Lupus nephritis in children at Chris Hani Baragwanath Academic Hospital, an observational study from January 2000 to December 2019.

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine in Paediatrics.

Johannesburg, August 2024

DECLARATION

I Ritshidze Mulaudzi, declare that this Research Report is my work. It is being submitted for the degree of Master of Medicine in Paediatrics at the University of the Witwatersrand, Johannesburg. The manuscript was submitted to the African Journal of Nephrology on the 20th of March 2024 and is currently being reviewed. It has not been submitted before for any degree or examination at this or any other University.

All authors have made a significant contribution to this study.

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Date:02/07/2024

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LIST OF ABBREVIATIONS

ANA: Anti-nuclear antibodies

Anti-dsDNA: Anti-double-stranded DNA antibodies

APLAs: Antiphospholipid antibodies (lupus anticoagulant (LA), anticardiolipin (aCL), and anti-beta-2 glycoprotein 1 (anti-β_2GP1)

CHBAH: Chris Hani Baragwanath Academic hospital

cLN: Childhood Lupus Nephritis

CKD: Chronic Kidney Disease

cSLE: childhood-onset systemic Lupus erythematosus

eGFR: estimated glomerular filtration rate

ISN/RPS: International Society of Nephrology/ Renal Pathology Society

KDIGO: Kidney Disease Improving Global Outcomes

LN: Lupus Nephritis

MAS: Microphage Activation Syndrome

NHLS: National Health Laboratory Service

SLE: Systemic Lupus Erythematosus

SLEDAI: Systemic Lupus Erythematosus Disease Activity Index

WHO: World Health Organisation

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ABSTRACT

Lupus nephritis in children at Chris Hani Baragwanath Academic Hospital, an observational study from January 2000 to December 2019.

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This is a retrospective study conducted at the Paediatric Renal Unit at Chris Hani Baragwanath Academic Hospital on patients under the age of eighteen years who had biopsy-confirmed lupus nephritis between January 2000 and December 2019.

This study reports the outcomes and complications of childhood Lupus Nephritis in a predominantly Black population in Low and Middle-Income Countries, with multiple indicators of severity.

Background:

Lupus nephritis (LN) is a severe manifestation of systemic lupus erythematosus resulting in worse outcomes than those without kidney involvement.

Methods:

We conducted a retrospective study reviewing records of children with biopsy-proven lupus nephritis, treated at the Paediatric Renal Unit of Chris Hani Baragwanath Academic Hospital from January 2000 to December 2019. Patients were identified using the renal biopsy register. Lupus nephritis was classified using the International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification system. Repeated biopsies from a single patient and those with insufficient records or no histology confirmation were excluded. Risk factors (initial clinical or laboratory manifestations, histo-pathological patterns and treatment modalities) for advanced chronic kidney disease were assessed.

Results:

A total of 833 percutaneous kidney biopsies were performed during the study period. Fifty-two (6.2%) were identified to have lupus nephritis, and 44 cases were analysed (seven had incomplete records and one child had inconclusive histology). The mean age at presentation was 11.8 (IQR, 11.3 to 12.1) years. Females accounted for the majority of cases at 33 (75%). Common renal manifestations at presentation were nephrotic range proteinuria seen in 26 (59.1%) patients, and elevated creatinine in seven (15.9%) patients. Common extrarenal manifestations

included dermatological in 31 (70.4%), musculoskeletal in 14 (31.8%), and neuro-lupus in 12 (27.3%). Anti-dsDNA antibody was positive in 36 (81%); antiphospholipid antibodies in 11 (n=30, 36.6%). The mean Systemic Lupus Erythematosus Disease Activity Index score was 14.6±1.2. Macrophage activation syndrome was diagnosed in seven (15.9%). Proliferative LN (class III, IV, and mixed) were seen in 28 (63.6%). Remission was achieved in 25 (56.8%). Two (4.5%) patients died and three (6.8%) patients developed advanced kidney disease (CKD 3,4 and 5). The mean (±SD) duration of follow-up was 52.5±6.9 months.

Conclusions:

Lupus nephritis presents commonly with proteinuria in this study. Class III LN is the dominant pathology. Screening for antiphospholipid and macrophage activation syndromes is recommended. Young age is associated with severe disease.

Keywords:

Lupus nephritis, children, Systemic lupus erythematosus, Renal biopsy, antiphospholipid, macrophage activation syndrome.

Main article

Introduction

Systemic lupus erythematosus (SLE) is a chronic multi-systemic autoimmune disease distinguished by the production of antinuclear antibodies [1]. Childhood-onset SLE (cSLE) is frequently more severe at diagnosis than adult-onset SLE due to renal involvement [2–4]. Lupus nephritis (LN) develops in 32 to 55% of children with SLE [5]. Globally, lupus nephritis (LN) varies in frequency, severity, and prognosis [6]. Patients of African ancestry are up to three times more likely to have LN and an increased severity of renal disease [6]. Prognosis is adversely affected by male sex, Black ethnicity, proliferative lupus nephritis (class III or IV LN), high Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score, acute kidney injury (AKI), anaemia, hypertension, and nephrotic syndrome at presentation [6–8]. Proliferative LN and disease progression may be predicted by detecting anti-double-stranded deoxyribonucleic acid (dsDNA) antibodies, proteinuria, haematuria, and positive antinuclear antibodies (ANA) [9]. Antiphospholipid antibodies (APLA) are present in up to 40% of SLE and 78% of childhood Nephritis (cLN) patients [10,11]. Childhood Lupus Nephritis (cLN) patients with positive APLa are more likely to have hypertension, increased serum creatinine levels, and stage 5 chronic kidney disease (CKD) [11]. Additionally, macrophage activation syndrome (MAS) has been reported in 0.9 to 4.6% of children with SLE, with mortality rates of up to 11% [12,13]. Advanced chronic kidney disease (CKD) and death are more

common in low and middle-income countries (LMICs), which may be impacted by socioeconomic, genetic, or ethnic predisposition [6]. Early identification and treatment are essential as LN may lead to the development of advanced CKD and mortality [14]. Even with the availability of more potent immunosuppressive medications, the response to treatment for LN is still suboptimal. At 24 months, only 20% of patients in LMICs and 60% of patients in high-income countries (HIC) achieve complete remission [6]. The rate of improvement in survival has plateaued in recent decades, with mortality rates of up to 9% and 14%, and advanced CKD rates of 15% and 30% in HIC and LMIC, respectively [6,7,15].

