CLINICAL PRESENTATIONS, GENOTYPIC SPECTRUM, AND OUTCOMES OF CHILDREN WITH PRIMARY HYPEROXALURIA AT CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL.

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A research report submitted to the faculty of Health sciences, University of Witwatersrand, Johannesburg in partial fulfilment of the requirements for the degree of Masters of Medicine in Paediatrics.

DECLARATION

I, Emilie Erasmus, declare that this research report is my original work. It is being submitted for the degree of Masters of Medicine in the branch of Paediatrics at the University of Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University. The submissible format of the research report will be submitted to The South-African Journal of Child Health.

Declarations of interest: None

Dr Emilie Erasmus, May 2023

DEDICATION

To all the children and their families living with this condition, and to my supervisors, family, and friends for their support.

ABSTRACT

Background:

Primary hyperoxalurias (PHs) are a rare group of autosomal recessive disorders involving the overproduction of oxalate which results in renal calculus, progressive nephropathy, and eventual renal failure.

Objectives:

This study describes the demographics, clinical presentation, genotypic spectrum, determinants of disease severity and causes of death in patients diagnosed with PH, at a tertiary hospital.

Methods:

Retrospective descriptive review of patients with PH at a single unit, over 20 years.

Results:

Sixteen patients were identified with a median age at presentation of 7.1 years. Clinical presentations included nephrolithiasis and urinary tract infections (UTI) in six and end-stage kidney disease (ESKD) in five. Eight had a homozygous mutation c.335C>A (p.A112D) while four had a heterozygous mutation. The twelve mutations found were all on the AGXT gene for PH1. The median age of presentation of the four patients with heterozygous mutations was 5.7 years compared to 7.5 years for the homozygous mutation. Thirteen patients (81,3%) received renal replacement therapy (RRT). Eight patients were listed for transplant and six underwent a combined liver and kidney transplant (CLKT). Four patients were still alive at the time of this report and two patients had demised.

Conclusion:

Nephrocalcinosis was present in all patients, all of whom had PH Type 1. The genotypic spectrum correlated with initial presentations involving the urinary tract. The median estimated glomerular filtration rate (eGFR) was 4.2 ml/min/1.72m2 describing ESKD, however only five presented clinically in ESKD. This emphasizes the importance of screening, education, and earlier detection in our population.

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LIST OF ABBREVIATIONS

PHs	Primary hyperoxaluria
ESKD	End-stage kidney disease
CLKT	Combined liver and kidney transplant
PH1	Primary hyperoxaluria type 1
PH2	Primary hyperoxaluria type 2
PH3	Primary hyperoxaluria type
AGT	Alanine: glyoxylate aminotransferase
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
KRT	Kidney replacement therapy
REDCap	Research Electronic Data Capture
eGFR	Estimated glomerular filtration rate
UTI	Urinary tract infections
HFA	Height-for-age
CKD	Chronic kidney disease
CKD-MBD	Chronic kidney disease-mineral and bone disorder
EF	Ejection fraction
FTT	Failure to thrive

INTRODUCTION

The primary hyperoxalurias (PHs) are a group of autosomal recessive disorders that involve the overproduction of oxalate, a dicarboxylic acid excreted almost entirely by the kidney. (1) Accumulation of oxalate leads to the formation of oxalate crystals in the renal tubules with resultant tubular toxicity and subsequent interstitial inflammation and fibrosis. (1) Three clinical subtypes of primary hyperoxaluria have been described: primary hyperoxaluria type 1 (PH1), primary hyperoxaluria type 2 (PH2) and primary hyperoxaluria type 3 (PH3). (1,2)

The clinical presentation of PH varies from asymptomatic patients detected on screening, recurrent urinary tract infection due to renal calculi or presentation with end-stage kidney disease (ESKD). (3) Phenotypic diversity accounts for diagnostic delays and, depending on the population group, ESKD is the commonest presentation in 20-59% of cases. (4) Systemic oxalate deposition can be identified in the walls of blood vessels, bones, joints, retina, skin, bone marrow, heart, and the central nervous system. (5) The clinical manifestations of PH1 are not inherently unique to the disease and thus contribute to diagnostic challenges. (6)

