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The clinical and demographic presentation of vitamin D deficiency rickets in Johannesburg, South Africa

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THE CLINICAL AND DEMOGRAPHIC PRESENTATION OF VITAMIN D DEFICIENCY RICKETS IN JOHANNESBURG, SOUTH AFRICA





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

Johannesburg, 2017

Declaration

I, Marisa Beretta, declare that this MMed is my own work. It is being submitted for the Degree of Master of Medicine in the branch of Paediatrics at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other university.

_____

Signature of candidate

_____ day of _____ 20_____in

_____

Abstract:

Overview: Over the past two decades a significant amount of research has been dedicated to vitamin D in an attempt to explore a contradictory reappearance of rickets despite a more extensive understanding of the predisposing factors and underlying pathophysiology of vitamin D deficiency, in addition to recommendations of vitamin D supplementation.

Aim: To describe the demographical and the clinical presentation of vitamin D deficiency rickets in children presenting to the Metabolic Bone Clinics at Charlotte Maxeke Johannesburg Academic hospital (CMJAH) and Chris Hani Baragwanath Academic hospital (CHBAH).

Results: A total number of 71 hospital files of children with vitamin D deficiency rickets were reviewed of which 35 (49.3%) were from CMJAH and 36 (50.7%) were from CHBAH. The majority of patients were black (97.2%). The mean age of presentation was 22.37 months (SD 12.51) and mean HAZ was -2.83 (1.64), WAZ was -1.33 (1.69) and BAZ was 0.62 (1.64). The most common presenting features were widened wrists (81.7%), rachitic rosary (80.3%) and genu varum deformities (65%). Comparisons between CMJAH and CHBAH showed that there was a greater number of patients residing in flats (91.2% vs 36.1%; $p< 0.05$) and in inner city areas (91.4% vs 30.5%; $p< 0.05$) attending the CMJAH. Nearly two-thirds of parents reported that their children had no sun-exposure and just over three-quarter of the children were breastfed; and there were no differences in these predisposing factors between the CMJAH and CHBAH patients.

Conclusion: Despite adequate sunlight exposure in Johannesburg, vitamin D deficiency rickets was noted in more than ninety percent of children from CMJAH who reside in crowded flats in the inner city compared to the non-urban patients at CHBAH. Preventative strategies such as education and public health awareness in urban areas may be introduced to limit the incidence of this disease that could easily be prevented in the South African environment by encouraging sun-exposure.

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List of Abbreviations

ALP	Alkaline Phosphatase
BAZ	Body mass index for age Z score
BMI	Body mass index
CHBAH	Chris Hani Baragwanath Academic Hospital
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
DBP	Vitamin D Binding Protein
FGF23	Fibroblast Growth Factor 23
HAZ	Height for age Z score
HIV	Human Immunodeficiency Virus
IOM	Institute of Medicine
IQ	Interquartile
IU	International Units
25(OH)D	25-Hydroxyvitamin D
NHLS	National Health Laboratory Service
NVDRE	Negative response element for vitamin D
PTH	Parathyroid Hormone
RANKL	Receptor activator nuclear κB ligand
RDA	Recommended daily allowance
RXR	Retinoid X
SD	Standard Deviation
SPF	Sun Protection Factor
TB	Tuberculosis
USA	United States of America

UV	Ultraviolet
VDR	Vitamin D receptor
WAZ	Weight for age Z score
WHO	World Health Organisation
XR	X-ray

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Chapter 1: Introduction and Literature Review

1.1 Introduction

First identified in the 1800s and immortalised by authors of the day in timeless characters such as Charles Dickens' Tiny Tim, rickets was thought to be relegated to the past as a disease of antiquity. However, the last two decades has seen resurgence in vitamin D deficiency rickets. This is contrary to the dramatic improvement in knowledge pertaining to the pathophysiology of the disease and in the identification of a number of associated predisposing factors; despite the advent of vitamin D fortified foods and routine supplementation. Research has stemmed from an interest in an attempt to determine the exact aetiology of this rise in a previously contained entity and secondary to discoveries of the extended actions of vitamin D beyond that on calcium and bone metabolism (1). A variety of both developed and developing counties have launched research into at risk populations and supplementation protocols following the findings gleaned from the last 40 years of investigation (1-10).

Over the last decade, studies on vitamin D deficiency have originated from an impressive variety of geographical locale that have varying economic and environmental predisposing factors. Representing the developing nations, in the subtropics with an average of 5.2 – 8.4 daily sunshine hours is Turkey, where an incidence of 1.67% to 19% of vitamin D deficiency was reported in children aged 1-3 years (6). From the developed nations, Sydney with an average of 6-8 daily sunshine hours, where there has been a steady increase in the number of cases per year with a doubling of cases from 2002 to 2003 (3). The temperate and limited UV sunlight zone of Glasgow, Scotland with an average of 3.24 daily sunlight hours reported twice as many cases in 2008 than in prior year (1). Finally, the extreme northern regions of Canada with an averages in the range of 5.05 – 5.32 daily sunshine hours showed an overall annual incidence rate of 2.9 per 100 000 children with vitamin D deficiency (11).

1.2 Vitamin D metabolism

Vitamin D is unique amongst the micronutrients defined as vitamins as it is converted into a hormone once it is in the metabolically active state (12). It has a characteristic flexibility of structure and can be manufactured by the human body independent of ingestion. It occurs naturally in 2 forms: vitamin D2, ergocalciferol originating from some plants and commercially produced via the irradiation of yeast and vitamin D3, cholecalciferol, produced in the skin following exposure to ultraviolent radiation and from animal sources (13-18). Few

natural foods contain vitamin D with sources limited to egg yolk (20IU), oily fish (250IU), cod liver oil (400-1000IU per teaspoon) and mushrooms exposed to irradiation (100IU - 1600IU). These non-fortified foods are both low in content and infrequently consumed. The majority of dietary vitamin D stems from foods fortified with vitamin D or from supplements (13, 17), in which either form of vitamin D or synthetic vitamin D analogs (doxercalciferol, paricalcitol and alfacalcidiol) may be used. Commonly fortified foods include infant formulas, milk, milk products and margarine. The metabolic pathway of both forms is identical although their metabolic clearance rates may be different (19).

Upon exposure to solar ultraviolet B radiation with a wavelength of 290-315nm, 7-dehydrocholesterol located in the epidermis and dermis absorbs the wavelength and is converted into pre-vitamin D (14, 15, 20) which following thermal rearrangement of the triene system is released into the circulation. Pre-vitamin D is converted in the plasma membrane to Vitamin D3 and enters successively the extracellular space and then the dermal capillary bed where it binds with vitamin D binding protein (DBP). Vitamin D2 and vitamin D3 absorbed from the gastro-intestinal tract are transported via chylomicrons and the lymphatic system into the superior vena cava (14, 15, 20). From both sources and in the bound form, the complex is conveyed to the liver, metabolised and catalysed via hydroxylation at C-25 to 25-hydroxyvitamin D (25(OH)D) by several cytochrome p450 dependent enzymes - 25 hydroxylases (21, 22), integrated membrane proteins of the mitochondria or smooth endoplasmic reticulum cells of the liver. Bound once again to DBP, 25(OH)D is conveyed to the kidneys and internalised via endocytosis with the assistance of the low-density lipoprotein, megalin. In the proximal renal tubule hydroxylation of C-1 by cytochrome p450 mono-oxygenase 25(OH)D 1α hydroxylase enzyme, located predominantly in the kidney but found in extra-renal sites, converts 25 (OH)D to 1,25 dihydroxyvitamin D (1,25(OH)2D) which is responsible for the majority of the biological activity of vitamin D (22). 1,25(OH)2D acts by binding to an intra-cellular receptor (VDR) and forms a hetero-dimer with retinoid X receptor (RXR), binding to the vitamin D response element in the promoter region of 200-2000 genes and triggering gene transcription (22, 23). 1, 25(OH) 2D regulates calcium, facilitating its effects via action in the bone and intestine (20, 22, 23).

