Bone health and body composition in paediatric patients with nephrotic syndrome managed with glucocorticoids.

A manuscript in submissible format in fulfilment of a Master of Medicine in Paediatrics

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Declaration

I, Noluthando Nokulunga Mkhize, student number: 0704512K / staff number: A0065845, declare that this research report is my own work. It is being submitted in partial fulfilment in the degree of Master of Medicine in Paediatrics at the University of the Witwatersrand, Johannesburg. It has not been submitted for any degree or examination at any other university.

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Date: 06/11/2024

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Dedication

I dedicate this work to my family who have been my support system.

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Bone health and body composition in paediatric patients with nephrotic syndrome managed with glucocorticoids

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Abstract

Background: Prolonged administration of glucocorticoids (GCs) is known to have deleterious effects on growth and bone integrity. This study aimed to assess the effects of GC therapy on bone health and body composition in South African children with primary nephrotic syndrome (NS).

Method: A retrospective record review of NS patients attending the Paediatric Renal Unit at Chris Hani Baragwanath Academic Hospital (CHBAH) between 1 January 2010 and 31 December 2019. Demographics, dual-energy x-ray absorptiometry (DXA) scans, biochemistry results and treatment history were analysed.

Results: NS was diagnosed in 346 patients; 145 met inclusion criteria. Histology showed 90 (62.1%) minimal change NS (MCNS). One hundred and twenty-four (85.5%) had steroid sensitive NS and 21 (14.5%) had steroid resistance. Sixty-two (42.7%) had at least one relapse with a median (IQR) time to relapse of 356 (179, 453) days. Thirty-three (22.8%) patients had DXA scans performed. Lumbar spine bone mineral density (BMD) Z score was -1.10 (-1.70, -0.30) and whole body less head BMD Z score was -0.81 (-1.86, -0.10). Four (12.1%) DXA patients had documented fractures. Ten (6.9%) patients had hypothyroidism treated with thyroxine; they were younger, stunted, had lower albumin, higher cholesterol and lower bone metabolism markers at diagnosis and at last clinic visit.

Conclusion: MCNS was the predominant histopathological diagnosis in a steroid sensitive less frequently relapsing population. BMD measurements were normal among the scanned patients, however fracture prevalence was 12.1%. We recommend screening all patients with NS for hypothyroidism and DXA screening for patients on prolonged GC therapy.

Keywords: Glucocorticoids, nephrotic syndrome, fractures, children, bone mineral density, South Africa

Word count: 241

Introduction

Nephrotic Syndrome (NS) is the most common kidney disease of childhood with normal renal function [1]. NS usually responds to high-dose glucocorticoid (GC) therapy (2 mg/kg/day) [1]. Childhood NS typically follows a relapsing and remitting course, often requiring recurrent courses of GCs [2]. Prednisone is the first line treatment of NS to induce remission. Prolonged administration of prednisone is known to interfere with growth and bone mineralization and has deleterious effects on bone integrity [2]. Glucocorticoid treatment results in reduction in bone formation because of decreased osteoblast differentiation and activity and increased osteoblast and osteocyte apoptosis [3]. Skeletal development in childhood is characterized by increases in bone mineral density (BMD) [1]. The growing skeleton may be especially susceptible to the decreased bone formation and bone resorption effects of GCs. The risk of fracture is increased in children with a history of frequent use of oral corticosteroids [4].

Urinary losses of low molecular weight proteins that are necessary for the transport of metals, drugs and hormones like thyroid binding globulin (TBG) and vitamin D binding globulin (VDBG) in addition to albumin, is a major complication in patients with NS [5]. The loss of TBG contributes to the resultant hypothyroidism seen in NS [6]. Hypothyroidism in childhood causes delayed skeletal development, growth retardation and short stature with impaired bone maturation due to defective endochondral ossification [7]. A Ugandan study reports hypothyroidism in 23% of NS patients, 81.2% of whom had subclinical hypothyroidism. The patients with hypothyroidism were found to have hypoalbuminaemia [8]. Children with nephrotic syndrome have been found to have a reduction in free 25(OH)D and serum calcium values, presumably related to a urinary loss of VDBG [5]

Urinary loss of proteins also results in intensified hepatic synthesis in an attempt to replace lost plasma proteins, generating a catabolic state [9]. As muscle is the main source of amino acids, the catabolic state existing in NS is manifested by a reduction in lean mass as measured by dual energy x-ray absorptiometry (DXA) [9,10].

Dual-energy X-ray absorptiometry (DXA) is a technique used to assess BMD. BMD is the amount of mineral mass per area or bone volume [2]. Other techniques are also available, but it is the gold standard technique due to short scan times, easy

set-up of patients for scanning, low radiation dose and good measurement precision [4]. There is evidence of a positive correlation between body mass index (BMI) and body composition as shown in a Chinese study assessing obesity and bone turnover [9]. This study further emphasized an inverse in this correlation once children displayed BMIs of greater than the 97th percentile [9]. While there is a paucity of data on this correlation in children with NS on GC therapy, a London study in children with Crohn's disease managed with long term steroids reported a positive correlation between BMI and BMD [10]. DXA analysis provides the opportunity to assess BMD and body composition in children with NS with presumably elevated BMI secondary to long term steroid use which is known to have deleterious effects on BMD.

Growth and bone health in NS is impacted by multiple factors related to the urinary protein loss of VDBG and TBG, but also due to the effects of GC therapy.

The purpose of this study was to determine the effects of disease modifying GC therapy on BMD or bone health and body composition in South African children with NS who are managed at the Chris Hani Baragwanath Academic hospital (CHBAH) Paediatric Renal Unit.

Methods

Study design and study site

This was a retrospective record review of patients younger than 18 years of age diagnosed with NS at the CHBAH Paediatric Renal Unit in Soweto, South Africa; serving a predominantly Black population [11].

The study included patients seen from 1 January 2010 to 31 December 2019. Patients were identified using the renal unit monthly admission lists.

Study population

A total of 346 patients were diagnosed with NS during the study period and 145 (41.9%) met inclusion criteria. Inclusion criteria included children between the ages of 2-17.9 years attending the paediatric renal clinic at CHBAH and diagnosed with NS. Exclusion criteria were conditions other than NS, infantile or secondary NS such as in systemic lupus erythematosus and HIV associated nephropathy, and underlying bone disease such as rickets, or a history of an oncologic condition. Two

hundred and one patients were excluded: 137 (68.2%) had secondary NS, 58 (28.8%) were under the age of two years, five (2.5%) were excluded due to missing clinical data and one patient was detected as a duplicate (0.5%). Of the 145 patients recruited into the study population, 136 (93.8%) patients presented to the last clinic visit during the study period. Thus a total of 9 (6.2%) were either lost to follow up, transferred out of the province or died. A sub analysis was further conducted on 33 (22.8%) patients who had records of DXA scans, Figure 1.

