Metabolic Bone Disease in Children with Idiopathic Nephrotic Syndrome

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Abstract: Children with idiopathic nephrotic syndrome (INS) may be at risk for metabolic bone disease (MBD) because of biochemical derangements caused by the renal disease, as well as the corticosteroid effects on bone. We studied 70 children with INS for clinical, biochemical, and radiological evidence of MBD. These patients were divided into two groups: 55 frequent relapsers (FR; group I), and 15 infrequent relapsers (IFR; group II). Thirty healthy children matched for age and sex constituted the control group. Bone mineral density (BMD) of these children was evaluated by the Achilles Express Quantitative Ultrasound (QUS) device. Univariate and multivariate analyses were performed to analyze factors predictive of low BMD T- score. We observed that nephrotic children had significantly lower mean BMD T- scores compared with controls (-1.99 ± 0.74 versus -0.39 ± 0.87; P = <0.0001). Also, children in group I were found to have significantly lower mean BMD T- scores compared with group II (-2.1± 0.67 versus -1.31± 0.64; P = <0.0001). We also observed that 32.7% of group I had osteoporosis compared to none of group II (P= 0.01). Significantly higher doses of steroids over longer duration of therapy were administered to group I compared with group II (P = < 0.0001 and 0.0004 respectively). On multivariate analysis, the only factor found to be predictive of a low BMD T-score was greater cumulative steroid dose (P= 0.02). We concluded that children with INS are at risk for MBD, especially those receiving higher doses of steroids. Regular BMD evaluation and appropriate therapeutic interventions are recommended for these children. The role of prophylactic therapy in such patients needs to be further investigated.

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1. Introduction

Children with idiopathic nephrotic syndrome (INS) may be at risk of metabolic bone disease (MBD) (*Gulati et al., 2003*). Patients with nephrotic syndrome and normal renal function frequently have abnormalities in calcium and vitamin D metabolism *(Grymonprez et al., 1995)*.

Glucocorticoid (GCS) drugs, mainly prednisone, remain the sheet anchor of therapy in children with INS. These drugs are often prescribed on a long-term basis, and are associated with a number of side effects. The most common and serious, however, is bone loss leading to osteoporosis *(Cohen and Adachi, 2004)*. In fact, they are considered the number-one cause of secondary osteoporosis *(Dore, 2010)*. Bone loss occurs even with low-dose GCS therapy, but is most rapid and extensive at prednisone doses ≥5 mg per day or equivalent. Loss of bone mass is most rapid during the first year of GCS therapy and significant reductions can be seen as soon as 3 months after starting therapy *(McIlwain, 2003)*. Up to 50% of chronic GCS users develop bone loss leading to fracture, especially of spine and neck of femur *(Saigal et al., 2006)*.

Thus, children with INS are prone to MBD because of both biochemical derangements caused by the renal disease itself, in addition to GCS therapy *(Gulati et al., 2003)*. However, the propensity of steroids to cause osteoporosis and fractures is often neglected *(Srinivasulu et al., 2010)*.

The data that have been published on BMD in children with nephrotic syndrome (NS) who have been treated with GCS are equivocal and there is still conflicting evidence on the risk of low bone mass in these children. Whether nephrotic children are prone to MBD is of therapeutic significance because these children would merit prophylactic therapy with calcium and vitamin D. Therefore, we conducted this study to evaluate the prevalence of MBD in children with nephrotic syndrome with normal renal function, and to study the effect of different variables, including cumulative dose of received corticosteroids, on the BMD of these children.

2. Patients and methods

This retrospective cross sectional study was conducted at the Pediatric Nephrology Clinic, Sohag University Hospital, Sohag, Egypt. We recruited 70 children (47 males, 23 females), aged 4- 14 years

previously diagnosed to have INS according to the criteria of the International Study of Kidney Disease in Children (ISKDC, 1981). The following patients were excluded: (1) those with abnormal renal function defined as GFR less than 90 mL/min/1.73m^2 calculated by the Schwartz formula *(Schwartz et al., 1987)*. (2) those who have a regular follow up period less than 12 months. (3) those who received corticosteroid therapy for less than 6 months period, or in a dose less than 5mg prednisone per day. (4) those with clinical evidence of malnutrition or systemic diseases. (5) those receiving calcium and vit.D supplementation. In addition, 30 children matched for age and sex with no history of renal disease were taken as controls. Informed consent was obtained from parents of all children prior to their participation in the study.

