Original Research Article

Urinary calcium and bone mineral density in children with nephrotic syndrome treated with glucocorticoids

Sharanagouda Patil, Sanjeev Reddy*

Department of Paediatrics, M R Medical College, Kalaburagi, Karnataka, India

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*Correspondence:
Dr. Sanjeev Reddy,
E-mail: sanjeevkamareddy@gmail.com

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ABSTRACT

Background: Mainstay of therapy in the idiopathic nephrotic syndrome is glucocorticoids. Glucocorticoid induced osteoporosis is considered as most prevalent type of secondary osteoporosis. Only limited studies are conducted in tropical nations. Therefore our study is undertaken with objectives to evaluate Glucocorticoid therapy impact on bone health in Nephrotic Syndrome (NS) children by 2 different tools, namely urinary calcium and bone mineral density (BMD) by Quantitative ultrasound (QUS) and compare both the tools.

Methods: Total 42 children with NS who completed minimum 12 weeks of Glucocorticoid therapy (6 weeks of daily regimen and minimum 6 weeks of alternate day regimen) were subjected to 24 hour Urinary calcium and Bone Mineral density by QUS at Tertiary health centre, Kalaburagi.

Results: Out of 42 cases, 45.2 % had Osteopenia and 2.4% had osteoporosis, so 47.6% of them had BMD measured by QUS. Hypercalciurea was seen in 10 out of 42 cases (23.8%). In normal BMD group only 0.5% had hypercalciurea, Osteopenia group had 47.4% of cases and all osteoporosis group had hypercalciurea.

Conclusions: Present study data concludes that children with NS treated with Glucocorticoids are at risk of Negative impact on bone health. Though both the tools detect impact of Glucocorticoids on bone health, BMD by QUS has better rate then urinary calcium in detecting negative effect of Glucocorticoid on bone health. As BMD by QUS decreases, Urinary calcium increases reflecting inverse relation between them.

Keywords: Bone mineral density, Glucocorticoids, Osteoporosis, Quantitative ultrasound, Urinary calcium

INTRODUCTION

All over the world, Nephrotic syndrome is a common kidney disease and also it is one of the important chronic disorders in children. It has incidence that is reported to be 2-3/100,000 children per year.1 The mainstay of therapy in idiopathic nephrotic syndrome is glucocorticoids. It has been observed that nephrotic syndrome itself is associated with bone and mineral metabolism, which may get further aggravated by steroid therapy.2 Children with idiopathic nephrotic syndrome may be at risk of metabolic bone disease (MBD) because renal disease, as well as steroid therapy are known to result in biochemical derangements.3 Glucocorticoids are known for their adverse effects on the bone health, highlighted in a large, epidemiological study describing increased extremity fracture rates among paediatric age groups treated with glucocorticoids for a variety of underlying conditions.4 Glucocorticoid induced osteoporosis (GIO) is considered as the most prevalent form of secondary osteoporosis.5

The data that have been published on BMD (bone mineral density) in nephrotic syndrome (NS) children who have been treated with glucocorticoids are equivocal and there are still conflicting evidences on the risk of low bone mass in these children. Whether children with nephrotic syndrome are prone to MBD is of therapeutic
significance because these children would merit prophylactic therapy with the vitamin D and calcium.\(^6\)

Quantitative ultrasound (QUS) methods are developed so that mineral status of bone in some of the peripheral skeletal sites like as calcaneum, hand phalanges and tibia can be assessed in the recent years. These techniques are safe, easy to use, free of radiation and devices are mobile, so that they are principally indicated in the measuring of mineral status of the bone in children.\(^7\)

So the study has been undertaken with primary objective to study the Glucocorticoid impact on bone health in Nephrotic Syndrome children by 2 different tools, namely urinary calcium and BMD by QUS and secondary objective as to compare both these tools.

