A RETROSPECTIVE STUDY OF THE BIOCHEMICAL AND RADIOLOGICAL PROFILE OF CHILDREN WITH GENETIC HYPOPHOSPHATEMIC RICKETS AND THEIR RESPONSE TO CONVENTIONAL TREATMENT

Candidate: Dr Nikhila Isaac

Student number: 301379

Department of Paediatrics, Faculty of Health Sciences, University of the Witwatersrand

Email: nikhila12isaac@gmail.com

Cell: 0848395740

Supervisor:

Adjunct Professor Kebashni Thandrayen

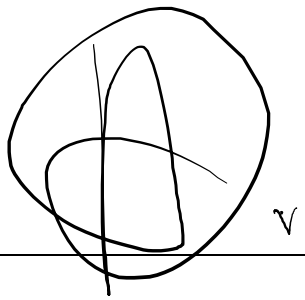
Department of Paediatrics, C.H Baragwanath Academic Hospital,

Faculty of Health Sciences, University of the Witwatersrand

DECLARATION

I, Nikhila Isaac, declare the following

- I confirm that the work of this Research report is my own unaided work. It is being submitted for the degree of Masters of Medicine in Paediatrics at the University of the Witwatersrand, Johannesburg.
- It has not been submitted before for any degree or examination at any other University.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- 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



Nikhila Isaac

Date: 22 November 2023

DEDICATIONS

I dedicate this MMed to:

-My parents, for always believing in my potential. Your boundless love and selflessness paved the way to all my achievements.

-My husband Naveen; for your endless support and unwavering encouragement that fuels my aspirations.

-My daughter Ameliah; may this accomplishment inspire your dreams and fill your heart with pride.

-Prof Thandrayen: Your mentorship, guidance and expertise in this journey have been

invaluable.

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LIST OF ABBREVIATIONS:

ALP: Alkaline Phosphatase

ANOVA: Analysis of Covariance

CHBAH: Chris Hani Baragwanath Academic Hospital

FGF23 : Fibroblast Growth Factor 23

HAZ: Height for Age z score

IQR: Interquartile Range

JEMDSA: Journal of Endocrine Metabolism and Diabetes of South Africa

MMed: Masters in Medicine

PHEX: Phosphate regulating endopeptidase homologous on the X chromosome

PTH: Parathyroid Hormone

SD: Standard Deviation

WAZ: Weight for Age z Score

XLH: X- Linked Hypophosphatemic rickets

$1.25(OH)_2D$: 1.25 Dihydroxyvitamin D

25(OH)D: 25 Hydroxyvitamin D

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AUTHORS GUIDELINES FOR INTENDED JOURNAL

This MMed is to be submitted for examination in a 'submissible" format. The manuscript is to be submitted to the Journal of Endocrine, Metabolism and Diabetes of South Africa (JEMDSA)

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MANUSCRIPT

ABSTRACT

Objectives

Assessing the biochemical and radiological profile of children with genetic hypophosphatemic rickets and their response to conventional treatment.

Design

Retrospective descriptive study.

Setting

Metabolic Bone clinic at Chris Hani Baragwanath Academic Hospital in Soweto, South Africa.

Subjects

Children less than 18 years, attending the Metabolic Bone clinic from 1st January 2006 to April 2020, with genetic hypophosphatemic rickets, commenced on conventional treatment.

Results

Seventy patients met the inclusion criteria. Majority of patients were black South African (n=54 (77%)). Positive family history seen in 32 (46%) patients. The patients were short statured with a mean height for age z score (HAZ) of - 3.4 ± 1.79. The mean calcium, phosphate, alkaline phosphatase, parathyroid hormone levels and median Thacher score was 2.3 ± 0.16 mmol/L, 0.84 ± 0.19 mmol/L, 776.6 ± 531 IU/L, 7.15 ± 4.8 pmol/L and 8 (4-8) respectively. Improvement on last follow up on treatment was seen in ALP (776 ±531 vs 525±232; p <0.001) and Thacher scores (8 (4-8) vs 2 (1-3.5); p =0.01) after 5 years, but no change in phosphate or HAZ.

Conclusion

Conventional therapy for treatment of hypophosphatemic rickets is not associated with an improvement in HAZ despite an improvement in radiology and ALP. Adherence is a major challenge for the majority of patients.

A RETROSPECTIVE STUDY OF THE BIOCHEMICAL AND RADIOLOGICAL PROFILE OF CHILDREN WITH GENETIC HYPOPHOSPHATEMIC RICKETS AND THEIR RESPONSE TO CONVENTIONAL TREATMENT

INTRODUCTION

Rickets is a bone disorder characterized by decreased mineralization of bone matrix at the growth plate, often resulting from disrupted calcium or phosphate homeostasis.
XLH is the most common inheritable form of rickets caused by a loss of function mutation in the phosphate regulating endopeptidase homologous on the X chromosome (PHEX) gene, leading to excess circulating fibroblast growth factor 23 (FGF23). This impairs phosphate reabsorption in the kidneys and reduces the synthesis of active vitamin D, resulting in renal phosphate wasting and hypophosphatemia. (1)

Phosphorus is crucial for cellular functioning and plays a vital role in bone formation. Phosphate deficiency can result from poor absorption in the gut or excessive loss through the kidneys. Phosphorus is absorbed in the gut and kidneys. Eighty to ninety percent of filtered PO_4 load is reabsorbed from the proximal tubules and the remainder is excreted in the urine. The main regulators of phosphate homeostasis are parathyroid hormone, FGF23, αKlotho, PHEX and 1.25 dihydroxyvitamin D ($1.25(OH)_2D$).
PTH mobilises phosphate from skeletal bones into the blood stream by enhancing osteoclastic bone resorption. (2) PTH decreases reabsorption of filtered phosphate along the proximal tubule.
FGF23 is a glycoprotein produced by osteoblasts and osteocytes and decreases renal phosphate reabsorption. FGF23 also decreases active vitamin D leading to decreased calcium and phosphate absorption in the gut and in the kidneys in humans. (3,4) PHEX is a protein that is expressed mostly in osteoblasts and osteoclasts of skeletal bones, and it regulates and restricts FGF23 expression. Inactivating mutations in PHEX increase circulating levels of FGF23 that result in phosphaturia, hypophosphatemia and suppresses the conversion of 25-hydroxyvitamin D (25(OH)D) to 1.25 dihydroxyvitamin D ($1.25(OH)_2D$) in the kidney. (5)
Although the main role of $1.25(OH)_2D$ is the absorption of calcium, it also causes phosphate absorption from the gut. Increased levels of $1.25(OH)_2D$ also suppresses the synthesis of PTH and enhances FGF23 production. (6)

Diagnosis of hypophosphatemic rickets (mainly XLH) involves three main settings:

1) Diagnosis of familial cases: About 85-90% of familial cases follow an X-linked dominant inheritance pattern. Affected fathers transmit the disease to all their daughters and none of their sons. Affected mothers have a 50% risk of having an affected daughter or son. (Babies born to affected parents should undergo biochemical screening, including measuring serum phosphate and alkaline phosphatase (ALP) levels. In suspected cases, the serum phosphate level is typically below the normal range for age, while ALP may be at the upper level of normal. (1)

2) Diagnosis of de novo cases of XLH: Children with XLH due to PHEX mutations without a family history but with clinical, radiological and biochemical suspicion should be evaluated. Clinical features include leg bowing, widening of the metaphyses in ankles and wrists, and dental issues. (6) Radiological signs include long bone deformities, abnormal growth plates, and dense appearing bone cortices. (7) Biochemically, serum phosphate levels are below normal for age, while ALP levels are above the upper limit of normal. PTH levels are usually in the normal or upper normal range and serum calcium is normal. Other disorders causing phosphate wasting and vitamin D or dietary calcium deficiencies must be ruled out. (8)

3) Genetic confirmation of XLH: The definitive confirmation of XLH is achieved through genetic analysis, which identifies mutations in the PHEX gene associated with the condition.