1.3 Vitamin D activity on bone

1,25(OH)2D induces an increase in serum calcium via osteoclastogenesis and bone resorption mediated via receptor activator nuclear κB ligand (RANKL), a member of the tumour necrosis α family, expressed in osteoblasts (24, 25). Interaction of RANKL with its receptor

RANK, located on precursor osteoclast cells and the expression of which is increased in response to VDR, initiates differentiation into multinucleated cells responsible for bone resorption (24, 25).

1.4 Regulation of vitamin D

Vitamin D is tightly regulated by a number of positive and negative feedback mechanisms that exert their effects via expression in the various hydroxylases involved in its metabolism. One of the first to be recognised was the direct negative feedback observed of vitamin D status mediated by 1,25(OH)2D on VDR, which upon forming a complex with the receptor down-regulates expression of the gene via interaction with the negative response element (NVDRE) through VDR interacting repressor (26, 27). Dietary and serum levels of both calcium and phosphate influence enzyme activity mediated through PTH and fibroblast growth factor 23 (FGF23) respectively, with a deficiency amplifying the activity of 1α hydroxylase. Regulation exerted by calcium is via the action of PTH on the promoter region encoding for 1α hydroxylase, which contains a region that renders its transcription sensitive to PTH, with the effects mediated by second messenger systems and limited exclusively to the proximal cells of the kidney. FGF23 is synthesised and secreted by bone osteocytes in response to elevated phosphate levels, acting as an indicator for the kidneys and parathyroid glands of phosphate levels and mediates its effects via inhibition of transcription of the 1α hydroxylase gene and reduction in the number of sodium-phosphate co-transporters in the brush border membranes of the proximal kidney leading to increased phosphate excretion in the urine. PTH and FGF23 are regulated by the levels of 1,25(OH)2D with PTH expression being supressed by and FGF23 production being inversely promoted by elevated 1,25(OH)2D levels (27-31).

1.5 Factors influencing vitamin D production resulting in vitamin D deficiency rickets in infants and young children

A number of factors influence the ability of the human body to produce vitamin D with the majority influencing exposure to adequate UV radiation for synthesis while others include dietary restrictions and those that influence vitamin D metabolism directly. With the skin being accountable for 90% of vitamin D production (21), any practise which reduces surface area exposure will proportionately reduce the production of vitamin D including clothing on an infant and religious or cultural apparel (32, 33). Exposing an infant's head and shoulders for as brief a period of 30 minutes twice weekly is adequate to maintain physiological levels of vitamin D (34). Evidence is currently under investigation that suggests that the age at

which direct sunlight exposure is introduced has an even greater relevance to the development of skin cancer than the duration of exposure with the American Association of Pediatrics (AAP) issuing directives that infants less than 6 months be kept out of direct sunlight. Protective clothing and sunscreen should be used on children of all age groups upon any exposure to UV radiation. Sunscreen of SPF 15 absorbs 99% of UV radiation and when applied correctly reduces production of vitamin D by 99% (35). Properties of the skin itself may alter production through melanin absorption of UV radiation, reducing synthesis capacity by up to 90% in pigmented infants (36) equating to a 10-15 time exposure required to synthesise similar quantities. Any practise that reduces exposure time to UV radiation will also reduce production including cultural or religious avoidance of exposure during daylight (32, 33). In addition, the absence of a safe outside environment in which to interact and receive adequate sun exposure which is most applicable to the urban setting of Johannesburg is another predisposing factor. Atmospheric factors reducing UV radiation are also accountable for reduced production of previtamin D_3 during the winter months with decreased production in the northern and southern hemispheres (37). of which the difference in production observed between the more southern located Cape Town and northern situated Johannesburg in South Africa during the winter period of May to August has been highlighted in a number of studies (13, 37-39). Atmospheric pollution may contribute significantly to vitamin D deficiency (40), particularly in informal settlements where the use of open fire is widespread,

Dietary factors responsible for inadequate intake of vitamin D in the infant include prolonged, unsupplemented and exclusive breastfeeding (41-43) or a lactating mother who is deficient in vitamin D (44, 45). Breast milk of a mother replete in vitamin D is estimated to contain a meagre 20-60IU/L with very few references regarding the content in a vitamin sufficient mother (46, 47). In the older child introduced to the adult diet, deficiency stems from unsupplemented vegetarian diets or a low calcium diet where cereals are the major staple constituent and diary is expensive which is easily recognised in South Africa (10). Factors that directly influence vitamin D metabolism include anti-epileptics and anti-retrovirals that interfere with metabolism which may also be considered an important factor in South Africa where HIV is prevalent (17) . South Africa with its' diverse ethnic background, genetic and ethnic differences in vitamin D metabolism (12, 48) that, although not yet actively explored in the South African population, as a risk factor for rickets, has been explored in other countries and cannot be excluded as a potentially important variable.

1.6. Definition and clinical presentation of vitamin D deficiency rickets:

Vitamin D deficiency rickets is defined as a disease of the growing child (21). It results from failure to mineralise growing bones secondary to impaired apoptosis of the hypertrophic cells of the growth plate and resultant impaired mineralisation of the growth plate and osteoid matrix (21). Rickets is divided into calciopenic and phosphopenic forms with the calciopenic variety originating from insufficient calcium intake or vitamin D deficiency. As vitamin D deficiency rickets manifests exclusively in growing children, the majority are identified during infancy, below the age of 18 months, and during the adolescent growth spurt (21).

Clinically rickets manifests predominantly as bone deformities in weight bearing limbs due to loss of structural support provided by poorly mineralised bone causing bowing of the arms in crawling infants and of the legs in walking children (15). The defects in chondrocyte maturation cause widening of the metaphyses presenting as widening of the distal ends of long bones, most commonly involving the wrist, and hypertrophy of the costochondral junctions with the development of the "rachitic rosary" or beading of the anterior ribs. Other clinical features include growth retardation, frontal bossing of the skull, delayed closure of the anterior fontanelle, craniotabes, Harrison's sulcus of the ribcage, delayed dentition, irritability arising from bone pain and hypotonia arising from muscle weakness with associated motor developmental delay. In the first year of life or early infancy period, the initial presentation may be that of hypocalcaemic seizures (21, 49-51).