All of the children with INS were treated according to the standard protocol according to Pais and Avner (2011). The initial therapy, consisted of daily prednisone 60 mg/ m^2 /day (80 mg daily max) for 4 weeks, followed by 40 mg/m^2/day given every other day as a single daily dose for at least 4 wks. The alternate-day dose was then slowly tapered and discontinued over the next 1-2 months. Relapses were treated with 60 mg/m^2/day in a single morning dose until the child entered remission (urine trace or negative for protein for 3 consecutive days). The prednisone dose was then changed to alternate-day dosing as noted with initial therapy, and gradually tapered over 4-8 wk. Patients who responded well to prednisone therapy but relapsed <4 times in a 12-mo period were considered infrequent relapsers (IFR; 15 patients), and those who relapsed ≥4 times in a 12-mo period were considered frequent relapsers (FR; 55 patients).

The medical charts of the patients were reviewed for date of diagnosis of NS, duration of the disease, duration of GCS therapy and cumulative dose of received steroids. A careful clinical history and physical examination were performed, and urine and blood samples were drawn from all children to measure serum albumin, calcium, phosphorus, alkaline phosphatase, calcitonin, 25(OH) vitamin D, intact parathyroid hormone (PTH), and urinary 24 hours proteins.

Bone scanning:

Although, the dual-energy x-ray absorptiometry (DXA) scan is considered the "gold standard" in measuring BMD, DXA scanning is not readily available to all patients. Quantitative ultrasound (QUS) is another bone scanning technique, introduced in the early 1990s. The Achilles Express Quantitative Ultrasound (QUS) device combines broadband ultrasound attenuation (BUA) and speed of sound (SOS) to reduce random measurement errors and provide better precision in estimating fracture risk. Tissue attenuation is determined by the change in ultrasound intensity measured between 2 transducers (National Kidney Foundation, 2003). The Achilles Express combines BUA and SOS into a measure called the stiffness index (SI), which is then compared with those in age-matched controls to determine a "T-score equivalent " (Achilles Express Ultrasonometer, 2001). The advantages of this technique over DXA scanning are: it is less expensive, it does not involve irradiation exposure, and is relatively rapid (DeHart and Gonzalez, 2004). Because of their portability, the QUS device might be a more convenient and clinically useful screening and monitoring tool compared with DXA (Grabe *et al.,* 2006).

Bone densitometry in this study was estimated by The Achilles Express Quantitative Ultrasound (QUS) device (Lunar Corporation, USA, 2001). Each one of the patients and controls received QUS of each calcaneus (QUS- dominant and QUS-non dominant) using the Achilles Express. QUS measurements were performed in duplicate by the same investigator, with the child's heel positioned in the system as required, and the mean value was calculated. According to the WHO diagnostic guidelines (1994), normal bone mass was defined as a T-score >-1, low bone mass (osteopenia) was defined as a T-score <-1 and >-2.5, and osteoporosis was defined as a T-score <-2.5.

Statistical analysis:

Statistical analysis was performed using STATA intercooled version 9.0. Data were presented as mean ± SD or number (%) when appropriate. Results were analyzed for statistical significance using Student's test for continuous variables and chi-square test for discrete variables. The correlation of BMD T-score with other parameters was studied by using Pearson's correlation coefficient method. Subsequently, multivariate analysis was performed using multiple regression analysis to evaluate factors predictive of a low BMD T-score. *P* value less than 0.05 was considered statistically significant.

3. Results:

The patients under study comprised 23 girls and 47 boys, with the means of age and age at the onset of nephrotic syndrome being 8.73 ± (2.98) yrs and 3.73 8.73 ± 2.98 yrs and 3.73 ± 1.84 yrs respectively. Thirty healthy children constituted the control group. The demographic, clinical and biochemical characteristics of both groups are presented in (Table 1). Out of all studied parameters, serum albumin was significantly lower, while serum PTH, 24 hours urinary proteins and BMD T-score were significantly higher in nephrotic patients than controls. Other clinical and biochemical parameters were similar in both groups.