Treatment should be initiated as soon as the diagnosis is made to prevent rachitic changes, leg bowing and short stature. Conventional therapy includes multiple daily doses of phosphate and vitamin D metabolites. (9) Since serum phosphate return to baseline concentrations within 1.5 hours, phosphate must be given as frequent doses of 4–6 times per day. Active vitamin D (calcitriol or alfacalcidol (1- hydroxycholecalciferol) is given in addition to oral phosphate supplements to counter-act calcitriol deficiency, prevent secondary hyperparathyroidism and increase phosphate absorption from the gut. (10)

In South Africa, there is little published data on hypophosphatemic rickets and XLH management and on the response to conventional treatment such as phosphate supplements and calcitriol or alfacalcidol. Burosumab is a fully human monoclonal IgG1 antibody that neutralizes FGF23. It has been approved for the treatment of XLH by The European Medicines Agency (EMA) in the European Union and by the US Food and Drug Administration (FDA) (9) It is not been approved for use in South Africa. Burosumab is an expensive drug, with an average annual drug cost of $129,780 to $1,168,196. (11) Burosumab is not available in South Africa thus conventional treatment is offered to

patients. We hypothesise that conventional therapy for the treatment of XLH rickets and the other genetic causes of hypophosphatemic rickets is not adequate, and burosumab should be made available to all patients with hypophosphatemic rickets in South Africa, if funding is possible as it is a very costly therapy.

METHODOLOGY

Ethical considerations

Protocol was approved by the Human Research Ethics Committee of the University of the Witwatersrand. Permission to access files and review them was obtained from the CEO of CHBAH. Then data was recorded using study numbers and the patients' details were kept anonymous and were only known to the researchers.

Aim of study

To assess the biochemical profile of genetic forms of hypophosphatemic rickets, including XLH at presentation and to monitor the biochemical and radiological response to conventional therapy.

Primary objectives

1. To assess the biochemical profile of genetic forms of hypophosphatemic rickets at baseline.
2. To evaluate the efficacy of conventional therapy in treating genetic hypophosphatemic rickets by assessing improvements in biochemical and serum bone markers (Calcium, Phosphorus, ALP and PTH) at 3, 6, 9 and 12 months and then every year until follow-up is completed or till the last follow-up visit.
3. To assess changes in the Thacher radiological score from baseline till 12 months after commencing treatment and till the last available radiological assessment.

Secondary objectives

1. To review medical records for documentation of compliance issues at 3, 6, 9 and 12 months and every year till follow-up is completed or till the last follow-up visit.
2. To assess improvement or changes in height for age z (HAZ) scores at 6, 12 and 24 months and yearly till follow-up is completed or till the last follow-up visit.
3. To assess family history in genetic hypophosphatemic rickets.

Study population and sample size

Children under the age of 18 years and attending the Metabolic Bone clinic at Chris Hani Baragwanath Academic Hospital from 1st January 2006 till 30th April 2020 and until follow up is completed or till the last follow-up visit.

Inclusion criteria

Children diagnosed with genetic hypophosphatemic rickets based on biochemical profile at baseline or prior to treating (normal calcium, normal PTH or not >2.5 X the upper normal limit) and low phosphate levels (below the normal reference limits for age), with or without a positive family history of hypophosphatemic rickets and commenced on conventional treatment of phosphate and alfacalcidol.

Exclusion criteria

1. Calciopaenic rickets
2. Hypophosphatemic rickets secondary to renal tubular acidosis (RTA). RTA is defined as a normal serum anion gap hyperchloremic metabolic acidosis and patients fulfilling these criteria will be excluded.
3. Hypophosphatemic rickets secondary to tumour-induced osteomalacia or fibrous dysplasia.
4. Patients already commenced on conventional therapy (> 3 months duration) at referral.
5. Hypocalcaemia or hypercalcaemia, defined as serum calcium levels outside the age-adjusted normal limits.
6. Evidence of hyperparathyroidism (PTH levels 2.5 X upper limit of normal)

Study design and methods

This was a retrospective descriptive study. The research involved reviewing patient files and entering data into a data collection sheet (appendix A) and analysing the data available. The biochemical profile at the first visit was compared to the profiles at 3, 6, 9, 12 months and longer till the last follow-up visit after starting treatment. The laboratory tests included for the study were serum phosphate, ALP, PTH and calcium. The Thacher score is a 10-point scoring system, where 10 is extreme degree and 0 is absence of rhacitary changes. The Thacher score was utilized to measure the severity of radiographic changes as well as to see radiographic response following treatment of rickets. (12) The Thacher severity score was

utilised by the researcher and confirmed by the supervisor at baseline and thereafter at 12 month intervals until the last available radiological assessment.

Statistical analysis

The statistical analyses was mainly descriptive. Data was captured into the data collection sheet (Appendix A) and then entered into an excel spreadsheet and analysed using Statistica (Statsoft USA, version 13.5). Categorical variables are reported as numbers and percentages. Continuous variables are reported as means and standard deviations or medians and interquartile ranges where applicable. ANOVA (analyses of covariance) was performed on the continuous data such as biochemical markers, radiological scores or HAZ scores at each of the different time points or follow-ups to assess for any improvements over these time points. The student- t test or Mann-Whitney U test was applied to assess changes in biochemical markers, radiological scores or HAZ scores from baseline to the 12 month follow-up and thereafter.

RESULTS

There were 70 patients that met the inclusion criteria.

Demographics

The majority of patients were black South African (n=54 (77%)). The male to female ratio was 1:1.7. The mean (SD) age of the patients was 59.1 (±44.6) months and there was a positive family history in 32 (46%) patients.

Anthropometry

The patients were short statured with HAZ of - 3.4 ± 1.79. The patients' weights were normal with a mean weight for age z score (WAZ) of -1.7 ± 1.7.

Clinical characteristics at presentation

For the majority of patients, the reason for referral was rickets. Majority of patients presented with genu varus deformities of lower limbs (n=57 (81%)), followed by widened wrists (n=51 (73%)), frontal bossing (n=44 (63%)) and rachitic rosary (n=42 (60%)). The rest of the clinical characteristics are summarized in Table 1.

Table 1: Clinical characteristics at presentation

Clinical features	**n**	**%**
Genu Varus	57	81%
Widened wrists	51	73%
Frontal bossing	44	63%
Rachitic rosary	42	60%
Genu Valgus	19	27%
Harrisons sulcus	16	23%
Dental caries	13	18.6%
Delayed motor milestones	10	14.3%
Wind swept deformity	8	11%
Delayed anterior fontanel closure	5	7.1%
Fracture on Xray	5	7.1%
Craniotabes	2	2.9%
Dental abscess	2	2.9%
Lordosis	2	2.9%
Scoliosis	1	1.43%
Kyphoscoliosis	0	0
Stridor	0	0
Delayed dentition	0	0

Biochemistry and radiological findings

The mean serum calcium at presentation was 2.3 ± 0.16 mmol/L, phosphate was 0.84 ± 0.19 mmol/L, ALP was 776.6 ± 531 IU/L and PTH level was 7.15 ± 4.8 pmol/L and the median Thacher score at baseline was 8 (4-8).

Follow-up progress

The patients were followed up from January 2006 till their last follow up visit. The biochemical profile and height were assessed at 3, 6, 12 months and then yearly. The Thacher

score was assessed yearly after being on treatment. There was improvement in Thacher scores (8 (4-8) vs 2 (1-3.5); p = 0.01) (Fig 1) from baseline to 5 years post baseline and ALP (Fig 2) 776 ± 531 IU at baseline vs 525 ± 232 IU at last follow up visit; p <0.001) There was no change in serum phosphate as baseline phosphate was 0.84± 0.19 mmol/L and at last follow up visit it was 0.91± 0.24 mmol/L The HAZ also did not improve from baseline mean -3.4(± 1.79) vs HAZ at last follow up visit -3.6(± -1.59) Nearly half of the patients (n=33(47%)) were noted to have poor adherence to treatment.

