

Calcium, Vitamin D, and Bone Derangement in Nephrotic Syndrome

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Abstract

Introduction. Derangement in calcium homeostasis is common in nephrotic syndrome (NS). It is postulated that low serum total calcium and vitamin D levels are due to loss of protein-bound calcium and vitamin D. It is unclear if free calcium and free vitamin D levels are truly low. The guideline is lacking with regards to calcium and vitamin D supplementation in NS. This study aims to examine calcium and vitamin D homeostasis and bone turnover in NS to guide practice in calcium and vitamin D levels supplementation.

Methodology. This is a prospective pilot study of ten patients diagnosed with NS, and eight healthy controls. Calcium, vitamin D, and bone turnover-related analytes were assessed at baseline, partial and complete remission in NS patients and in healthy controls.

Results. NS patients had low free and total serum calcium, low total 25(OH)D, normal total 1,25(OH)D levels and lack of parathyroid hormone response. With remission of disease, serum calcium and vitamin D metabolites improved. However, nephrotic patients who do not attain complete disease remission continue to have low 25(OH)D level.

Conclusion. In this study, the vitamin D and calcium derangement observed at nephrotic syndrome presentation trended towards normalisation in remission. This suggested calcium and vitamin D replacement may not be indicated in early-phase nephrotic syndrome but may be considered in prolonged nephrotic syndrome.

Key words: Vitamin D deficiency, hypocalcaemia, bone loss, immune-mediated nephrotic syndrome

INTRODUCTION

Derangement in calcium homeostasis is common in nephrotic syndrome. It is postulated that the low serum total calcium and vitamin D levels in nephrotic syndrome are due to the loss of protein-bound calcium and vitamin D respectively. However, it is not clear if free calcium and free vitamin D levels are truly low.¹⁻⁵ Low serum free calcium had been shown in some studies in nephrotic syndrome^{3,5,6} but not in others.^{2,4} Whereas, low vitamin D level had been shown to be due to the loss of 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25-(OH)D], which are mostly protein-bound, in the urine.⁷⁻⁸

Also, patients with immune-mediated nephrotic syndrome are frequently treated with immunosuppressants such as steroids, cyclosporine, or tacrolimus that could affect bone resorption and formation. The current guidelines⁹⁻¹⁰ with regards to calcium and vitamin D metabolism and supplementation in nephrotic syndrome, with normal renal function, is lacking. There is variable practice in the monitoring of calcium and vitamin D levels and supplementation.

This study aims to examine the calcium and vitamin D homeostasis and the impact on bone turnover in patients with nephrotic syndrome during treatment and remission.

METHODOLOGY

Patients

This was a single-centre, prospective pilot study of ten adult patients diagnosed with nephrotic syndrome, and eight healthy controls. The diagnosis of nephrotic syndrome was based on clinical presentation and renal biopsy findings, including that from light microscopy, immunofluorescence, and electron microscopy. The exclusion criteria included: (a) patients with acute kidney injury, (b) stage 3B and above chronic kidney disease (i.e., estimated GFR <45 ml/min), (c) diabetic nephropathy, (d) use of corticosteroids, vitamin D or calcium supplements in the last 6 months prior to study commencement. Duration of nephrotic syndrome was defined as time of study recruitment to time of complete remission, or time of study cessation (28 February 2017) if not in remission by then. We conducted this study over 3 years and 8 months. The study was approved by National Healthcare Group domain specific review board

ethics committee (reference number: NHG DSRB Ref: 2013/00590). All study subjects provided written consent before enrolment into the study.

Biochemistry

Serum and urine biochemical markers were assessed (i) at baseline prior to starting treatment, (ii) during partial remission, as defined by reduction in proteinuria by 50% or sub-nephrotic range proteinuria, about 2 to 6 weeks into the treatment with immunosuppressant, and (iii) during complete remission as defined by proteinuria <0.3 g/day using 24 hr urinary collection or urine protein to creatinine ratio. The timing of assessment for partial & complete remission could be variable depending on the clinical course of each nephrotic patient.

