) was determined by radioimmunoassay using a kit supplied by Diagnostic System Laboratories Inc. (Cat. No. 171246).

Determination of serum creatinine, calcium, phosphorus, and alkaline phosphatase levels was carried out by standard laboratory techniques using Hitachi 771 autoanalyzer (Boehringer Mannheim).

Measurement of bone mineral density (BMD) was done for all patients and controls using quantitative computed tomography (QCT). This was made using G.E. prospeed plus with 512 x 512 matrix and solid state phantom containing 0 mg/cc, 75 mg/cc and 150 mg/cc of calcium hydroxyapatite. The density of the selected area of interest within a slice through a vertebral body is measured in Houndsfield units (HU), also known as CT number where water = 0 HU and air = -100 HU. Conversion to gm/cm^3 is made by comparing the CT number of the trabecular bone to that of the compartment of the calibration standard. The calculated densities of the vertebrae are averaged and compared to those of a normal population[10].

Statistical Analysis

The data of the study were tabulated and analyzed by SPSS (Statistical Package for Social Science) under windows (version 11). Data were expressed as mean ± standard deviation (Mean ± SD) for parametric variables. Comparisons between groups were done by student's t-test (parametric) and chi-square test (non-parametric) when appropriate. For measurement of linear relations, Pearson's correlation coefficient was considered. The level of statistically significant difference was defined as $p < 0.01$.

RESULTS

The results of this study are reported in Tables 1 to 3. The total amount of prednisone taken ranged from 548 - 12,476 mg (4312 ± 2305), and the average prednisone dose (mg/day) ranged from 7.5 - 45 (28.12 ± 11.50).

As shown in Table 2, nephrotic children had significantly lower serum levels of alkaline phosphatase (189.9 ± 15.4 IU/l), and osteocalcin (1.7 ± 1.5 ng/ml) compared with controls (226.6 ± 85.5 IU/l and 4.8 ± 1.1 ng/ml, respectively), $p \leq 0.05$ for each. Also, BMD was significantly lower in patients (118.2 ± 26.5 gm/cm^3) compared with controls (150.3 ± 33.5 gm/cm^3), $p \leq 0.05$. No significant differences were detected between patients and controls as regard serum calcium, phosphorus or parathormone levels.

As shown in Table 3, we found significantly positive correlation between BMD and serum osteocalcin levels (R: 0.447, p: < 0.05), and significantly negative correlation between BMD and the total dose of prednisone (R: -0.546, p: < 0.01). No significant correlation was found between BMD and serum calcium, alkaline phosphatase or the disease duration.

Table 1: Data from 30 children with steroid-dependent and frequently-relapsing nephrotic syndrome.

	Mean ± SD	Range
Age (years)	7.85 ± 3.24	3.8 - 12
Sex: M/F	21/9	-
Duration of therapy (years)	2.33 ± 1.52	0.83 - 4.32
Cumulative dose of steroids (mg)	4312 ± 2305	548 – 12,476
Average prednisone dose (mg/day)	28.12 ± 11.50	7.5 - 45

Table 2: Mean ± standard deviation (M ± SD) of serum calcium, phosphorus, alkaline phosphatase, calcitonin, PTH and BMD in nephritic children and controls.

	Patients n = 30	Controls n = 15	p
	Mean ± SD	Mean ± SD	
Calcium *(mg/dl)*	9.07 ± 0.80	9.95 ± 0.80	NS
Phosphorus *(mg/dl)*	5.19 ± 0.73	4.95 ± 0.68	NS
Alk. phosph. *(IU/l)*	189.9 ± 15.4	226.6 ± 85.5	< 0.05
Osteocalcin *(ng/ml)*	1.7 ± 1.5	4.8 ± 1.1	< 0.05
PTH *(pg/dl)*	16.4 ± 8.4	12.1 ± 6.1	NS
BMD *(gm/cm^3)*	118.2 ± 26.5	150.3 ± 33.5	< 0.05

Table 3: Correlation between bone mineral density and some variables in nephrotic children.

	R	p
Calcium	0.026	0.781 (NS)
Alkaline Phosphatase	0.216	0.260 (NS)
Osteocalcin	0.447	< 0.05
Disease Duration	- 0.290	0.113 (NS)
Total dose of steroids	- 0.546	< 0.01

DISCUSSION

In the present study, we found that serum levels of calcium and phosphorus didn't differ significantly between patients and controls, while serum levels of osteocalcin and alkaline phosphatase were significantly lower in nephrotic children as compared to healthy controls. Our results are in accordance with the results of El-Moselhy et al.[11], who found no significant difference between the patients and controls as regards the mean values of serum calcium and phosphorus, while the mean value of serum alkaline phosphatase (marker for bone formation) was significantly lower in patients compared to the controls, thus indicating suppressed bone formation in nephrotic patients. Wojnar et al.[12] found that serum alkaline phosphatase was significantly lower in nephrotic patients after 6 months of steroid therapy compared to pretreatment values, and suggested that corticosteroids influence bone metabolism through suppression of bone formation rather than induction of bone loss. Similar findings were reported by Mishaela et al.[13] who stated that the cardinal feature of glucocorticoid-induced osteoporosis on skeletal dynamics is a reduction in bone formation, and reported that biochemical markers of bone formation, osteocalcin and bone-specific alkaline phosphatase, are suppressed.