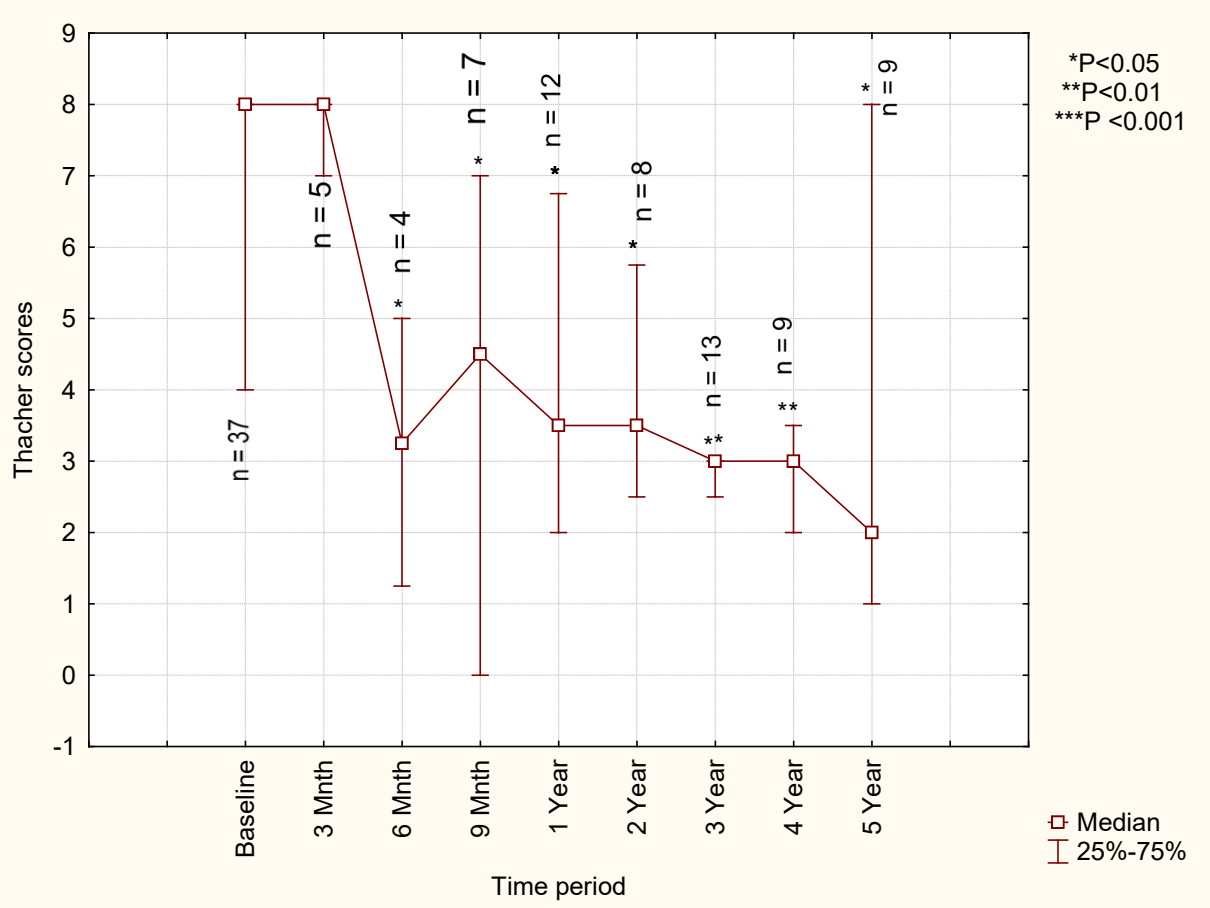

Fig 1: Thacher from baseline till 5 year follow up

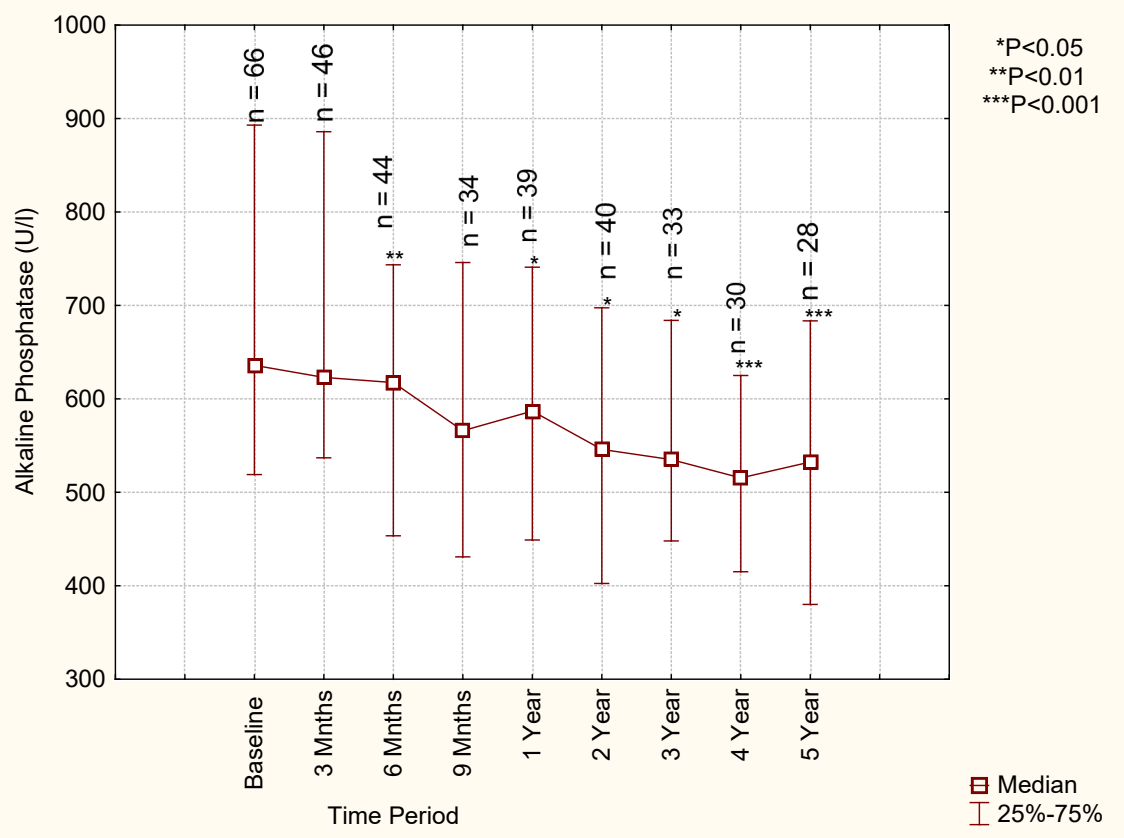

Fig 2. ALP from baseline till 5 year follow-up

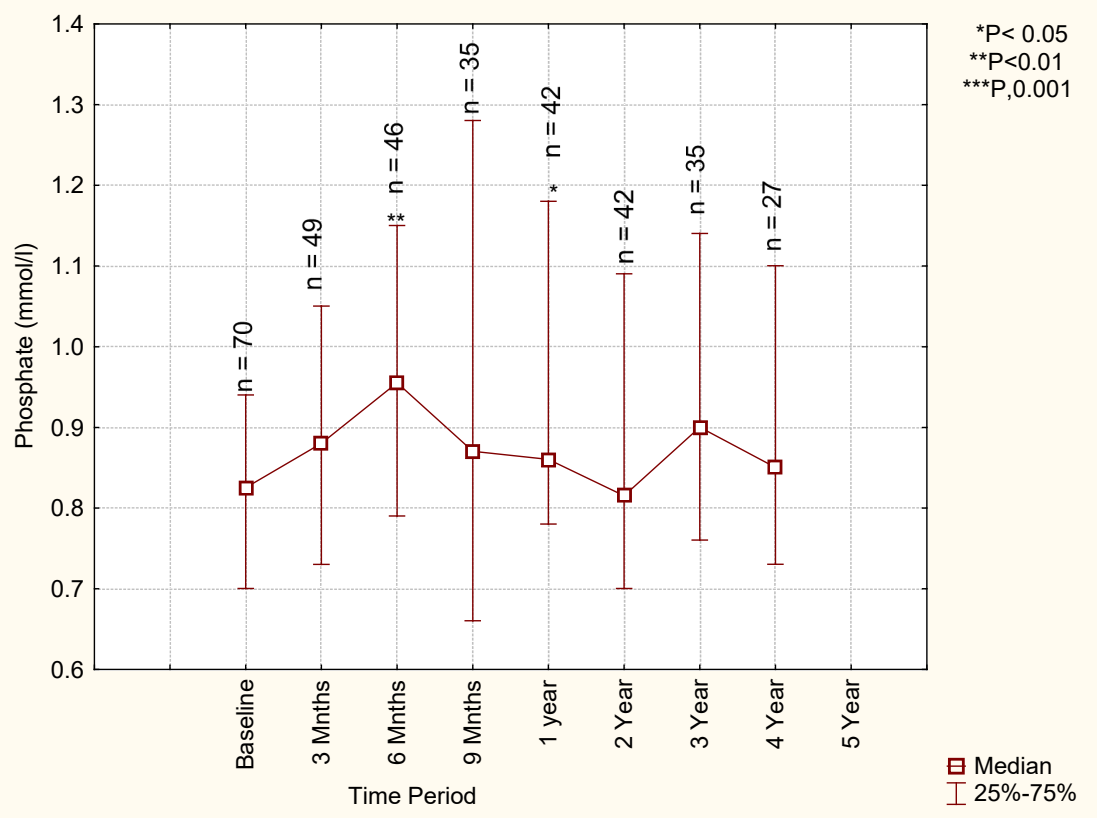

Fig 3. Phosphate from baseline till 5 year follow up

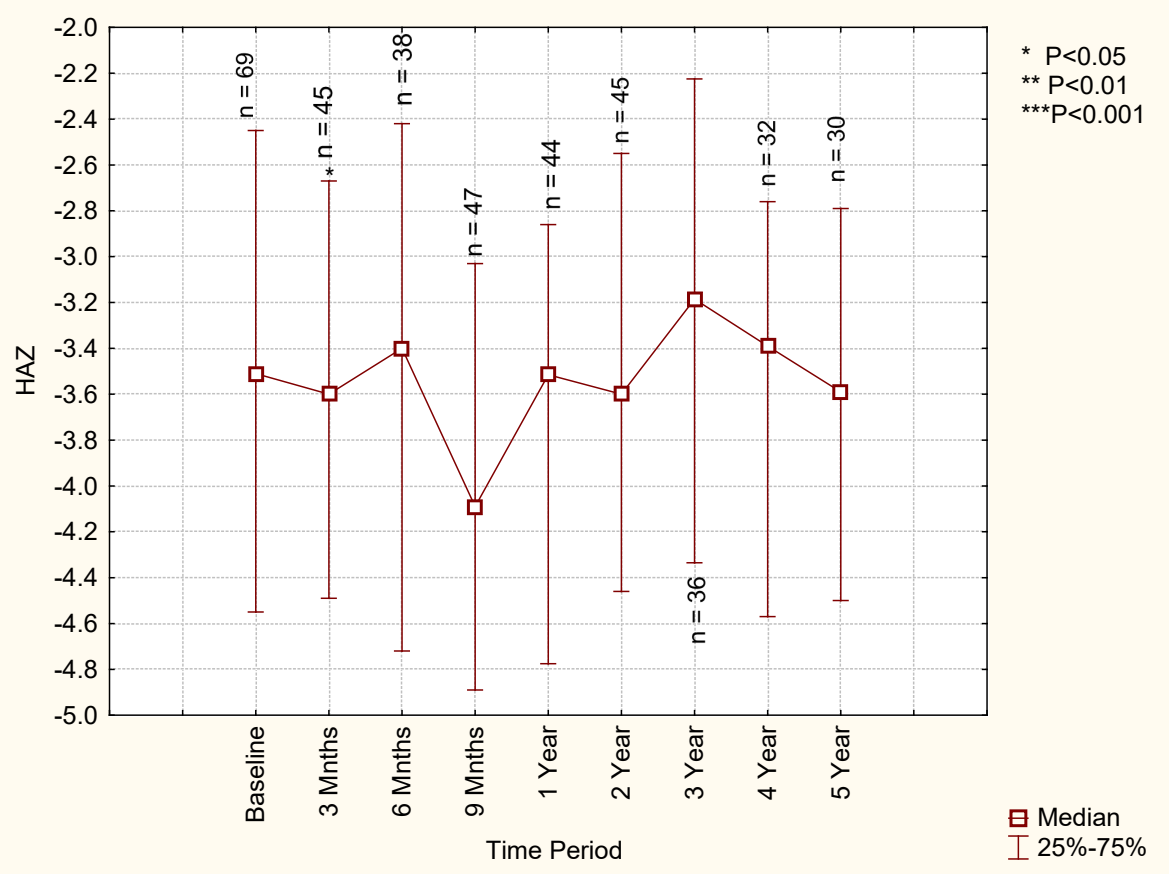

Fig 4. HAZ from baseline till 5 year follow up

DISCUSSION-

XLH is the most common form of inherited rickets and therapy should be aimed at counteracting consequences of FGF23 excess. Females were seen to be affected more than males as is what would be expected in X linked dominant conditions. (13) There was a positive family history in only 32 (46%) patients which means that the sporadic cases were more common than the hereditary type. Sporadic cases tend to be more prevalent in the black population in South Africa as previously reported by Basu et al. (13) The current treatment available in South Africa in the public setting is conventional treatment which comprises of oral phosphate supplementation with multiple daily dosing to compensate for renal phosphate wasting and active vitamin D analogues to counter the $1.25(OH)_2D$ deficiency. The initial end point for treatment showing healing of rickets is normalization of ALP and radiological symptoms. (14) Our study shows that although conventional treatment improves some aspects of rickets, the renal wasting of phosphate and final height does not improve.

Burosumab is a monoclonal antibody that targets FGF23 in patients with XLH. It is shown to be more effective in treating rickets and improving hypophosphatemia and height in patients with XLH. (10)

It has been shown that earlier treatment with conventional therapy yielded better results. (14) Active rickets present as bone deformities and disharmonic growth retardation which are major manifestations in the paediatric age. Dental abscesses and mineralization defects in teeth are also common findings. (6) The majority of our patients presented with bone deformities and short stature, with only a few (2.9%) presenting with dental issues.
The HAZ for all the patients was low on presentation even though the WAZ was normal thus indicating that nutrition is not a cause of the short stature. The HAZ at presentation was -3.4 ±1.79 as compared to the height at last follow up on treatment -3.74 ±1.59 showing that conventional treatment did not improve height and the patients remained short for their age. A study on the use of Burosumab, has shown that after a year on treatment, the height of patients increased from −3.56 to −0.46 SD (10); suggesting that Burosumab is a better modality of treatment in improving final height as compared to conventional treatment.

