Original Article

Osteocalcin Level in Children with Nephrotic Syndrome.

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ABSTRACT

Introduction: Nephrotic syndrome is the predominant glomerular disorder in pediatric patients. Increased permeability across the glomerular filtration barrier is a consequence of renal disorders. It is often distinguished by four clinical characteristics: The presence of proteinuria, hypoalbuminemia, edema, and hyperlipidemia within the nephrotic range.

Aim of the Study: The objective of this study was to measure serum osteocalcin (S-OC) level in children with nephrotic syndrome attending Pediatric Nephrology Clinic & General Pediatric Clinic at our University Hospital from March 2021 to March 2022.

Methods: This case – control study was done at our University Hospital on 60 patients with nephrotic syndrome and 20 matched healthy children as control group who were between 2 to 12 years old, blood sample was collected in sterile EDTA tubes and shifted to the hospital laboratory to be separated and measured by ELISA technique. Normal S-OC level is 50–150ng/mL in children.

Results: we studied 60 children with nephrotic syndrome on steroid therapy,40 (66,7%) were males. There were lower level of total , ionized calcium and higher level of S-OC, positive correlation with p-value <0.05 between steroid duration of treatment and patients weight.

Conclusion: The increase in osteocalcin levels is exclusively determined by the dosage of glucocorticoids, rather than the specific kind of glucocorticoids used. This finding potentially has significant therapeutic significance and has the potential to mitigate bone-related adverse effects. In the majority of instances, the growth parameter of height remains unaffected by many sessions of steroid medication, mostly owing to the administration of vitamin D supplements.

Use of S-OC as screening tool is not recommended in children on steroid therapy because the increasing in serum osteocalcin level may be due to bone turnover or bone formation.

Keywords : Nephrotic syndrome, Proteinuria, Osteocalcin.

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INTRODUCTION

Nephrotic syndrome (NS) is the prevailing glomerular disorder seen in pediatric populations. The prevalence of NS is estimated to range from one to 16 instances per 100,000 children [1], with variations seen across different ethnicities and regions.Nephrotic syndrome (NS) is distinguished by the presence of three main symptoms: severe proteinuria, hypoalbuminemia (2.5 g/dL), and widespread edema [1]. The overflow of serum proteins into the urine leads to a condition of hypercoagulability, heightened susceptibility to infection, and disruption of water equilibrium [2].

Idiopathic nephrotic syndrome accounts for about 90% of cases in children with nephrotic syndrome. Idiopathic nephrotic syndrome has many histologic subtypes, with minimal change nephrotic syndrome (MCNS) being the predominant form, accounting for around 85% of all study cohorts examining nephrotic syndrome in pediatric patients. Corticosteroid treatment has a response rate of over 95% in children with minimal change illness [3]. The prevalence of idiopathic nephrotic syndrome is higher in males compared to females, with a ratio of 2:1. This condition often manifests between the ages of 2 and 6 years. However, there have been documented cases as early as 6 months of age and persisting into adulthood [4].

Approximately 80% of pediatric patients diagnosed with nephrotic syndrome exhibit steroid-sensitive nephrotic syndrome, which demonstrates a favorable response to early medication and is linked to a favorable long-term outcome [5]. Although a significant number of children have steroid-sensitive nephrotic syndrome, approximately 80% of them will experience at least one relapse. Furthermore, over half of these children will develop a recurring pattern of relapses and/or dependence on steroids, which requires frequent use of corticosteroids and the associated side effects [1]. A minority of individuals who do not show improvement with steroids are categorized as steroid-resistant nephroyic syndrome (SRNS). In pediatric patients, the management of medical conditions often necessitates extended use of steroids and perhaps the administration of numerous immunosuppressive drugs. Each of these drugs has notable adverse effect profiles, such as growth failure in the case of steroids and bone marrow suppression in the case of mycophenolate mofetil, tacrolimus, and azathioprine [6]. The occurrence of bone loss and short-term growth degradation is contingent upon the specific kind and dosage of corticosteroid administered, with the most pronounced effects seen during the first six months of therapy. The occurrence of development failure produced by corticosteroids may also be attributed to direct impacts on the growth plate. The administration of corticosteroids into the growth plate results in a transient decrease in the pace of leg development and has the potential to disturb the vasculature of the growth plate [7]. The use of high dosage corticosteroids leads to a reduction in bone formation due to alterations in alkaline phosphatase levels. As the cumulative dosage of corticosteroid increases, the alterations in biochemical bone markers become more pronounced [8].