Fasting venous blood was collected in a heparinised blood gas syringe and sent on ice for ionised calcium measurement within 15 minutes of collection to ensure sample integrity. Serum total calcium, magnesium, phosphate, albumin, creatinine were measured on the Advia 2400 analyzer (Siemens Healthcare Diagnostics, Erlangen, Germany). Serum ionized calcium was measured using ion selective electrode on Siemens Rapidlab 1265. Serum total 1,25(OH) D was measured by liquid chromatography-tandem mass spectrometry (LCMS) assay at Mayo Medical Laboratories. Serum total 25(OH)D was measured by: (a) liquid chromatography-tandem mass spectrometry assay at Shimadzu Laboratory, Singapore and (b) competitive protein binding immunoassay on Roche Elecsys e411. Plasma fibroblast growth factor-23 (FGF-23) was measured on ELISA assay at Mayo Medical Laboratories. Serum vitamin D binding protein was measured by quantitative sandwich enzyme immunoassay using the Quantikine ELISA kit. The 24-hour urinary total calcium, magnesium, phosphate, creatinine and protein was measured on the Advia 2400 analyzer (Siemens Healthcare Diagnostics, Erlangen, Germany). One of the nephrotic subjects declined 24-hour urinary collection and only agreed to proceed with a spot urine protein, creatinine, calcium, magnesium, and phosphate.

Serum C-terminal telopeptides (CTX), plasma intact parathyroid hormones (iPTH), and serum alkaline phosphatase (ALP), were evaluated as markers of bone turnover. Serum iPTH was assessed using 2nd generation chemiluminescent immunoassay on Beckman DXI 800 (Beckman Coulter, Inc., USA) from April 2014; the initial 5 iPTH samples were measured on Advia Centaur analyser. Serum CTX was measured on Roche Cobas E411-based on ECLIA (Electro-ChemiLuminescence Immunoassay) and serum ALP on Beckman DXI 800 (Beckman Coulter, Inc., USA).

Lifestyle survey

Both subjects with nephrotic syndrome and healthy volunteers were interviewed using a standardized questionnaire (Appendix 1). This provided information on their dietary calcium and vitamin D content and sun exposure.

Statistical methods

For sample size calculation, utilizing paired t test to compare assumed mean ionised calcium difference of 0.1mmol/L between nephrotic syndrome at presentation

and in remission, assuming standard deviation of the difference of ± 0.1 mmol/L (based on our previous study),⁶ with an alpha of 0.05, power of 80%, and an allocation ratio of 1:1, a total of 10 people per group should be recruited.¹¹ We compared the biochemical markers across the 3 time points of nephrotic syndrome status (baseline, partial remission, complete remission) using a repeated measure fixed-effect model with a generalised estimating equation (exchangeable correlation structure, Gaussian identity, with robust variance). Parametric variables were reported as mean with standard deviation, and compared using t-test; non-parametric variables were reported as median with range and compared using Mann-Whitney U test. We compared the biochemical markers between baseline nephrotic syndrome state and healthy volunteers, as well as complete remission state and healthy volunteers using T-test. Categorical variables were reported in frequency (percentage) and compared using Fisher's exact chi-square test. A 2-tailed p-value <0.05 was considered statistical significance. All analysis was performed using Stata software (Version 15.1; StataCorp, Texas, USA).

RESULTS

Baseline characteristics of subjects

The 10 nephrotic patients had proteinuria (mean urinary total protein 8.2 ± 3.4 gm/day), with hypoalbuminaemia, and normal glomerular filtration rate (median 73 ml/min, range 55 to 136 ml/min). (Table 1) Majority (80%, 8/10) of the patients received immunosuppressants as monotherapy or combination therapy as clinically indicated. Nine of the nephrotic patients were newly diagnosed on study recruitment, whereas 1 nephrotic patient had nephrotic syndrome relapse for 3 years on study recruitment.

