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Original Article

Bone Mineral Density and Bone Turnover Markers in Children with Primary Nephrotic Syndrome

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ABSTRACT

Background: Children with PNS may be at risk for metabolic bone disease because of biochemical derangement caused by the renal disease as well as steroid therapy. Few studies have been done for the evaluation of the magnitude of osteopenia in children with nephrotic syndrome but the results were conflicting.

Objectives: This study was conducted to highlight the prevalence as well as the contributing factors responsible for the development of osteopenia in children with primary nephrotic syndrome (PNS).

Methods: Measurement of bone mineral density (BMD) in the lumbar spine region (L2-L4) using dual energy x-ray absorprometry (DEXA) was done in 42 patients with PNS (28 males and 14 females, aged 3 to 15 years) and 352 healthy age- and sex-matched Egyptian children. Serum levels of osteocalcin, bone specific alkaline phosphatase (BAP), parathyroid hormone (PTH) and 25-OH cholecalciferol (25-HCC) and urinary deoxypyridinoline (Dpd) were measured in 14 patients out of the studied children and 12 healthy age- and sex-matched controls.

Results: Osteopenia was observed in 13 patients (30.9%), 7 of them had non-severe osteopenia (Z-score between -1 and -2.5) and 6 had severe osteopenia (Z-score more negative than -2.5). Compared to controls patients showed significantly lower 25-HCC [median = 17.5, range 11-33.3, vs 66, range 61-69 ng/ml; $p = 0.0001$] and higher urinary Dpd [median = 81.1, range 76.5-174.9 vs 47.1, range 29.0-58.1 nmol/l; $p = 0.001$]. No significant differences were observed in serum osteocalcin, BAP and PTH between patients and controls. A significant negative correlation was observed between Z-score and the number of relapses ($r = -0.35$, $p = 0.02$). No correlation was observed between Z-score and total dose of corticosteroid, age or sex ($p > 0.05$).

Conclusions: Osteopenia is evident in about one third of children with PNS. This osteopenia is associated with increased evidences of bone resorptive markers and normal osteoblastic markers and is related to the number of relapses. These patients may get benefits from vitamin D supplementation and bone anti-resorptive medications.

INTRODUCTION

Bone remodeling occurs constantly throughout life with removal of old bone and replacement with new one. Osteoclasts are responsible for reabsorbing bone, whereas osteoblasts are responsible for laying down new bone[1]. The main bone minerals are calcium and phosphorous[2].

The cells that accomplish this bone remodeling are closely regulated as are the absorption and excretion of the bone minerals used in this remodeling[1]. The main hormonal regulators of bone remodeling activity are parathyroid hormone (PTH), vitamin D and calcitonin[3].

Children with PNS may be at risk for metabolic bone disease because of biochemical derangement caused by the renal disease as well as steroid therapy[4,5]. Few studies have been done for the evaluation of the magnitude of osteopenia in children with nephrotic syndrome but the results were conflicting[4-7].

AIM OF THE WORK

This study was conducted to highlight the prevalence of osteopenia and contributing factors for this problem in children with PNS.

SUBJECTS AND METHODS

This study was conducted on 42 patients with PNS (28 males and 14 females) aged 4-15 years. Patients were recruited consecutively from the Pediatric Nephrology Unit, Mansoura University Children's Hospital, Mansoura, Egypt in the period between June 2002 and January 2003. Patients were diagnosed according to the criteria submitted by the International Study of Kidney Diseases in Children (ISKDC)[8]. All patients had normal kidney functions.

Assessment of bone mineral density was done in all studied children. Assessment of the osteoblastic activity (by measuring serum osteocalcin and bone specific alkaline phosphatase) and osteoclastic activity (by measuring urinary deoxypyridinoline) and serum PTH and 25 OH cholecalciferol were done in 14 out of studied patients and 12 age- and sex-matched healthy children.

1) Assessment of bone mineral density (BMD): By using dual energy X-ray absorprometry (DEXA, Lunar DPX-IQ system, USA).

   BMD of lumbar spinal region (L2-L4) of the patients was assessed in the Pediatric Endocrinology and Diabetes Unit, Mansoura University Children's Hospital, Mansoura, Egypt. The results of BMD testing of the patients were compared to BMD of 352 healthy age- and sex-matched Egyptian healthy children. BMD results were expressed as Z-scores which were calculated from the following equation; Z-Score = [BMD (g/cm^2) of patient – BMD predicted for age and sex]/SD for BMD (age- and sex-matched)]. Patients were considered as osteopenic if Z-score was < -1.0 and if Z-score was ≤ -2.5 patient was considered to have severe osteopenia.