Biomarkers of bone turnover include osteocalcin and alkaline phosphatase (ALP), which are assessed in individuals afflicted with illnesses linked to heightened bone resorption, such as

osteoporosis, fracture susceptibility, and bone metastases. Additionally, these biomarkers are used in the evaluation of therapy efficacy, particularly in relation to bisphosphonates [9]. Osteocalcin is a noncollagenous protein consisting of 49 amino acids. It is produced by osteoblasts and the majority of it is integrated into the bone matrix, while a lesser portion is released into the bloodstream during the process of osteogenesis [10]. Osteocalcin acts as a bone mineralization inhibitor by preventing the formation of calcium salts from complete solutions [11]. Elevated levels of osteocalcin in the bloodstream serve as an indicator of either accelerated bone growth, often seen throughout adolescence, or heightened bone turnover, leading to less mineralization and therefore a diminished binding substrate for osteocalcin, as shown in cases of osteoporosis [12,13]. Reduced bone turnover is linked to low levels of osteocalcin [13]. The S-OC level in children ranges from 50 to 150ng/mL, whereas in healthy adults it ranges from 12 to 20ng/mL [14].

AIM OF THE STUDY

In this study, we aim to assess the bone turnover marker, serum osteocalcin, in children diagnosed with Initial episode, infrequent relapse (IFRNS), Frequent Relapse (FRNS), Steroid Dependent (SDNS), and Steroid-Resistant Nephrotic Syndrome (SRNS) who are undergoing long-term steroid therapy. We will compare these children to a control group consisting of healthy individuals. Additionally, we will evaluate whether there is a significant impact on the growth of these patients in terms of height, weight, and BMI.

METHODS

This case-control study was conducted at our University Hospital. It included 60 patients with nephrotic syndrome from Outpatient Nephrology Clinic and 20 matched healthy children as control group from General Pediatric Clinic , samples were collected during 1 year from March 2021 to March 2022.They were classified into three groups based on their response to steroid therapy on follow-up: Group I (40 children with Steroid Dependent (SDNS) and Steroid Resistant (SRNS) , group II : 20 children with Steroid Sensitive (SS) and infrequent relapse(IFRNS) and group III : 20 matched healthy children of the same age and sex as control group.

The inclusion criteria for this study were children aged 2 to 12 years, of both genders, who were diagnosed with nephrotic syndrome and were receiving care at an outpatient nephrology clinic. Exclusion criteria included those with a documented medical history of chronic disease or those who were prescribed drugs known to impact bone turnover, except those with renal impairment, renal tubular dysfunction, or congenital nephrotic syndrome.

Every patient underwent a thorough history-taking process, which involved collecting data on their age of onset, duration of illness, number of relapses, presence of hypertension and hematuria, symptoms suggestive of osteoporosis (such as back pain, spinal deformity, recurrent long bone fractures, etc.), history of calcium and vitamin D supplementation throughout the disease, detailed information about therapy modalities and steroid response patterns, history of taking immunosuppressant drugs like

cyclophosphamide, cyclosporine, or MMF, and duration of treatment in children with steroid dependent and steroid resistance nephrotic syndrome.

The clinical examination encompasses a comprehensive assessment, with particular attention given to height and weight in relation to age percentiles based on Egyptian growth curves. This evaluation aims to determine the growth status of the children, as well as to assess blood pressure, the presence of edema, and body mass index (BMI).