This retrospective study evaluated the clinical characteristics of patients with childhood LN at Chris Hani Baragwanath Academic Hospital in South Africa over 20 years to identify prognostic markers and overall outcomes in a LMIC setting. Chris Hani Baragwanath Academic Hospital is the world's third-largest hospital and the largest in the Southern Hemisphere, serving a population of over 3.5 million people [16,17]. This is a predominantly Black population, a legacy of Apartheid [16,17]

Methods

This retrospective study was conducted at the Paediatric Renal Unit at Chris Hani Baragwanath Academic Hospital. Patients under the age of eighteen years who had biopsy-confirmed lupus nephritis, between January 2000 and December 2019, were included in the study. Repeated biopsies from a single patient and those with insufficient

records or no histology confirmation were excluded. Patients were identified using the biopsy register from the renal unit kept by the head of the unit. Archived patient records were used to gather anonymized data on patient demographics, treatment, and laboratory and clinical characteristics. The estimated glomerular filtration rate (eGFR) was calculated using the original Schwartz estimation equation when the National Health Laboratory Service (NHLS) used the Jaffe reaction for serum creatinine measurement and the modified Schwartz formula in the period when the enzymatic method was initiated at the NHLS [18]. Advanced CKD (stages 3, 4, and 5) was classified using the Kidney Disease Improving Global Outcomes (KDIGO) classification [19]. Kidney biopsy specimens were examined under light microscopy, immunofluorescence, and electron microscopy [20]. Histological LN was classified according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) [21]; kidney biopsies performed before 2004 were reviewed and reclassified according to the latest ISN/RPS classification by the histopathologist at National Health Laboratory Service (NHLS) [19]. The SLEDAI scoring system was used to assess disease activity at presentation [22]. Systemic Lupus International Collaborating Classification criteria (SLICC) was used to diagnose the clinical components of SLE [3]. Treatment of paediatric lupus nephritis was standardised for induction and maintenance therapy [19]. For the induction phase, patients received intravenous methylprednisolone 0.5g/m^2 on alternate days for three dosages and intravenous cyclophosphamide(CYC) 0.5g/m^2 monthly for six months [19]. The maintenance therapy included oral prednisone at the start of 2 mg/kg (max. 80 mg) for at least two years followed by tapering to 0.5mg/kg/day

if satisfactory improvement in kidney and extrarenal disease was achieved. In addition to prednisone, intravenous cyclophosphamide 0.5g/m^2 every three months for 18 months was administered. When mycophenolate mofetil (MMF) became available in 2008, the cyclophosphamide(CYC) maintenance protocol was changed to MMF 1.2g/m^2/day. Rituximab was added for those with refractory disease. Refractory lupus nephritis was defined as failure to attain clinical remission after induction with intravenous methylprednisolone and other immunosuppressive therapy (CYC or MMF) [23]. Outcomes included disease remission at the last visit, death, transfer to the adult unit, and CKD staging at 24 months. Remission was defined as the return of serum creatinine to the previous baseline, plus no haematuria and proteinuria on the urinary dipstick. Hypertension was defined as any patient requiring antihypertensives other than Angiotensin-converting enzyme (ACE). The diagnosis of Macrophage activation syndrome (MAS) met the criteria mentioned in the European League Against Rheumatism and the American College of Rheumatology (ACR/EULAR) classification [13].

Ethics clearance was obtained from the University Human Research and Ethics Committee, clearance certificate number M210898.

Statistical analysis

Data analysis was performed using Statistica software (Statsoft USA, version 13.5) [24]. Descriptive statistics were used for demographic and baseline clinical data analysis. The binary logistic regression model was used to identify the risk factors associated with a poor outcome; p-values of <0.05 were considered statistically significant.

Results

A total of 833 percutaneous kidney biopsies were performed during the study period. Fifty-two (6.2%) were identified to have lupus nephritis, and 44 cases were analysed (seven had incomplete records and one child had inconclusive histology). Repeat biopsies for LN were not included in the analysis, [figure 1].