The commonest and most severe form is PH1 caused by deficiency or loss of activity of liver-specific peroxisomal alanine: glyoxylate aminotransferase (AGT) involved in the final step of glyoxylate metabolism. (7) AGT catalyses the conversion of glyoxylate to glycine, and in its absence, glyoxylate is converted to oxalate, forming insoluble calcium salts. (6) There are few reports on African patients with PH1: Chang et al described a series of 20 patients in Soweto, South Africa, six of whom had diagnoses confirmed with genetic mutations. (9,8)

PH2 has a less severe clinical course and accounts for 10% of patients diagnosed with PH, however, this proportion may indeed be higher as many patients may be undiagnosed due to the milder clinical presentation. (7) PH3 often presents with recurrent urolithiasis during the first decade of life, has less severe progression than PH1 and may become clinically silent later in life. (1) Although the hyperoxaluria with or without hypercalciuria does not disappear, nephrocalcinosis and ESKD are uncommon and systemic involvement has not been documented in these patients. (1,7)

Few patients with known genetic mutations have been described in Africa or South Africa. (9)

The aim of this study was to document the genetic mutations found in patients with PH at CMJAH and to describe the associated phenotype for these patients.

METHODS

Study design and population

This is a descriptive retrospective review of medical records of patients diagnosed with PH. We reviewed all patients diagnosed with PH, including active patients in the paediatric renal clinic at CMJAH, from 1 January 2000 to 30 June 2020.

Methodology

Children under 16 years diagnosed with PH were included in the study. The diagnosis was made based on clinical and radiological criteria in combination with a high urine oxalate, either on spot analysis or 24-hour collection of urine. This was confirmed where possible with genetic analysis or with evidence of oxalate crystal deposition on kidney or bone marrow biopsy. Genetic testing for PH is done by the National Health Laboratory Services where they perform gene sequencing for the detection of the common p.A112D mutation but also offer full gene sequencing if the common mutation is not found. (17) These samples are sent to a laboratory in Cape town. (17)

Current management protocols in high income countries recommend that patients with ESKD receive intensive daily haemodialysis to clear the oxalate. (1) In Johannesburg, patients are offered intermittent haemodialysis three times per week alternating with home peritoneal dialysis, with the aim of reducing serum oxalate levels prior to a staged combined liver and kidney transplant (CLKT). At the time of this study, the standard operating procedure was that patients would first receive a liver transplant whilst maintaining their intensive dialysis regime, further reducing serum oxalate levels to minimise the risk of progressive systemic accumulation. This was then followed by a kidney transplant at least six months later when the risk of renal parenchyma oxalate deposition is mitigated. Demographics, genetic mutational analysis, laboratory values, clinical presentations, and outcome, were entered into a Research Electronic Data Capture Database (REDCap™) and then further analysed. Patients whose files were substantially incomplete were excluded. Patients who did not return for treatment despite efforts to contact them were considered to have abandoned therapy.

Data analysis

The data were analysed using simple descriptive statistics. Categorical data were presented using percentages and the continuous data as medians with ranges.

Ethical consideration

Approval for the study was obtained from the Human Research Ethics Committee of the University of the Witwatersrand (ref. no. M201175). Signed permission from the Clinical Manager and Hospital Chief Executive Officer were obtained to allow the study to be performed at Charlotte Maxeke Johannesburg Academic Hospital.

RESULTS

During the 20-year study period, 808 new patients were seen in the paediatric renal clinic. Of these, 16 were diagnosed with PH and met criteria to be included in this study.