**METHODS**

It is a Hospital based Prospective Study. Initially 48 children were assessed for the eligibility of which 4 children were those in exclusion criteria and 2 children declined participation by parents for study. We were able to recruit 42 cases of Nephrotic syndrome from October 2017 to July 2019.

**Inclusion criteria**

- Aged between 6 months to 18 years.
- Nephrotic syndrome [Nephrotic syndrome is clinical manifestation of glomerular diseases associated with nephrotic-range proteinuria (3.5 g/24 hr or a urine protein: creatinine ratio >2)].

**Exclusion criteria**

- Nephrotic syndrome not responding steroid treatment
- Participants with illnesses or on medications unrelated to steroid-sensitive nephrotic syndrome that may impact growth, nutritional status, pubertal development or bone accrual.

Children with NS who were reviewed or followed up in Paediatric Outpatient department (previous admitted and treated in the institution or other outside health setups) after completing minimum 12 weeks of steroid therapy (6 weeks of initial daily regimen and minimum 6 weeks of alternate day regimen) were subjected to Urinary calcium and bone mineral density measurement by QUS . Prior to the test, parents were counselled regarding Nephrotic syndrome and possible impact of Glucocorticoids therapy on bone health of their children, the need for early diagnosis and intervention.

Informed consent as obtained from the parent or guardian. The study was approved by Institute Ethical Committee. A detailed history of children was elicited including time of start of steroid therapy, total duration on therapy, other drug history in previous 3 months having impact on bone health like calcium, vitamin D3.

A detailed clinical examination was done with special emphasis to weight (measured by digital weighing machine), height (measured by stadiometer), body mass index, blood pressure to look for hypertension, clinical signs of periorbital oedema, abdominal distension, pedal oedema and other significant findings if any. As per IAP guidelines, Height below -2 standard deviation for age, sex taken as short stature and BMI between +1 to +2 standard deviation for age, sex taken as overweight and BP between 90 to<95th centiles taken as elevated BP and >95th centile taken as hypertension as per America Academy of paediatrics guidelines 2019.

Previous Reports while diagnosing as Nephrotic syndrome like CBC, serum Albumin, serum Cholesterol, urine Albumin, urine sugar, urine pus cells, urine RBC levels were collected and entered in proforma. Weight, height, body mass index were interpreted in terms of IAP Z score standard deviation for age and sex. All the relevant details were entered in the proforma prepared for the purpose.

**Urinary calcium levels estimation**

Cases were advised to collect urine for 24 hours at home, and submit sample for testing next day morning.

24 hr urine sample was examined by Arsenazo III method at lab and reports were obtained after 3 to 7 days. Reports collected were analysed and any values above 6 mg/kg/day (laboratory standard reference) was considered as abnormal urine calcium levels.

**Bone densitometry by QUS**

After submitting 24 hour urine sample to laboratory, patient subjected to measure bone mineral density by Quantitative ultra sound performed with FURUNO CM-200 ultrasound bone densitometer (Paltech systems, Tamil nadu, India). QUS measurements were performed in duplicate by the same investigator, with child sitting comfortably on chair and apply gel to a Right heel and foot positioned in the system as required, and the T score by machine based on speed of sound and attenuation property of calcaneum and results were analysed as per WHO guidelines.

According to the WHO diagnostic guidelines (1994), \(^8\)

- Normal bone mass defined as T-score >-1,
- low bone mass (osteopenia) defined as a T-score <-1 and > -2.5,
- Osteoporosis defined as a T-score < -2.5.

**Statistical analysis**

Data was entered into Microsoft Excel (Windows 7; Version 2007) and analyses were done using the Statistical Package for Social Sciences (SPSS) for Windows software (version 20.0; SPSS Inc, Chicago).
Descriptive statistics such as mean and standard deviation (SD) for continuous variables, frequencies and percentages were calculated for categorical Variables were determined. Association between Variables was analyzed by using Chi-Square test for categorical Variables. Comparisons of mean of quantitative variables were analyzed using ANOVA and Pearson correlation coefficient.