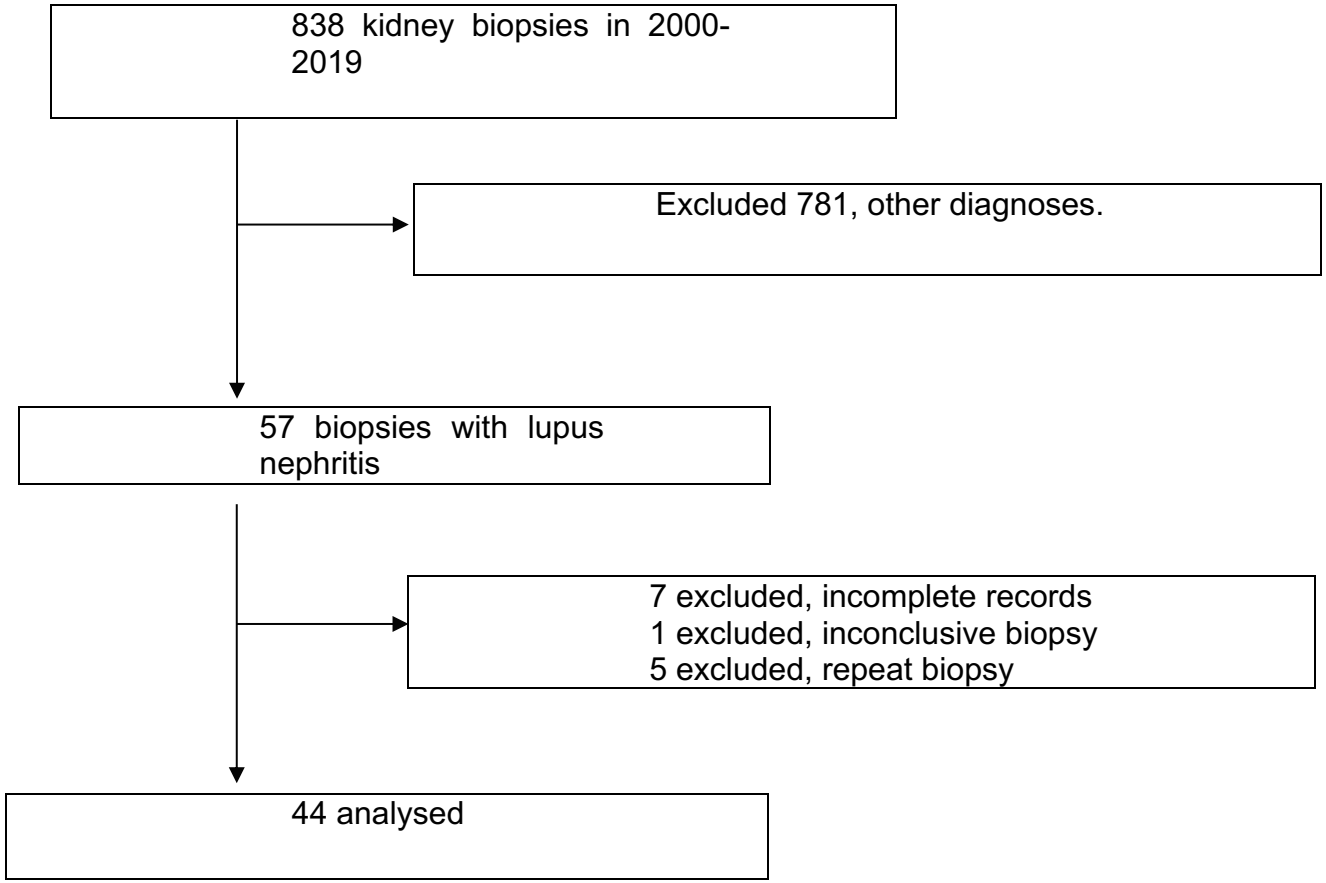

Figure 1: Study design

Presenting features

The mean age at diagnosis was 11.8±1.3 years. The youngest patient was eight years old. Females accounted for 33 (75%). The most prevalent extra-renal clinical manifestations were dermatological in 31 (70.5%) patients, musculoskeletal in 14 (31.8%) patients, and cardiovascular in nine (20.5%) patients. The mean SLEDAI score was 14.6±1.2. Seven (15.9%) patients had a diagnosis of MAS, [Table 1]. One of these seven patients (14.2%) died, but none of the other six patients who had MAS developed CKD at the time of the last visit before December 2019. All seven patients received cyclophosphamide, while four also received cyclosporin.

Table 1: Baseline extra-renal clinical features of the patients

Clinical feature (N=44)	**n (%)**
Dermatological: Lupus malar rash, bullous lupus, toxic epidermal necrolysis variant of SLE, maculopapular lupus rash, photosensitive lupus rash, classic discoid rash, diffuse thinning, or hair fragility.	31(70.5)
Musculoskeletal: any synovitis ≥ 2 joints, swelling, or effusion.	14 (31.8)
Central nervous system: (seizures, psychosis, multiple mononeuropathy, or acute confusional state).	12 (27.3)
Cardiovascular system: serositis, pericarditis, myocarditis, and endocarditis	9 (20.5)
Macrophage activation syndrome: temperature >38 ˚C and high ferritin>684µg/L, plus any two of laboratory criteria: (thrombocytopenia <140 x 10^9 /L, aspartate transaminase >48U/L, triglycerides >4mmol/L, and fibrinogen <1.5g/L), or evidence of haemophagocytosis on bone marrow aspirate.	7 (15.9)

The median eGFR at diagnosis was 115 (IQR 103 to 148.5; range 3.1 to 227) ml/min/1.73m^2. Proteinuria was present in 40 (90.9%) patients, with a nephrotic range in 26 (59.1%) patients; hypertension was diagnosed in 27 (61.4%) patients. Five (11.4%) patients received peritoneal dialysis at diagnosis, [Table 2].

Table 2: Baseline renal clinical features

Renal presentation, n =44	**n (%)**
Proteinuria, any positive dipstick	40 (90.9)
Hypertension	27 (61.4)
Nephrotic range proteinuria, UPCR >0.2 g/mmol	26 (59.1)
Dipstick haematuria, any positive	19 (43.2)
Peritoneal dialysis	5 (11.4)

UPCR: spot urine protein to creatinine ratio

Positive anti-nuclear antibodies (ANA) (IQR: 320 to 640) and low C3 were present in all patients. Anti-double-stranded DNA antibodies (IQR: 65 to 320) were present in 36 (81.1%) patients; APLA were positive in 11 (36.6%) patients. Anaemia was present in 30 (69.8%) patients, [Table 3].

Table 3: Baseline laboratory parameters

Characteristics	**n (%)**
ANA >1:40	44/44 (100)
Anti-ds DNA >1:20	36/44 (81.1)
C3 <0.9 g/L	44/44 (100)
C4 <0.10 g/L	26/44 (59)
Thrombocytopenia, platelets <100×10^9/L	11/44 (25.6)
Anaemia, Hb < 12 g/dL	30/44 (69.8)
Leucopenia, leucocyte < 4.0 x 10^9/L	10/44 (23.3)
ESR >20 mm/hr	26/40 (65)
Anti-RNP >25 U/ml	24/41(58.5)
Anti-Smith antibody	19/41(46.3)
Anti-phospholipid antibodies	11/30 (36.6)
Anti-SS-A/Ro	9/40 (22.5)
Anti-SS-B/La	2/40 (5)

ANA: anti-nuclear antibody; ds-DNA: double stranded DNA; C3: complement 3; C4: complement 4; Hb: haemoglobin; ESR: erythrocyte sedimentation rate; Anti-RNP: Anti-Ribonucleoprotein; Anti-SS-A (Ro): Anti-Sjögren Syndrome A; Anti-SS-B (La): Anti-Sjögren Syndrome B

Histopathologic characteristics

Of the 44 kidney biopsies analysed, 28 (63.6%) showed proliferative LN class III/IV with or without class V (21 had pure class III/IV and seven children had class III/IV with class V). No cases of class VI were reported. Pure class III was the most prevalent (n=16,36.4%), [Figure 2].