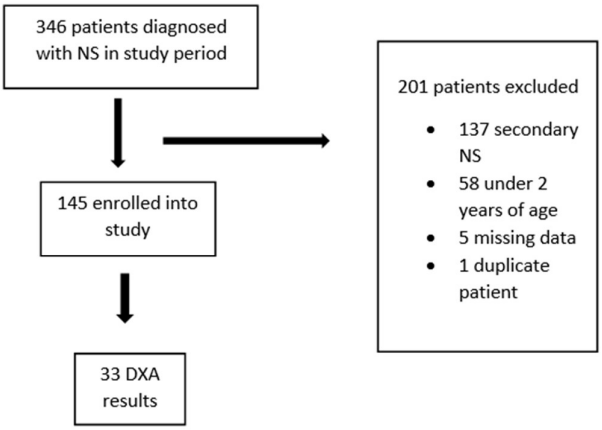

Figure 1. Process of study population determination.

Data sources and variables

Biochemical results such as urea, creatinine, electrolytes, calcium, phosphate, alkaline phosphatase (ALP), 25 hydroxyvitamin D (25(OH)D), parathyroid hormone (PTH), thyroid function tests (TFTs), cholesterol and albumin were obtained from files at diagnosis, at time of DXA scan visit, and at the last clinic visit which was defined as the patients last presentation to the unit within the study period. The estimated glomerular filtration rate was calculated using the Schwartz formula [12].

Histopathological diagnosis was collected from biopsy results documented in patient files as well as sourced through results captured in the national health laboratory service using patient details. Steroid sensitivity was documented in patient files

according to the CHBAH Paediatric Renal Unit guidelines derived from the following International Paediatric Nephrology Association (IPNA) guidelines [12]:

Steroid-sensitive nephrotic syndrome (SSNS): Complete remission within 4 weeks of GC (prednisone) at standard dose (60 mg/m2/day or 2 mg/kg/day, maximum 60 mg/day)

Steroid-resistant nephrotic syndrome (SRNS): Lack of complete remission within 4 weeks of treatment with GC at standard dose

Steroid-dependent nephrotic syndrome (SDNS): A patient with SSNS who experiences 2 consecutive relapses during recommended GC therapy for first presentation or relapse or within 14 days of its discontinuation

Relapse: Urine protein excretion was measured by urine dipstick and considered a relapse if urine dipstick ≥ 3 + (≥ 300 mg/dl) or Urine protein creatinine ratio (UPrCr) ≥ 200 mg/mmol on a spot urine sample on 3 consecutive days, with or without reappearance of oedema in a child who had previously achieved complete remission

Frequently relapsing nephrotic syndrome (FRNS): ≥ 2 relapses in the first 6-months following remission of the initial episode or ≥ 3 relapses in any 12 months

Infrequently relapsing nephrotic syndrome: < 2 relapses in the 6 months following remission of the initial episode or fewer than 3 relapses in any subsequent 12-month period.

A sub analysis of patients with DXA scans was conducted. This subgroup consisted of 33 patients, 23 were enrolled in a prospective study where patients scanned had at least 3 months exposure to GC [13] The remaining ten patients had DXA scans performed at the discretion of the clinician. Documented history of patient fractures, BMD measurements, and lateral vertebral assessments of DXA scans performed during GC therapy was obtained from files. The fractures were either identified through documentation in the files or noted on the lateral vertebral assessments of the DXA reports. A subgroup of patients previously enrolled in a cross-sectional study evaluating fracture history, BMD, body composition and fractures present on lateral vertebral assessment were also included in this study [13].

DXA uses parallel beam radiation that passes through the soft tissue and bone and are captured by a sensor located oppositely [3]. Therefore, it depicts the bone in two-dimensional form. The limitations of DXA are the inability to assess bone geometry, distinguishing trabecular bone from cortical bone and requiring comparison with healthy individuals of the same age, sex, and ethnicity, analysed by similar equipment and software in paediatric assessments. DXA measurements of the whole body less head (WBLH), lumbar spine (LS), lateral vertebral assessments, fat mass (FM) and lean mass (LM) were obtained from scans using a Hologic Discovery machine (Software version Apex 4.0.2). Measurements were performed for LS and WBLH BMD Z-scores, adjusted for height, using the Zemel equation calculator (Pediatric Z-Score Calculator | Pediatric Bone Density) [14].

DXA is one of the most common body composition assessment techniques, providing acceptable accuracy in measuring body composition among children.

The masses derived from DXA were then used to calculate fat mass index (FMI) and lean mass index (LMI) reported in kilograms per metre². Biochemical results obtained at the time of DXA scan for patients with confirmed fractures were analysed to assess for any potential associations with DXA measurements.

Statistical analysis

Anonymised patient information and treatment records were collected from patient renal files. Data were entered into an Excel spreadsheet then imported into Statistica 14.0.0.15 (USA Statsoft) and Stata (USA StataCorp version 15.1) for data analysis. Categorical data were analysed as frequencies and percentages, while continuous data as means with standard deviations (SD) or medians with interquartile ranges (IQR) depending on the distribution of the data. The comparison between those with and without fractures was compared using the Mann-Whitney U test for skewed data and continuous variables. Fisher exact or Chi-square tests were performed for comparisons between categorical data. The paired student t-test for dependent samples was used to compare variables from diagnosis until last clinic visit. Partial correlations and 2D scatterplots were performed to correlate BMD with LMI and FMI.

Ethics

The study was performed in partial completion of Master of Medicine in Paediatrics at the University of the Witwatersrand. Permission was obtained from the University's Human Research Ethics Committee, clearance number M210668 (MED21-04-115). This study was conducted in conformance with the Helsinki Declaration, Good Clinical Practice and within the laws and regulations of South Africa.

Results

Demographics and clinical characteristics

Displayed in Table 1, the median (IQR) age of the study population was 6.5 (3.8, 9.4) years. Males accounted for 97 (66.9%) patients. Percutaneous kidney biopsy was performed in 140 (96.6%) patients after clinical diagnosis to attain a histopathological diagnosis. The biopsies were performed within a median (IQR) of six (2, 11) days. The histological diagnosis was minimal change nephrotic syndrome (MCNS) in 90 (62.1%), focal segmental glomerulosclerosis (FSGS) in 43 (29.6%), membranoproliferative glomerulonephritis (MPGN) in four (2.8%) and primary membranous nephropathy in three (2.1%) patients. Steroid sensitive nephrotic syndrome (SSNS) was seen in 124 (85.5%) of whom 20 (16.1%) were steroid dependent (SDNS). Twenty-one (14.5%) patients had steroid resistant nephrotic syndrome (SRNS). Sixty-two (42.7%) patients had at least one relapse during the study period. The median (IQR) time in days to relapse was 356 (179, 453). Of the 62 patients, 41 (66.2%) had SSNS and this group continued to predominate the relapsing population. Four (6.5%) patients with initial SRNS responded to methylprednisone and monthly intravenous cyclophosphamide; and relapsed once during the study period.