Laboratory examinations include many parameters such as serum urea, creatinine, serum albumin, serum calcium, albumin/creatinine ratio in urine, urine analysis, and serum osteocalcin (Bone Gla Protein) analysis, venous blood was collected in sterile EDTA tubes, centrifuged for 10 min at 4C to separate plasma (0.5 to 1ml) which was collected in plastic tubes and stored at -20C until analysis, it was measured by an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with human OC antibody. OC present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human OC Antibody is added and binds to OC in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated OC antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human OC. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Statistical analysis

The data that was collected and encoded was entered into Microsoft Access in order to facilitate data manipulation. The data analysis was performed using the Statistical Package for Social Science (SPSS) software version 22 on Windows 7, which was created by SPSS Inc. in Chicago, IL, USA. In this research, a descriptive analytic technique is used, whereby qualitative data is represented using numerical values and percentages. Furthermore, arithmetic means are used as a metric for determining central tendency, while standard deviations are applied to evaluate the variability of quantitative parametric data.

In the setting of quantitative parametric data, the independent samples t-test was used to compare quantitative measures between two distinct groups. In contrast, the one-way analysis of variance (ANOVA) test was used to assess and compare quantitative measurements across numerous independent sets of quantitative data.

In quantitative non-parametric data, the Kruskal-Wallis test is used to compare several independent groups.The Mann-Whitney test is used to compare two groups that exhibit homogeneity.

The Chi-square test is used for the purpose of comparing across many qualitative groups in the context of qualitative data analysis. A bivariate Pearson correlation test is used to analyze the association between variables. The P-value was found to have a statistical significance of 0.05.

RESULTS

The average age of the 60 patients was 7.07±3.2 years, with a range of 2-12 years. Furthermore, 66.7% of the patients were male, while 33.3% were female. The average age of the control group was 6.7±3.2 years old, with a range of 2-12

years. There was no statistically significant difference between the cases and controls in terms of age, sex, family history, and consanguinity, as shown by a p-value greater than 0.05. The SDNS/SRNS group had an age of onset of 5.2±2.8 years, whereas the SSNS/INFRNS group had an age of onset of 5.05±2.6 years. The SDNS/SRNS group had 2 relapses over the whole duration of the illness, whereas the SSNS/INFRNS group experienced 1 relapse.

There were no statistically significant differences seen in the anthropometric parameters of the patients compared to the control group, specifically in terms of height, weight for age percentile, and BMI (p-value >0.05) **Table 1**.

The studied participants had notably reduced concentrations of total and ionized calcium, together with elevated levels of osteocalcin, in comparison to the control cohort. The observed differences were associated with p-values of 0.001, 0.01, and 0.03, respectively **Table 2**. A statistically significant increase in the proportion of patients treated with Cyclosporin, MMF, Capoten, and Vitamin D supplementation in group I was seen when comparing drugs and therapies between the SDNS/SRNS group and the SSNS/INFRNS group, with a p-value of less than 0.05. Furthermore, group I exhibited a statistically significant longer duration of steroid therapy, as shown by a p-value of 0.01 **Table 3**. When comparing laboratory studies across various case groupings, it was shown that the SDNS/SRNS group exhibited a statistically significant increase in osteocalcin levels and a decrease in total calcium levels, with p-values of 0.005 and 0.01 respectively **Table 4**.

A significant positive correlation ($p < 0.05$) was observed between the duration of steroid treatment and patients' weight. Additionally, a negative correlation was found between the duration of steroid treatment and patients' ionized and total calcium levels. Furthermore, a significant positive correlation ($p < 0.05$) was observed between the duration of vitamin D supplementation during treatment and patients' BMI and level of osteocalcin **Table 5**.

Table 1 : Comparisons of Anthropometric Measures in Different Study Groups.