Bar charts and pie charts were used for visual representation of the analyzed data. At 95% confidence interval (CI), a probability value ("p" value) of less than or equal to 0.05 was considered to be statistically significant. Level of significance was set at 0.05.

RESULTS

Among 42 children, 28 belonged to 2-6 years age group accounting for 66.7%, followed by 14 belonging to 7-10 years age group; with mean age of 5.84±2.48 years. There was male preponderance accounting for 69% of study population. Rest 31% was females. Hence male to female ratio was 2.23:1 (Table 1).

**Table 1: Distribution of study subjects according to their age and sex (N = 42).**

<table>
<thead>
<tr>
<th>Age (in Years)</th>
<th>Males</th>
<th>Percent</th>
<th>Females</th>
<th>Percent</th>
<th>Total</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-6</td>
<td>19</td>
<td>45.2</td>
<td>9</td>
<td>21.5</td>
<td>28</td>
<td>66.7</td>
</tr>
<tr>
<td>7-10</td>
<td>10</td>
<td>23.8</td>
<td>4</td>
<td>9.5</td>
<td>14</td>
<td>33.3</td>
</tr>
<tr>
<td>&gt;10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>69</td>
<td>13</td>
<td>31</td>
<td>42</td>
<td>100</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>5.84 (2.48) year</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>2.0-10.0 year</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Among 42 cases prior to steroid therapy, 76.2% (n=32) cases had Anaemia with mean Haemoglobin of 10.18±1.36 g/dl; 45.2%(n=19) cases had leucocytosis; 28.6%(n=12) of them had Thrombocytosis; 73.8% (n=31) had hypoalbuminemia, with mean albumin of 2.35±0.74 mg/dl; 92.9%(n=39) had hypercholesterolemia, with mean of 300.33 (±121.18SD) mg/dl for total study group. 45.2 % of cases with symptoms, prior to steroid treatment had Pyuria (>5 pus cells/hpf) and 7.1% of them had Microscopic hematuria. In 42 NS children after steroid therapy, 11.9% cases had height < -3 SD, 14.3% were between -2 to -3SD, rest had weight normal for age; 35.7% (n=15) of cases had short stature (height < -2SD) with 16.7% of them with height less than -3 SD whereas rest (n=27) of them had normal height (-2 to±1 SD). Most of cases(n=33) had normal BMI ,where as 4.8% (n=2)of cases were severely thin (BMI less than -3 SD ), 4.8%(n=2) of cases were thin (BMI between -2 to -3 SD ) and 11.9%(n=5) of cases were overweighing for age and sex (+1 to +2 SD ). Upon Chi-Square Test (p value=0.28) association between gender and BMI was not significant.

Majority (61.9%) of cases had BP between 50th to 90th centile for age, sex & height .1 case had Elevated BP while 19% of cases had Hypertension (BP > 95th). P Value = 0.666, Not Significant infers no association between gender and BP in NS treated with steroids. Among 42 cases, 10 children (23.8%) had Hypercalciuera, with mean calcium excretion for all cases of 4.50±2.33 mg/kg/day (Figure 1). p value of 0.056 implying Urinary calcium levels is not associated with Height. No association between Urinary calcium levels and BMI (p Value of 0.092).

![Figure 1: Distribution of urinary calcium.](image)

![Figure 2: Distribution of BMD (T-Score) measured by QUS.](image)
Osteoporosis 2.40%

Osteopenia

Figure 3: Bar diagram depicting distribution of BMD (T-Score) measured by QUS bone densitometry.

Table 2: Distribution of study subjects according to comparison between urine calcium and BMD (T-Score) measured by QUS bone densitometry (N=42).