Table 1. Clinical characteristics of patients with childhood nephrotic syndrome on GC therapy at CHBAH paediatric renal unit.

Characteristics	Patients N= 145
Age (years), median (IQR)	6.5 (3.8, 9.4)
Sex n (%)	
M	97 (66.9)
F	48 (33.1)
Histology n (%)	
MCNS	90 (62.1)
FSGS	43 (29.6)
MPGN	4 (2.8)
Primary Membranous	3 (2.1)
Presumed MCNS	5 (3.4)
Days to renal biopsy (days), median (IQR)	6.0 (2.0, 11.0)
Steroid sensitivity n (%)	
SSNS	124 (85.5)
SDNS	20/124 (16.1)
SRNS	21 (14.5)

MCNS = minimal change nephrotic syndrome; FSGS = focal segmental nephrotic syndrome; MPGN = membrano- proliferative glomerulonephritis; SSNS = steroid sensitive nephrotic syndrome; SDNS = steroid dependent nephrotic syndrome; SRNS = steroid resistant nephrotic syndrome

Anthropometry and biochemical results

Table 2 shows the anthropometry and serum biochemical results for the study population at diagnosis and at last clinic visit. The weight for age (WFAZ) and height for age (HFAZ) z scores were normal at diagnosis and at last visit with no significant increase in WFAZ [0.07 ±1.26 vs -0.05 ±1.37; *P* = 0.19], and similarly in HFAZ [-0.70 ±1.41 vs -0.62 ±1.92; *P* = 0.56]. The body mass index for age z (BMIZ) score

showed a significant decrease from diagnosis to last clinic visit but remained normal [0.72 ±1.46 vs 0.35 ±1.38; *P* = 0.01].

The mean corrected calcium at diagnosis was 1.98 ±0.27 and improved to 2.19 ±0.34; *P<0.*001. Similarly, serum albumin increased from 16 ±5.9 at diagnosis to 28.8 ±12.8; *P<0.001*. Total cholesterol levels decreased from 11.57 ±3.5 to 6.32 ±4.4; *P<0.001*. Urine protein creatinine ratio at diagnosis was 0.74 ±0.67 and decreased to 0.45 ±0.55; *P = 0.04.* PTH and 25(OH)D testing were not routinely performed.

Table 2. Anthropometric and biochemical measurements at diagnosis and last clinic visit.

	n	**Diagnosis Mean ± SD**	**Last clinic visit Mean ± SD**	***p-value***
WFAZ	136	0.07 ±1.26	-0.05 ±1.37	*0.19*
HFAZ	136	-0.70 ±1.41	-0.62 ±1.92	*0.56*
BMIZ	136	0.72 ±1.46	0.35 ±1.38	*0.01*
Corrected Calcium (2.19- 2.64 mmol/L)	68	1.98 ±0.27	2.19 ±0.34	*0.00*
Phosphate (1.45- 1.80 mmol/L)	67	1.67 ±0.40	1.67 ±0.61	*0.96*
ALP (93- 345 U/L)	28	161.8 ±68.7	189.7 ±113.7	*0.15*
PTH (1.2- 6.9 pmol/L)	5	22.17 ±36.9	40.8 ±50.7	*0.13*
GFR (ml/min/1.73m²)	91	153.23 ±68.7	125.66 ±56.6	*0.00*
Albumin (29- 42g/L)	58	16.0 ±5.9	28.8 ±12.8	*0.00*
Cholesterol (<4.4 mmol/L)	47	11.57 ±3.5	8.3 ±4.4	*0.00*
UPrCr	23	0.75 ±0.66	0.44 ±0.55	*0.04*

(<0.2g/mmol)				

WFAZ: weight for age z score; HFAZ: height for age z score; BMIZ: BMI for age z score; ALP: Alkaline phosphatase; PTH: Parathyroid hormone; 25(OH)D: 25 hydroxyvitamin D; GFR: Glomerular filtration rate; TSH: Thyroid stimulating hormone; T4: Thyroxine; UPrCr: Urine protein creatinine ratio.

Bone mineral density and body composition

A total of 33 (22.8%) patients had DXA scans performed. The median (IQR) age at time of DXA scan was 9 (7, 11) years. Analysis of steroid sensitivity within the subgroup showed 32 (96.6%) had SSNS of whom 11 (33.3%) were steroid dependent, with a single patient (3%) with SRNS. Twenty-five (75.8%) patients in the subgroup had experienced at least one relapse. Anthropometric measurements as per WHO classification are shown in table 3 and remained within normal limits. The WBLH and LS BMD Z scores were -0.81 (-1.86, -0.10) and -1.10 (-1.70, -0.30) respectively. FMI and LMI were within normal reported ranges and interpreted according to NHANES [15] and LEAD [16] published percentile charts. We found a positive correlation between WBLH BMD and LMI (r = 0.59; *P* =0.001) and FMI (r = 0.46; *P* = 0.015) as shown in figure 2 and 3 respectively. Biochemistry of the 33 patients with DXA measurements were similar to the overall study population. Four (12.1%) of the DXA scanned patients were identified to have fractures post initiation of GC therapy for NS.