Variables	Cases (N=60)	Control (N=20)	P-value	Sig.
	Mean ± SD	Mean ± SD		
Height (cm)	117.6±20.6	119.6±19.7	0.7	NS
Weight (kg)	24.9±9.9	25.9±10.6	0.7	NS
BMI (kg/m^2)	17.3±2.6	17.4±2.8	0.8	NS

BMI: body mass index.

Table 2 : Comparison of Calcium and Osteocalcin Levels in Different Study Groups.

Variables	Cases (N=60)		Control (N=20)		P-value	Sig.
	Mean±SD	Median /IQR	Mean±SD	Median /IQR		
Ionized calcium, mg/dl	4.30±0.59	4.4/0.7	4.64±0.41	4.65/0.63	**0.01**	**S**
Total Calcium,mg/dl	9.01±0.87	8.9/ 1.8	9.7±0.53	10/ 0.57	**0.001**	**HS**
Osteocalcin, µg/dl	30.9±27.1	24.75/29.1	22.5±28.8	7.9/22.4	**0.03**	**S**

Table 3: Comparison of Medications and Treatments in Different Cases Subgroups.

Variables	Group I (N=40)		Group II (N=20)		P-value	Sig.
Type of treatment	**No**	**%**	**No**	**%**		
Steroids	40	100%	20	100%	---	----
Cyclosporin	21	52.5%	0	0%	**<0.001**	**HS**
MMF	13	32.5%	0	0%	**0.003**	**HS**
Cyclophosphamide	2	5%	0	0%	0.5	NS
Capoten	22	55%	2	10%	**0.001**	**HS**
Vitamin D supplementation	23	57.5%	17	85%	**0.04**	**S**
Calcium	5	12.5%	3	15%	0.9	NS
Duration of treatment (M)	**Mean**	**SD**	**Mean**	**SD**		
Steroids	11.9	7.6	4.6	4.8	**0.01**	**S**
Cyclosporin	7.3	6.04	---	---	---	---
MMF	4.8	4.6	---	---	---	---
Cyclophosphamide	4	0	---	---	---	---
Capoten	7.4	6.5	1.5	0.71	0.07	NS
Vitamin D supplementation	5	3.6	3.4	2.7	0.6	NS
Calcium	3.2	1.8	2.7	2.1	0.2	NS

MMF: Mycophenolate mofetil

Table 4 : Comparison of Laboratory Investigations in Different Cases Subgroups.

Variables	Group I (N=40)	Group II (N=20)	P-value	Sig.
	Mean ± SD	**Mean ± SD**		
Ionized calcium, mg/dl	4.2±0.57	4.4±0.62	0.3	NS
Total Calcium, mg/dl	8.8±0.84	9.4±0.81	0.01	S
Osteocalcin, µg/l	37.7±29.7	17.2±13.01	**0.005**	**HS**

Table 5 : Correlation Between Treatment Duration with Anthropometric Measures and Laboratory Investigations Among Cases.

Variables	Treatment duration (m)					
	Steroid		Vidrop		Calcium	
	r	P-value	r	P-value	r	P-value
Anthropometric measures						
Height (cm)	**0.13**	**0.3**	-0.09	0.5	0.18	0.7
Weight (kg)	**0.44**	**0.001**	-0.00	0.1	0.08	0.8
Body Mass Index (BMI)(kg/m^2)	0.01	0.9	**0.41**	**0.008**	0.34	0.4
Laboratory investigations						
Ionized calcium, mg/dl	**-0.26**	**0.04**	-0.09	0.6	0.51	0.2
Total Calcium, mg/dl	**-0.34**	**0.008**	-0.19	0.2	0.29	0.5
osteocalcin, µg/l	0.14	0.3	**0.35**	**0.02**	-0.29	0.5

DISCUSSION

The most often seen glomerular disorder in pediatric patients is nephrotic syndrome (NS) [1]. Steroid-sensitive nephrotic syndrome (SSNS), a condition characterized by a rapid response to therapy and a favorable long-term prognosis, impacts around 80% of pediatric patients diagnosed with nephrotic syndrome [5]. The findings of our research indicate that there were no statistically significant differences observed between the cases and control groups in terms of age, sex, family history, consanguinity, and height percentile.