<table>
<thead>
<tr>
<th>BMD</th>
<th>No.</th>
<th>Urinary calcium(mg/kg/day)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;6</td>
</tr>
<tr>
<td>Normal</td>
<td>22</td>
<td>21(95.5%)</td>
</tr>
<tr>
<td>Osteopenia</td>
<td>19</td>
<td>10(52.6%)</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>1</td>
<td>1(100%)</td>
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<td>p value &lt;0.001, Significant</td>
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Higher mean urinary calcium levels (8.25 mg/kg/day) are observed in osteoporotic cases than in Osteopenia cases (5.68 mg/kg/day) whereas; cases with normal BMD have normal mean urinary calcium levels (Figure 4).

Pearson correlation coefficient was -0.535 and p value <0.001. Pearson correlation coefficient ranges from -1 to +1. In this case its -0.535, negative sign indicates inverse relation and value 0.535 indicates moderate correlation. As BMD decreases, Urinary calcium increases (Figure 5).

DISCUSSION

All over the world, prevalence rate is 16 per 100000 and incidence of nephrotic syndrome is estimated to be 2-7 cases per 100000 per year below 16 years age.9

Children with nephrotic are prone to biochemical derangements in vitamin D and calcium metabolism caused by the disease as well as Glucocorticoid
Many studies worldwide have reviewed about demographics of children presenting with nephrotic syndrome. The study population consisted of 42 children, who were treated for nephrotic syndrome with Glucocorticoids. In our study, majority of patients enrolled belonged to 2-6 years and 7-10 years.

Mean age at onset of NS in our study was 5.84±2.48 years (66.7% cases in 2-6 years and 33.3% in 6-10 years) which was comparable to other studies conducted worldwide. B Prasun et al in their study on 88 children for 1 year found mean age on onset of NS to be 5.9±2.2 years while Bakhiet et al had 5.3±2.8 years as mean age of onset of NS in their study.11,12

Our study male to female ratio was 2.23:1; El Bakkali et al13 study had male to female ratio of 2.9:1 while Other prospective study of 163 children done by Bakhiet et al 12 in Johannesburg, South Africa found out as 1.5:1, less compared to our study. This study was done in exclusive black race children and might have confounding effect.

Statistically 35.7% of cases had short stature, while others had normal height. Mohan KR et al in study on 35 NS cases found that as steroid cumulative dose raises, statistically significant changes seen in height SD score, thus confirming worsening of growth retardation.14

Most of cases had normal BMI, where as 4.8% of cases were severely thin, 4.8% of cases were thin and 11.9% of cases were overweighing for age and sex. None of them had obesity after steroid treatment. Study by Nina Lestari et al reported that overall obesity prevalence was 22%, inferring no significant association between steroid regimen and obesity risk in NS children.15

Majority (61.9%) of cases had normal BP, 1 case had Elevated BP while 19% of cases had Hypertension. No association between gender and BP in NS treated with Glucocorticoids. Klepikov et al reported that 25.9% of NS cases had hypertension with steroid therapy.16

Glucocorticoid impact on bone health; evaluation by QUS

QUS is a radiation free technique that can be used as valid tool in locations where there is a narrow availability of DEXA (Dual Energy X ray Absorptiometry) data for the estimation of subjects at risk of fractures in future.17

In our study, 45.2% had Osteopenia and 2.4% had osteoporosis and rest 52.4% had normal BMD. So total 47.6% of cases had abnormal BMD measured by QUS. It indicates BMD by QUS was able to detect negative impact of steroids on bone.

Our results on BMD measured by QUS were compared to other studies on BMD after steroid therapy. El-Mashad GM et al revealed Osteopenia in 44% patients and osteoporosis in 8% cases by DEXA scan.18 Gulati et al reported 61 % of their cases had osteopenia, and 22 % had osteoporosis by DEXA scan.