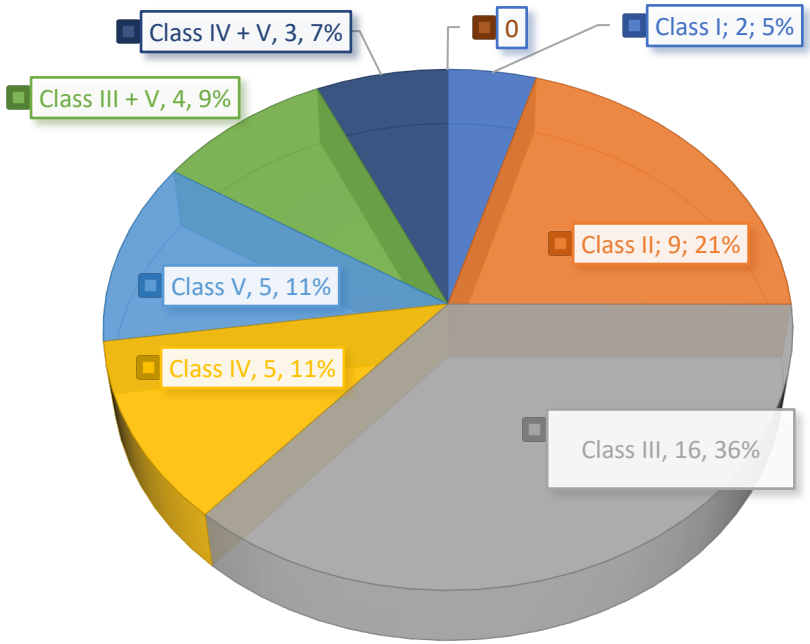

Figure 2. LN classification as per ISN/RPS (International Society of Nephrology/Renal Pathology Society)

Follow-up and prognosis

The mean (±SD) duration of follow-up was 52.5±6.9 months; 25 (56.8%) achieved remission at the last visit. To analyse factors associated with remission, we divided the 44 children into two groups: a group in remission (complete or partial) on the last date of follow-up, and a group without remission. Haematuria (OR =0.12, 95% CI 0.02 to 0.80) at presentation was linked with a reduced chance of establishing remission, while positive anti-Ro antibodies (OR =26.0, 95% CI 1.6 to 410.6) was associated with achieving remission, [Table 4].

Table 4: Factors associated with remission

Variables	N=44	Remission		Univariate analysis			Multivariate analysis	
		No, *n (%)*	**Yes,** *n (%)*	**P-value**	**Unadjusted OR (95% CI)**	**P-value**	**Adjusted OR (95% CI)**	**P-value**
Haematuria								
No	24	5(28.8)	19(79.2)		*Reference*		*Reference*	
Yes	20	14(70.0)	6(30)	<0.001	0.11 (0.03-0.45)	<0.001	0.12 (0.02-0.80)	0.03
Ro								
No	27	15 (55.6)	12 (44.4)		*Reference*		*Reference*	
Yes	9	1 (11.1)	8 (88.9)	0.02	10.0 (1.1-91.4)	0.04	26.0 (1.6-410.6)	0.02
Elevated creatinine								
No	37	13 (35.1)	24 (64.9)		*Reference*		*Reference*	
Yes	7	6 (85.7)	1 (14.3)	0.01	0.09 (0.01-0.83)	0.03	0.17 (0.01-2.63)	0.20
Peritoneal dialysis								
No	39	14 (35.9)	25 (64.1)		*Reference*		*Reference*	
Yes	5	5 (100.0)	0 (0.0)	0.01	-	1.00	-	-

p<0.05=statistically significant; OR=Adjusted odds ratios for remission

At 24 months after diagnosis, 38 patients were available for analysis; three (7.9%) developed CKD stage 3, and one (2.6%) developed CKD stage 5. The eGFR was normal in 34 (89.5%) patients. Of the six (13.6%) patients who were not seen at 24 months, two died, one defaulted, and three were transferred to adult services. The two patients died within one month of diagnosis, one with neuro-lupus and the other with MAS. Analysis of risk factors for death was not possible due to small numbers.

Table 5: Risk factors for advanced chronic kidney disease

Variables	**Univariate analysis**			**Multivariate analysis**	
	No CKD	**Stages 3-5**	**P-value**	**OR (95% CI)**	**P-value**
Haematuria					
No, *n (%)*	20 (100.0)	0 (0.0)		Reference	
Yes, *n (%)*	14 (80)	4 (20)	0.02	**-**	1.00
Age (years), *Mean (SD)*	12.0 (1.1)	10.3 (2.2)	0.01	0.37 (0.15-0.93)	0.04
Creatinine, *Mean (SD)*	53.9 (15.5)	260.3 (154.3)	<0.001	-	1.00

p<0.05=statistically significant; OR=unadjusted odds ratios for CKD

Risk factors for CKD

For analysis of CKD risk factors at the 24-month visit, we compared those with advanced CKD stages 3 to 5, to those without CKD. Risk factors for advanced CKD identified on univariate analysis were haematuria, young age, and elevated serum creatinine at diagnosis. On multivariate analysis, the only independent risk factor was age at diagnosis; younger age at presentation was associated with worse outcomes. The average age of patients with advanced CKD was 10.3 years, which was two years younger than those without CKD [Table 5].

Assessment of overall therapy

All patients received oral prednisone as maintenance therapy for the duration of follow-up and intravenous methylprednisolone as induction therapy at diagnosis. In addition to steroids, all patients received other immunosuppressant therapy; 34 of 44 (77.2%) received cyclophosphamide with a mean of 4.5 dosages. Twenty-one (61.7%) patients received monthly cyclophosphamide treatment for six months as part of induction therapy (six dosages or fewer). Nine (26.5%) patients received three monthly cyclophosphamides (more than seven dosages) for maintenance therapy, while 22 (50%) received Mycophenolate mofetil. Five (11.4%) patients received cyclosporin for MAS, while two (4.5%) received rituximab for refractory disease and immune thrombocytopenic purpura respectively.