In the current research, it was observed that male patients constituted a greater proportion (66.7%), consistent with a prior study that reported male patients

accounting for 66.7% and 63.3% of patients diagnosed with SDNS/FRNS and SRNS, respectively [15], Furthermore, another survey revealed that 65% of patients were male [16].

During our investigation, we observed that the levels of ionized and total calcium were significantly lower (p-values of 0.01) and 0.001, respectively, while the levels of serum osteocalcin were higher (p-value of 0.03) in the cases compared to the control group, This finding aligns with previous research that examined the linear growth and bone turnover indicators, such as serum osteocalcin, in children diagnosed with steroid-dependent/frequently relapsing and steroid-resistant nephrotic syndrome (NS). The study compared these findings with a control group that was matched, and found that serum osteocalcin levels were significantly elevated in both the SDNS/FRNS and SRNS groups compared to the control group. Additionally, the study revealed a negative correlation between S-OC levels and serum calcium levels in the entire patient population (P value = 0.0001) [15].

Our study revealed a statistically significant positive correlation ($p < 0.05$) between the duration of steroid treatment and patient weight. Specifically, we observed that both groups of patients who received longer durations of vitamin D supplementation treatment had higher BMI. This finding aligns with a previous study that reported higher weight for age percentile among patients with SDNS/FRNS compared to the control group [15]. This finding is consistent with another research that observed a higher body weight and increased body fat percentage in children diagnosed with NS compared to the control group [18].

Our investigation revealed no statistically significant disparity in height between the cases and controls. However, we did observe that 64% of our patients fell inside the 50th percentile or lower. All nephrotic patients had normal height, which was shown to be associated with the duration of steroid administration. The observed phenomenon might perhaps be attributed to the administration of low-dose steroids to certain patient cohorts, namely SDNS/FRNS and SRNS, in conjunction with cyclophosphamide, cyclosporine, and an extended duration of vitamin D treatment. The research group consisted of just eight patients who exhibited symptoms of osteoporosis, including widespread bone pain. Based on their medical history, it was determined that none of these patients had any vitamin D or calcium supplements, and some of them were on an irregular vitamin D regimen. This finding aligns with a prior investigation that examined a cohort of 64 children diagnosed with severe depressive symptoms (SDNS) who had extended treatment with cyclosporine and steroids. The research was a retrospective analysis. The height standard deviation score (HSDS) stayed within the normal range in 47 patients during the course of the 10-year follow-up period, whereas it fell below -2 SD in 17 individuals. Out of the patients in his research group, only 3 individuals (5%) had bone fractures, which were confirmed by plain X-ray, while the illness was ongoing and they were receiving steroid therapy. According to historical records, two individuals were given an irregular dosage of calcium without the inclusion of vitamin D, while one individual did not get any kind of calcium or vitamin D supplementation [19].

This finding contradicts a research that documented a negative connection between height according to age percentile and the frequency of relapses. Negative relationships were seen between height for age percentile and the duration of therapy with steroids, cyclophosphamide, and cyclosporine. Both the SDNS/FRNS and SRNS patient groups had a lower height for age percentile in comparison to the control group **[15].**

Osteocalcin is a protein found in the bone matrix that is not composed of collagen. It serves as a reliable biomarker of bone growth and is very sensitive to the inhibitory effects of corticosteroids **[20].**

In the present study, it was observed that both study groups exhibited elevated levels of S-OC in comparison to the control group. Additionally, the patient group (SDNS/FRNS and SRNS) demonstrated higher S-OC levels in comparison to the group (SSNS-IFRNS) that received high dose steroid treatment. Furthermore, a negative correlation was found between OC levels and serum calcium levels in the entire patient population. According to research, it has been shown that the administration of a solitary oral dosage of 2.5 mg of prednisone results in an almost instantaneous impact on the S-OC level **[21]**, and also agrees with another study that found a negative influence of long term corticosteroid therapy on the osteocalcin level in children with nephrotic syndrome. Upon categorizing the 60 patients in their research based on their condition of remission or relapse throughout the study period, it was observed that the average S-OC level in relapsed patients was 20.3 ± 16.6 ng/mL, which was comparatively lower than the mean level in remission patients (30.2 ± 16 ng/mL). The observed disparity has statistical significance **[22]**.