There were eight healthy controls in this study with normal serum creatinine (median 62 μ mol/L, reference range 60-107 μ mol/L). The healthy volunteers were younger and were mainly female as compared to nephrotic patients though the median age and gender of nephrotic patients were not statistically different as compared with healthy volunteers. On review of lifestyle factors, both the nephrotic patients and healthy volunteers had low median daily vitamin D and calcium intake.

Biochemical variables with disease progress

Both free calcium and total calcium were significantly lower at nephrotic disease presentation compared to controls. (Table 2). However, there was a lack of iPTH response to hypocalcaemia. Both free and total calcium improved significantly with complete disease remission.

Total 25(OH)D and 1,25(OH)D were significantly lower at baseline compared to healthy controls. These improved significantly with complete disease remission (Figure 1). With complete disease remission, the serum vitamin D binding protein and serum albumin increased significantly from baseline. At baseline, total 1,25(OH)D correlated with total 25(OH)D level measured by mass spectrometry ($r=0.6624$, p 0.04), and total 25(OH)D level measured by immunoassay ($r=0.930$, p 0.01).

Out of the 10 nephrotic patients, 4 had persistent nephrotic syndrome for more than 6 months duration, and 5 did not attain complete disease remission by the end of the study.

Table 1. Characteristics of patients and healthy controls

Characteristics	Nephrotic Syndrome patients (n=10)	Healthy controls (n=8)	P value
Age at study entry (years) [median (range)]	56.4 (25.6 – 70.3)	39.8 (25.9 – 57.6)	0.131
Gender			0.637
Males	6 (60.0%)	3 (37.5%)	
Females	4 (40.0%)	5 (62.5%)	
Ethnicity			1.000
Chinese	6 (60.0%)	5 (62.5%)	
Malay	1 (10.0%)	0 (0%)	
Indian	1 (10.0%)	1 (12.5%)	
Others	2 (20.0%)	2 (25%)	
Duration of Nephrotic Syndrome (months) [median (range)]	4.54 (1.78 – 27.35)		
Etiology of Nephrotic Syndrome			
Membranous nephropathy	5 (50.0%)		
Minimal change disease	4 (40.0%)		
Focal segmental glomerulosclerosis	1 (10.0%)		
Immunosuppressant therapy received ^a			
Prednisolone	8 (80.0%)		
Cyclosporin	5 (50.0%)		
Cyclophosphamide	3 (30.0%)		
Tacolimus	1 (10.0%)		
Rituximab	1 (10.0%)		
Lifestyle factors			
Dietary vitamin D intake per day (IU) [median (range)]	233 (21 – 1798)	384 (8 – 3037)	0.753
Dietary calcium intake per day (mg) [median (range)]	523 (244 – 1009)	273 (152 – 840)	0.133
Sun exposure per week (hours) [median (range)]	2.7 (0 – 8.0)	2.0 (0 – 7.3)	0.247

^aSome patients had received several types of immunosuppressant and were included in multiple categories.

Vitamin D level is lower in nephrotic syndrome patients with nil or partial remission as compared to those who attain complete remission (p 0.04) as illustrated in Figure 2.

The serum intact-parathyroid hormone (iPTH), serum magnesium, serum phosphate, serum fibroblast growth factor-23 (FGF-23), serum c-terminal telopeptide (CTX), and alkaline phosphatase (ALP) levels did not differ significantly at baseline when compared to healthy controls, and did not change significantly with treatment.

Bone turnover markers in relation to calcium, vitamin D, and albumin status

In nephrotic syndrome at disease presentation, lower total 25(OH)D level (measured by mass spectrometry) was associated with higher iPTH ($r=-0.636$, $p<0.05$) and CTX levels ($r=-0.590$, $p=0.073$, trend towards significance), and lower ALP levels ($r=0.555$, $p=0.096$, trend towards significance). This is likely related to increased bone resorption at diagnosis, but the 25-OH vitamin D level improved with steroid therapy and disease remission.