Discussion

This study describes a cohort of childhood lupus nephritis in a tertiary hospital in South Africa. The female predominance and median age at diagnosis are similar to other case series [4,15]. The prevalence of extrarenal manifestations has not changed from previous reports from this site, with dermatological manifestations being the most common [4,8,14,25]. The mean SLEDAI score of 14.6±1.2 at presentation in our cohort indicates substantial organ damage; a score greater than nine is associated with LN and death in both high- and low-income populations [8]. Hypertension due to disease and steroid treatment is common in patients with LN, similarly, demonstrated in this study [4]. Since previous serum creatinine levels were not known at the time of presentation, we were unable to define AKI at diagnosis [19]. However, the dialysis rate at presentation is similar to other African cohorts [8]. Although our remission percentage (56.8%) is lower than that of the Indian cohort (94%), there were significant differences in the patient's clinical presentation, demographics, and therapeutic regimens [15]. We report MAS in this cohort with favourable survival rates. We could find no published report on MAS in African children with SLE, but an American study reported a mortality rate of 11% [12].

We also report a significant number of patients with antiphospholipid syndrome (APLA); other studies have shown micro-vascular involvement leading to poor patient and renal survival in APLA-positive patients [10,26]. All patients had positive ANA and low C3 which is expected in LN. Anti-ds DNA antibodies is a documented indicator of LN severity,

present in 81% of cases. Another indicator of the severity of LN is the histopathological class, with proliferative lupus nephritis as the most severe and also the most common finding in this group, similar to other reports [6,8,15,25]. The remission rate at the last visit is similar to reports from HIC [6]. As observed in other research, the presence of haematuria, high creatinine, and dialysis therapy at presentation lowers the chance of achieving remission [4,8,9]. In this study, the advanced CKD prevalence is similar to the Hong Kong children and the PULSE cohorts in Cape Town [7,8]. While several reports have indicated that haematuria, young age, and elevated creatinine are predictors for the development of chronic kidney disease (CKD) stage 3-5, this study found that an age of 10.3 years at presentation is associated with the development of advanced CKD, two years younger than those without advanced CKD [4,6, 8].

Contrary to several adult case studies, the presence of anti-Ro antibodies in this study is associated with remission; we hypothesize that this finding could be attributable to cases with incomplete data on anti-Ro status, creating a non-sampling error [27]. Induction therapy using intravenous cyclophosphamide is preferred because the availability and adherence to oral medication are not guaranteed at this site. Rituximab was not often administered, as it is a recent addition to treatment protocols [19,28]. Despite the multiple indicators of disease severity, we observed mortality rates comparable to other paediatric cohorts [4,6,8].

Limitations

This study is limited by the retrospective design and small sample size. Archived paper records were not available for all cases of LN. Also,

because this is a single-center study, it lacks generalisability. Despite the limitations, this study reports the outcome and complications of childhood LN in a population in a LMIC, with multiple indicators of severity.

Conclusion

The observed renal and patient survival rates despite the multiple indicators of severity demonstrate the heterogeneity of this disease. Macrophage activation and antiphospholipid syndromes are documented complications at presentation in this population. Future studies should analyse the psycho-social impact, medication adherence, and the effect of Ro antibodies in childhood LN. In addition, genetic testing may shed some light on the observed differences.

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APPENDIX

Appendix A: Research protocol

Lupus nephritis in children at Chris Hani Baragwanath Academic Hospital, an observational study from January 2000 to December 2019.

A study protocol by publication in partial fulfilment of Mmed (Paed) at the University of the Witwatersrand**.**

Investigator:

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Supervisors:

Adjunct Professor Karen Petersen

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Dr Anees Laher

Department of Paediatrics and Child Health

University of the Witwatersrand

1. LIST OF ABBREVIATIONS

ANA: antinuclear antibodies

Anti-dsDNA: Anti double-stranded DNA antibodies

CHBAH: Chris Hani Baragwanath Academic hospital

CKD: Chronic Kidney Disease

cSLE: childhood-onset systemic Lupus erythematosus

cLN: Childhood Lupus Nephritis

eGFR: estimated glomerular filtration rate

ESKD: end-stage kidney disease

ISN/RPS: International Society of Nephrology/ Renal Pathology Society

KDIGO: Kidney Disease Improving Global Outcomes

LN: Lupus Nephritis

SLE: Systemic Lupus Erythematosus

SLEDAI: Systemic Lupus Erythematosus Disease Activity Index

WHO: World Health Organisation

2. LITERATURE REVIEW

Introduction

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease characterised by the formation of antinuclear antibodies[1]. The incidence of 0.3 to 0.9 per 100 000 children per year, and a prevalence of 3.3 to 24 per 100 000 children has been reported[2]. It is more common in females[3]. Lupus Nephritis (LN) is more common in patients with African ancestry[2,4]. Childhood-onset SLE (cSLE) is often more severe at diagnosis than adult-onset SLE, because patients are more likely to have renal involvement[4,5]. Renal involvement is seen in more than 70% of children with SLE[5,6]. Children with SLE in Egypt showed a 5-year survival rate of 88 % in patients with nephritis compared to 96% for those without nephritis[5]. A Cape Town study in children with LN reported a mortality rate of 9.7% and a 5-year survival rate of greater than 97%[7].

Pathogenesis

The aetiology of Lupus nephritis is still not well understood[8]. SLE develops when there is a loss of immune tolerance to nuclear autoantigens, which becomes clinically evident by the presence of diverse autoantibodies and immune complex deposition that result in kidney injury[1,8,9]. Autoantibodies are present in 88% of SLE patients, namely antinuclear antibodies (ANA), anti-double-stranded(ds) DNA, anti-Smith, anti-Ro, anti-La and anti-phospholipid antibodies[10]. An increase in anti-ds DNA titre is strongly associated with the onset of renal

disease[10]. Immune complex deposition in the mesangial and subendothelial space results in glomerulonephritis[8].

Clinical aspects

SLE is diagnosed on clinical and laboratory features based on the Systemic Lupus International Collaborating Clinics (SLICC) classification[11]. This requires at least four of 17 criteria, including at least one clinical and immunologic criterion, or biopsy-proven lupus nephritis (Appendix 1)[11]. The organs most commonly affected are the skin and kidneys, and it is often associated with non-specific constitutional systems[3]. The cardiac, gastrointestinal, respiratory, haematological, and central nervous systems are affected less commonly[3]. Local data at Chris Hani Baragwanath Academic hospital (CHBAH) on childhood SLE showed that 77% of patients presented with cutaneous features, 55.5 % presented with non-specific constitutional features and 44% with renal disease[3]. The most common histological finding was either class III or IV in 76% of the patients with renal disease, like other reports[3,6]. The CHBAH data also concluded that class III and IV nephritis indicate a poor prognosis3.