At presentation serum phosphate levels were below the normal threshold for age 0.84 ± 0.19 mmol/L, which is expected with renal phosphate wasting. On the last follow up on conventional treatment, the phosphate levels remained low (phosphate was 0.91± 0.24 mmol/L); showing that renal phosphate wasting continues on conventional treatment.
Serum level of ALP is a reliable marker of rickets activity. Bone specific ALP represents 80-90% of ALP in children and when rickets is undertreated the ALP remains high. In the last follow up after conventional treatment had been started, the ALP had improved (776 ±531 IU vs 525 ±232 IU) indicating that the rickets activity were improving on the conventional treatment.
PTH levels were in the upper normal range on presentation. Oral supplementation of phosphate alone can promote secondary hyperparathyroidism further causing renal wasting of phosphate and worsening hypophosphatemia. Active vitamin D analogue supplementation is thus important to help prevent this. All the patients in the study were supplemented with active vitamin D analogues. The PTH at presentation was 7.15 ± 4.8 pmol/L and at last visit was 6.61±3.81 pmol/L which shows that the patients were adequately supplemented with active vitamin D analogues and that secondary hyperparathyroidism is not a cause of the hypophosphatemia. In the study done on burosumab, the use of burosumab for one year lead

to an increase in serum phosphate levels (mg/dL) from 2.10 ± 0.39 to 3.33 ± 0.24 and ALP had also showed improvement from (IU) 628 ± 267 to 525 ± 419. (10)

Radiographic examinations revealed evidence of rickets in the patients, with X-rays showing long bone deformities, widened and frayed metaphysis, and increased bone density. In our study, two observers assessed the X-rays at presentation and yearly thereafter. The median Thacher score at baseline was 8 (4-8), while the Thacher score at 5 years improved to 2 (1-3.5) on conventional treatment.

Due to the multiple dosing of phosphate, compliance was seen to be poor (47%). In patients with good compliance (51%), the ALP improved from 858 ± 646 IU to 477 ± 211 IU ($p = 0.02$). In the poor compliance group, the ALP did not improve with a mean of 678 ± 333 IU in the beginning of treatment to 574 ± 248 IU (p value = 0.34), indicating that compliance played a role in improvement. However, phosphate did not improve in the good compliance group with a mean of 0.88 ± 0.20 mmol/L at the start and remained low at 0.93 ± 0.26 mmol/L ($p = 0.10$) on treatment. The height also did not improve on the compliant group as patients were still noted to be short with a mean HAZ of –3.32 ± -1.26 ($p= 0.67$) after being on treatment. It is also important to take into consideration that in the compliant group, because the patients claimed to be compliant it does not mean they necessarily were compliant. Urine phosphate measured as a 24-hr collection should parallel the daily intake of phosphate supplementation and would have been a good tool to confirm compliance. Burosumab however is known to be well tolerated and the two weekly dosing regimens make it more convenient to administer thus improving compliance. (10)

Due to the retrospective nature of the study, we were unable to investigate other reasons of poor compliance such a poor accessibility of hospital and financial constraints. Since CHBAH is a tertiary hospital with referrals from a wide area, it is possible that another reason for poor compliance would have been inaccessibility to attend hospital visits. Conventional treatment is also not always available in their base hospital which could have also contributed to the low compliance.

Burosumab is an expensive alternative and the ethical considerations that needs to be taken into consideration when prescribing this therapy would be the cost to benefit ratio. However, given that in the long term, patients with XLH usually require surgery and prolonged

admission (13) and this adds to the overall costs of treating the disease; it may be beneficial to advocate for the use of Burosumab in low=middle income countries that cannot afford this therapy to lessen the burdens of this chronic condition. Self-administration of Burosumab have been approved in some countries such as Japan, the European Union and the UK, this may also help prevent the financial burden on patients with decreasing the multiple hospital visits and hospital staff can be redirected to other areas of need. The extent of the physical disability and short stature that impacts these patients psychosocial well-being as well as their ability to secure a job in the long term or to do daily activities such as long distance walking or driving can be negated with the superior therapy of Burosumab and thus it is important to advocate for the use of Burosumab in resource constrained settings.

Limitations of study

1) Patients may claim compliance, but this may not be true.
2) Since there is no DNA testing performed in South Africa to confirm XLH or other genetic causes of hypophosphatemia, patients were chosen based on the biochemical profile, radiological findings; with or without a positive family history of hypophosphatemia and commenced on conventional treatment after diagnosis.
3) Physical ability (6-minute walk test) and patient-reported pain and functional disability could not be assessed due to the retrospective nature of the study

Further research that can be done

1) Improvement of subjective parameters such as pain and disability on conventional treatment
2) Studies exist as to the effect of Burosumab on XLH and hereditary hypophosphatemia however, there is little data as to whether it will help in other diseases associated with an excess in FGF23 such as tumour-induced osteomalacia.

CONCLUSION

The use of conventional therapy for the treatment of rickets shows an improvement of ALP indicating improvement in rachitic activity. It also shows an improvement in radiological changes evidenced by the improvement in Thacher severity score. However, it does not improve serum phosphate, nor does it improve the height of patients with genetic hypophosphatemic rickets. Adherence to therapy is also a major challenge for the majority of patients, with the multiple dosing of phosphate being one component contributing to poor

compliance. On the other hand, burosumab has shown to improve ALP, serum phosphate and height. Burosumab should therefore be made available in the public sector for the treatment of genetic hypophosphatemic rickets.

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APPENDIX

Appendix 1: Data collection sheet

Study no: ……

A. Demographics

Male O Female O

Age at diagnosis or at initial presentation to the Metabolic Bone clinic_____

Area of residence:

Gauteng O

North west O

Limpopo O

Mpumalanga O

Western Cape O

Eastern Cape O

North Cape O

KwaZulu Natal O

Northern Province O

Other country ……

Reason for referral: …….

Referred by:

1. Clinic…….
2. Peripheral hospital…….
3. Paediatrician…….
4. General Practioner (GP)…….
5. Other…….

C. Clinical Presentation at initial visit or consult

Weight …… kg

Height ……. Metres

Head circumference: ……cm

SIGNS & SYMPTOMS	YES	NO
Genu Varus		
Genu vulgus		
Wind-swept deformity		
Widened wrists		
Rachitic rosary		
Harrison's sulcus		
Frontal bossing		
Delayed anterior fontanel closure		
Craniotabes		
Dental caries		
Delayed dentition		
Dental abscesses		
Delayed motor milestones		
History of bone fracture		
Confirmed fracture on x-rays		
Scoliosis		
Kyphosis		
Kyphoscoliosis		
Stridor		
Family history of rickets: Whom is affected?		

Myopathy with normal deep tendon reflexes		
Previous corrective orthopaedic interventions		

Biochemical results, radiological scores and HAZ measurements (from baseline till 3, 6, 9 ,12 month and follow-ups yearly till the last visit.

Biochemical Marker	On referral	Follow-up 1 (3/12)	Follow-up 2 (6/12)	Follow-up 3 (9/12)	Follow-up 4 (1yr)	Follow-up 5 (2yrs)	Add more as needed	Follow-up ???? Till last visit
Date:								
Age (months)								
Calcium (ionised) mmol/L								
Total Calcium (corrected) mmol/L								
Phosphate mmol/L								
Alkaline phosphatase (IU)								
Parathyroid hormone (pmol/L)								
25(OH)D (nmol/L)								
1, 25-dihydroxy-vitamin D (calcitriol)								
Thacher Score		--------	--------					
Height for age z score (HAZ)		---------						
Weight for age z score (WAZ)								

Surgical interventions								
Lenolax (mg/kg/d)								
One alpha (µg/kg/d)								
Compliance Yes/No/ NR								

Treatment on referral: Yes O No: O

If yes, what treatment: 1) Medication ……..

Dose……..

2)Medication…….

Dose……..

1. Medication prescribed:

Drug: ……

Dosage: …….

2. Medication prescribed:

Drug: …….

Dosage: …….

Final diagnosis:

Sporadic Hypophosphatemic rickets O.