Table (1): Demographic, clinical, and biochemical parameters of cases and controls.

	Cases (n = 70)	Controls (n=30)	*P* value
Sex			
Female	23 (32.9%)	14 (46.7%)	0.19
Male	47 (67.1%)	16 (53.3%)	
Age (year)	8.73 ± 2.98	9.07 ± 2.99	0.60
Age of onset of NS (year)	3.73 ± 1.84		
Duration of disease (year)	5.03 ± 2.78		
Duration of therapy (year)	3.31 ± 2.19		
Cumulative dose of steroids (gm)	15.16 ±10.34		
S. calcium (mg/dl)	9.25 ± 0.66	9.45 ± 0.40	0.13
S. phosphorus (mg/dl)	4.67 ± 0.74	4.95 ± 0.48	0.06
S. alkaline phosphatase (IU/l)	176.89 ± 60.82	193.07 ± 60.08	0.22
S. 25(OH) vit. D (nmol/l)	68.1 ± (23.9	73.67 ± 29.54	0.32
S. parathormone (pg/ml)	53.01 ± 28.03	28.75 ± 14.60	**<0.0001***
S. calcitonin (pg/ml)	7.36 ± 2.11	7.41 ± 3.29	0.89
S. albumin (gm/dl)	3.95 ± 0.65	4.65 ± 0.55	**<0.0001***
24 hours urinary proteins (gm/24hrs)	0.57 ± 0.35	0.29 ± (0.15)	**0.0001***
T- score	-1.99 ± 0.74	-0.39 ± 0.87	**<0.0001***

Data are expressed as no (%) and mean ± SD

Of 70 nephrotic children, 55 children were frequent relapsers (FR; group I) and 15 were infrequent relapsers (IFR; group II). There were no differences between the two groups in regard to age, age of onset of NS, sex distribution, and duration of the disease. FR children received higher cumulative doses of steroids over longer duration of therapy (Table 2). Serum albumin was lower, while serum PTH and 24 hours urinary proteins were higher in group I. However, serum calcium, phosphorus, alkaline phosphatase, 25(OH) vit.D and calcitonin were similar in both groups. BMD T-score was greater in group I (mean -2.1) than group II (mean -1.3) *(P* = <0.0001). Eighteen of 55 patients in group I (32.7%) had osteoporosis compared to none of 15 patients in group II (*P*=0.01). Osteopenia was similarly observed in both groups.

Table (2): Demographic, clinical, and biochemical parameters of FR and IFR children.

	Group I (FR; n = 55)	Group II (IFR; n=15)	*P* value
Sex			
Female	17 (30.9%)	6 (40%)	0.51
Male	38 (69.1%)	9 (60%)	
Age (year)	8.85 ± 3.05	8.27 ± (2.76)	0.50
Age of onset of NS (year)	3.74 ± 1.90	3.63 ± (1.65)	0.93
Duration of disease (year)	5.15 ± 2.93	4.57 ± 2.10	0.48
Duration of therapy (year)	3.78 ± 2.19	1.60 ± 1.03	**0.0004***
Cumulative dose of steroids (gm)	17.75 ± 10.09	5.64 ± 3.16	**<0.0001***
S. calcium (mg/dl)	9.18 ± 0.68	9.51 ± 0.53	0.09
S. phosphorus (mg/dl)	4.65 ± 0.81	4.74 ± 0.39	0.66
S. alkaline phosphatase (IU/l)	175.09 ± 58.93	182.47 ± 62.61	0.67
S. 25(OH) vit. D (nmol/l)	69.94 ± 23.30	64.00 ± 21.76	0.38
S. parathormone (pg/ml)	58.36 ± 29.11	33.4 ± 8.99	**0.002***
S. calcitonin (pg/ml)	7.24 ± 2.16	7.66 ± 1.50	0.48
S. albumin (gm/dl)	3.73 ± 0.48	4.77 ± 0.50	**<0.0001***
24 hours urinary proteins(gm/24hrs)	0.65 ± 0.35	0.27 ± 0.14	**0.0001***
T- score	-2.1 ± 0.67	-1.31 ± 0.64	**<0.0001***
T-score < -2.5 (osteoporosis)	18 (32.7%)	0 (0%)	**0.01***
T-score <-1 and >-2.5 (osteopenia)	30 (54.5%)	8 (53.3%)	0.93