Diagnosis of Lupus Nephritis

A renal biopsy is the gold standard for diagnosis of LN[12,13.] Different classes have different prognosis and treatment[12,13]. The International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification system is used (Table 1)[12,13.]

Table 1: ISN/RPS Classification of LN[12,13]

Class I Minimal mesangial Lupus Nephritis: Normal glomeruli by light microscopy, but deposition of immune complexes is detectable by immunofluorescence techniques.
Class II Mesangial Proliferative Lupus Nephritis: Mesangial hyper cellularity with immune deposits detectable by light microscopy.
Class III Focal Lupus Nephritis: Focal, segmental or global proliferative glomerulonephritis involving <50% of all glomeruli.
Class IV Diffuse Lupus Nephritis: Diffuse, segmental or global proliferative glomerulonephritis involving ≥50 % of all glomeruli
Class V Membranous Lupus Nephritis: Global or segmental subepithelial immune deposition It can occur in combination with class III or IV and it can manifest advanced sclerosis.
Class VI Advanced sclerosis Lupus Nephritis: There's a global sclerosis in ≥90% of the glomeruli.

Complications:

Patients with SLE experience disease exacerbations or relapses of different severity which increases the risk for end organ damage[14,15]. The Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) has been used in clinical settings to evaluate relapses of lupus in children, but it lacks precision in excluding infection[14,15,16]. Infections causes a

average of 25% mortality in SLE patients[15]. It is important to exclude infections in all patients that present with a flare as management for the patient will differ[15]. However, differentiating between a lupus flare and infection is a clinical challenge due to non-specific biomarkers[15]. In addition, there can also be treatment related adverse events like infections, secondary malignancies, stunting, osteopenia and depression[17]. Other life-threatening complications that are due to SLE include increased risk of thrombosis associated with antiphospholipid syndrome[18,19], and excessive immune activation causing macrophage activating syndrome[20].

Outcome of LN

Lupus Nephritis is a severe manifestation of SLE resulting in worse outcomes than those without kidney involvement[5]. Complications of LN may include hypertension, nephrotic syndrome, chronic kidney disease (CKD) and death[2,5]. In Cape Town, 13 % developed end stage kidney disease (ESKD) compared to <1% in North American patients, and mortality was reported to be higher in South African patients[7]. Nephritis was the only strong marker of mortality in South African population compared to North American deaths where infection was a common cause[7]. However, differences in race, age, disease duration, antibody profile and treatment protocol were reported between the two groups[7].

Treatment

The aim of treatment is to achieve complete or partial remission and limit the complication of the disease and its treatment[12]. Standard treatment guidelines by Kidney Disease Improving Global Outcomes (KDIGO) are

available according to different LN classes[17,12]. Treatment for LN include induction and maintenance therapy with immunosuppressive medication[2,12,17]. Combinations of corticosteroid therapy and alkylating agents are recommended[12,17].

Assessing treatment response

Response is associated with good long-term outcomes. Patients may have complete, partial or no response [12](Table 2).

Table 2: Treatment Response assessment[12]

Criteria	Definition
Complete response	Return of serum creatinine to previous baseline, plus a decline in the uPCR to <500 mg/g (<50 mg/mmol).
Partial response	Stabilization (±25%), or improvement of SCr, but not to normal, plus a >50% decrease in uPCR.
Deterioration/No response	A sustained 25% increase in serum creatinine is widely used but has not been validated.

Considering there has been major advances in the treatment available to patients with LN during the last two decades, this study will describe the characteristics, morbidity, and mortality of LN in our population. This study will contribute to the understanding of risk factors and prognostic markers at disease onset associated with CKD/ESKD.

3.AIMS AND OBJECTIVES

3.1 To describe the clinical and laboratory parameters at presentation.

3.2 To describe complications and outcome of patients with LN.

3.3 To identify risk factors associated with chronic kidney disease and mortality associated with LN.

4.METHODS

4.1 Study design

A retrospective record review of patients diagnosed with Lupus Nephritis treated by the Paediatric Renal Unit at Chris Hani Baragwanath Academic Hospital over a 20-year period from January 2000 to December 2019.

4.2 Study population

Patients diagnosed with Lupus Nephritis will be selected from a database(REDCap) and data will be collected from patient's files. Anonymity will be ensured with the use of study numbers. A sample size of approximately 40 is anticipated (based on the estimation of two patients per year over 20 years)

4.2.1. Inclusion criteria

Children ≤18 years of age

Confirmed diagnosis of LN on biopsy

Patients diagnosed and managed during the time period 01 January 2000 to 31 December 2019.

4.2.2. Exclusion criteria

Patients with inadequate records (including clinical and laboratory information as well as follow up information).

4.3. Variables

See appendix 2- Data collection sheet for all variables that will be included for the data collection. Clinical and laboratory parameters will be collected at baseline from the time of first biopsy and eGFR will be calculated at 2 years, 5 years, and 10 years from diagnosis. The number of relapses and hospitalisations per patient will be captured. Progression of CKD will be defined according to eGFR categories using KDIGO guideline[9]. The eGFRs will be calculated using the original Schwartz estimation equation when the NHLS used the Jaffe reaction for creatinine measurement, and the modified Schwartz formula in the period when the enzymatic method of creatinine measurement was initited at the NHLS. Progression of histological LN classes will be documented for patients that had repeated biopsy. Treatment received and complications of treatment such as osteoporosis and sepsis episodes will be documented. Complications of the disease such as macrophage activation syndrome, antiphospholipid syndrome, hypertension, and chronic kidney disease will be documented. Relapses will be assessed using a SLEDAI scoring system[10].

4.4. Definitions

Cardiovascular involvement will be described as serositis, pericarditis, myocarditis, and endocarditis[21].

CNS involvement will be described as any patient with seizures, psychosis, multiple mononeuropathy or acute confusional state[21].

Dermatological manifestation will be described as Lupus malar rash, bullous lupus, toxic epidermal necrolysis variant of SLE, maculopapular lupus rash, photosensitive lupus rash, classic discoid rash, diffuse thinning, or hair fragility with visible broken hair[21].

Fever will be any temperature reading above 38 ˚C[21].