1.7. Biochemical markers of vitamin D deficiency:

Vitamin D deficiency is defined based on the assessment of serum 25 (OH) D measurements which, with a half-life of 3 weeks, is the most accurate reflection of vitamin D stores and incorporates dietary and supplemental sources in addition to cutaneous production thus reflecting the vitamin D status rather than vitamin D function (49). Vitamin D stored in other body tissues is not reflected in this measurement. Vitamin D deficiency resulting in bone disease has been defined in 2010 by the Institute of Medicine (IOM) as levels below 30nmol/L (52). Vitamin D insufficiency is an intermediary level between levels manifesting bone disease and optimal levels postulated to be associated with other disease outcomes and are defined as levels of 30-50nmol/L (52). The optimal or vitamin D replete level has been defined as above 50nmol/L (52). Multiple methods exist for measurement of 25(OH)D including radioimmune assays, competitive protein biding assays (CPBA), high pressure liquid chromatography (HPLC) and liquid chromatography tandem mass spectrometry (LC-MS/MS). These methods may yield an inter-assay variation of 25% and intra-assay variation

of 10% due to the assay differences in affinity for vitamin D2 and D3 leading to lower measured vitamin D serum levels where vitamin D2 is the dominant supplement in fortified foods. Laboratory findings in vitamin D deficient rickets include a depressed 25 (OH)D serum concentration, an elevated alkaline phosphatase and elevated PTH serum concentrations. Calcium measurements usually remain normal in vitamin D replete and deficient children, only dropping when the calcium stores of the skeleton have been exhausted in advanced stages of the disease process (13, 17, 21). Vitamin D deficiency causes an increase in PTH in an attempt to induce 1-alpha-hydroxylation stimulating bone resorption and release of calcium to maintain serum concentrations in addition to increasing calcium absorption from the gastro-intestinal tract. PTH also degrades sodium-dependent phosphate co-transporter protein of the proximal renal tubules leading to increased phosphate losses and reduced serum phosphate concentrations (22, 29).

1.8. Radiological features of vitamin D deficiency rickets:

Radiographic features of rickets are observed at the growth plates of rapidly growing bones, the zone of provisional calcification, where the mineralising metaphyses meets the cartilaginous physis. This results in an increase in longitudinal thickness and an expansion of the radiolucent area between metaphysis and epiphysis where the zone of provisional calcification is partly or completely obscured. The metaphysis in rickets may be frayed or irregular and the calcified margin concave and spread transversely with a cup or "champagne-glass" appearance (53). Other features include curving of long bones, delayed appearance of ossification centres, greenstick fractures and generalised osteopenia. The Thacher scoring system implements a 10 point scoring of radiographs of the wrists and knees grading radiological changes of rickets in the radius and ulna, femur and tibia (Table 1)) (53).

Table 1: Ten-point radiographic scoring method of rickets (Sourced from Thacher TD, Fischer PR, Pettifor JM, Lawson JO, Manaster BJ, Reading JC. Radiographic scoring method for the assessment of the severity of nutritional rickets .)(53)

WRIST – score both radius & ulna separately		
Grade		Radiographic features
1		Widened growth plate, irregularity of metaphyseal margin but without concave cupping
2		Metaphyseal concavity with fraying of the margins
2 bones x 2 points = 4 points possible		
KNEE – score both femur & tibia separately		
Multiply the grade in A by the multiplier in B for each bone, then add femur & tibia scores together		
A:	Grade	Degree of lucency & widening of zone of provisional calcification
	1	Partial lucency, smooth margin of metaphysis visible
	2	Partial lucency, smooth margin of metaphysis not visible
	3	Complete lucency, epiphysis appears widely separated from distal metaphysis
B:		Multiplier
	0.5	</= 1 condyle or plateau
	1	2 condyles / plateaus
2 bones x 1 point x 3 points = 6 points possible		
		Total 10 points possible Score the worst knee & worst wrist

1.9. Treatment of vitamin D deficiency rickets:

Vitamin D deficient rickets responds to vitamin D2 or D3 in therapeutic doses of 1500-6000IU/day and is continued until biochemical abnormalities resolve, usually for a period of 2-3 months (21). Large dose, single dose therapy has been used in situations where compliance is questionable (23, 54). For prevention most fortified foods have, at most, 100IU per serving. Recently the Institute of Medicine guidelines suggest supplementation with 600IU daily in children over 1 year to prevent vitamin D deficiency (18). Dark skinned infants on exclusive breastfeeding are at an increased risk and should be supplemented with 400IU per day, a notion supported by studies of the nationwide program in Turkey showing a reduction in the prevalence of rickets from 6% in 1998 to 0.1% in 2008 (12,1).

Chapter 2: Study Description

2.1. Justification for the study:

Studies originating in South Africa are limited with none involving the Gauteng region, which is responsible for provision of health care to a large populace. Specialty services are limited and as such, Chris Hani Baragwanath Academic Hospital (CHBAH) and Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) receive referrals from within and outside of the Gauteng area. The purpose of this study is to investigate the presentation of vitamin D deficiency rickets in children, to assess their demographic patterns of presentation and to provide an insight into possible reasons for rickets in these children presenting at these two

large academic hospitals in Johannesburg, Gauteng. By identifying risk factors, preventative strategies may be introduced and encouraged to limit the incidence of a disease that could easily be eliminated in the South African environment. By reviewing the clinical presentation it may also assist practitioners in identification of the features with increased awareness for referral to specialist care.

2.2. Hypothesis of the study:

Despite adequate sunlight exposure in Johannesburg, vitamin D deficiency is not uncommon in children living in the inner city and the risk factors are due to poor sunlight exposure and dark-skinned, breastfed, high risk children residing in overcrowded, urban flats.

2.3. Methods

2.3.1. Aim of the study:

To describe the demographic as well as the clinical and biochemical presentation, the management and outcome of vitamin D deficiency rickets in children presenting to the Metabolic Bone Clinics at CHBAH and CMJAH.

2.3.2. Objectives

1) To determine and compare the demographics and referral patterns of patients presenting with vitamin D deficiency rickets to CHBAH and CMJAH
2) To determine the predisposing factors to vitamin D deficiency
3) To describe the clinical signs of vitamin D deficiency rickets present on referral
4) To determine if there is a correlation between the biochemical results and the radiological findings at the time of referral

2.3.3. Study Population and Sample Size

The files of all patients with vitamin D deficiency rickets presenting to CHBAH and CMJAH's Metabolic Bone clinics during the period of 2006 to 2012 were audited. No sampling technique was implemented. Seventy one files or records were available for review.

2.3.4. Inclusion Criteria

1) All patients with vitamin D deficiency based on a combination of biochemical findings in keeping with vitamin D deficiency rickets (low serum calcium and/or phosphorus, elevated alkaline phosphatase and parathyroid hormone values) and;

2) patients with evidence of resolution of biochemical and radiological findings after being were treated with vitamin D.

2.3.5. Exclusion Criteria

1) Patients whose initial presentation and diagnosis occurred at over the age of 5 years as their cause of vitamin D deficiency might be due to an underlying medical condition rather than nutritional rickets or lack of sun-exposure.
2) Patients with other causes of rickets such as phosphopenic rickets due to X-linked hypophosphataemic rickets or Fanconi syndrome

2.4 Study Design and Methods

This is a retrospective clinical audit. Metabolic Bone clinic is a specialty discipline with all patients having been reviewed by paediatricians. All files of patients managed at the two clinics were reviewed and those with a diagnosis of vitamin D deficiency rickets were further analysed. Patients' files were reviewed for information on demographics, (especially age of onset of symptoms, age at presentation, feeding history, type of residence and location), clinical presentation, therapy implemented and response to therapy and the information was entered on the data collection sheet (see appendix). Radiological findings were not included as part of the inclusion criteria for vitamin D deficiency rickets as the study cohort included infants less than 6 months in whom radiological findings are less likely to be present at this age.