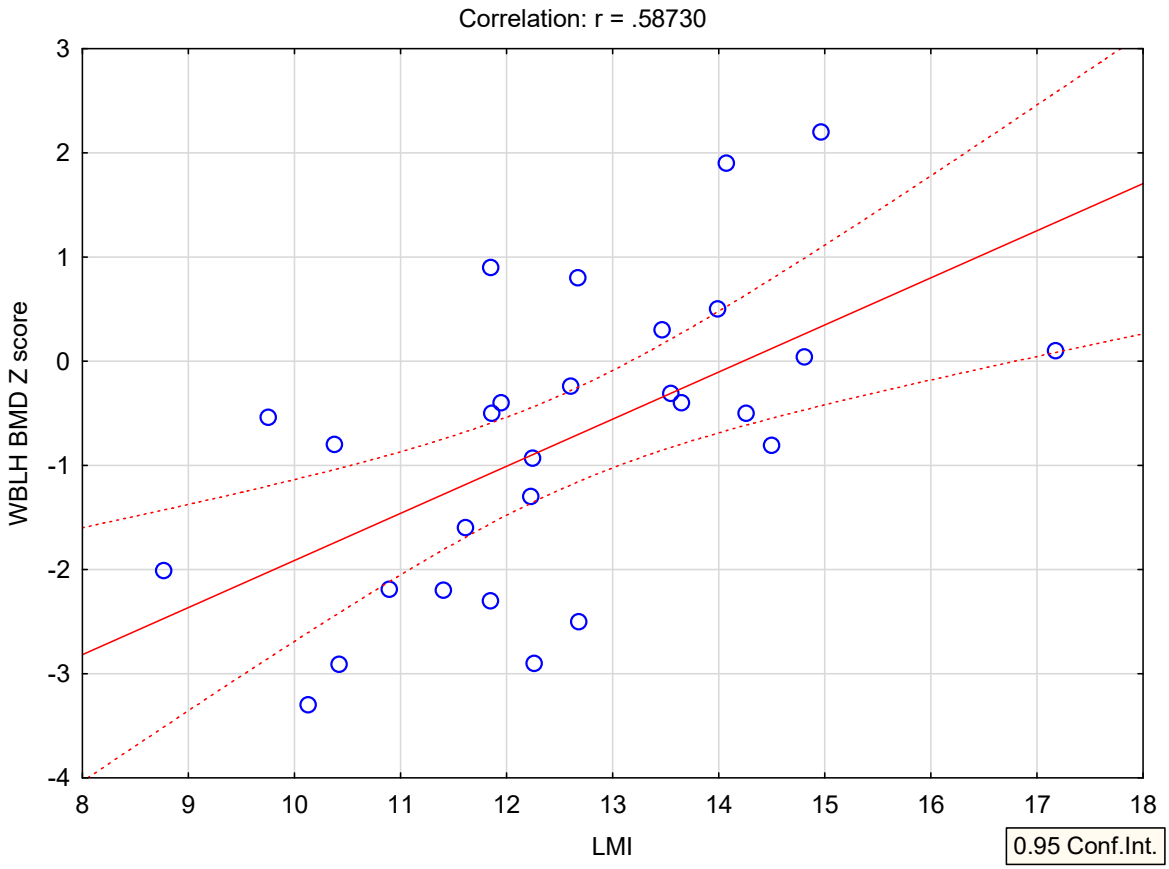

Figure 2. Correlation between WBLH BMD Z score and LMI (n=28).

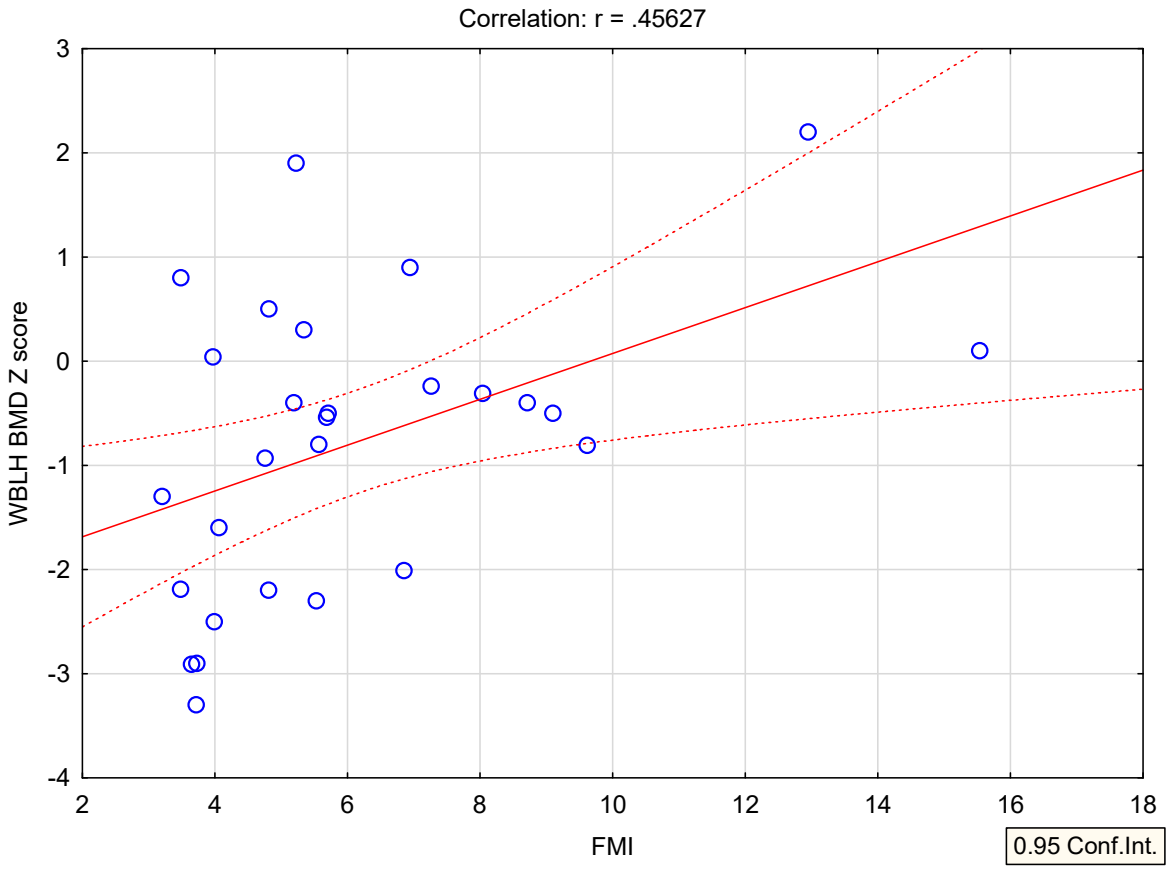

Figure 3. Correlation between WBLH BMD Z score and FMI (n=28).

The number of days from diagnosis to DXA scans was a median (IQR) of 564 (192, 1053) days. Fifteen (45.4%) of the scanned patients had GC exposure documented and of these patients three belonged to the fracture group. The months on GC prior to DXA scan for fracture and non-fracture group were 20 (5.0, 24.0) and 23.5 (13.0, 40.5); *P* = 0.29 respectively, showing no significant difference between the two groups. Paradoxically, the fracture group in this small population had a lower cumulative GC dose in mg/kg/day in the month prior to DXA scan than the non-fracture group 0.43 (0.29, 0.54) vs 0.76 (0.50, 1.09); *P* = 0.10 however this was not a statistically significant difference. There were no significant differences in anthropometry, DXA measurements nor biochemistry between those with and without fractures but the numbers were too small to appreciate a meaningful comparison (Table 3). Of the four patients with fractures, all had SSNS of which two

(50%) patients had SDNS. Three had at least one relapse prior to the fracture. With the exception of one patient being vitamin D insufficient at DXA scan, all four had normal anthropometry, BMD measurements and biochemistry results (Table 3).

Table 3: Demographics, anthropometry and DXA measurements of patients that had DXA scans with and without fractures.