Data are expressed as no (%) and mean ± SD

Nephrotic children had significantly lower T-scores compared to the control children (-1.99 ± 0.74 versus -0.39 ± 0.87, *P*= <0.0001) (Table 1). Among the patients group, T-scores were significantly lower in FR (group I) than IFR (group II) (-2.1± 0.67 versus -1.31± 0.64, *P*= <0.0001) (Table 2). T-scores of the three studied groups are presented in Figure (1).

Multivariate analysis was performed to identify factors predictive of a low BMD T-score among our nephrotic children. We observed that the only factor found to be predictive of a low BMD T-score was greater cumulative steroid dose (*P*= 0.02) (Table 3). The correlation between T- score and cumulative dose of steroids is presented in (Figure 2).

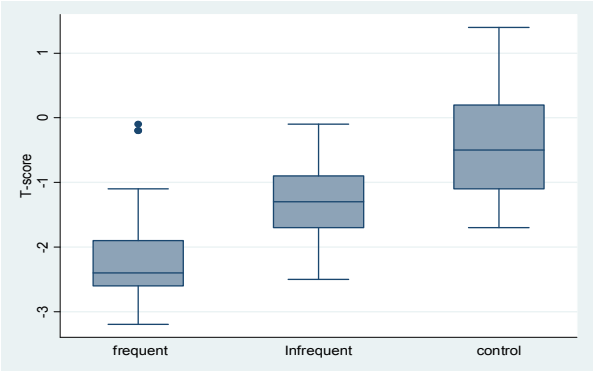

Figure (1): Comparison of T-scores in FR, IFR nephrotic children and controls.

Table (3): Multivariate Analysis for Factors Predictive of Low BMD T- Score

Predictor	Unstandardized Coefficient	t	P	[95% Conf. Interval]	
				lower	upper
Male sex	0.20	1.14	0.26	-0.15	0.54
Age	0.15	0.69	0.49	-0.28	0.57
Age at onset	-0.20	-0.89	0.37	-0.66	0.25
Duration of disease	-0.13	-0.59	0.56	-0.56	0.30
Duration of therapy	0.10	0.73	0.47	-0.17	0.37
Cumulative dose of steroids	-0.06	-2.49	**0.02***	-0.12	-0.01

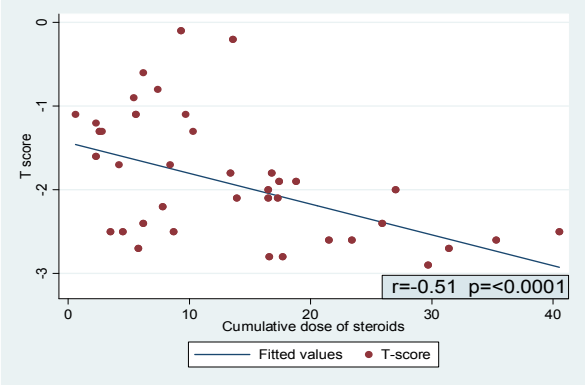

Figure (2): Correlation between T-score and cumulative dose of steroids.

4. Discussion:

This study revealed that nephrotic children had significantly higher levels of PTH compared to the control group (*P*= <0.0001). Raised PTH levels were reported in adult nephrotic patients with normal renal function by Goldstein *et al.* (1977). In nephrotic children, Freundlich *et al.* (1986) reported modest hyperparathyroidism, but Grymonprez *et al.* (1995) found no difference in PTH levels between nephrotic children and controls. However these studies included children with nephrotic syndrome not receiving GCS that have a proved effect on PTH. GCS suppress intestinal calcium absorption and decrease renal calcium reabsorption, resulting in the development of secondary hyperparathyroidism (Iwamoto *et al.* 2005). Among nephrotic children, FR patients were found to have significantly higher PTH levels than IFR patients which can be explained by the longer duration of disease and higher doses of GCS received by the FR children. This result is consistent with that observed by Mohamed and Abdel-Latif (2011), but contrasted to that reported by Gulati and colleagues (2003).