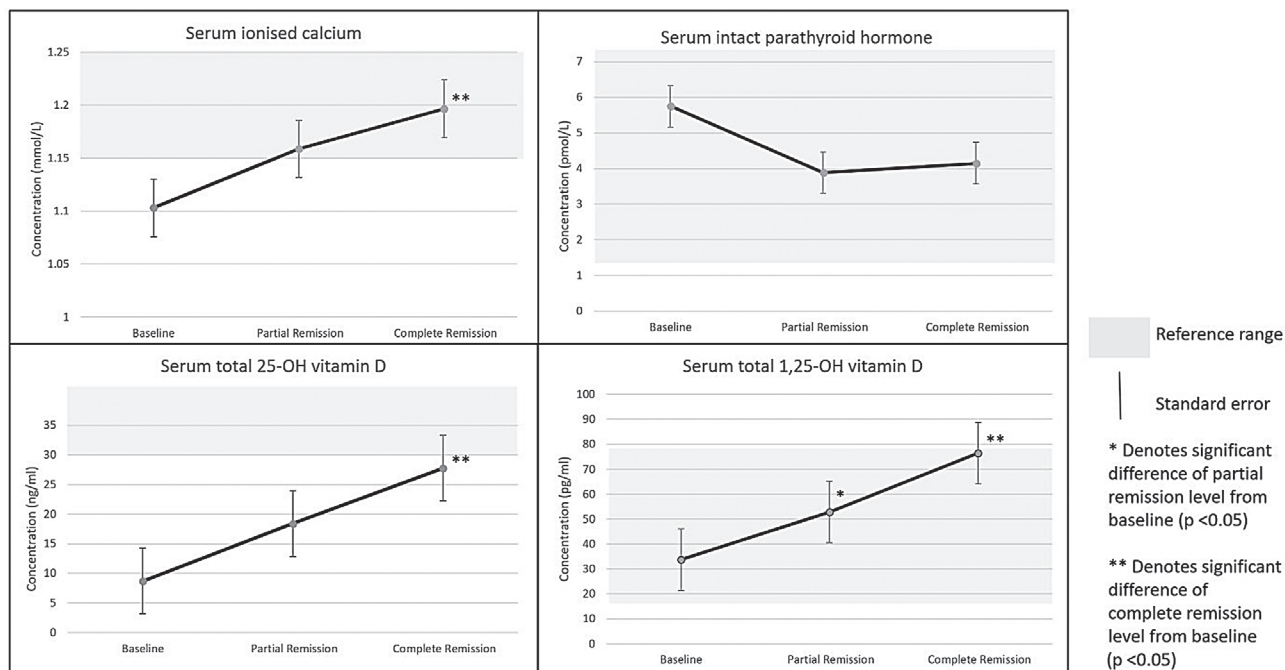


Figure 1. Calcium, parathyroid hormone, 25(OH)D (by mass spectrometry), and 1,25(OH)D levels with nephrotic syndrome remission.

Table 2. Comparison of biochemical variables in 3 phases of nephrotic syndrome (NS) and healthy control