	N	Total (N = 33) Median (IQR)	n	Fractures (n=4) Median (IQR)	n	No fractures (n= 29) Median (IQR)	p-value
Demographics							
Age (years)	33	9.00 (7.00, 11.00)	4	11.00 (10.00, 11.00)	29	9.0 (6.0, 10.0)	0.03
Anthropometry							
WFAZ	32	0.57 (-0.56, 0.95)	4	0.1 (-0.64, 1.16)	28	0.69 (-0.56, 1.03)	0.33
HFAZ	32	-0.34 (-1.60, 0.34)	4	-0.05 (-0.18, 0.54)	28	-0.46 (-1.63, 0.34)	0.21
BMI z score	32	0.72 (-0.07, 1.72)	4	0.3 (-0.88, 1.27)	28	0.79 (0.19, 2.02)	0.11
DXA measurements							
WB BMD Z score	32	-0.81 (-1.86, -0.10)	4	-1.05 (-1.50, -0.26)	28	-0.67 (-2.01, -0.10)	0.89
LS BMD Z score	33	-1.10 (-1.70, -0.30)	4	-0.9 (-1.25, -0.51)	29	-1.10 (-1.50, -0.20)	0.73
FMI (kg/m2)	28	5.28 (3.98, 7.10)	3	5.34 (3.21, 9.62)	25	5.23 (3.99, 6.94)	0.94
LMI (kg/m2)	28	12.25 (11.51, 13.82)	3	13.47 (12.23, 14.50)	25	12.25 (11.40, 13.65)	0.35
Biochemistry							
Corrected Calcium (2.19- 2.64 mmol/L)	30	2.27 (2.00, 2.37)	4	2.33 (2.16, 2.41)	26	2.26 (2.00, 2.37)	0.43
Phosphate (1.45- 1.80 mmol/L)	29	1.60 (1.37, 1.94)	4	1.70 (1.45, 1.87)	25	1.60 (1.37, 2.00)	1.00

ALP (93- 345 U/L)	24	189 (143, 281)	4	230 (165, 262)	20	175.50 (143.50, 281.0)	0.97
PTH (1.2- 6.9 pmol/L)	19	2.00 (1.80, 4.10)	4	2.95 (2.10, 4.45)	15	1.90 (1.5, 4.10)	0.26
25(OH)D (deficient <30; insufficient 30- 50 sufficient > 50 nmol/L)	18	59.32 (31.87,101.60)	4	78.43 (48.40, 121.69)	14	59.32 (26.10, 91.00)	0.44
Albumin (29-42g/L)	9	31 (14.00, 40.00)	0	-	9	31 (14.0, 40.0)	-
cGFR (ml/min/1.73m²)	18	125.20 (106.5, 159.9)	3	117.60 (106.50,154.30)	15	127.34 (104.40,164.00)	0.65

WFAZ: weight for age z score; HFAZ: height for age z score; BMIZ: BMI for age z score; WB BMD Z: whole body less head bone mineral density; LS BMD: lumbar spine bone mineral density; ALP: Alkaline phosphatase; PTH: Parathyroid hormone; 25(OH)D: 25 hydroxyvitamin D; cGFR: Calculated glomerular filtration rate

In table 4, all four patients with fractures had MCNS and were SSNS. Three of the four SSNS patients had steroid dependence, of which two had frequently relapsing NS. These three patients also had incidental vertebral fractures on DXA scan. The remaining one patient sustained an ulna-radial fracture after a fall.

Table 4. Summary of clinical, anthropometric and DXA derived BMD data of 4 NS patients diagnosed with fractures during GC therapy.

	Age	Histology	Steroid sensitivity	Relapses prior to fracture	WFAZ	HFAZ	BMIZ	Fracture site	WBLH BMD Z score	LS BMD Z score
1	11	MCNS	SDNS	4	1.74	1.07	1.65	T5 Wedge	-0.5	0.51
2	9	MCNS	SDNS	3	-0.38	-0.34	-0.29	T5 Wedge	-1.7	-1.4
3	8	MCNS	SDNS	1	-0.9	-0.02	-1.46	T5 Wedge	-1.3	-0.31
4	8	MCNS	SSNS	1	0.58	0.01	0.89	Ulna/ Radius	0.3	-0.7

MCNS = minimal change nephrotic syndrome; SSNS = steroid sensitive nephrotic syndrome; SDNS = steroid dependent nephrotic syndrome; WFAZ: weight for age z score; HFAZ: height for age z score; BMIZ: BMI for age z score; WBLH BMD = whole body less head bone mineral density; LS BMD = lumbar spine bone mineral density

Ten (6.9%) patients in this study were treated with thyroxine for either sub-clinical or overt hypothyroidism. Thirty-three (22.8%) patients had normal TFTs. The remaining 102 (70.3%) patients with abnormal TFTs were either euthyroid sick or had indeterminate results but were monitored and not commenced on treatment. The hypothyroid group was marginally younger than the euthyroid group at diagnosis [3.64 (2.95, 5.39) vs 7.41 (3.84, 10.92); $P = 0.06$] and at last clinic visit [5.93 (4.89, 11.05) vs 9.78 (7.82, 12.59), $P = 0.06$]. The thyroxine group was stunted with HFAZ -2.17 (-2.88; -1.49) at diagnosis and -3.23 (-3.45; -1.55) at last visit showing poor linear growth. The hypothyroid group also showed significantly lower corrected calcium and marginally lower ALP levels at diagnosis compared to the euthyroid group ($P = 0.03$ and $P = 0.06$ respectively). In the hypothyroid group, albumin remained low, whereas the euthyroid group showed normalising from a median albumin of 16.0 (13.0; 18.0) to 42.0 (32.5; 46.0); $P = 0.01$, from diagnosis to last clinic visit. The hypothyroid group also had considerably higher cholesterol levels at diagnosis compared to last clinic visit [16.86 (11.2, 17.4) vs 8.25 (6.29, 10.21); $P = 0.07$].

An analysis of steroid sensitivity in the group of 10 patients managed for hypothyroidism showed that three had SRNS and seven had SSNS. The predominant histological diagnosis in this group was FSGS found in eight patients (80%) with only two patients with MCNS. Similarly, the euthyroid group was also predominantly steroid sensitive (93.9%) but in contrast MCNS was seen in 24 (72.7%) of the euthyroid patients. A clinical overview of these ten patients showed several variables potentially responsible for the stunting in this group. Eight patients had a concurrent diagnosis of pulmonary tuberculosis (PTB), one of whom had multidrug resistant PTB (MDR PTB). Three patients were on prolonged steroid therapy, defined as more than 8 weeks of therapy, three received intravenous methyl prednisone, and five patients received monthly cycles of intravenous cyclophosphamide, an immunomodulatory agent known for its deleterious effects on

growth [12]. Three patients had repeat TFT at the last visit, and all three were still hypothyroid. The remaining seven patients had no documented result of repeat TFT, four of whom were lost to follow up.

Discussion

This South African study of paediatric patients with predominantly SSNS and low relapse recurrence, managed with GCs has highlighted that GC therapy does not significantly alter these patients BMD measurements or body composition.

A fracture prevalence of 12.1% amongst the scanned patients in our study mirrored findings in both a Canadian and South African study assessing fracture prevalence in children managed with oral GCs [13,17]. The Canadian study further emphasised the need for detection of incidental vertebral fractures in children on prolonged oral GCs and proposed ways to allow for early detection by risk assessment and symptom review. Although a very small study population, our fracture group also consisted predominantly of patients with incidental vertebral fractures.