The data collection sheet was designed using retrospective analysis of a small proportion of the Metabolic Bone Clinic files with the information for inclusion being that most commonly included in the files. The blood results on referral, at presentation and following interventional therapy were reviewed. The laboratory tests included for the purposes of this study were serum calcium, phosphate, alkaline phosphatase, parathyroid hormone and 25(OH)D levels performed at the NHLS laboratories based at CHBAH and CMJAH. The radiographs at presentation were interpreted and scored in accordance with the Thacher scoring system (53) by the researchers (M.B and supervisor, K.T). Outcome of treatment was assessed by the resolution of biochemical markers to normal reference ranges, resolution of radiological Thacher score to zero and weeks to discharge. The two cohorts from CMJAH and CHBAH were compared with the intent of comparing an inner-city population dominated by multi-storey, high density dwellings represented at CMJAH to a semi-urban population of mostly formal and informal housing represented by CHBAH.

2.5 Statistical Analysis

Information from the data collection sheets were entered into Microsoft Excel and subsequently into Statistica statistical software version 10 (Stat soft, USA). Continuous variables were defined with means and standard deviations or medians and interquartile ranges where applicable. Categorical variables were defined using percentages and frequencies. For comparison between the findings of the patients at CHBAH and CMJAH, data was interpreted via the chi-squared test for categorical variables or student t-test or Mann-Whitney U test for continuous variables depending on the normality of the data. Pearson correlation coefficients were used to describe the relationship between certain biochemical markers and also between biochemical markers and the Thacher score.

2.6. Ethical Considerations

The protocol was submitted to the University of Witwatersrand Committee for Research on Human Subjects for ethical approval which was received with an ethical certificate issued (ethics number - M120727). Permission to conduct the study was obtained from the CEOs of CHBAH and CMJAH hospitals. The data was recorded using a patient study number, whose identity was known only to the researcher.

Chapter 3: Results

Seventy one files or hospital records of children with vitamin D deficiency rickets were reviewed of which 50.7% (n=36) of the patients were from CHBAH and 49.3% (n=35) were from CMJAH.

The diagnosis of vitamin D deficiency in these children was based on the following criteria:

- Biochemical findings in keeping with vitamin D deficiency rickets
- Resolution of biochemical and radiological findings after treatment with vitamin D

3.1. Demographics

3.1.1. Ethnicity and gender:

The majority of the patients were black (97.2%). Fifty eight percent of the cases were male, and forty two percent (n=30) were female. CHBAH had 19 males and 17 females; while CMJAH had 22 males and 13 females with a greater number of females presenting to CHBAH compared to CMJAH which was not statistically significant ($p<0.39$).

3.1.2. Chronological age and anthropometric measurements at presentation:

As shown in Table 3.1 below, the mean age of the patients at initial presentation to CHBAH was significantly lower compared to the patients presenting to CMJAH (18.3 months versus 26.5 months, $p<0.01$). The weight for age Z score (WAZ) and BMI for age Z (BAZ) scores were significantly lower in the CHBAH cohort. The patients in both hospitals were stunted but there was no significant difference in height for age Z score (HAZ) when comparing the patients between these two hospitals.

Table 3.1: Chronological age and anthropometric measurements at presentation

	Both Hospitals		CHBAH		CMJAH	
	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
Chronological age (months)	71	22.4 (12.5)	36	18.3 (12.6)*	35	26.5 (11.2)
Weight	70	9.8 (2.9)	36	8.7 (3.2)	34	11 (2)
WAZ	70	-1.3 (1.7)	36	-1.8 (2)**	34	-0.9 (1.2)
Height	70	74.9 (10)	36	71.4 (11.6)	34	78.6 (6.3)
HAZ	70	-2.8 (1.6)	36	-3 (1.9)	34	-2.7 (1.4)
BAZ	70	0.6 (1.6)	36	0 (1.9)*	34	1.3 (1.1)
Head circumference	30	47.2 (3.8)	17	45.5 (4)	13	49.4 (2.4)

*p<0.01; **p<0.05; WAZ, HAZ, BAZ calculated using the WHO Anthroplus software

3.1.3. Area of Residence:

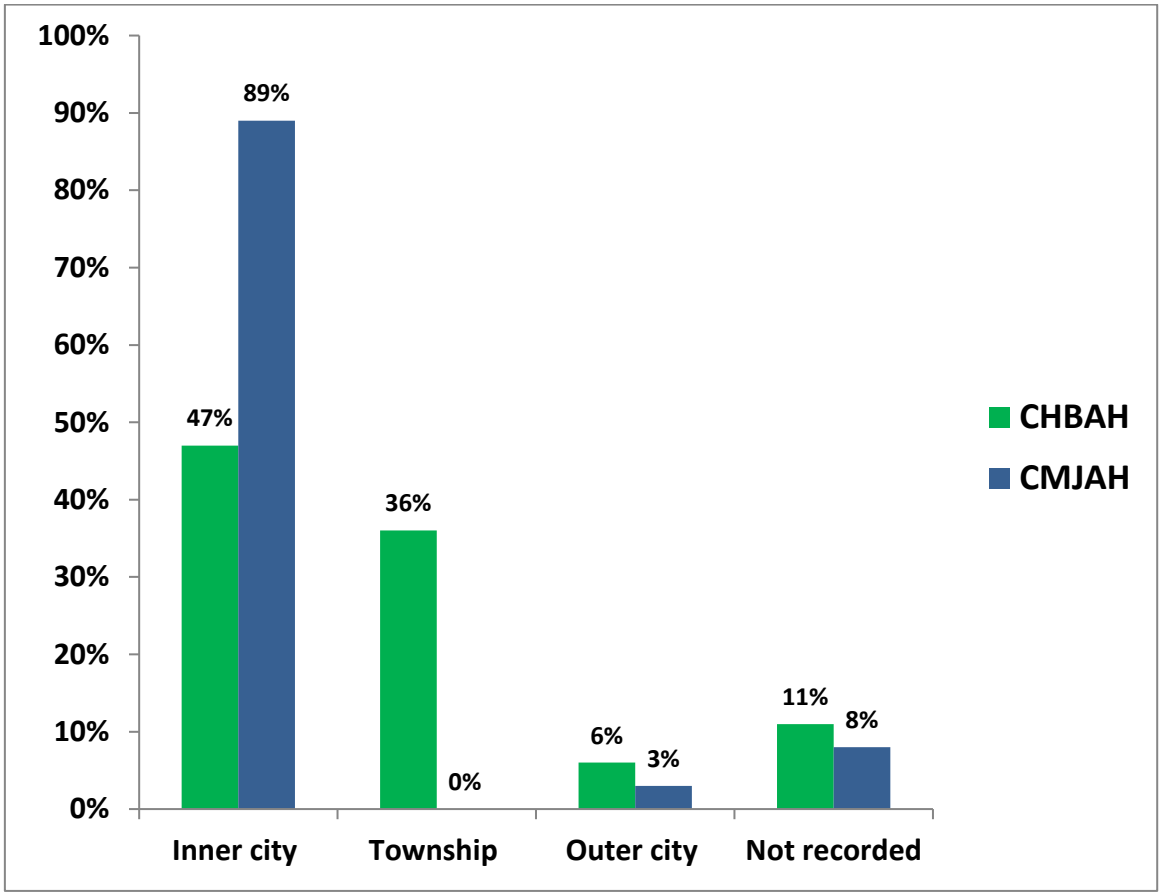
Of the 71 patients, 64 (90%) of the patients resided in Gauteng and 3 (4.2%) resided in the North West province. The remaining 5 (5.8%) did not have a residential province recorded.