Suspected X-linked Hypophosphatemic rickets (with positive family history) O

Suspected autosomal dominant O

Suspected hereditary hypophosphatemic rickets with hypercalciuria O

Other causes O

APPENDIX 2 FACULTY APPROVAL OF PROTOCOL

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

01 July 2020

Dr N Isaac
Chris Hani Baragwanath Academic Hospital
Department of Paediatrics and Child Health

Sent by email to: nikhila12isaac@gmail.com

Dear Dr Isaac

Re: **Protocol Ref No:** M1911123
Protocol Title: A retrospective study of the biomedical and radiological profile of children with genetic hypophosphatemic rickets and their response to conventional treatment
Principal Investigator: Dr N Isaac
Protocol Amendments

This letter serves to confirm that the Chairman of the Human Research Ethics Committee (Medical) has approved the amendments for the abovementioned protocol, as detailed in your letter, dated 02 May 2020.

Thank you for keeping us informed and updated.

Yours Sincerely,

UNIVERSITY OF THE WITWATERSRAND
OFFICE OF THE DEPUTY VICE CHANCELLOR RESEARCH
01 JUL 2020
RESEARCH OFFICE HREC (Medical)
JOHANNESBURG

.....
Miss Mapula Ramaila
Administrative Officer
Human Research Ethics Committee (Medical)

Research Office Secretariat: Faculty of Health Sciences, Phillip Tobias Building, 3rd Floor, Office 302, Corner York Road and 29 Princess of Wales Terrace, Parktown, 2193 Private Bag 3, Wits 2050 I **T**+27 (0)11-717-1234/2656/2700/1252 **E**: Mapula.Ramaila@wits.ac.za I **Office E** HREC-Medical.ResearchOffice@wits.ac.za IWebsite: www.wits.ac.za/research/about-our-research/ethics-and-research-integrity/

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr N Isaac

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M1911123

NAME: (Principal Investigator)	Dr N Isaac
DEPARTMENT:	School of Clinical Medicine Department of Paediatrics and Child Health Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	A study of the biochemical profile of children with hypophosphataemic rickets and their response to conventional therapy
DATE CONSIDERED:	2019/11/29
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Professor K Thandrayen
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2020/03/04

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 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 will therefore reports and re-certification will be due early in the month of **November** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE CLEARANCE CERTIFICATE NUMBER IN ALL ENQUIRIES

Appendix 4 : Turnitin Report

00100697:Isaac_MMed_Final_Turnitin_Oct2023.docx

ORIGINALITY REPORT

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SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

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Appendix 5: Protocol.

A RETROSPECTIVE STUDY OF THE BIOCHEMICAL AND RADIOLOGICAL PROFILE OF CHILDREN WITH GENETIC HYPOPHOSPHATEMIC RICKETS AND THEIR RESPONSE TO CONVENTIONAL TREATMENT

Candidate: Nikhila Isaac

Student number: 301379

Department of Paediatrics, Faculty of Health Sciences, University of the Witwatersrand

Supervisor:

Adjunct Professor Kebashni Thandrayen

Department of Paediatrics, C.H Baragwanath Academic Hospital,

Faculty of Health Sciences, University of the Witwatersrand

Index page

Background

Rickets is a disorder characterised by decreased mineralization of bone matrix at the growth plate. It can occur due to decreased calcium or phosphate **(**PO_4) homeostasis. For the purpose of this study, this research will concentrate on genetic hypophosphatemic rickets and the `response to conventional therapy in children with this metabolic bone condition.
Significant research has been done on hypophosphatemic rickets and the response to Burosumab therapy, a new and expensive therapeutic agent.(1) Little is known about the response to conventional therapy in children with hypophosphatemic rickets residing in low-middle income countries and where Burosumab is currently not affordable.(2)

Phosphate homeostasis

Phosphorus is essential for numerous cellular molecules such as nucleic acids, proteins and lipids. It is also involved in acid base regulation and cellular physiology and plays a critical role in bone formation.
The body contains 700g of phosphorus: 85% is in the skeletal bones and teeth, 14% in soft tissue, and 1% is in the extracellular fluid. In plasma, 16% of the phosphorus is bound to proteins and lipids. The rest is present either as orthophosphate or as free phosphate and is filtered into the Bowman's capsule of the glomerulus.
PO_4 deficiency can occur due to poor absorption or renal wasting. Thirty percent of phosphorus absorption in the gut is controlled by active 1.25 dihydroxyvitamin D ($1.25(OH)_2D$). Phosphate is transported across the brush border membranes of the small intestine mediated by the sodium (Na) dependant PO_4 cotransporter IIb (NaPi-IIb) facilitating transport in the gut. (2) The rest of the phosphate is reabsorbed by the kidneys.
Phosphate passes freely through the glomerular filtration barrier. Eighty to ninety percent of filtered PO_4 load is reabsorbed from the tubular lumen, mainly the proximal convoluted tubules and the remainder is excreted in the urine. Reabsorption in the proximal convoluted tubules is a unidirectional transcellular process. On the apical brush border membranes of the epithelium of proximal tubules are transport proteins, NaPi cotransporters which facilitate transport against the tubular concentration.(3)
The NaPi transporters to be noted are type IIa cotransporter, type IIc co-transporter and type III symporter.

Regulation of phosphate

The key hormonal and gene regulators of phosphate homeostasis are parathyroid hormone, fibroblast growth factor 23 and αKlotho, Phosphate regulating endopeptidase homologous on the X chromosome (PHEX) and $1.25(OH)_2D$.

PTH mobilises PO_4 from skeletal bones into the blood stream by enhancing osteoclastic bone resorption.(5) It induces the expression of 1 alpha hydroxylase in the proximal tubule of the kidney and generation of the $1.25(OH)_2D$ in a variety of tissues. PTH binds to receptors on the proximal tubular epithelial cells leading to diminished apical proximal tubular NaPi-IIb cotransporters and decreased reabsorption of filtered PO_4 along the proximal tubule and decreases reabsorption through inhibition of the basolateral Na-K-ATPase which removes intracellular sodium in exchange for potassium.(3)

FGF23 is a glycoprotein produced by osteoblasts and osteocytes. Increased phosphate and $1.25(OH)_2D$ stimulates the synthesis of FGF23. Signalling by the FGF23 decreases sodium-dependant phosphate reabsorption. FGF23 also increases 1 alpha hydroxylase and increases 24 hydroxylase activity both of which decreases the availability of $1,25(OH)_2D$ decreasing active vitamin D leading to decreased calcium absorption in the gut and in the kidneys in humans.(6, 7)

PHEX is a protein that comprises 18 exons and codes for type II membrane proteins. It is expressed in osteoblasts and osteoclasts of skeletal bones, teeth and parathyroid glands as well as lung, brain, ovary, testicles and muscles but not in kidneys. It binds to matrix extracellular phosphoglycoprotein (MEPE) and relieves the inhibitory effect of these proteins on bone mineralization. The interaction between PHEX, dentin matrix protein-1 (DMP1) and a5b3-integrin, regulates and restricts FGF23 expression, whereas acidic serine- and aspartate-rich peptides (ASARM) derived from MEPE and other bone and dental matrix proteins, competitively inhibit the trimeric complex and increase FGF23 expression.

Inactivating mutations in PHEX lead to an accumulation of ASARM peptide, a substrate for PHEX and a strong inhibitor of mineralization, and increases circulating levels of FGF23 that result in phosphaturia, hypophosphatemia, and suppression of 25-hydroxyvitamin D (25(OH)D) to $1.25(OH)_2D$.(3)

Although the main role of $1.25(OH)_2D$ is the absorption of calcium, it also causes phosphate absorption from the gut by enhancing the expression of NaPi-IIb cotransporters. Increased levels of $1.25(OH)_2D$ also suppresses the synthesis of PTH and enhances FGF23 production.(8)

Causes of hypophosphatemic rickets

1) Nutritional: dietary insufficiency of phosphate, phosphate binders
2) FGF23- independent hypophosphatemic rickets
   - mutations in renal NaPi co transporter: Fanconi syndrome
   - Hereditary Hypophosphatemia with Hypercalciuria
3) FGF23- mediated rickets
   - X-Linked Hypophosphatemic rickets (PHEX)
   -Autosomal dominant Hypophosphatemic rickets (HR) (FGF23)
   -Autosomal recessive HR-1(DMP1)
   -Tumour induced osteomalacia
   -Autosomal recessive HR -2
   -Autosomal recessive HR -3
   -Fibrous dysplasia (9)

X-linked hypophosphatemia (XLH)

XLH will be discussed in further detail as the other genetic forms of hypophosphatemic rickets are uncommon and not well described in the literature.