Biochemical variables	Reference range	Baseline NS (mean, SD) [n=10]	Partial remission NS (mean, SD) [n=8, includes data of the 5 NS who attain complete remission, when they were in partial remission phase]	Complete remission NS (mean, SD) [n=5]	Healthy control (mean, SD) [n=8]	Correlation in NS patients across 3 stages of disease state (Baseline, partial, & complete remission NS) (p value)	Baseline NS vs healthy control (p value)	Complete remission NS vs healthy control (p value)
Calcium and related analytes								
Ionised serum calcium (mmol/L)	1.15 - 1.35	1.10 (0.08) ↓	1.16 (0.09) ↔	1.20 (0.04) ↔	1.17 (0.02) ↔	<0.001	0.034	0.066
Total serum calcium (mmol/L)	2.15 - 2.55	1.98 (0.15) ↓	2.23 (0.12) ↔	2.35 (0.13) ↔	2.26 (0.05) ↔	<0.001	0.0001	0.113
Serum phosphate (mmol/L)	0.85 - 1.45	1.30 (0.35) ↔	1.00 (0.25) ↔	1.19 (0.32) ↔	1.15 (0.10) ↔	0.301	0.209	0.759
Intact-parathyroid hormone (pmol/L)	1.3 - 9.3	5.8 (2.9) ↔	3.9 (1.1) ↔	3.9 (1.3) ↔	6.1 (1.5) ↔	0.065	0.780	0.020
Serum magnesium (mmol/L)	0.75 - 1.07	0.78 (0.13) ↔	0.84 (0.04) ↔	0.80 (0.05) ↔	0.84 (0.06) ↔	0.532	0.225	0.309
Serum albumin (g/L)	38 - 48	22.6 (5.4) ↓	35.1 (6.0) ↓	40.4 (4.8) ↔	44.6 (2.6) ↔	<0.001	2.83x10 ⁻⁸	0.061
Serum creatinine (umol/L)	60 - 107	83 (22) ↔	78 (22) ↔	76 (23) ↔	62 (11) ↔	0.502	0.022	0.256
24 hour-urinary calcium (mmol/day)	2.5-10.0	1.1 (0.8) ↓	3.3 (2.0) ↔	3.8 (1.9) ↔	- ↔	<0.001	-	-
24 hour-urinary magnesium (mmol/day)	6-10	2.4 (1.5) ↓	3.0 (1.0) ↓	3.0 (1.5) ↓	- ↔	<0.001	-	-
24 hour-urinary phosphate (mmol/day)	5-50	14.0 (7.2) ↔	17.4 (6.0) ↔	14.2 (9.0) ↔	- ↔	0.986		
Vitamin D-related analytes								
Total serum 25(OH)D by mass spectrometry (ng/ml)	Deficient: < 20 Insufficient: 20 - 29 Sufficient: 30 - 100	8.7 (4.7) ↓	18.4 (8.8) ↓	27.8 (7.2) ↓	18.0 (6.4) ↓	<0.001	0.004	0.027
Total serum 25(OH)D by immunoassay (ng/ml)	Deficient: < 20 Insufficient: 20 - 29 Sufficient: 30 - 100	6.4 (4.5) ↓	8.1 (3.9) ↓	15.7 (5.1) ↓	15.6 (6.4) ↓	<0.001	0.008	0.964
Total serum 1,25(OH)D by mass spectrometry (pg/ml)	Male: 18-64 Females: 18-78	33.7 (17.0) ↔	52.9 (11.0) ↔	81.2 (51.6) females ↔ males ↑	59.9 (13.5) ↔	<0.001	0.002	0.280
Vitamin D binding protein (ug/ml)	55.9 - 473.0	174.5 (66.2) ↔	244.6 (71.6) ↔	256.2 (46.2) ↔	253.5 (114.4) ↔	0.005	0.114	0.955
Bone turnover analytes								
C-terminal telopeptide (ug/L)	Pre-menopausal: 0.070 - 0.670 Menopausal: 0.080 - 0.810 Male: 0.154 - 0.885	0.487 (0.204) ↔	0.572 (0.288) ↔	0.667 (0.439) ↔	0.363 (0.224) ↔	0.439	0.243	0.123
Alkaline phosphatase (U/L)	40 - 130	78 (18) ↔	69 (21) ↔	92 (33) ↔	66 (13) ↔	0.784	0.127	0.066

The arrows indicate if the variables are normal, low, or high with respect to the reference ranges. Shaded p values are ≤ 0.05.