The areas of residence of patients were then classified according to inner city, comprised of the areas of Yoeville, Bertrams, Troyeville, Braamfontein, Jeppestown, Berea, Hillbrow, Newtown, Fordsburg and Vredorp, township represented by the semi urban areas of Soweto and outer city.

Almost two-third (n=43/66) of the patient population resided in the inner city areas. The remaining 35% resided in the outer city or township areas. Only 5 patients had no area of residence defined.

Figure 3 shows the comparison of inner city, township and outer city residential areas of the patients at CHBAH and CMJAH. At CMJAH, there was a greater number or percentage of

patients residing in the inner city residential areas and at CHBAH the number or percentage of patients residing in the township areas was significantly greater compared to CMJAH ($p<0.05$).



*p<0.05

Figure 3.1: Comparison of the residential areas of the patients at CHBAH and CMJAH

Results further indicate that the referrals to CHBAH are characterized by 36% (n=13/36) flat dwellers, 33% (n=12/36) formal housing residents, 3% (n=1/36) in a shack and in the remaining 28% (n=10/36) of cases the type of residence was not recorded.

Referrals to CMJAH were characterized by 91% flat dwellers (n=32/35) which was greater than CHBAH ($p<0.05$). One patient was residing in a formal house (3%) and two other patients (6%) had no type of residence recorded in the files.

3.2. Referral

3.2.1 Referral origin

More than two-third of the patients were referred by orthopaedic surgeons and paediatricians to the Metabolic Bone clinics. The remaining third were mainly referred by the peripheral hospitals and local clinics (Table 3.2). CMJAH had a greater number of referrals from the orthopaedic surgeons compared to CHBAH ($p<0.05$).

Table 3.2: A comparison of the referral origins of patients between CMJAH and CHBAH

Referral Origin	All patients n = 71 n (%)	CHBAH n = 36 n (%)	CMJAH n = 35 n (%)
Orthopaedic surgeon	26 (36.7%)	9 (25%)	17 (48.8%)*
Paediatrician	23 (32.4%)	12 (33.3%)	11 (31.4%)
Peripheral hospital within Gauteng province	5 (7%)	5 (13.9%)	0 (0%)
Peripheral hospital outside Gauteng province	4 (5.6%)	4 (11.1%)	0 (0%)
Local clinic	4 (5.6%)	2 (5.6%)	2 (5.7%)
General practitioner	1(1.4%)	1 (2.8%)	0 (0%)
Metabolic	1 (1.4%)	1 (2.8%)	0 (0%)
Not recorded or missing	7 (9.9%)	2 (5.6%)	5 (14.3%)

*$p<0.05$

3.2. Reasons for referral

More than 50% of patients were referred for suspected rickets or further management of rickets and the other reasons for referral were for the investigation of lower limb deformities, abnormal gait, delayed motor milestones and hypocalcaemic seizures (Table 3.2). There was a greater number of patients referred for hypocalcaemic seizures at CHBAH (n=6/6) than at CMJAH (n=0/6) ($p<0.05$)

Table 3.3: A comparison of reasons for referral between CHBAH and CMJAH

Reason for referral	All patients n = 71 n (%)	CHBAH n = 36 n (%)	CMJAH n = 35 n (%)
Rickets or suspected rickets	38 (53.5%)	17 (47.2%)	21 (60%)
Hypocalcaemic seizures	6 (8.5%)	6 (16.7%)*	0 (0%)
Bowed legs or lower limb deformities	10 (14.1%)	5 (13.9%)	5 (14.3%)
Abnormal gait and delayed milestones	6 (8.4%)	5 (13.9%)	1 (2.9%)
Not recorded	8 (11.3%)	2 (5.5%)	6 (17.1%)
Widened wrists	1 (1.4%)	0 (0%)	1 (2.9%)
Fractures	1 (1.4%)	0 (0%)	1 (2.9%)
Screening of sibling	1 (1.4%)	1 (2.8%)	0 (0%)

*$p<0.05$

3.3. Predisposing factors:

The major predisposing factors for rickets were that of breastfeeding and lack of sun exposure in both hospitals. More than two thirds of the patients were breastfed at both hospitals (69% at CMJAH and 86% at CHBAH). Of the patient's breastfed, the mean duration was 12.96 months and a standard deviation of 6.8 months. Only 44% of the patients reported sun-exposure at CHBAH and a lower percentage of patients (23%) reported sun-exposure at CMJAH. As shown in Figure 4 below.

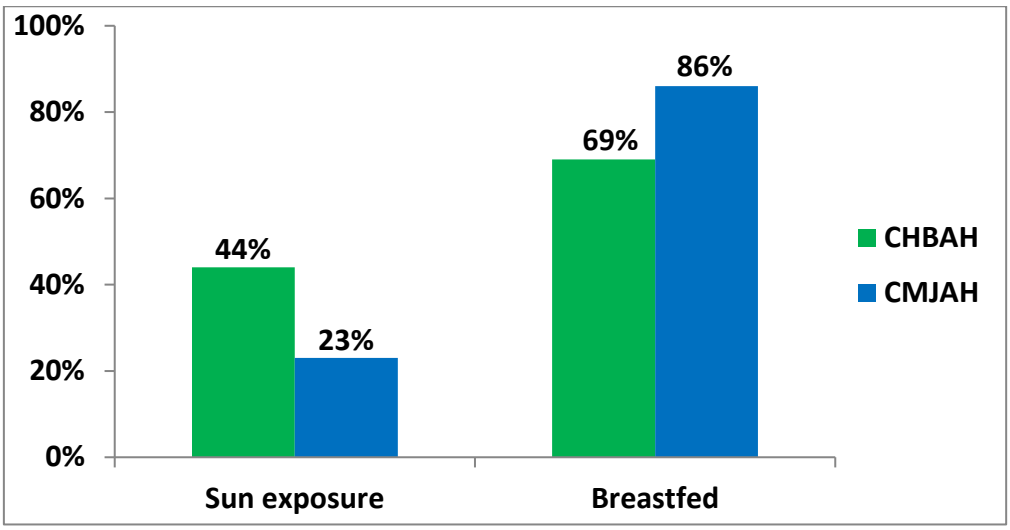


Figure 3.2: Comparisons of the predisposing factors for rickets in patients presenting at CHBAH and CMJAH

Other predisposing factors:

3.3.1. Dark skin:

The majority of patients at both hospitals are of black ethnicity and therefore dark-skinned. Thus, being dark-skinned, was not recorded in 96% of the hospital files. The remaining two patients (4%) were documented not to be dark-skinned.

3.3.2. Purdah:

In both hospitals, 97% (n=35/36 at CHBAH and n=34/35 at CMJAH) of the cases, the practice of purdah was not recorded. One patient was not practicing purdah at CHBAH whilst at CMJAH only one patient was recorded to have practiced purdah.

3.3.3. Crèche:

Sixty two percent (n=44/71) of the patients had no record as to whether the child was attending a crèche or not, 16.9% (n=12/71) were attending a crèche, and 21.1% (n=15/71) were at home. There is no significant difference between the hospitals in terms of crèche attendance and residing at home**.**

Of the 12 patients attending a crèche, more than two-thirds (n=8/12) indicated that the crèche location was a flat. The remaining third had no record of the location of the crèche in the hospital files.

3.3.4. Calcium in diet and other milk feeds:

Nearly more than a quarter of the patients (26.8%) reported a diet insufficient in calcium intake whilst 45.1% of the patients reported a diet sufficient in calcium intake and 28.2% of the patients had no records of whether their diet was adequate in calcium intake or not.