X-linked hypophosphatemia is a genetic disorder of renal phosphate wasting and is the most common inheritable form of rickets.(10) It is caused by a loss of function mutation in the PHEX gene resulting in excess circulating FGF23. Since FGF23 is the primary regulator of phosphate homeostasis and controls the kidney reabsorption of phosphate, excess FGF23 will impair proximal renal phosphate reabsorption. It also causes reduced 1α hydroxylation of 25(OH)D and thus decreases synthesis of $1.25(OH)_2D$, the active metabolite of vitamin D.(11)

Diagnosis:

Disease awareness by physicians is of utmost importance for diagnosis. Early diagnosis and early treatment initiation leads to better outcomes such as improved linear growth and final height, bone mass accrual, fewer bone deformations and better dental health. Three diagnostic settings need to be distinguished.

1) Diagnosis of familial cases

About 85-90% of familial cases of hypophosphatemic rickets are associated with *PHEX* gene mutations and follows an X-linked dominant inheritance pattern. Affected fathers transmit the disease to all their daughters and none of their sons. Affected mothers have a 50% risk of having an affected daughter or son. In babies born to XLH affected parents, biochemical

screening should take place at birth or within the first three months of age. Screening includes serum phosphate, creatinine and alkaline phosphatase (ALP), and urinary phosphate and creatinine. In suspected XLH, the serum phosphate level is below the normal range for age and renal phosphate wasting occurs and is documented using the calculated renal phosphate tubular reabsorption rate. The serum phosphate and ALP concentrations should be interpreted based on reference ranges for newborns and infants as these are physiologically higher than those for adults. Although clinical and radiological signs of rickets are often lacking in those babies, ALP may be found at the upper level of normal. To be noted, is that the serum phosphate level at birth being normal does not exclude XLH. If there is history of familial XLH, the biochemical screening should be repeated at approximately no later than 3 months of age. The genetic diagnosis, i.e. *PHEX* sequencing, confirms the diagnosis. It may be done on cord blood or on a sample drawn after birth at centres that can perform the tests.(11)

2) Diagnosis of *de novo* cases of XLH

Children with XLH due to *de novo PHEX* mutations with no family history need to be assessed clinically, radiologically and biochemically.

Clinical features:

Any leg bowing (*genu varum* or *valgum*) whether or not associated with poor statural growth, widening of the metaphysis (ankles and wrists) should lead to a radiological and biochemical work-up. Tooth abscesses or facial cellulitis occurring on apparently healthy teeth suggest poor dentin mineralization.(8)

Radiological signs:

Radiographs of the hand, knees and lower limbs showing long bone deformities, abnormal growth plates with widened and frayed metaphysis are suggestive of hypophosphatemic rickets. Bone cortices appear dense as opposed to other forms of rickets. Fractures are uncommon in children and adolescents.(12)

Biochemical criteria:

Serum phosphate below the normal threshold for age associated with renal phosphate wasting, e.g. reduced calculated maximal tubular reabsorption of phosphate as a function of glomerular filtration rate. The fractional tubular resorption of phosphate (TRP) value may be within the normal range in children with XLH, and in the presence of hypophosphatemia the TmP/GFR is diagnostic.

ALP levels above the upper limit of normal for age, indicating rickets/osteomalacia. In children, ALP is however not so high as in the case of vitamin D deficiency, defects in calcitriol synthesis or calcitriol receptor mutations.

PTH levels in the normal or upper normal range. Normal serum calcium, and low urinary calcium excretion; exclusion of other proximal or distal tubular wasting disorders, and otherwise prior correction, of vitamin D or dietary calcium deficiency.

In summary, the key to correct diagnosis of *de novo* XLH cases is good knowledge of the clinical signs and symptoms and the correct use of age-adjusted biochemical markers.

3) Genetic confirmation of XLH

The final confirmation of XLH can be obtained by genetic analysis. This identifies the mutations in the *PHEX* gene.

Treatment of hypophosphatemic rickets

Treatment should be initiated as soon as the diagnosis is made in order to prevent rachitic changes, leg bowing and short stature. Conventional therapy for X-linked hypophosphatemia or other causes of hypophosphatemic rickets is multiple daily doses of phosphate salts and vitamin D metabolites.(13)

Phosphate supplements are given in dosages of 40-60mg/kg/day from birth to 10 years in 4 divided doses for at least a year and 30-50mg/kg/day between 10 and 15 years of age.(11) Serum phosphate levels increase rapidly after oral intake but return to baseline concentrations within 1.5 hours, therefore phosphate has to be given as frequent doses, 4–6 times per day. Oral phosphate supplements does not restore fasting phosphate levels. (13) Phosphate supplements are available as oral solutions, capsules or tablets containing sodium- based and/or potassium- based salts. Dosages should be based on elemental phosphorus because the phosphorus content largely differs between the available phosphate salts. Phosphate should not be given together with calcium supplements or foods with high calcium content, such as milk, as precipitation in the intestinal tract reduces absorption. (13)

Active vitamin D (calcitriol or alfacalcidol) is given in addition to oral phosphate supplements to counter act calcitriol deficiency, prevent secondary hyperparathyroidism and increase phosphate absorption from the gut.(15) Vitamin D alfacalcidol dosage is 1-2ug/day from birth to 10 years and 1.5-3ug/day between 10 and 15 years of age, in a single daily dose. The optimal dose varies from patient to patient. Requirements are generally higher during early childhood and puberty and the dose can be adjusted on the basis of serum levels of ALP and PTH and urinary calcium excretion.(15) Large doses of active vitamin D promote growth and bone healing but are associated with an increased risk of hypercalciuria and nephrocalcinosis. Insufficient doses of active vitamin D is associated with low intestinal

calcium absorption, low urinary calcium excretion, persistent rickets and high ALP and/or PTH levels. (15)

Early treatment with oral phosphate supplementation and active vitamin D heals rickets, limits dental abscess formation and prevents progressive growth failure. In a substantial proportion of patients, treatment is unsuccessful and/or associated with adverse effects such as persistence of renal phosphate wasting and hypophosphatemia. (13)
Long-term $1.25(OH)_2D$ deficiency can result in secondary hyperparathyroidism and this is worsened by chronic phosphate supplementation.(16) Secondary hyperparathyroidism might aggravate phosphaturia and promote bone resorption; especially in patients not treated with active vitamin D. Suppressed PTH levels secondary to excessive vitamin D therapy and/or insufficient oral phosphate intake might decrease bone turnover and compromise rickets healing and growth. Therefore, therapies should be adjusted to keep PTH levels within the normal range (10–65 pg/ ml) (16).
The use of vitamin D analogs may also result in hypercalciuria and nephrocalcinosis. Conventional therapy further stimulates FGF23 levels and thereby renal phosphate wasting, resulting in a vicious circle, which might limit its efficacy. The multiple dosing in a day can lead to poor compliance. (12)

Burosumab (International studies)
Burosumab, a fully human monoclonal IgG_1 antibody that neutralizes FGF23, was approved in 2018 by health authorities for the treatment of patients with hypophosphatemic rickets. Treatment has shown to promote growth, reduces bone pain, progressively corrects leg deformities and improves dental health.(14) If infants are diagnosed before they develop bone changes, Burosumab can be used to prevent rickets.(14) The European Medicines Agency (EMA) granted a conditional marketing authorization in the European Union for Burosumab for the treatment of XLH with radiographic evidence of bone disease in children ≥1 year of age and in adolescents with a growing skeleton.(15) In April 2018, the US Food and Drug Administration (FDA) granted approval of Burosumab to treat adults and children ≥1 year with XLH.(16) These decisions were based on the results of trials testing Burosumab in children with severe XLH and in adults with skeletal pain associated with XLH and/or osteomalacia. Serum levels of phosphate, TmP/GFR, the severity of rachitic lesions in children (based on radiography images) and osteomalacia in adults (based on radiography images and bone histomorphometry) were chosen as primary end points. In children, the dose

of burosumab was initially titrated against serum levels of phosphate, targeting empirical levels ranging from 1.1 to 1.6 mmol/l, whereas adult patients received a fixed weight-related dose.(15) Currently, only the data submitted to the regulatory agencies and published in peer review journals are available. Burosumab is an expensive drug and data on cost-effectiveness and long-term outcome are pending. Thus, conclusive recommendations on the use of Burosumab are premature.