Haematological characteristics will be defined as follows: leucopenia as < 4000/mm3, anaemia as Haemoglobin (Hb) < 13g/dL for males, Hb< 12 g/dL for females, elevated CRP/ESR (CRP>40 mg/L / ESR >20 mm/hr), low C4 as <0.10 g/L, low C3 as <0.9 g/L, hypertriglyceremia >3 mmol/L, hypofibrinogemia <1.5g/L and elevated serum ferritin >500µg/L [21]. Elevated LDH>190 u/L, low haptoglobin <0.30 g/L and elevated reticulocyte count > 2%.

Hypertension will be described as any patient on antihypertensives.

Musculoskeletal involvement will be described as synovitis involving ≥ 2 joints, characterized by swelling or effusion[21]. Splenomegaly is defined as a palpable splenic edge felt >2 cm below the left costal margin.

Lupus Nephritis will be defined as hematuria (>5 RBCs per field), Proteinuria (urine protein creatinine ratio >0.05 g/mmol) or biopsy in keeping with Lupus Nephritis[21].

Various antibodies to be defined as Positive ANA (>1:40) Positive Anti-ds DNA (>1:20), positive anti-RNP (>25 U/ml), any positive for; Anti-Ro, Anti-La, Lupus Anti- anticoagulant (LA), Anticardiolipin(aCL), Anti-Beta 2 Glycoprotein 1 (β2GP1)[21].

5.DATA ANALYSIS

Data will be obtained retrospectively from patients' files, which are kept at the paediatric renal clinic at the Chris Hani Baragwanath Academic Hospital. It will be captured in a data collection sheet (see appendix 2). Collected data will be entered and managed in Microsoft Excel database prior to analysis. Data will be checked for incompleteness and inconsistencies and analysed using STATISCA (Statsoft USA, version 13.5).

For normally distributed, the student's *t*-test will be used to compare means; for skewed data, the Mann-Whiteney U test will be used to compare medians. The chi-squared test will be used when we want to see if two categorical variables are related.

5.1 for demographic profile, a categorical variables will be represented using percentages and bar graphs. Continuous variables will be represented using means and standard deviation if they are normal distribution, medians and interquartile ranges will be used in skewed data.

5.2 To describe the clinical and laboratory parameters at presentation.

Categorical variables will be represented using percentages and frequencies. Continuous variables will be represented using means and

standard deviation if they are normal distribution, medians and interquartile ranges will be used in skewed data.

5.3 To describe complications and outcome of patients with LN.

Categorical variables will be represented using percentages and frequencies. Pie charts and bar charts will be used to give a pictorial view.

5.4 To identify risk factors associated with chronic kidney disease (disease flare leading to relapses or admission and progression to ESKD) and mortality associated with LN.

CKD will be used as a binary response variable, frequencies and percentages will be used to summarise it. To determine the risk factors associated with CKD, a logistic regression method will be used . Similarly, to the mortality outcome (died/alive) a logistic regression method will be used to determine the factors associated with mortality.

Renal survival will be calculated at time of diagnosis, 2 years, 5 years and 10 years using a Kaplan-Meier survival method and cox regression if suited. The outcome will be classified as remission, CKD staging according to KDIGO guideline, defaulted, receiving renal replacement therapy, died, and transferred to another unit for transplant or adult unit. For all analyses, a p value of < 0.05 will be considered significant.

6.TIMELINE

Chris Hani Baragwanath Academic Hospital and HREC submission: July 2021

Protocol submission: July 2021, Protocol assessment: August 2021

Anticipated commencement of the study: October 2021

	Nov-20	Dec-20	jan-21	Mar-21	Apr-21	May-21	Jun-Jul-21	Aug 21	Sep - 21	Oct - 21	Nov-Dec 21
Literature review											
Protocol preparation											
Protocol submission and assessment											
Ethical clearance											
Postgraduate committee clearance											
Data collection											
Data analysis ,Writing up and submission											

7. ETHICS

Permission to access files and to conduct a study will be requested from the Head of the Renal unit and Head of Department for Paediatrics and the CEO of Chris Hani Baragwanath Academic Hospital. Patient identifiers will not be used in the data sheet as patients will be given a study number. The completed data collection sheet will be stored and only accessible by me and supervisors.

Online approval from the medical Human Research Ethics Committee of the University of Witwatersrand will be requested. Permission will be requested from the Department of Health. A study will commence once permission and approval from all the authorities at Chris Hani Baragwanath Academic Hospital has been obtained.

Plagiarism will be avoided during this study

Funding

Funds for publication will be requested from the MMed fund. Printing and any additional costs will be incurred by the investigator.

8. LIMITATIONS

Anticipated problems may include missing data from patients' files during data collection. There may also be a number of patients who were lost to follow up and patients who were non-compliant.

9. Strengths of the study:

This study will document the long-term follow-up of patients, specifically to define the prognosis and morbidity in South African children with lupus nephritis.

10. Outcome of research:

To publish the data in a journal accredited by the DHET.

Appendix B: Ethics Clearance

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Ritshidze Mulaudzi

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M210898

NAME: (Principal Investigator)	Dr Ritshidze Mulaudzi
DEPARTMENT:	Paediatrics Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Lupus nephritis in children at Chris Hani Baragwanath Academic Hospital, an observational study from January 2000 to December 2019
DATE CONSIDERED:	Ad hoc
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof K. Petersen and Dr A. Laher
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	21/09/2021

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions.Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.** The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **September** and will therefore be due in the month of **September** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

22/09/2021
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix C: African journal of nephrology guidelines

Instructions for Authors

Aim and scope of the Journal

The *African Journal of Nephrology* (*Afr J Nephrol*) is the official journal of the African Association of Nephrology (AFRAN) and publishes peer-reviewed papers relating to clinical or basic research in nephrology and hypertension, as well as nephrology education. The Journal considers submissions of original and review articles, case reports, technical reports, practice guidelines, images in nephrology, teaching points, proceedings of national or regional congresses, and letters to the Editor.

General

Manuscripts may only be submitted online, via the Journal website. Manuscripts should not be under consideration by another journal and should not have been published previously except as a poster or abstract. We recommend that manuscripts be scanned with a plagiarism checker before submission. Permission from the copyright holder must be submitted with manuscripts containing previously published material such as figures or tables.