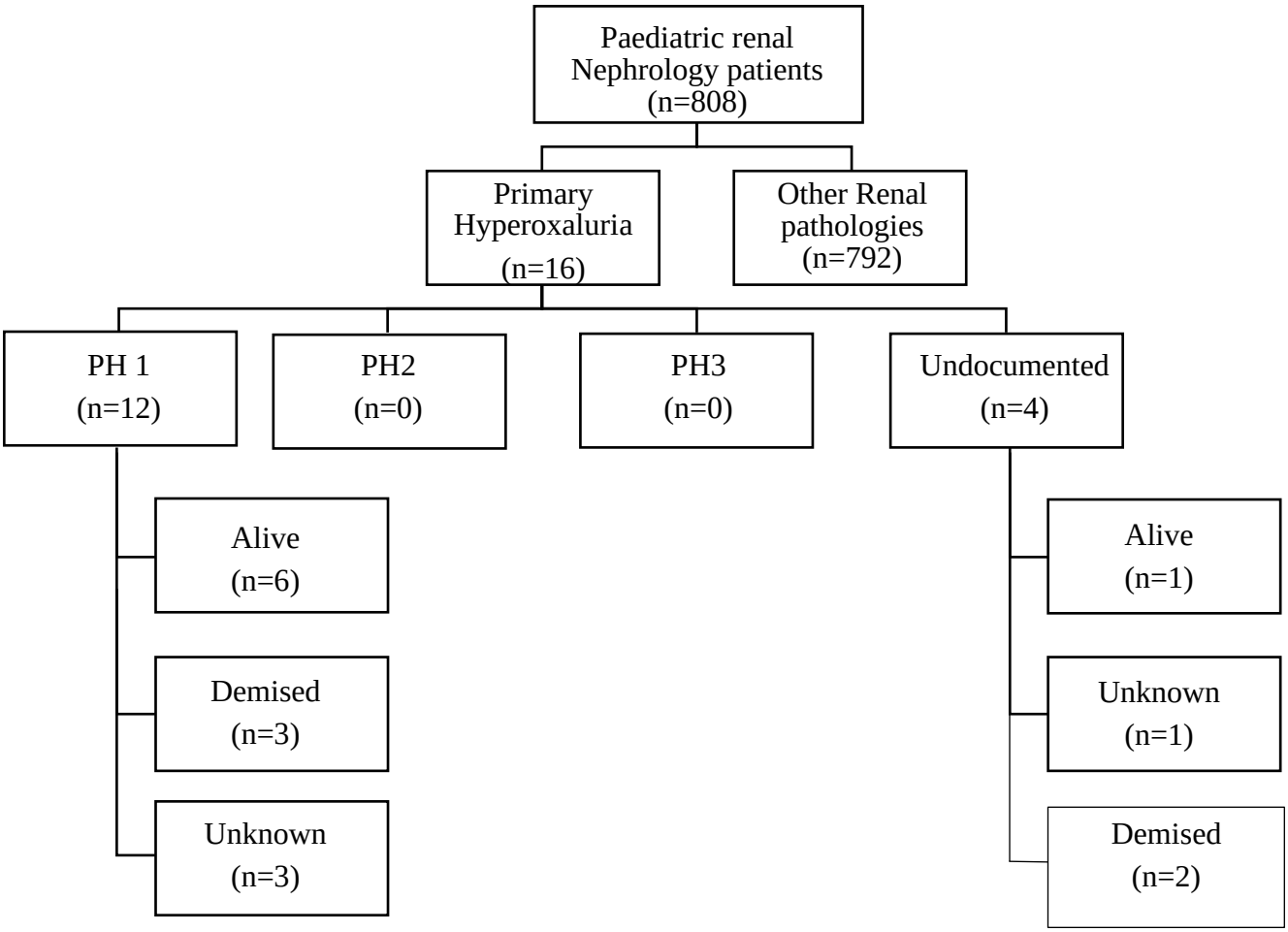

Figure 1: Flow diagram of patient recruitment

Table 1: Socio-demographic, genetic and clinical diagnoses of study participants

Category	**Number of patients**	
Sex		**Percentages**
Male	7	43.8%
Female	9	56.3%
Genetic mutation		
Homozygous	8	66.6%
Heterozygous	4	33.3%
c.198C>G(p.Tyr66*)	1	
c.335C>A(p.Ala112Asp)	3	
c.198C>G (p.Tyr66Ter)	2	
c.335C>A (p.A112D)	1	
c.473C>T (p.S158L)	1	
Primary Hyperoxaluria type		
1	12	75.0%
2	0	0.0%
3	0	0.0%
Unknown	4	25.0%
		Range
Median age at diagnosis (years)	7.1	2.7-13.1
Median duration from diagnosis to transplant listing (months)	4.4	1.7-14.4
Median age at transplant (years)	10	5.2-14.2
Median eGFR at diagnosis (ml/min/1.73m2)	4.2	2.7-132.8
Median creatinine at diagnosis (umol/L)	1188.0	32-2021
Types of Presentation		**Percentages**
Nephrolithiasis	6	37.5%
End stage kidney disease	5	31.25%
Urinary tract infection	6	37.5%
Haematuria	2	12.5%

Presentation:

The median age at diagnosis was 7.1 years (range 2.7-13.1). Nine of the 16 patients were female and seven were male. One patient came from Lesotho for treatment, and the remainder were from South Africa (10 from Gauteng, two from the North-West province and two from Mpumalanga). The clinical presentation included nephrolithiasis in six (37,5%) and UTI in six (37,5%), while five patients (31.25%) presented in ESKD at the time of diagnosis. Twelve patients presented with Stage 5 chronic kidney (CKD) with an eGFR <15mL/min/1.73m2. Of these, only five (31.25%) had clinical features of ESKD, namely oligoanuria and hypertension. The rest presented with nephrolithiasis in six (37.5%) and UTI in six (37.5%).

The median eGFR was 4.20 mL/min/1.73 m2 (range 2.7-132.8) with a median creatinine of 1880 µmol/L (range 32-2021) at the time of their diagnosis. Of those that did not present in ESKD, the median eGFR was 57.4 ml/min/1.73m2 compared to an eGFR of 4.0 ml/min/1.73m2 for the 12 who presented in ESKD.