The most common histopathological diagnosis in this study is MCNS, drawing similarity to findings in a previous Johannesburg study whilst contrasting to the historical findings of FSGS predominance in Black African patients [18]. The proportion of FSGS is similar to other reports from international studies [18].

This study found no significant differences in WFAZ and BMIZ from diagnosis to last clinic visit for the study population, as well as no growth parameter faltering as per WHO classifications. Instead BMIZ decreased likely due to resolution of oedema in patients in remission at last clinic visit. Assessments of FMI and LMI also proved to be within normal published reference ranges, but it is noted that the DXA study population was a small sample size. We found a positive correlation between WBLH BMD and LMI as well as FMI. Despite this chronic illness, these findings were similar to those of a meta-analysis in healthy children that supported a positive association between BMD and LMI [19]. A possible explanation to the normal findings of bone health and body composition in our study population could be the higher rates of SSNS and lower overall relapse rates compared to other studies [20].

The DXA sub-analysis group had normal BMD, metabolism markers and anthropometry. These findings may be due to our small sample size, but it also supported by similar findings of another study in the United Kingdom [4]. In contrast, an Indian study showed a decrease in BMD with GC treatment and the beneficial

role of calcium and vitamin D supplementation, even during the first episode of NS [21]. The reason for this difference is unclear as this study was conducted in New Delhi, India, also a developing country with similar sun exposure to South Africa. Differences in diet and/or nutrition are postulated but not assessed in both studies.

In this study population, the hypothyroid patients remain stunted despite thyroxine treatment in comparison to euthyroid patients. However, this group of patients had exposure to confounders as they had prolonged steroid exposure, concurrent PTB and received cycles of cyclophosphamide all impacting final height. Furthermore, many patients were lost to follow up and/or had no TFT results at last clinic visit limiting assessment of progression or resolution of hypothyroidism over time. In addition, albumin levels remained low in the hypothyroid group.

This study is limited by the retrospective collection of data and missing data or biochemical tests not performed for the purpose of this study. DXA scans are not routinely performed in patients with NS and limited the sample size of the DXA scan group. In view of the small sample size and the retrospective design of the study, multiple regression analyses could not be performed to account for confounding factors affecting BMD and body composition. Strengths of the study is that it was over a ten-year study period.

Conclusion

Bone disease and body composition in NS is the result of a complex interplay of the effects of the disease and its treatment. No significant abnormalities of bone health or body composition were identified likely due to the predominant histological type of MCNS with sensitivity to GC therapy and the low frequency of relapses. NS patients with hypothyroidism displayed significant growth stunting and persistent hypoalbuminaemia despite receiving treatment, but many variables contributing to growth faltering were also identified in this group including lack of remission. We recommend screening all patients with NS for hypothyroidism and referral to endocrinology if indicated. DXA scan should be considered in SDNS patients treated with prolonged GC. Enquiring about symptoms of backache suggestive of vertebral fractures and a history of fractures should be routine clinical practice. The role of vitamin D could be explored in future studies.

Conflicts of interest

The authors have no conflicts of interest to declare.

Acknowledgements

A special thank you to my supervisors, Prof K Thandrayen and Prof KL Petersen for their guidance and support. I would also like to extend my gratitude to the clerical workers at Chris Hani Baragwanath Academic Hospital, the WITS Faculty of Health Sciences Research courses and the Paediatric Department for the provision of protocol leave and a dedicated research block to assist in the completion of this body of work.

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Appendix 1: Approved research protocol

Bone health and body composition in paediatric patients with nephrotic syndrome managed with glucocorticoids.

A research proposal by submissible format/ publication in partial fulfilment for MMed (Paed) at the University of the Witwatersrand.

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Literature Review

Nephrotic Syndrome (NS) is the most common chronic renal disease of childhood with normal renal function (1). NS is defined by the presence of nephrotic-range proteinuria (≥40 mg/m2/hour or urine protein/creatinine ratio ≥200 mg/mL or 3+ protein on urine dipstick), hypoalbuminemia (<25 g/L) and oedema (2). NS usually responds to high-dose glucocorticoid (GC) therapy (2 mg/kg/day) (1). Childhood NS typically follows a relapsing and remitting course, often requiring recurrent courses of GCs (3). During remission, the underlying disease remains quiescent without detectable alterations in cytokine levels (1). The absence of systemic inflammation during remission in childhood NS treated with a GC, makes it the best clinical model for studying the relationship between the use of GC and bone mineral density or bone health in children treated with GCs. This statement is in part the hypothesis of our proposed research.

GCs are steroid hormones physiologically produced by the adrenal glands and synthesized for intermittent or continuous systemic use. GCs are recognized for their significant anti-inflammatory and immunomodulatory activities, to control the pathogenesis of autoimmunity and/or inflammation in a variety of diseases (4). In our study, we will focus on the use of GCs for the management of idiopathic NS.

Prednisone is the first line treatment of NS to induce remission, preventing relapses and adverse effects of the disease. Prolonged administration of prednisone interferes with growth and bone mineralization and has deleterious effects on basic cellular mechanisms that are important in the development and maintenance of bone strength (3). Bone remodelling is a tightly controlled mechanism in which osteoblasts, the cells responsible for bone formation; osteoclasts, the cells specialized for bone resorption; and osteocytes, the multifunctional mechano-sensing cells embedded in the bone matrix, are the main actors. The remodelling process is highly active throughout life and the complexity of this process can lead to many pathologies including osteoporosis (5). Studies have consistently shown that GCs resulted in sustained reductions in bone formation because of decreased osteoblast differentiation and activity and increased osteoclast and osteocyte apoptosis (6).

Bone mineral density (BMD) is the amount of mineral mass per area or bone volume (7).
Three major techniques are used to assess BMD: dual-energy X-ray absorptiometry (DXA), quantitative computed tomography (QCT), and quantitative ultrasound. DXA, whose results are expressed in grams/cm², uses parallel beam radiation that passes through the soft tissue and bone and are captured by a sensor located oppositely (7). Therefore, it depicts the bone in two-dimensional form. The limitations of DXA are the inability to assess bone geometry, distinguishing trabecular bone from cortical bone and requiring comparison with healthy individuals of the same age, sex, and ethnicity, analysed by similar equipment and software in paediatric assessments (8). However, it is still the most used technique as it has short scan times, easy set-up of patients for scanning, low radiation dose and good measurement precision.

In a one-year observational study to assess bone mineral density, bone metabolism markers and vitamin D levels in children with idiopathic nephrotic syndrome, significant decreases of total body-BMD Z-score (from −0.24 ± 1.34 to −0.74 ± 1.31, $p < 0.05$) and 25(OH)D_3 serum level (from 31.7 ± 16.3 to 23.7 ± 9.3; $p < 0.05$) were observed (9).