LIMITATIONS OF THE STUDY

The research has notable limitations. It was conducted in a hospital setting, resulting in a restricted number of cases and a comparatively smaller sample size compared to the study results. Additionally, the study was not multicentric, which poses a considerable risk for publishing. The study was biased and did not focus on a specific neighborhood. Additionally, the calculation of the cumulative dosage of steroids was not conducted to validate our findings.

RECOMMENDATIONS

Use of osteocalcin as screening tool is not recommended in children on steroid therapy because the increasing in serum osteocalcin level may be due to bone turnover or bone formation.

CONCLUSION

Based on our investigation, it can be inferred that the increase in osteocalcin levels is only influenced by the dosage of glucocorticoids, rather than the specific kind of glucocorticoids used. This finding potentially has significant therapeutic significance and has the potential to mitigate bone-related adverse effects. In the majority of instances, the growth parameter of height remains unaffected by many sessions of steroid medication, mostly owing to the administration of vitamin D supplements.

Children diagnosed with nephrotic syndrome who undergo corticosteroid treatment have a potential risk of experiencing bone mass loss.

ABBREVIATIONS

FRNS	Frequent relapse nephrotic syndrome
IFRNS	Infrequent relapse nephrotic syndrome
SDNS	Steroid-dependent nephrotic syndrome
SRNS	Steroid-resistant nephrotic syndrome
S-OC	Serum Osteocalcin
NS	Nephrotic syndrome
MMF	Mycophenolate mofetil
GLA	Y-carboxyglutamic acid
BMI	Body mass index
HSDS	Height standard deviation score