El-Khodary et al.[14] and Meeran et al.[15] found that serum osteocalcin was markedly and significantly reduced after 1 and 3 months of steroid therapy respectively, compared to control subjects with no difference in alkaline phosphatase, and concluded that serum osteocalcin is an early and more sensitive marker for decreased bone formation. The longer duration of steroid therapy in our study explains this discrepancy as regards serum alkaline phosphatase levels.

On the other hand, Abu Al-Hassan et al.[16] found normal serum osteocalcin and bone-specific alkaline phosphatase levels in their nephrotic children and reported that osteopenia was associated with increased evidence of bone resorptive markers.

As regards PTH, although its serum levels were higher in our patients compared to controls, the difference was not statistically significant. It was previously shown that patients with nephrotic syndrome and normal renal function have elevated blood levels of PTH[17]. This state of secondary hyperparathyroidism was attributed to the decrease in the blood levels of ionized calcium[18], and Malluche et al.[19] reported that the states of vitamin D deficiency and secondary hyperparathyroidism are associated with bone disease manifested by defective mineralization of osteoid and enhanced bone resorption. Patients in our study had normal serum calcium as compared to control subjects and this may explain the contradiction with previously mentioned studies.

Our results are in agreement with Tessitore et al.[20] and Mittal et al.[21]. Abu Al-Hassan et al.[16] found similar results and concluded that the normal serum PTH levels in such patients suggest that hyperparathyroidism may not share in the pathogenesis of bone changes occurring in primary nephrotic syndrome. Data indicating that PTH levels are not changed by glucocorticoid administration come from studies in

normal, healthy subjects receiving prednisone (either short or long courses)[13], as well as in specific medical illnesses requiring steroid therapy[22-25]. The expectation that PTH levels should have been elevated in these studies, given increases in urinary calcium excretion and decreases in intestinal calcium absorption is not confirmed by the data. It remains possible that skeletal responsiveness to PTH is differentially affected by glucocorticoids without any change in PTH concentrations. Thus, even a normal PTH level might elicit significant physiological effects, such as an elevation in the level of cAMP[26,27].

In the present study, we found that BMD (measured by QCT) was significantly lower in our patients compared with controls, and there was a significant negative correlation between BMD and the total doses of prednisone the patients received. This is in agreement with Fujita et al.[28] who reported that high-dose glucocorticoids rapidly decrease patients' basal BMD, and found that BMD of the lumbar spine (measured by DEXA) significantly decreased in a 3-month treatment group compared with the pretreatment group, and BMD in the long-term treatment group decreased continuously. Lettgen et al.[29] using peripheral QCT, found that cortical and total bone density were inversely correlated with the cumulative dose of steroid treatment. They found a decrease in BMD in children with idiopathic nephrotic syndrome with high cumulative doses of steroids compared with controls and with children with a low cumulative steroid dose. Gulati et al.[30] conducted a study in India using DEXA to determine the areal bone mineral density of the spine in 100 children with the nephrotic syndrome, concluding that the majority had osteopenia. Sixty-one percent of their subjects were given a diagnosis of osteopenia (areal bone mineral density z score, less than -1), and 22 percent received a diagnosis of osteoporosis (z score, less than -2.5). The deficits in areal bone mineral density and cumulative exposure to glucocorticoids were greater in the children with glucocorticoid-dependent nephrotic syndrome, frequent relapses, or glucocorticoid-resistant nephrotic syndrome than in those with infrequent relapses.

Also, our results are in accordance with El-Khodary et al.[14], who found that BMD as measured by QCT was significantly reduced in their children treated with prednisone in the usual dose (1-2 mg/kg, maximum 60 mg, for 1 month) compared to the control group. El-Moselhy et al.[11] found that BMD of 29 nephrotic children (assessed by DEXA) was significantly reduced compared to controls. Similar results were reported by Takeda[31] using QCT. Abu Al-Hassan et al.[16] found osteopenia in 30.9% of their patients with primary nephrotic syndrome. Glucocorticoids do seem to have adverse skeletal effects, even in the nephrotic syndrome[32].

On the other hand, The study by Leonard et al.[33] examined the skeletal effects of glucocorticoid therapy in a homogeneous group of children with glucocorticoid-sensitive nephrotic syndrome. The children had received an average of 23,000 mg of prednisone over a mean of 4.4 years, three times as much as the daily dose of 5 mg that represents the adult threshold for bone loss. Despite long-term glucocorticoid

therapy, the bone mineral content of the lumbar spine and whole body was similar among children with the nephrotic syndrome and controls, after adjustment for age, sex, bone area, maturity (Tanner stage), and race. Children with the nephrotic syndrome had a lower bone mineral content than the controls only after correction for body-mass index and only at the spine[33]. The earliest changes of glucocorticoid-induced bone loss are indeed seen in the lumbar spine because of its high bone mineral content[30]. Differences in nutritional status between Egyptian and American children may contribute - in part - to differences in bone health. Also, it is not possible to exclude the possibility of bone loss or decreased rates of mineral accrual in individual patients in that study as the data were cross-sectional.

The present study provides further evidence that repeated courses of prednisone used for treatment of children with steroid-dependent and frequently-relapsing nephrotic syndrome lead to decreased bone mineral density with decreased serum levels of osteocalcin and alkaline phosphatase, while serum PTH remains normal in absence of hypocalcemia. If we care for patients on steroid therapy, we have to consider both the problem of steroid-induced osteoporosis, and to find answers to some questions about its pathogenesis and clinical management. These include identification of the optimum BMD threshold at which to intervene with bone-active drugs, the dose or duration of exposure to steroid therapy that warrants intervention, and the presence of risk factors as low BMD at baseline.

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