2) Measurement of serum osteocalcin: By using The Biosource Human Osteocalcin-EASIA (ELIZA-Biosource, Europe S.A., Belgium) which is a solid phase enzyme amplified sensitivity immunoassay (EASIA) performed on microtiter plate. The assay uses monoclonal antibodies directed against distinct epitopes of human osteocalcin.

3) Measurement of serum bone specific alkaline phosphatase: By using the Alkphase-B (ELIZA-MetroBiosystems Inc, CA, USA) which is an immunoassay in a microtiter strip format utilizing a monoclonal anti-BAP antibody coated on the strip to capture BAP in the sample. The enzyme activity of the captured BAP is detected with a p-nitrophenyl phosphate substrate.

4) Measurement of urinary deoxy-pyridoxine (Dpd) cross-links: By using the pyrilinks-D assay (ELIZA-Metro-Biosystems Inc, CA, USA) which is a competitive enzyme immunoassay in a microtiter stripwell format utilizing a monoclonal anti-Dpd antibody coated on the strip to capture Dpd.

5) Measurement of serum PTH: By using PTH-EASIA (ELIZA-Biosource Europe S.A., Belgium) which is a solid phase

enzyme amplified sensitive immunoassay performed on in serum plate to measure serum intact human PTH.

6) Measurement of serum 25-OH cholecalciferol: By using 25 OH-VIT.D3-RIA-CT (RIA-Biosource Europe S.A, Belgium) which is a radioimmunoassay for the quantitative measurement of 25 OH-Vit.D3 in serum.

Statistical Analysis:

Analysis of data was done using Statistical Package For the Social Sciences (SPSS) for windows version 10. Distribution of data was tested by using Kolmagorov-Smirnov test. Data were expressed as median and interquartile range (IQR). Non-parametric tests were applied. Mann-Whitney u test was used for comparison between groups. Spermann rank and Kendall's tau-t tests were used for the assessment of correlation between variables.

RESULTS

Fig. 1 shows that osteopenia was observed in 13 patients (30.9%), 7 of them had non-severe osteopenia (Z-score between -1 and -2.5) and 6 had severe osteopenia (Z-score more negative than -2.5).

Compared to controls, patients showed significantly lower 25-HCC [median = 17.5, range 11-33.3, vs 66, range 61-69 ng/ml; p = 0.0001] and higher urinary Dpd [median = 81.1, range 76.5-174.9 vs 47.1, range 29.0-58.1 nmol/1; p = 0.001]. However no significant differences were observed in serum osteocalcin, BAP and PTH between patients and controls [Table 1].

A significant negative correlation was observed between Z-score and the number of relapses (r = -0.35, p = 0.02). No correlation (p > 0.05) was observed between Z-score and total dose of corticosteroid, age or sex [Table 2].

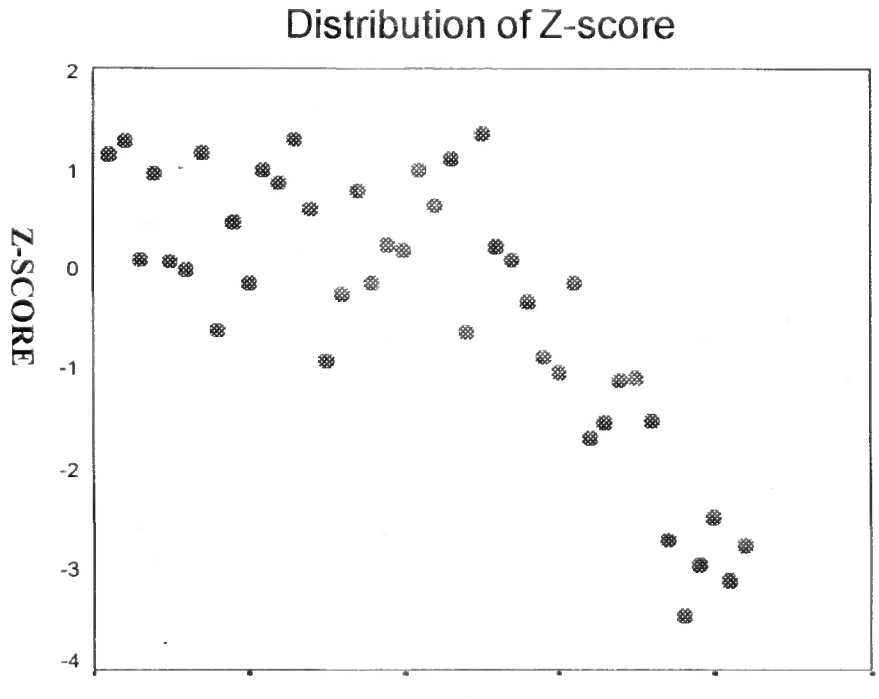

Fig. 1: Z-score values of the studied children (lines presents normal range).