A prospective study in patients with chronic kidney disease (CKD) supported the proposed use of measuring BMD through DXA to identify patients at risk for fracture (7). This study reported that fracture risk increased with low BMD determined by DXA scan.

Yadav et al. showed a decrease in BMD with GC treatment and the beneficial role of calcium and vitamin D supplementation, even during the first episode of NS (10). As fractures lead to significant morbidity and mortality, we believe that there is a role for BMD evaluation in CKD programs. Future research is needed on the utility of serial measures of CKD bone markers and BMD to predict fractures in these patients (10).

Nephrotic syndrome affects thyroid function and presents with low circulating thyroid hormone concentration through the insufficient binding of thyroxine to carrier proteins (11). Therefore, patients with nephrotic syndrome are at risk of hypothyroidism due to loss of the protein thyroid binding globulin in urine. Hypothyroidism in childhood causes delayed skeletal development, growth retardation and short stature with impaired bone maturation due to defective endochondral ossification (12).

Pathophysiological studies have indicated that nephrotic syndrome is associated with a hypercatabolic state. This is attributed to a urinary loss of albumin and an increased fraction of protein catabolized in renal tubular cells resulting in intensified hepatic synthesis in attempt to replace lost plasma proteins. As muscle is the main source of amino acids, the catabolic state existing in nephrotic patients is manifested by a reduction in lean tissue (13). Matyjek et al. determined that children with severe nephrotic syndrome were in a hypercatabolic state with loss in lean mass that they have inferred to be a nephrotic protein energy wasting. Severe nephrotic syndrome was defined as nephrotic range proteinuria (> 3.5 g/day) with serum albumin

concentration ≤ 2.5 g/dL and oedema in physical examination (13). DXA is one of the most used body composition assessment techniques, providing acceptable accuracy in measuring body composition among children (14). Steroid sensitive nephrotic syndrome is the ideal model for studying the effects of glucocorticoids on growth and body composition because there are minimal confounding effects by underlying disease activity (14). An assessment of nephrotic protein energy wasting can be assessed with the aid of analysing fat and lean mass from DXA scans.

Studies show that the prevalence of obesity among steroid sensitive nephrotic syndrome is around 20-41% (15). Vidianty et al. found that in children with frequently relapsing and steroid dependent nephrotic syndrome the prevalence of obesity was 23% (15). This was determined through body weight, height and body fat mass measurement.

The purpose of this study is to highlight the effects of disease modifying GC therapy on BMD, or bone health and body composition in South African children with NS who are managed at Chris Hani Baragwanath Academic hospital (CHBAH) paediatric renal unit. The findings of this study can be used to draft recommendations for prophylactic mineral (calcium and vitamin D) supplementation and BMD screening and treatment thereafter, if warranted, with bisphosphonates. The assessment of body composition is intended to yield information on the prevalence of nephrotic protein energy wasting in children as well as the prevalence of obesity in patients with nephrotic syndrome.

Aim of the study

To investigate the prevalence and characteristics of bone disease and body composition in South African children, on oral glucocorticoid therapy for the management of nephrotic syndrome.

Objectives

1. To evaluate glucocorticoid (GC) use in children with nephrotic syndrome (NS), assessing overall duration of treatment and frequency of relapses.
2. To assess the renal function of children with NS on GC by means of calculating glomerular filtration rate (GFR) using the Schwartz formula (16) at baseline, at DXA scan and at last follow-up (at least 18 months post diagnosis).
3. To assess the number and prevalence of fractures (vertebral and non-vertebral) in children with NS on GC therapy.
4. To assess the total body less head (TBLH) lumbar spine (LS) bone mineral density measurements as well as fat and lean mass in children with NS on GC > 1 week duration and compare those with and without fractures.
5. To assess the vitamin D levels, albumin and thyroid function in patients with NS at baseline and on GCs; and to correlate with fractures or BMD measurements.

Methods

All children, between the ages of 2 - 17.9 years, with idiopathic nephrotic syndrome will be enrolled into the study. This is a retrospective review of NS patients' renal files seen at the paediatric renal clinic at CHBAH between 1 January 2010 and 31 December 2019 to allow for a minimum of 18-month follow-up period of patients for data retrieval, collection and analyses.

Study population and sample size

An estimated sample size of 50 patients based on 5 newly diagnosed nephrotic syndrome patients seen in renal clinic per year.

Inclusion criteria

1. Children between the ages of 2-17.9 years attending the paediatric renal clinic at CHBAH and diagnosed with NS.

Exclusion criteria

1. Patients attending the CHBAH paediatric renal clinic for renal conditions other than nephrotic syndrome.

2. Patients with secondary causes of nephrotic syndrome e.g., systemic lupus erythematosu, HIVAN
3. Children with underlying primary bone disease such rickets.
4. Children with a history of previous or current oncologic conditions such as malignancy, leukaemia, and lymphoma.
5. Children with familial infantile nephrotic syndrome (< 2 years of age)

Children diagnosed with nephrotic syndrome during the study period will be identified using the renal unit Redcap database. Anonymised patient information will be collected from patient renal files. Results from DXA scans performed at any time during GC therapy will be retrieved from the patient's renal files and interpreted. Relevant biochemical tests such urea, electrolytes, albumin, calcium, magnesium, phosphate, 25 hydroxyvitamin D levels (25(OH)D), thyroid function tests, alkaline phosphatase and creatinine will also be documented. Biochemical results from the time of diagnosis, time of DXA scan visit and at the last clinic visit will be retrieved from the files. Biochemical results obtained at the time of DXA scan will be analysed to assess for any potential associations between DXA measurements, 25(OH)D levels and thyroid functions. DXA findings will also be analysed in relation to frequency of relapses. In this study we will look at DXA body composition assessment seeking to determine increased fat mass and correlate with documented frequency of relapses and anthropometry. We will also explore the potential association between hypothyroidism secondary to nephrotic syndrome on bone mineral health.

Data collection sheet (appendix A) will be completed to formulate a database of all the recruited patients with nephrotic syndrome on GC.

Information will be collected to include the following:

1. Age (Years and months)
2. Gender
3. Primary diagnosis: Idiopathic nephrotic syndrome with or without differentiation of histological diagnosis

4. Date of diagnosis
5. Weight, height and anthropometry or pubertal status
6. Date of DXA scan
7. DXA scan bone mineral density and body composition measurements
8. Biochemical blood test results: specifically, calcium, phosphate, ALP and 25(OH)D levels, TFT and GFR . Results will be documented at diagnosis, at date of DXA scan and at last clinic visit during the study period.
9. Start and end date of prednisone course(s), including dosing used and number of relapses.
10. Vitamin D and calcium supplementation and other medication prescribed.
11. Co-morbidities or complications

Dual energy X-ray absorptiometry (DXA) scans have been performed at Developmental Pathways for Health Research Unit (DPHRU). Black South African children have similar LS DXA measurements to that of US white children (17). TBLH and LS BMC and BMD measurements together with lean and fat mass and lateral vertebral fracture assessment have been obtained using the Hologic Discovery DXA machine which uses a US paediatric reference for white American children (18).