Two open-label uncontrolled trials testing the use of Burosumab in 65 children aged 1–12 years with severe XLH demonstrated that in the short term (12–16 months), Burosumab resulted in positive outcomes. These were the positive outcomes:

1. A significant increase in TmP/GFR and consequently raised serum phosphate levels into the lower end of the age-related normal range, with increased 1,25(OH)2D levels
2. A significant reduction in the severity of rickets (as measured by the Rickets Severity Score (RSS) and the Radiographic Global Impression of Change (RGI- C));
3. A significant improvement in physical ability (as measured by walking distance in the 6MWT) and;
4. A significant reduction in patient-reported pain and functional disability (as measured using the Pediatric Orthopedic Society of North America Outcomes Data Collection Instrument). (16)

The most common adverse reactions observed with Burosumab were injection- site reactions, headache and pain in the extremities. (16)

Burosumab is administered every 2 or 4 weekly subcutaneously and improves renal tubular phosphate reabsorption thus increasing serum phosphorus levels and increasing serum $1.25(OH)_2D$.(2, 13) Two weekly doses were superior to four weekly doses with respect to normalization of serum levels of phosphate and radiological improvement of rickets.(15) However, in South Africa it is not available in the public health sector.

Justification for study

In South Africa, there is little published data on hypophosphatemic rickets and XLH management and on the response to conventional treatment such as phosphate supplements and calcitriol or one alphacalcidiol. Burosumab is costly and not freely available to the public health system and thus conventional treatment is offered to patients. We hypothesise that conventional therapy for the treatment of XLH rickets and the other genetic causes of hypophosphatemic rickets is not adequate, and Burosumab should be made available to all patients with hypophosphatemic rickets in South Africa.

Aim of study

To assess the biochemical profile of genetic forms of hypophosphatemic rickets, including XLH at presentation and to monitor the biochemical and radiological response to conventional therapy.

Primary objectives

4. To assess the biochemical profile of genetic forms of hypophosphatemic rickets at baseline.
5. To evaluate the efficacy of conventional therapy in treating genetic hypophosphatemic rickets by assessing improvements in biochemical bone and serum markers (Calcium, Phosphorus, ALP, and PTH) at 3, 6, 9 and 12 months and then every year until follow-up is completed or till the last follow-up visit.
6. To assess changes in the Thacher radiological score from baseline till 12 months after commencing treatment and till the last available radiological assessment.

Secondary objectives

4. To review medical records for documentation of compliance issues at 3, 6, 9 and 12 month and every year till follow-up is completed or till the last follow-up visit.
5. To assess improvement or changes in height for age z (HAZ) scores at 6, 12 and 24 month and yearly till follow-up is completed or till the last follow-up visit.
6. To assess family history in genetic hypophosphatemia

Study population and sample size

Children under the age of 18 years and attending the Metabolic Bone clinic at Chris Hani Baragwanath Academic Hospital from 1st January 2006 till follow up is completed or till the last follow-up visit.

Inclusion criteria

Children diagnosed with genetic hypophosphatemic rickets based on biochemical profile at baseline or prior to treating (normal calcium, normal PTH or not >2.5 X the upper normal limit) and low phosphate levels (below the normal reference limits for age), with or without a positive family history of hypophosphatemic rickets and commenced on conventional treatment of phosphate and one alphacalcidol.

Exclusion criteria

7. Calciopaenic rickets
8. Hypophosphatemic rickets secondary to renal tubular acidosis (RTA). RTA is defined as a normal serum anion gap hyperchloremic metabolic acidosis and patients fulfilling this criteria will be excluded.
9. Hypophosphatemic rickets secondary to tumour-induced osteomalacia or fibrous dysplasia.
10. Patients already commenced on conventional therapy (> 3 months duration) at referral.
11. Hypocalcaemia or hypercalcaemia, defined as serum calcium levels outside the age-adjusted normal limits
12. Evidence of hyperparathyroidism (PTH levels 2.5 X upper limit of normal)

Study design and methods

This will be a retrospective descriptive study. The research will involve reviewing patient files and entering data into a data collection sheet (appendix A) and analysing the data available. The biochemical profile at the first visit will be compared to the profiles at 3, 6, 9,12 months and longer till the last follow-up visit after starting treatment. The laboratory tests included for the study will be serum phosphate, ALP, PTH and calcium. The Thacher radiological rickets severity score (12) will be utilised by the researcher and confirmed by the supervisor for assessing the radiological changes of rickets at baseline and thereafter at 12 months and the last available radiological assessment.

Statistical analysis

The statistical analyses will be mainly descriptive. Data will be captured into the data collection sheet (Appendix A) and then entered into an excel spreadsheet and analysed using Statistica (Statsoft USA, version 13.5). Categorical values will be reported as numbers and percentages. Continuous variables will be reported as means and standard deviations or medians and interquartile ranges where applicable. ANOVA (analyses of covariance) will be

performed on the continuous data such as biochemical markers, radiological scores or HAZ scores at each of the different time points to assess for any improvements over these time points. The student- t test or Mann-Whitney U test may be applied to assess changes in biochemical markers, radiological scores or HAZ scores from baseline to the 12 month follow-up and thereafter. The Kaplan-Meier survival analysis will be applied to investigate the effect of conventional therapy on the increase in PO_4 levels and decrease in ALP levels and radiological scores from baseline and over the specified time periods to reach a normal age limit value (PO_4 and ALP) or improved radiological score of <1.5.

Budget

The cost of printing the protocol, ethics forms and data collection sheets will be covered by the researcher.

Ethical consideration

Protocol will be submitted for ethics approval to the Human Research Ethics Committee of the University of the Witwatersrand. Permission to access files and review them will be obtained from the CEO of CHBAH. Then data will be recorded using study numbers and the patients' details will be kept anonymous and will only be known to the researchers.

Study Timeline

The period March - July 2020 files will be reviewed and entry of data collection sheets completed. Data analysis, compilation and write up of the thesis will begin in June 2020 and be completed by May 2021 as shown in Gnat chart below.

	Oct-Dec 2019	Jan 2020	Feb 2020	Mar 2020	Apr 2020	May 2020	Jun 2020	July 2020	Aug 2020	Sept-Dec 2020	Jan-May 2021
Literature review											
Preparing protocol											
Protocol assessment											
Ethics application											
Collecting data											
Data analysis											
Writing up thesis											
Writing paper											

Limitations of study

1) Patients may claim compliance but this may not be true.
2) Since there is no DNA testing performed in South Africa to confirm XLH or other genetic causes of hypophosphatemia, patients were chosen based on the biochemical profile, radiological findings; with or without a positive family history of hypophosphatemia and commenced on conventional treatment after diagnosis.
3) Physical ability (6-minute walk test) and patient-reported pain and functional disability could not be assessed due to the retrospective nature of the study
4) Lab methods have changed over the years and delay in processing specimens may affect phosphate levels

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