REFERENCES

1. Noone DG, Iijima K & Parekh R. Idiopathic nephrotic syndrome in children. Lancet. 2018;392: 61–74.
2. Kaneko K, Tsuji S, Kimata T, Kitao T, et al. Pathogenesis of childhood idiopathic nephrotic syndrome: a paradigm shift from T-cells to podocytes. World J Pediatr. 2015; 11:218.
3. Ferri and Fred F. Ferri's Clinical Advisor 2018 E-Book: 5 Books in 1. Elsevier Health Sciences.p.2017; 889.ISBN 9780323529570.
4. Kher, Kanwal, Schnaper, H. William, Greenbaum, et al. Clinical Pediatric Nephrology, Third Edition. CRC Press.p.2016; 307.ISBN 9781482214635.
5. Ijima K, Sako M & Nozu K. Rituximab for nephrotic syndrome in children. Clin Exp Nephrol.2017; 21: 193–202.
6. Smith JM, Stablein DM, Munoz R, Hebert D, et al.Contributions of the transplant registry: The 2006 annual report of the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS). Pediatr Transplant.2007; 11: 366–373.
7. Freedberg, Irwin M. et.al, ed. Fitzpatrick's dermatology in general medicine (6th ed.). New York, NY [u.a.]: McGraw-Hill. p. 659.ISBN.2003; 0-07-138076-0.
8. Kelly, Christopher R, Landman & Jaime. The Netter Collection of Medical Illustrations - Urinary System e-Book.Elsevier Health Sciences.p. 101.ISBN.2012; 1455726567.
9. Shetty S, Kapoor N, Bondu JD, Thomas N, et al. Bone turnover markers: emerging tool in the management of osteoporosis. Indian J Endocrinol Metab.2016;20:846–52.
10. Zoch ML, Clemens TL & Riddle RC. New insights into the biology of osteocalcin. Bone.2016 ;82: 42–9.
11. Willems BA, Vermeer C, Reutelingsperger CP & Schurgers LJ. The realm of vitamin K dependent proteins: shifting from coagulation toward calcification. Mol Nutr Food Res.2014; 58(8): 1620 -35.
12. Boivin, G. Y., Chavassieux, P. M., Santora, A. C., Yates, J.,et al. Alendronate increases bone strength by increasing the mean degree of mineralization of bone tissue in osteoporotic women. Bone.2000; 27(5), 687–694.
13. Szulc, P., and Bauer, D. C. Biochemical markers of bone turnover in osteoporosis. In R. Marcus, D. Dempster, J. A. Cauley, D. Feldman, & M. Luckey (Eds.), Cambridge, MA: Academic Press. Osteoporosis.2013; (pp. 1573–1610).
14. Luo Y, Ma X, Hao Y, et al. Relationship between serum osteocalcin level and carotid intima-media thickness in a metabolically healthy Chinese population. Cardiovasc Diabetol. 2015; 14:82.
15. Ghobrial EE, Abdel-Aziz H, El Sayed RH & Abdel-Aliem AM. Osteocalcin Level in Children with Steroid Dependent/Frequently Relapsing and Steroid Resistant Nephrotic Syndrome. Iranian Journal of Pediatrics. Dec 1.2017;27(6).
16. Hammad A, Yahia S, Gouida MS, Bakr A, et al. Low expression of glucocorticoid receptors in children with steroid resistant nephrotic syndrome. Pediatr Nephrol.2013 ;28(5):759–63.
17. Shah SS, Akhtar N, Sunbleen F, ur Rehman MF, et al. Histopathological Patterns in Paediatric Idiopathic Steroid Resistant

Nephrotic Syndrome. J Ayub Med Coll Abbottabad. 2015;27(3):633–6.
18. Moon RJ, Gilbert RD, Page A, Murphy L, et al. Children with nephrotic syndrome have greater bone area but similar volumetric bone mineral density to healthy controls. Bone. 2014; 58:108– 13.
19. Leroy V, Baudouin V, Alberti C, Guest G, et al. Growth in boys with idiopathic nephrotic syndrome on long-term cyclosporine and steroid treatment. Pediatr Nephrol. 2009;24(12):2393– 400.
20. Clemens TL and Karsenty G. The osteoblast: an insulin target cell controlling glucose homeostasis. J Bone Miner Res.2011;26(4):677–80.
21. Saag KG. Glucocorticoid-induced osteoporosis. Endocrinol Metab Clin North Am. 2003;32(1):135–57.
22. Biyikli NK, Emre S, Sirin A & Bilge I. Biochemical bone markers in nephrotic children. Pediatr Nephrol.2004;19(8):869–73.
23. Yang M, Pan X, Liang Z, Huang X, et al. Clinical features of nephrotic syndrome with cerebral hemorrhage. Medical science monitor: international medical journal of experimental and clinical research.2019 ;25:2179.

AUTHORS' CONTRIBUTIONS

The submitted manuscript is the work of the author & co-author.
All authors have contributed to authorship, have read, and approved the manuscript.
Conception and design of study: SKH &ASK
Acquisition of data: GAO and DYA
Analysis &/or interpretation of data: GAO and DYA
Drafting the manuscript: SKH and ASK
Revising the manuscript critically for important intellectual content: SKH and ASK
Approval of the version of the manuscript to be published: all authors.

STATEMENTS

Ethical approval and written consent to participate.

This study protocol and the consents were approved and deemed sufficient by the Ethical Committee of Faculty of Medicine, Fayoum University (register number M534) and informed consent was obtained in every case from their legal guardians.

Consent for publication

The contents and material of the manuscript have not been previously reported at any length or being considered for publishing elsewhere.

Availability of data and material

"Not applicable"

Conflict of interest

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