A normal height with height-for-age (HFA) was documented in 13 patients (81.3%) and the remaining patients were moderately stunted. None of the patients were severely stunted.

Anaemia was the most common complication of CKD on presentation (14, 93.3%) (Table 2) and the median haemoglobin of the group was 8.6g/dl (range 5.8-14.0)

Table 2: Complication of Chronic Kidney Disease in the PH Study Group

Complication	**Number of patients**	**%**
Anaemia	14	93%
Metabolic acidosis	10	66%
Electrolyte abnormalities	9	60%
Hypertension	7	46%%
Chronic kidney disease-mineral and bone disorder	3	20%
Malnutrition	3	20%

Diagnosis:

Patients were referred from multiple hospitals, with differing diagnostic approaches. The majority (n=11) were screened with spot urine oxalate: creatinine ratios and four patients underwent a 24-hour oxalate collection and quantification.

The diagnosis was confirmed by genetic testing in 12 patients, while the diagnosis was based on clinical presentation and radiological findings in the remaining four patients. The patients who did not have genetic mutational analysis performed were either too ill at presentation and died prior to a definitive diagnosis being made or genetic analysis was not available in the public sector at the time of diagnosis.

The median urine oxalate: creatinine ratio was 196 µmol/mmol (range 53-424). Normal values for spot urine oxalate: creatinine ratios vary with age: 11-120 µmol/mmol from 1-5 years, 60-150 µmol/mmol from 5-12 years and 2-80 µmol/mmol for age above 12 years. (18)

Two patients were suspected to have bone marrow involvement based on refractory anaemia and had bone marrow aspirate and trephine biopsies performed, with one patient's sample demonstrating oxalate deposition.