In this study, lower BMD T-scores were observed among nephrotic children compared to the controls (*P* = < 0.0001). This finding is supported by results of Lettgen *et al.* (1994), Fujita *et al.* (2000), Gulati *et al.* (2003) and Basiratnia *et al.* (2006). It has been shown that NS results in a number of biochemical and metabolic disturbances that may occur even with normal renal function and before GCS therapy (Grymonprez *et al.* 1995). Moreover, children treated with GCS are prone to the complex direct and indirect effects of these drugs on bone formation and resorption. These effects are much more complicated than was previously thought. GCS

reduce bone formation through decreasing osteoblast number and stimulation and decreasing synthesis of matrix constituents. They also increase bone resorption through decreasing serum calcium, osteoprotegerin and adrenal androgens and increasing PTH levels (Saigal *et al.*, 2006). Now, reduced bone formation rather than increased bone resorption is thought to be the predominant effect of glucocorticoids on bone turnover (Dore, 2010).

Our result contrasted with those of Polito *et al.* (1995), Morin *et al.* (1996) and Esbjorner *et al.* (2001). Although Esbjorner and coworkers found no difference in BMC between their patients and the control group, they observed reduced bone turnover and lower growth rate in nephrotic patients compared to the controls. Also, Leonard and associates (2004) reported that GCS therapy was not associated with bone loss at the lumbar area after correction for body mass index. However, they were in agreement with the effect of steroids on bone mineral and quality not captured by bone mineral content. This discrepancy might be explained by the fact that some of these studies included patients not receiving GCS at the time of investigation, and others included patients receiving calcium and vitamin D treatment which might have counteracted the demineralization effect of steroids.

Comparing BMD values in the two groups of nephrotic children, we observed that FR children had significantly lower T-scores compared to IFR children (P = <0.0001). Although mean duration of disease was similar in both groups, duration of treatment and cumulative dose of administered steroids were significantly higher in FR patients (P = 0.0004 and < 0.0001 respectively). Gulati *et al.* (2003) reported that FR nephrotic children had significantly lower z scores compared with IFR children. They also observed that osteoporosis affected 20 of 70 FR children (28.6%) compared to 6.7% of IFR children, a result which is consistent with ours.

On multivariate analysis, we observed that the only factor that was predictive of a low BMD score was the cumulative dose of steroids (P =0.02). Patients' age, sex, age of onset of nephrotic syndrome, duration of disease and duration of steroid therapy were not correlated to BMD T-scores. This result is supported by that of Gulati and coworkers (2003) who reported that cumulative dose of steroids was predictive of a low BMD score. However, older age at onset of nephrotic syndrome was also a predictive factor for low score in their study. Our result is also consistent with Basiratnia and colleagues (2006) who found that higher cumulative steroid dose was associated with lower BMD in relapsing nephrotic children. The correlation of low BMD score with steroid therapy has been also well documented in patients with asthma and rheumatoid arthritis (Bouvard *et al.*, 2010).

Our result is contrasted with those of Leonard (2007) who reported that intermittent treatment with high-dose GCS during growth was not associated and maturation in SSNS. GCS-induced obesity was associated with increased whole-body BMC and maintenance of spine BMC. It is also contrasted to the results of Mishra and associates (2009) who found that the majority of patients had normal BMD and was uninfluenced by cumulative dose of prednisolone, when other co-variants were adjusted.

Thus, we concluded that children with INS who are on steroid therapy are at risk for MBD. Children received higher doses of steroids had significantly higher PTH levels, and lower BMD T- scores. Greater cumulative steroid dose was the only factor observed to be predictive of low BMD T-scores (P =0.02). Regular BMD evaluation and appropriate therapeutic interventions are recommended for these children. The role of prophylactic therapy in such patients needs to be further evaluated.

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