15.5% of the patients were formula fed and 18.3 % of the patients that presented at the clinics were fed cow's milk; and many patients were receiving mixed feeds with breastmilk.

3.3.5. Prior Admissions:

Seventeen (21.1%) patients had prior admissions. Of these 17 patients, 8 (47%) had bronchopneumonia or tuberculosis and 3 out of the 8 were failing to thrive. Five (29%) presented with hypocalcaemic seizures, two (12%) patients presented with femur fractures and another two (12%) patients presented with malnutrition.

3.4. Presentation

3.4.1. Clinical features of rickets on presentation:

The majority of patients (more than 80%) presented with widened wrists and rachitic rosary followed by genu varum deformities (65%), frontal bossing (44%) and thereafter approximately a third of the patients presenting with delayed motor milestones as shown in Figure 3.3 below.

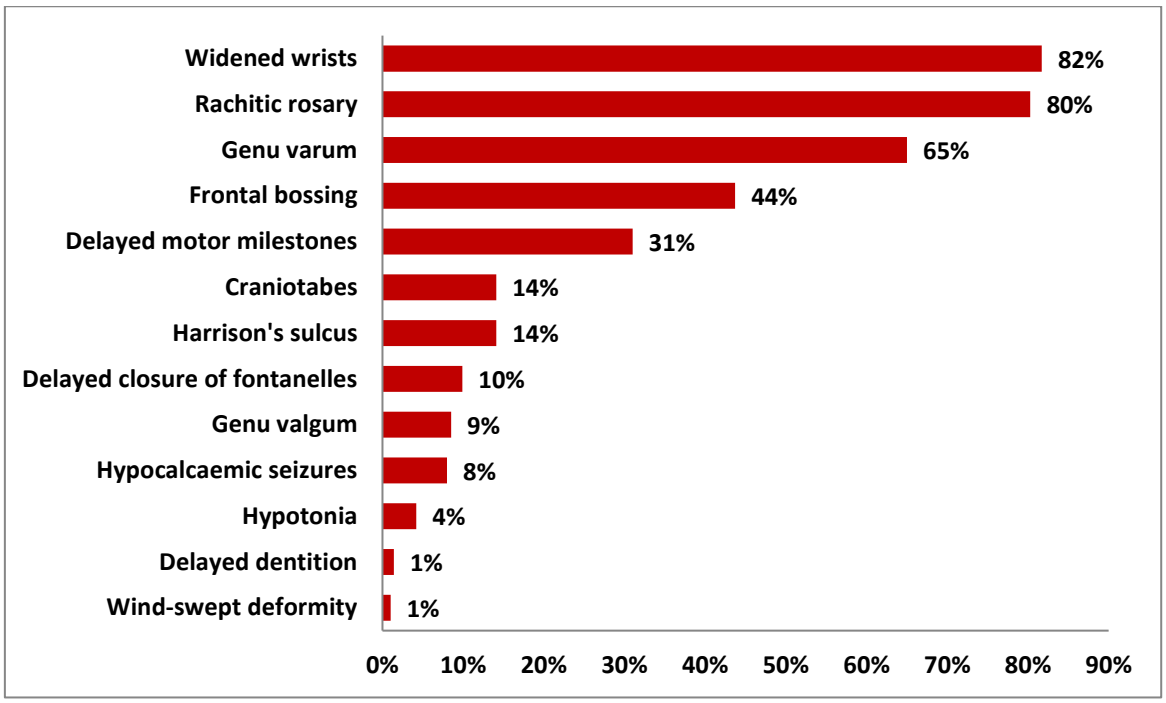


Figure 3.3: Percentages of clinical characteristic features of rickets on presentation

A comparison of the clinical presenting features of rickets between CHBAH and CMJAH are shown in Table 3.4 below. Widened wrists were found in a greater number of patients presenting to CMJAH compared to CHBAH ($p<0.05$). There were no other significant differences in the clinical presentation between the two hospitals.

Table 3.4: Comparisons of the initial presenting clinical features of rickets in patients at CHBAH and CMJAH

Clinical features	All patients n = 71 n (%)	CHBAH n = 36 n (%)	CMJAH n = 35 n (%)
Genu Varum	46 (65%)	20(56%)	26 (74%)
Genu Valgum	6 (9%)	3 (8%)	3 (9%)
Wind-swept deformity	1 (1%)	0 (0%)	1 (3%)
Not recorded/No lower limb deformities	18 (25%)	13 (36%)	5 (14%)
Widened wrists	58 (81.7%)	25 (69.4%)	33(94.3%)*
Rachitic rosary	57 (80.3%)	30 (83.3%)	27 (77.1%)
Harrisons sulcus	10 (14.1%)	8 (22.2%)	2 (5.7%)
Frontal bossing	31 (43.7%)	15 (41.7%)	16 (45.7%)
Delayed anterior fontanelle closure	11 (15.5%)	8 (22.2%)	3 (8.6%)
Craniotabes	10 (14.8%)	7 (19.4%)	3 (8.6%)
Delayed dentition	1 (1.4%)	1 (2.8%)	0 (0%)
Hypotonia	3 (4.2%)	3 (8.3%)	0 (0%)
Delayed motor milestones	22 (31%)	10 (27.8%)	12 (34.3%)
History of bone fracture	4 (5.6%)	2 (5.6%)	2 (5.7%)
Confirmed fracture on X-rays	3 (4.2%)	2 (5.6%)	1 (2.9%)

*$p<0.05$

3.4.1.1. Milestone Delay:

At CHBAH, 28% (n=10/36) of the patients displayed a delay in motor milestones which was similar at CMJAH with 34% (n=12/35) displaying a delay in motor milestones.

Of the total number of patients (n=71), 11.3% of the patients exhibited crawling as the delayed milestone, a further 11.3% reported walking as a delayed milestone, 2.8% sitting as a delayed milestone, with 1.4% of the patients reporting each of the following combinations of motor milestones as being delayed: crawling and standing; sitting and crawling; sitting and walking; and standing alone.

3.4.1.2. Tetany, stridor, laryngospasm and spinal deformities:

No patients presented with tetany, stridor, laryngospasm or either scoliosis or kyphoscoliosis.

3.4.2. Biochemical findings on initial presentation:

The biochemical findings shown in Table 3.5 were that of marginally low calcium levels, low phosphate levels with high alkaline phosphatase and PTH levels in keeping with vitamin D deficiency rickets. The median 25(OH)D levels were in the insufficient range (30-50nmol/l) in the patients from CHBAH and in the deficient range (<30nmol/L) in the CMJAH confirming vitamin D deficiency or insufficiency. The 25(OH)D levels in the group of patients from CMJAH were significantly lower compared to the group of patients in CHBAH hospital.