Of the 14 patients who had echocardiograms, 13 had normal results while one patient had an ejection fraction (EF) less than 40%, possibly due to oxalate deposition in the myocardium. Nephrocalcinosis was detected in 15 patients on abdominal X-ray, while one patient had a normal X-ray. Bilateral nephrocalcinosis was demonstrated in all 16 patients using abdominal ultrasound, seven of whom had atrophic kidneys. Four patients had ophthalmologic examination performed as part of routine screening, but no abnormalities were detected.

Genetics:

Twelve patients were diagnosed with PH Type 1, eight had a homozygous mutation of c.335C>A (p.A112D), while four had heterozygous mutations as shown in Table 3. Two patients had the same heterozygous mutation but presented differently: one in Stage 1 CKD and the second in Stage 5 CKD. Three patients presented in extremis and did not have genetic testing performed while the fourth patient abandoned therapy before testing could be performed.

Table 3: Heterogeneity of clinical presentation and genotype and distribution.

	Homozygous mutation	**N=8**	**%**	**Heterozygous mutation**	**N=4**	**%**	**Unknown**
Mutation	c.335C>A(p.A112D)	8	100	c.198C>G(p.Tyr66*) c.335C>A(p.Ala112Asp) c.198C>G(p.Tyr66Ter) c.335C>A(p.A112D) c.473C>T(p.S158L)	1 3 2 1 1	12.5 37 25 12.5 12.5	
Median age	7.5			5.7			
Stage 5 CKD	7		87.5	2		50	

Outcomes:

Thirteen patients (81%) received Kidney replacement therapy (KRT) with the median age of initiation of KRT being 7,1 years. Three patients did not receive KRT (18,8%): two of these patients had heterozygous mutations and the third did not have genetic testing done. Two of the three patients presented in Stage 1 CKD and the third with Stage 2 CKD. Two of these patients abandoned therapy and the third still has a preserved renal function.

Of the patients included in this study, five (31.2%) had demised at the time of study completion, four patients (25%) had abandoned therapy (25%) and seven (43.7%) were still alive. Five patients who demised presented with Stage 5 CKD (100%).

Eight patients were listed for transplant and six (40%) underwent a CLKT. The majority of the patients received dialysis for 6-24 months prior to transplantation with the most common mode of dialysis being combined peritoneal and haemodialysis (n=8, 61.5%). Median age at transplantation was 10 years (range 6.7-13.7). Two patients were not transplanted, one died while awaiting transplant and the other was still listed for transplant at the time of this report.

Among the six patients transplanted, four were still alive at the time of this report and two patients demised, one from salmonella septicaemia and the other from ESKD secondary to kidney allograft failure. Two of the transplanted patients have chronic kidney rejection and one had acute rejection post-transplant. The median age of death was 13,3 years.

DISCUSSION

KRT and transplantation are scarce sources in Southern Africa and place a burden on clinicians who have access to these lifesaving interventions to assist and treat a bigger population. We face many challenges in the prevention and early detection of diseases causing ESKD in South-Africa. Further education and statistics around these rare diseases, such as PH, are imperative. Patients are referred to specialist facilities such as like CMJAH from all over the country requiring immediate interventions. The majority of these patients present with advanced disease, in urgent need of KRT. Reasons for this include lack of access to specialised facilities, and poverty. This cohort of patients, in whom we have attempted to correlate genetic and phenotypic characteristics, is currently the largest reported case series from Africa. PH is a rare inborn error of metabolism of which three subtypes have been described at a molecular level. (3) From European and North American surveys, we have seen its clinical importance as it accounts for 2% of patients starting KRT before the age of 15. (3) The aim of this study was to provide data around the clinical presentation, genotypic and phenotypic features, and the outcomes of these patients in South-Africa.