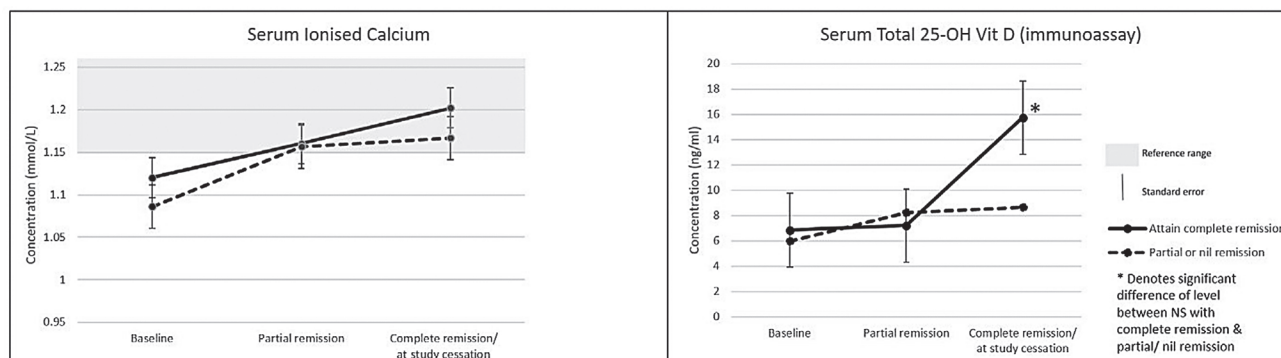


Figure 2. Calcium and 25(OH)D levels between nephrotic syndrome with complete remission and partial or nil remission.

In the eight nephrotic patients who received steroid therapy, there was no significant differences in iPTH, CTX, and ALP levels between treatment period and baseline. Increased 24-hour urinary calcium excretion was observed as the disease progressed from baseline (mean 1.1 ± 0.8 mmol/day) to partial remission (mean 3.3 ± 2.0 mmol/day) and complete remission (mean 4.2 ± 1.9 mmol/day).

DISCUSSION

In this study, we showed that nephrotic patients had low free and total serum calcium, associated with low total 25-OH vitamin D, normal total 1,25(OH)D levels and lack of iPTH response to hypocalcaemia. With remission of disease, the free and total serum calcium, total 25(OH)D and total 1,25(OH)D levels improved significantly. However, nephrotic patients who do not attain complete disease remission continue to have low 25(OH)D level.

As circulating 25(OH)D and 1,25(OH)D are bound to vitamin D binding protein (85 – 90%) and albumin (10 – 15%), with less than 1% of circulating vitamin D in its free form,¹² the low total 25(OH)D was likely attributed to urinary losses of vitamin D binding protein-bound vitamin D and albumin-bound vitamin D. The urinary losses of vitamin D in nephrotic syndrome had been demonstrated in previous studies.^{7,8} Hoof et al., had showed low total 1,25(OH)D (measured by radioreceptor assay) and low free 1,25(OH)D (measured by symmetric dialysis) in 16 nephrotic patients, as compared to 12 healthy volunteers.¹³ From our data, in nephrotic patients, the baseline total 1,25(OH)D level was positively correlated with the baseline total 25(OH)D level supporting urinary loss of vitamin D metabolites and reduced 25(OH)D substrates. Even though the total 1,25(OH)D was normal, free 1,25(OH)D could have been low leading to reduced intestinal absorption of calcium contributing to low free serum calcium. In our previous study, we had demonstrated marked impairment of intestinal absorption of calcium amongst nephrotic patients, in whom faecal calcium equalled or exceeded dietary calcium.¹

Previous studies have shown variable levels of serum free calcium, total 25(OH)D, total 1,25(OH)D, and iPTH.²⁻⁵ Low serum free calcium in 6 nephrotic patients was observed by Malluche et al.,³ and in our study. In the Tessitore et al., study,⁵ only 55.6% of 29 nephrotic patients with normal renal function had low free calcium (In a larger study of 30 nephrotic patients with normal renal function, Mittal et al.,² only observed low free calcium in 6.7%. These studies used similar ion selective electrode assay for ionized calcium ascertainment. These studies had utilised immunoassays for measurement of total 25(OH)D²⁻⁵ and Tessitore et al.,⁵ measured total 1,25-OH vitamin D using radioimmunoassay (RIA). Some of these vitamin D immunoassays are no longer in use currently. The contemporary 25(OH)D immunoassays have analytical limitations, including poor precision at low concentrations of 25(OH)D.¹⁴ The measurement of vitamin D metabolites using LCMS techniques is now considered the gold standard.¹⁵ In our study, we measured total 25(OH)D and total 1,25(OH)D by mass spectrometry. Contrary to Hoof et al.,¹³ we had demonstrated normal total 1,25(OH)D levels in nephrotic patients, similar to previous studies.^{2,4,5} Consistent with previous studies,²⁻⁵ we had noted low

total 25(OH)D levels, by both immunoassay and mass spectrometry methods, in nephrotic patients. The month of vitamin D measurement was not stated as the study was conducted in a country with tropical climate, having minimal fluctuations in temperature and sun exposure over the course of the year.¹⁶