Table 3.5: Biochemical findings of rickets on presentation at CHBAH and CMJAH

Biochemical results	n	Both hospitals Median (IQ range)	n	CHBAH Median (IQ range)	n	CMJAH Median (IQ range)
Calcium (2.12 – 2.64 mmol/L)	70	2.18 (1.96-2.32)	36	2.15 (1.95-2.34)	34	2.19 (2-2.3)
Phosphate (1.45 – 1.78 mmol/L)	70	1.14 (0.93-1.33)	36	1.15 (0.93-1.48)	34	1.14 (0.92-1.22)
Alkaline phosphatase (75 – 345 U/L)	61	995 (692-1644)	33	980 (721-1644)	28	1043 (653-1622)
Parathyroid hormone (1.6 – 6.9 pmol/L)	51	24.2 (17.7-49.3)	29	22.2 (17.7-33)	22	44.6 (18.6-66.8)
25 (OH) D (deficient < 30 nmol/L; insufficient 30 – 50 nmol/L)	41	35 (26-45)	23	43 (31-48)	18	*27.5 (14-42)

*$p<0.05$

There was a negative correlation between the calcium and PTH levels and between phosphate and PTH levels ($p<0.05$) as shown in Figures 6a and 6b below.

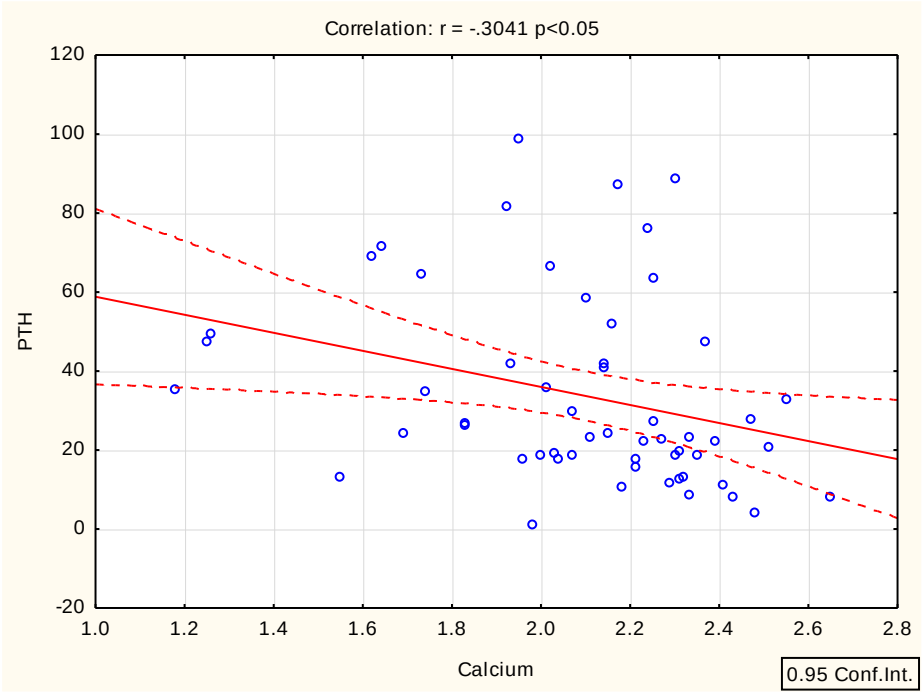


Figure 3.4a: Correlation between calcium and PTH levels on initial presentation

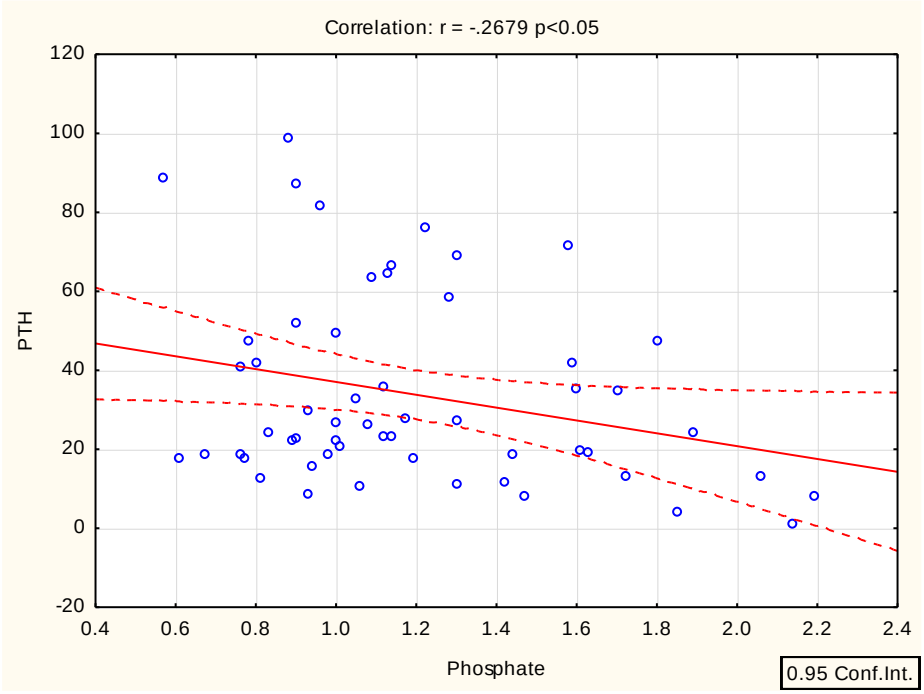


Figure 3.4b: Correlation between phosphate and PTH levels on initial presentation

3.4.3. Radiological findings of rickets on initial presentation:

Fifty six (79%) of the patients' x-rays from initial presentation and before treatment was commenced, was available for scoring by the researchers. The initial median Thacher score of the radiological findings were the same between the two hospitals and is shown in Table 3.6

Table 3.6 Thacher score of the radiological findings of rickets at CHBAH and CMJAH

	n	Both hospitals Median (IQ Range)	n	CHBAH Median (IQ Range)	n	CMJAH Median (IQ Range)
Thacher score	56	8 (4.5-10)	27	8 (4-10)	29	8 (6-10)

3.4.4. Association between biochemical findings and the Thacher scores (both hospitals combined) [Figures 3.5a-d]:

There was a negative correlation between phosphate levels and the Thacher score at initial presentation (Figure 3.5b). A positive correlation was noted between ALP and the Thacher score (Figure 3.5d).

No correlations were found between calcium and the Thacher score (Figure 3.5a) and no correlation between 25(OH)D levels and the Thacher score (Figure 3.5c) at initial presentation.

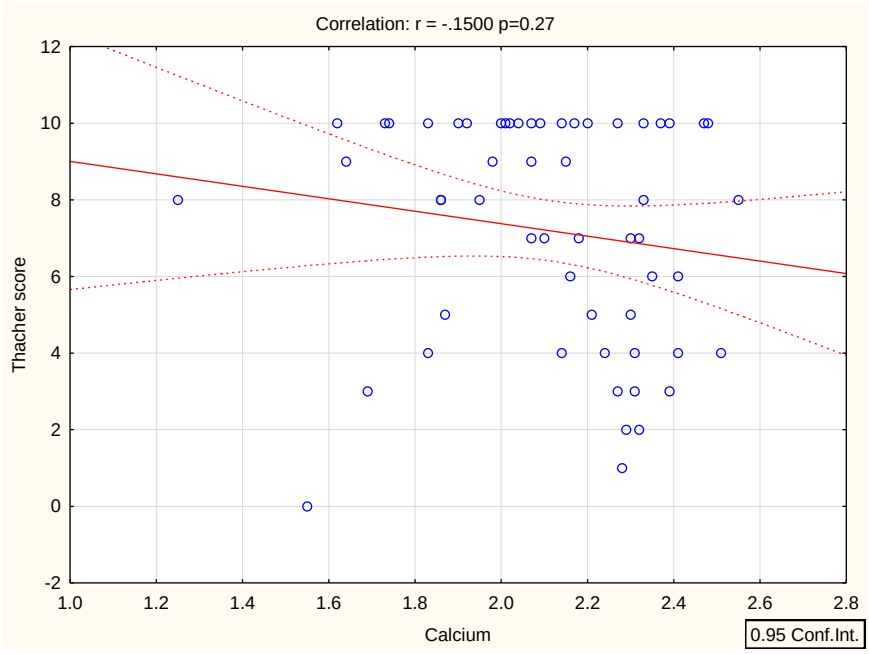


Figure 3.5a: Correlation between calcium levels and the Thacher score on initial presentation