Table 1: Comparisons of urinary deoxypyridinoline (Dpd) and serum bone specific alkaline phosphatase (BAP), osteocalcin, PTH and 25-HCC between patients and controls.

	Patients (n = 14)		Controls (n = 12)		p
	Median	IQR	Median	IQR	
Dpd (nmol/L)	81.1	76.5-174.9	47.1	29.0-58.1	0.001
BAP (U/L)	78.6	46.2-93.5	110.6	71.9-122.4	0.08
Osteocalcin (ng/ml)	84.1	53.1-88.0	78.3	72.4-82.1	0.44
PTH (pg/ml)	27.1	18.2-32.2	26.1	23.5-33.5	0.47
25-HCC (ng/ml)	17.5	11-33.3	66.0	61.0-69.0	0.0001

Table 2: Correlations between Z-scores and the numbers of relapses, total dose of steroid, age and sex.

	Z-Score	
	R	p
No of relapses	-0.35	0.02
Total dose of steroid	0.02	0.9
Age	-0.28	0.7
Sex	-0.09	0.5

DISCUSSION

The osteoblastic activity of bone could be denoted by markers as BAP as well as osteocalcin which are secreted in large amounts by active osteoblasts[9]. The breakdown of bone matrix by osteoclasts results in collagen peptides that have pyridinoline structures such as Dpd which can be assayed in urine as a measure of bone resorption rates[9]. DEXA is the most readily available and the most commonly used technique for the measurement of BMD in children as it has short scan time, very low radiation dose, excellent precision and the ability to assess bone mineral content (BMC) and (BMD) at both axial and appendicular skeletal sites[10].

In this study osteopenia, assessed by DEXA, was observed in 13 PNS patients (30.9%). Out of them 7 (16.7%) had non-severe osteopenia while 6 (14.3%) had severe osteopenia. Twenty two patients of 100 children with PNS (22%) had osteopenia when their BMD of the lumbar spines was examined by DEXA[11]. Fujita et al.[12] who studied BMD of lateral lumbar spines in 23 patients with PNS using DEXA, observed osteopenia in 10 patients.

Osteopenia was diagnosed in 30% of chronic nephrotic children who studied by Chlebna-Sok and his colleagues[13]. BMD of the total body and spines was assessed in these patients by DEXA. Takeda[14] demonstrated a significant decrease in BMD of lumbar vertebrae assessed by quantitative computed tomography in sixteen children with idiopathic nephrotic syndrome. However Polito et al.[6] and Esbjorner et al.[15] reported no significant decrease in BMC in children with PNS compared to healthy controls. This discrepancy may be related to the small number of patients assessed in these two studies.

Our study showed high urinary Dpd and normal serum osteocalcin and BAP levels. These observations point to the increased osteoclastic activity and the normal osteoblastic activity in patients with PNS. Jwojnar et al.[16] showed high serum carboxyterminal pyridinoline crosslinked telopeptide of type I collagen in patients with PNS under steroid therapy. High urinary pyridinoline were reported in rats[7] and children[13] with nephrotic syndrome. Fujita et al.[12] showed high urinary Dpd levels in patients with PNS. These levels decreased significantly after a 3 month treatment with etidronate. Bone histology in patients with PNS and normal renal functions showed isolated osteomalacia in 17-56.7% of patients and 7-10% had an increased bone resorption in addition to defective mineralization[17,18].

Low serum 25 HCC and normal PTH levels in children with PNS were reported in the present study. Barragry et al.[19], Tessitore et al.[17] and Mittal et al.[18] showed lower serum levels of 25 HCC in patients with PNS. These low levels could be explained by the enhanced urinary excretion and the decreased plasma binding capacity of vitamin D3 metabolites in patients with PNS[20]. Normal serum PTH levels in children with were reported by other investigators[17,18]. The normal serum PTH levels suggest that hyperparathyroidism may not share in the pathogenesis of bone changes occurring in PNS.

In our study osteopenia was correlated with the number of relapses of the disease while there was no correlation between osteopenia and the cumulative dose of steroids. Polito et al.[6] demonstrated no significant relation between BMC in nephrotic patients and the amount of prednisone taken or the duration of therapy. Freundlich et al.[21] stated that abnormalities of mineral and bone metabolism in nephrotic children occurred irrespective of steroid therapy. The presence of a significant correlation between the numbers of relapses and the degree of osteopenia indicates that corticosteroid is not the only factor responsible for osteopenia in these patients but other factors may contribute as nutritional deficiency and hypoproteinemia[22] and immobilization[23].

In conclusion, osteopenia is evident in about one third of children with PNS. This osteopenia is associated with increased evidences of bone resorptive markers and normal osteoblastic markers and is related to the number of relapses. These patients may get benefits from vitamin D supplementation and bone anti-resorptive medications.

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