The median age of presentation was 7.1 years, comparable to a large European study that showed the median age of diagnosis to be 8 years. (10) Data from a single centre study done in Iran that had 18 patients diagnosed with PH, saw that the mean age of diagnosis was 4.4 years, lower than the patients in our cohort. (5) This contrast in median age is likely due to the increasing number of infantile cases diagnosed internationally over the past few years, with up to half being diagnosed in the infantile period. In our population, the higher median age of presentation is likely to be related to delayed diagnosis of the condition due to either paucity of diagnostic tools available, or due to a low index of suspicion for this diagnosis amongst public health care providers.

The initial symptoms varied, with the majority presenting with nephrocalcinosis and UTI. This is similar to other studies in America, Netherlands and Iran showing urolithiasis and nephrocalcinosis to be the first manifestation of the disease. (4) (3,10) In our cohort 93,7% of the patients had abdominal X-rays done showing bilateral nephrocalcinosis. The one patient in this cohort noted to have a normal abdominal x-ray, still had preserved renal function and a history of passing a stone. All the patients in this cohort had abdominal sonars done showing bilateral nephrocalcinosis. This highlights the importance of the utilisation of more than one imaging modality when there is clinical suspicion of this condition.

A large European study looked at clinical features seen on patients diagnosed with PH on family screening that also showed the majority to have urinary tract involvement, 31 % urolithiasis, 12% nephrocalcinosis and 24 % for both nephrocalcinosis and urolithiasis. A recent study published in 2022 in South-Africa that looked at the patients with PH at Chris Hani Baragwanath Academic Hospital also reported that most patients presented with urolithiasis, UTIs and nephrocalcinosis. (9) This emphasises the importance of further investigations for any child that presents with a history of passage of renal stones or recurrent UTIs. (11)

In our study, 75% of the patients presented with an eGFR of < 15 mL/min/1,73m2, with the median eGFR of 4.2 mL/min/1.73 m^2 (Range 2.7-132.8) at the time of diagnosis. This contrasts with similar data from America and Europe which reported much higher mean eGFR at the time of presentation than ours did. An American study published in 2005 reported a mean eGFR of 99 mL/min/1.73m2 at the time of presentation, while data from the Rare Kidney Stone Consortium in 2015 reported a median eGFR of 73 mL/min/1.73m^2 at the time of presentation (14,15). Data from Europe showed that only between 23% and 30% of their patients with PH presented with well-established ESKD (10). The Iranian cohort of 18 patients described 33% with clinical presentations of ESKD at diagnosis and only five with bilateral nephrocalcinosis. (5). Our findings of extremely low eGFR at presentation could be related to delays in diagnosis and referral, partially attributed to the poor socio-economic status of most patients in South-Africa, with restricted access to quality healthcare.

Nephrocalcinosis and recurrent urolithiasis cause progressive inflammation of the renal parenchyma leading to renal impairment. Renal excretion of oxalate falls once the eGFR falls below 30-50mL/min per 1.73m2 leading to lead to increased plasma oxalate with resultant systemic deposition. (16) The skeletal system acts as the biggest reservoir for calcium oxalate, manifesting as pain and growth retardation. (12) Patients in our cohort were not markedly stunted. This finding seemed unusual but the current studies available do not mention specific growth parameters found, only failure to thrive (FTT), and a study in the Netherlands showed only 11% to have FTT compared to 44% in an Iran cohort (5,13).

Anaemia was the most common complication of CKD on presentation, followed by metabolic acidosis and electrolyte abnormalities. The mean haemoglobin was 8.6g/dl; most likely of multifactorial aetiology due to ESKD, compounded by ongoing oxalate deposition

in the marrow with resultant refractory anaemia. (3) The most common electrolyte abnormality found was hyperkalaemia while nearly half the patients also had hypertension.

Genetic results were available for 12 patients although the initial diagnosis of PH was based on spot urine oxalate: creatinine ratios (n=11) with a median of 196 µmol/mmol. It is suggested that in all suspected patients a fresh urine sample should be collected and sent for spot urine oxalate: creatinine ratios. If these samples results are borderline or elevated, a 24-hour urine for oxalate should be performed as there can be variability of oxalate excretion from day to day. (3) Fourteen patients had echocardiograms performed, and only one patient had a low EF that was likely attributed to cardiomyopathy from systemic oxalate deposition.