Gulati et al inferred that, on follow-up, NS children with higher steroid doses were likely to have decline in bone mineral density.19 Our results are also in agreement with study results by Gabriella Aceto et al which reports in 2014 that, in steroid sensitive NS children long-term steroid treatment results in a status of lower mineralization of bone, as per evaluation by phalangeal QUS and lumbar DEXA, that is related to steroid duration as well as dosage.20 Results of QUS were comparable to results of DEXA, concurring that phalangeal QUS is a reliable diagnostic tool to assess health status of bone in children.

Basiratnia et al found that greater cumulative steroid dose was associated with lower BMD in children with relapsing nephrotic syndrome.21

In a study of 60 children with nephrotic syndrome and 195 control subjects, Leonard et al reported that steroid treatment was not associated with bone loss of lumbar area after correction for BMI.22 However, they agreed effect of GC on bone mineral and quality not captured by bone mineral content. This discrepancy can be explained by the fact that some of these studies included patients not receiving steroids at the time of evaluation, and others included cases receiving vitamin D and calcium treatment that might have neutralized the demineralising action of steroids.

Glucocorticoid impact on bone health; evaluation by 24 hour urinary calcium

Hypercalciuria was noted in 23.8% NS cases on steroids, Mean 24 hour urinary calcium excretion of 4.50+ 2.33 mg/kg/day in our study suggests increase in urinary calcium excretion in some of NS children treated with glucocorticoids indicating 24 hr urinary calcium estimation would indirectly reflect the adverse impact of glucocorticoids on bone health. No association between height, BMI and urine calcium levels.

In the literature, researches on effect of steroids on calcium levels in urine in children, only a limited number of studies were available. GÜngör SS et al reported that Urinary calcium/creatinine ratio was raised in nephrotic syndrome group after steroid therapy, and one-month prednisolone therapy significantly increased urinary calcium levels suppressing bone osteoblastic activity.23 Koşan et al reported that increase in urine calcium excretion during 4th and 12th weeks of steroid treatment in children with NS.24
Comparison and association between urinary calcium and BMD by QUS

Among NS children treated with glucocorticoid, only 4.5% had hypercalciuria in normal BMD group, where as Osteopenia group had 47.4% of cases with hypercalciuria and all osteoporosis group had hypercalciuria, p value (<0.001) is Statistically significant indicating strong association between BMD and urinary calcium levels. There is a higher mean urinary calcium level (8.25mg/kg/day) in osteoporotic cases than in osteopenic cases (5.68 mg/kg/day) whereas, cases with normal BMD have lower mean urinary calcium levels.

From the results we can concur that both the tools detect the bone resorption effect of glucocorticoid on bone health in cases of NS treated with glucocorticoid. BMD by QUS is better (47.6% cases) than urinary calcium (23.8% cases) in rate detecting negative effect of glucocorticoids on bone health. With p value of <0.001, there is statistically significant association between BMD by QUS and urinary calcium levels. With Pearson correlation coefficient of -0.535 and P value <0.001, It implies As BMD by QUS decreases inversely Urinary calcium increases.

Limitations of the study include small sample size and no control group. We could not perform BMD by QUS and 24 hour urinary calcium prior to start of steroid therapy to know baseline health status. In addition, reliable height and weight data before starting glucocorticoid therapy were not available for subjects.

CONCLUSION

On conclusion, children with NS treated with Glucocorticoids are at risk of Negative impact on bone health i.e. a status of lower bone mineralization seen in some children, based on assessments by both urinary calcium and bone mineral density by QUS. Though both the tools detect the impact of Glucocorticoids on bone health in cases NS, BMD by QUS has better rate then urinary calcium in detecting negative effect of Glucocorticoids on bone health.

As BMD by QUS decreases, Urinary calcium increases reflecting inverse relation between them. Regular follow up of NS children on steroids to monitor bone health, to prevent fracture risks. The role of prophylactic therapy of calcium and vitamin D in such patients needs to be considered or further evaluation recommended. Additional longitudinal studies are needed to further explore QUS and urinary calcium as clinical tools for predicting bone loss in Nephrotic syndrome treated with Glucocorticoids.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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