The duration of prednisone course as well as the number of relapses per patient will be used as markers for overall GC therapy.

Height for age (HAZ), weight for age (WAZ) and body mass index for age (BAZ) z-scores will be calculated using WHO AnthroPlus.Ink version 1.0.4.

Measurements for LS and WBLH BMC and BMD Z-scores, unadjusted and adjusted for height, using the Zemel equation calculator will be performed. (https://zscore.research.chop.edu/bmdCalculator.php) [22].

Anthropometry will be documented at birth, at time of diagnosis, at time of DXA scan and at the last documented clinic visit within the study period.

Statistical analysis

Data will be entered into an Excel spreadsheet on a password protected computer accessible only to the principal investigator, then imported into Statistica (USA

Statsoft) and Stata (USA StataCorp version 15.1) for data analysis. Categorical data will be presented as frequencies and percentages and continuous data as means with standard deviations or medians with interquartile ranges depending on the distribution of the data. The comparisons between those with and without fractures will be compared using the student-t test for continuous normally distributed data or the Mann-Whitney U test for skewed data and Chi-square or Fisher exact tests for comparisons between categorical data. Spearman's correlation tests will be performed to assess the relationship between 25(OH)D levels and thyroid function status with bone mass measurements. Correlations between body composition measurements and the cumulative doses of GCs will also be analysed. Linear regression models will be utilised to determine the predictors for an increase in fat mass or BMI as well as for a decrease in bone mineral measurements.

Ethics

This protocol will be submitted to the Human Research Ethics Committee of the University of the Witwatersrand, and to the CEO of Chris Hani Baragwanath Academic Hospital for approval prior to commencing the study. This is a study that will be a review of the renal clinic files of patients diagnosed with NS from Jan 2010 till December 2019 to allow for a minimum of 18-month follow-up period of patients. The study participants will remain anonymous, identified only by their assigned study number.

The information gained from the study will help to better understand the adverse effects of GC on bone mineral health and body composition, as the GCs are the mainstay management for patients with NS.

Timing

Gant Chart: Proposed timing of research report

	Apr-Sept 2020	May 20	Jun 20	Jul 20	Aug 20	Sep 20	Oct 20	Nov 20	Dec 20	Jan 21	Feb 21	Mar 21	Apr-June 21	July-Dec 21	Dec 21-Feb

															2022
Literature Review															
Protocol preparation															
Protocol assessment															
Ethics application															
Data Collection and analyses															
Write Up															

Funding

Breakdown of costs:

1. Printing of data collection sheet and research report sheets: R1000
2. Publication costs of paper: R7 000-R40000 which can be supported by grants.

Limitations

Data may be incomplete in patients who have missing hospital records or lack routine blood tests. Not all patients will have a DXA scan done. DXA scans are not

routinely done in children less than 5 years of age as it is difficult to interpret, and the scanner does not have software to generate Z scores in this age group.

Research outcomes

A paper entitled: “Bone health and body composition in patients with nephrotic syndrome managed with glucocorticoids” will be published in a medical journal.

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Appendix 2: Ethics certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research and Innovation)

TO:	Dr NN Mkhize School of Clinical Medicine Department of Paediatrics and Child Health Medical School University E-mail: noluthandomkhize152@gmail.com
CC:	Supervisor: Professors K Thandrayen and KL Petersen <Kabashni.Thandrayen@wits.ac.za> and <HREC-Medical Research Office@wits.ac.za>
FROM:	Mr Iain Burns Human Research Ethics Committee (Medical) Tel: 011 717 1252 E-mail: Iain.Burns@wits.ac.za
DATE:	2021/09/06
REF:	R14/49
PROTOCOL NO:	**M210668** (This is your ethics application reference number. Please quote it in all enquiries, oral or written, relating to this study.)
PROJECT TITLE:	*Bone health and body composition in children with nephrotic syndrome managed with glucocorticoids*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to Government funding of the University.

MSWorks2000/Iain0007/Clearscan.wps

Appendix 3: Plagiarism declaration

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Noluthando Mkhize (Student number: A0065845) am a student registered for the degree of MMED Paediatrics in the academic year 2024.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature: N. N. Mkhize Date: 12/07/2024

Appendix 4: Turnitin Report

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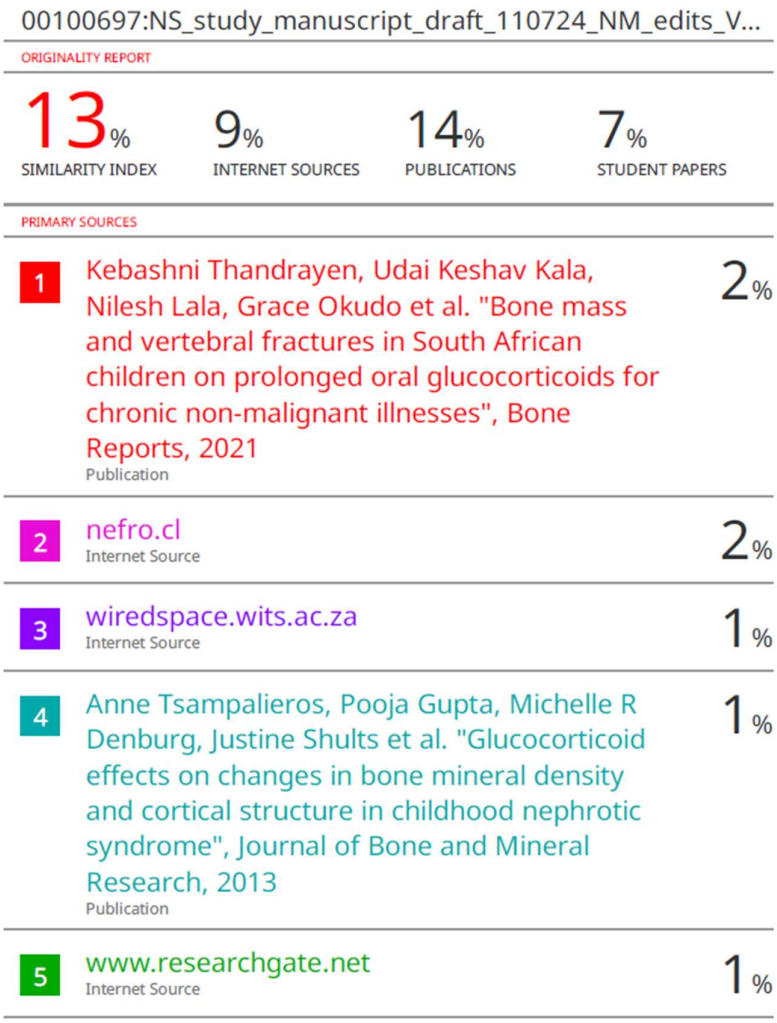

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