The twelve genetic tests done all tested positive for PH1, 66,6% with the homozygous mutation for c.335C>A (p.A112D), 100%. PH2 AND PH3 have a less severe clinical course, and these patients may remain undiagnosed or may not be referred to specialist clinics.

Of the four patients with heterozygous mutations, two had the same heterozygous mutation but had very different clinical presentations, one presented with anaemia and Stage 5 CKD while the other had both a normal haemoglobin and eGFR. This emphasises the clinical heterogeneity that exists in PH. (6).

Thirteen patients received KRT (81.3%) and, of these, eight were listed for transplant and six patients underwent a CLKT at a median age 10 years. The clinical heterogeneity of this disease is further emphasised where half the patients with heterozygous mutations required RRT while the remaining half had preserved renal function. European and North American studies have suggested a better survival with CLKT as the only way to replace both the causative organ (liver) and the most biochemically affected organ, (the kidney). (10,14) The majority of patients required intensive KRT with peritoneal and haemodialysis. Conventional haemodialysis is inadequate as it is unable to overcome the continuing oxalate production, leading to ongoing systemic deposition of oxalate with higher morbidity and mortality. (14) Four of the six transplanted patients were still alive at the time of this report. The crude mortality rate of this cohort was 31.2%, but could possibly be higher as four patients abandoned therapy.

A large European registry reported that patients with PH were younger at the initiation of RRT compared to non-PH patients and noted that patients with PH had a three-fold higher risk of death at five years compared to non-PH patients. (10)

Nephrocalcinosis was detected on all patients in this cohort. Clinicians should thus suspect PH in a patient found to have nephrocalcinosis on ultrasound or X ray. In family members of patients known to have PH, screening should be diligently performed.

The limitations of this study are attributed to the small sample size and the retrospective nature. The differences in clinical presentation also means that some patients are not referred as they are too well and others present in extremis, which will also contribute to lost data. Nevertheless, this is a relatively large case series of patients with an extremely rare condition and adds valuable insights into the disease in an African population. Nation-wide or even regional collaborative studies would add more insights into genetic mutations associated with PH in this population.

CONCLUSION

Patients with PH experience multiple severe morbidities and have a higher risk of mortality than many other patients with renal pathologies. The majority of patients in this small series had homozygous mutations, and all presented with nephrocalcinosis. Patients with PH Type 1 with homozygous mutations appeared to present at an earlier age than those with heterozygous mutations, but small numbers preclude firm conclusions. The high rate of treatment abandonment warrants further investigation.

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APPENDIX A: ETHICS CLEARANCE CERTIFICATE

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R49 Dr E Erasmus

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M201175

NAME: (Principal Investigator)	Dr E Erasmus
DEPARTMENT:	School of Clinical Medicine Depatment of Paediatrics and Child Health Medical School University
PROJECT TITLE:	*Clinical presentations, genotypic spectrum and outcomes of primary hyperoxaluria at Charlotte Maxeke Johannesburg Academic Hospital* ***Change of study title noted on 2021/03/29***
DATE CONSIDERED:	2020/11/27
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr S Irusen and Professor J Geel
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2020/12/10

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

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 submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **November** and therefore reports and re-certification will be due in the month of **November** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator Date

APPENDIX B: TURN-IT-IN REPORT

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APPENDIX C: PLAGIARISM DECLARATION CERTIFICATE

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Emilie Erasmus (Student number: 2377249) am a student registered for the degree of Masters of Medicine in the academic year 2023.

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.
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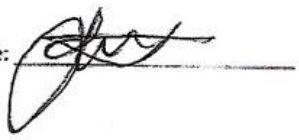
Signature:  Date: 26/6/2023

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