Manuscripts which fail to satisfy the requirements set out in this document may be rejected without further review.

Authors are referred to the uniform requirements for manuscripts submitted to biomedical journals at http://www.icmje.org/.

Language policy

Authors are invited to submit articles written in English or French. All French articles must also be accompanied by an English title, abstract and list of keywords. Please contact us if assistance is required.

Authorship

All authors must have made a significant contribution and must have approved the final manuscript submitted for publication. A signed authorship statement must be submitted by each author to confirm that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Lesser contributions should be indicated in an Acknowledgments paragraph after the Conclusions. Persons mentioned in the Acknowledgments must have agreed to be acknowledged in this way.

Ethics

All studies must comply with institutional and international regulations such as the Declaration of Helsinki. Human and animal studies require ethics committee or institutional review board approval, and human studies require informed consent. Compliance with these requirements must be documented at the end of the Methods section of the manuscript.

Acknowledgements

Manuscripts should include an Acknowledgements section, if appropriate. This follows the Conclusions and should provide detail on funding sources and other contributions to the paper that do not merit authorship.

Conflict of interests

The manuscript and cover letter must include a declaration that the authors have no conflict of interests, or a statement that details the potential conflict.

Covering letter

All manuscripts must be accompanied by a 1-page cover letter which specifies the type of article and summarizes the contribution it makes to the scientific literature. It should confirm that the manuscript is not under consideration by another journal and has not been previously published. It should contain an author contribution statement as well as a statement on any potential conflict of interests. The cover letter can be uploaded with the supplementary files.

Types of contributions

Original articles

These articles present new research results and should be limited to 4000 words and no more than 30 references. Section headings should include Abstract, Introduction, Methods, Results, Discussion, Conflicts of interest, Acknowledgements and References.

Review articles

Review articles are usually invited. Authors who wish to submit a review article should first liaise with the editor before manuscript submission. These articles should usually be limited to 5000 words and include no more than 50 references.

Reports of clinical trials

Reports of clinical trials should comply with the CONSORT guidelines and include a participant flow diagram. See http://www.consort-statement.org/ for details and for a template of the flow diagram.

The study should be included in a registry of clinical trials before commencement and the Methods section of the manuscript should cite the registration number. The Pan African Clinical Trials Registry (http://www.pactr.org/) is an example of such a registry.

Case reports

Concise reports of unusual or informative cases should be no more than 800 words (excluding the abstract and references). The abstract should not exceed 100 words. One table, up to two images, and up to 15 references may be included. All case reports must include a statement on ethics approval or a statement that permission for publication was granted by the patient or family.

Images in Nephrology

One or two high resolution images of exceptional interest may be submitted. These may be made available by the Journal to readers as PowerPoint slides. The accompanying text should be no more than 300 words, have a very brief abstract, and have a maximum of 6 references.

Congress proceedings

Abstracts of presentations at national or regional congresses can be submitted for publication in the Journal. Congress organisers are responsible for ensuring that the abstracts are correctly formatted and checked for errors. They will be published as received and are not sent for peer review. Congress proceedings are published as a single, searchable PDF document. The first page(s) should include the name, place and dates of the congress, and a concise list of abstract titles and authors.

Letters to the Editor

Letters should be no more than 250 words and relate to papers recently published in the Journal. They will be sent to the authors of the original paper for reply.

Manuscript layout

Submissions should be submitted as Microsoft Word files. UK English spelling should be used. The text should be Arial, font size 12. Pages should be A4 size and should be numbered consecutively, commencing with the title page.

Abbreviations must be spelled out the first time they are used, followed by the abbreviated form in parentheses.

Units of measurement. Système International (SI) units must be used e.g. kg, g, µg, cm, mm, etc. An exception is blood pressure which is reported in mmHg.

Trade names. Non-proprietary (generic) names should be used. The source of any new or experimental preparation should be given.

Manuscript sections

Title page:
Include the article title (use an initial capital letter only i.e. sentence case) and avoid abbreviations within the title, list all authors and their institutional affiliations at the time of the study, provide a short running title of up to 50 characters, the word count (not including the abstract, tables and references) and the name, address and email address of the corresponding author.

Abstract and keywords:
Abstracts should be structured into Background, Methods, Results and Conclusions, and should not exceed 250 words in original or review articles, and 100 words in case reports. A French translation of the abstract may be submitted as an addition. Abstracts should not contain any figures, tables or references. List a maximum of 6 keywords below the abstract.

Main text:
Original articles should include Introduction, Methods, Results and Discussion sections, each beginning on a new page. Statistical methods must be included in the Methods section. Methods not in common use should be described fully or supported by references. A statement on ethical approval should be included at the end of the Methods section.

Tables

Tables should not be submitted separately but should be included in the manuscript file directly after the paragraph in which it is first cited. All tables should be editable and not pasted into the manuscript as an image. They should be numbered consecutively and each must have a brief caption placed above the table. Place footnotes below the table.

Figures

Figures should be submitted as separate, high- resolution images. Low-resolution copies may be embedded in the manuscript file. Multipanel figures must be provided as a single file. Figures should be numbered in the order they are mentioned in the text. Figure titles and legends should be included in the main manuscript, not in the graphic files.

Photomicrographs should have a scale bar and the legend should include details of the staining technique. Patients shown in photographs should have their identity concealed or should have given their written consent to publication.

References

References should be relevant and not exceed 50 for review articles, 30 for original articles, 15 for case reports, 6 for images in nephrology and 5 for letters to the editor.

We strongly recommend the use of bibliographic software tools, such as EndNote or Mendeley, for reference management and formatting. For EndNote users, an *Afr J Nephrol* style file is available. The *PLOS* output style can also be used.

Citations in the text are indicated by the reference number(s) in square brackets, without using any spaces, e.g. [1,5-7].

At the end of the article, the references should be numbered in the order in which they first appear in the text. They should give the names of all authors unless there are more than six, when only the first six should be given, followed by "et al". This should be followed by the title of the article, the abbreviated journal name, the year of publication, the volume number and the full first and last page numbers. Consult PubMed for journal name abbreviations -

https://www.ncbi.nlm.nih.gov/nlmcatalog/journals/.

References to books should give the title, followed by the place of publication, the publisher, the year and the page numbers.

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