Most of the previous studies had measured iPTH using first generation iPTH assays³⁻⁵, except for Mittal et al.,² and our study, where second generation iPTH assay was used. Only 1 previous study by Malluche et al., showed elevated iPTH levels in nephrotic patients.³ Similar to most previous studies,^{2,4,5} our study observed inappropriately-normal iPTH levels in nephrotic patients, despite the presence of hypocalcaemia and low vitamin D level, reflecting a lack of PTH response. We postulate the following reasons for this inappropriate PTH response to hypocalcaemia. First, the degree of hypocalcaemia might be mild and insufficient to stimulate hyperparathyroidism. Second, inhibitor of PTH might be present in the nephrotic syndrome. Given that our patients had normal serum magnesium and phosphate, the inhibiting factor for PTH is unknown at present. The low ionised calcium could be contributed by low free 1,25(OH)D leading to reduced intestinal calcium absorption, as well as inadequate PTH response.

Previous studies evaluated skeletal impact of nephrotic syndrome through bone biopsy morphology, reporting features of normal finding, osteomalacia, increased bone turnover or mixed findings.¹⁻⁵ Using bone turnover markers assessment, Fujita et al.,¹⁷ showed that urinary deoxypyridinoline (bone resorption marker) increased significantly but serum osteocalcin (bone formation marker) fell significantly in 9 nephrotic patients within 3 months treatment with steroids. There are case series of steroid-induced vertebral fractures and low bone mass in nephrotic syndrome patients treated with steroids with or without cyclosporine.^{18,19} However, our study did not find any significant difference in CTX and ALP with treatment of nephrotic syndrome, duration of disease, and between nephrotic patients and healthy controls. This was despite our cohort having a median duration of disease of 4.54 months (range 1.78 – 27.35 months), with majority of patients having received immunosuppressant. The increasing urinary calcium excretion of NS patients in our study with disease remission could be a result of steroid-related hypercalcaemia.

Given the bone histology evidence,^{1-3,5} bone turnover markers changes¹⁷ reported from previous studies, it is likely that the metabolic bone consequences of nephrotic syndrome could heighten the risk of osteoporosis.²⁰ However, our study did not show any adverse skeletal biochemical parameters in nephrotic syndrome patients.

This study has several strengths. We had studied nephrotic subjects across several time points during their course of disease allowing for paired comparison. These were also compared with that in healthy individuals. The total 25(OH)D and total 1,25(OH)D were evaluated using mass spectrometry.

The small sample size is a limitation of this study. There is a risk of having false negative results (beta error) from inadequate sampling distribution to make inference. The

healthy volunteers were different from nephrotic patients with the former having lower median age and proportion of males. The median age and gender of nephrotic patients were not statistically different as compared with healthy volunteers due to the small sample size. In addition, we did not measure tissue magnesium level that would have excluded magnesium deficiency more definitively. All the healthy volunteers had vitamin D insufficiency or deficiency, and the nephrotic patients' vitamin D levels were compared against these levels. At complete remission phase, most nephrotic patients still had vitamin D insufficiency or deficiency but were assessed to have no statistical difference in levels compared to our cohort of healthy volunteers.

CONCLUSION

Even though initial nephrotic syndrome stage is associated with low serum calcium and 25(OH)D levels, these resolve with nephrotic disease remission with lack of adverse bone turnover parameters. However, nephrotic patients who do not attain remission continue to have low 25(OH)D level. This suggested calcium and vitamin D replacement may not be indicated in early-phase nephrotic syndrome but may be considered in prolonged nephrotic syndrome.

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Statement of Authorship

All authors certified fulfilment of ICMJE authorship criteria.

Author Disclosure

The authors declared no conflict of interest.

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