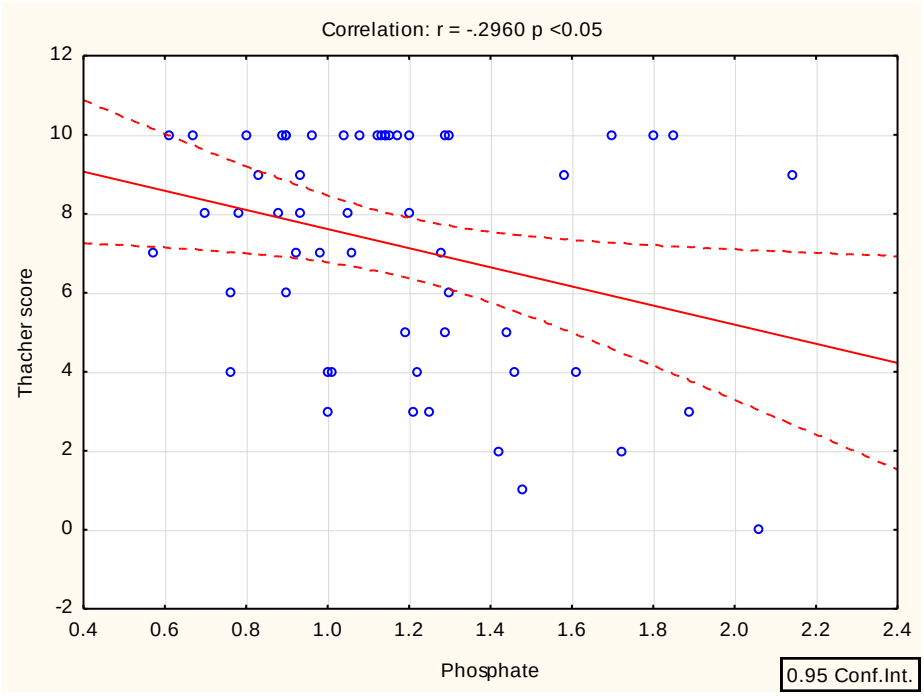


Figure 3.5b: Correlation between phosphate levels and the Thacher score on initial presentation

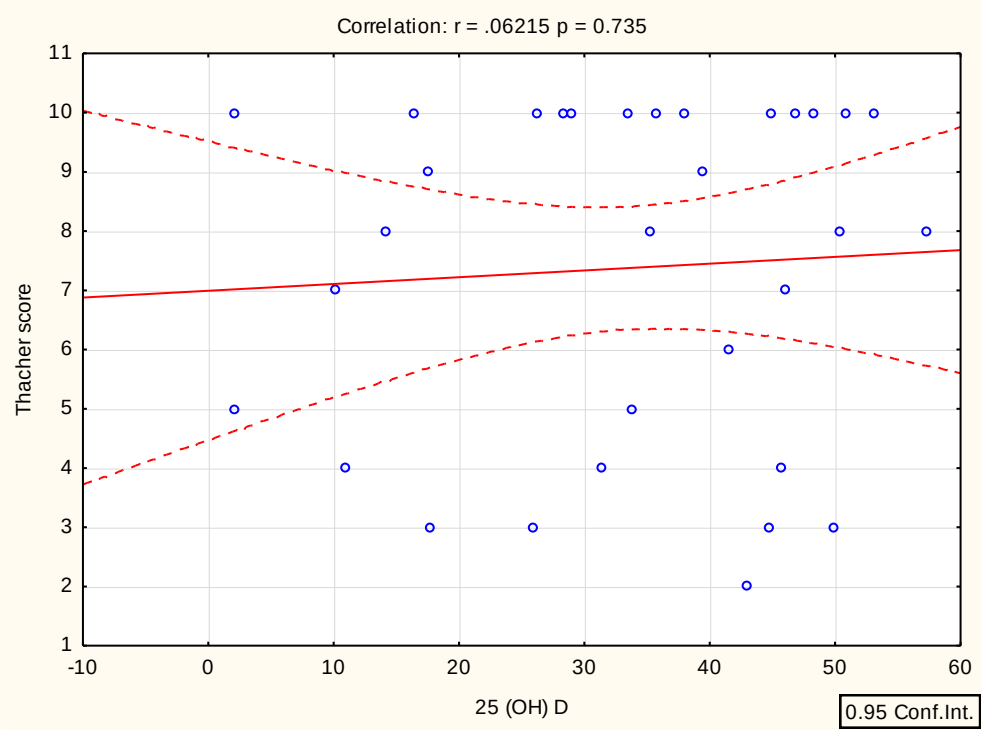


Figure 3.5c: Correlation between the 25(OH)D levels and the Thacher score on initial presentation

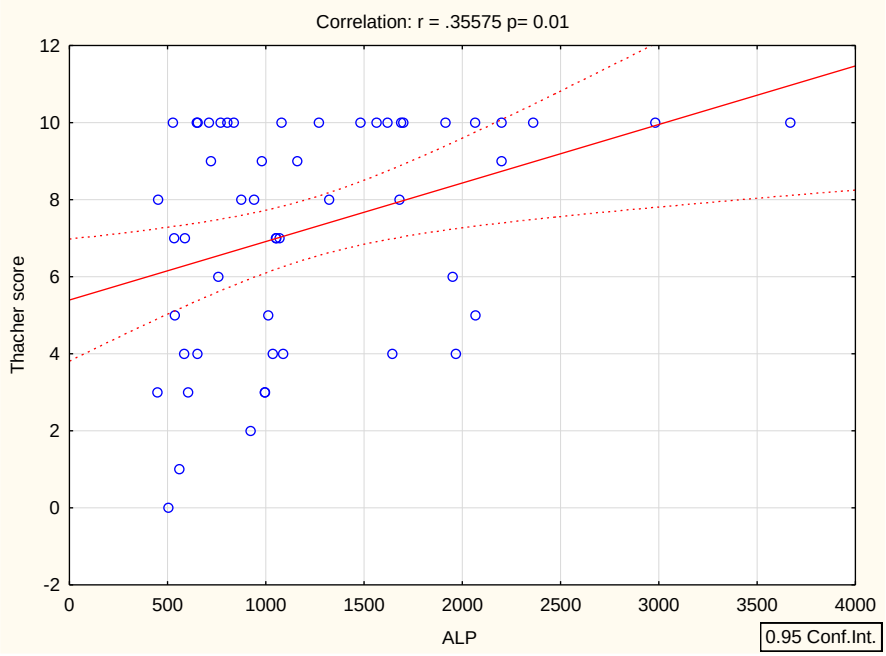


Figure 3.5d: Correlation between ALP and the Thacher score on initial presentation

3.5. Treatment

3.5.1. Treatment on referral

Sixteen out of 70 (23%) patients were on vitamin D treatment on referral, ranging between 500- 5000IU per day. There is no significant difference in the number of patients referred on vitamin D treatment between CHBAH and CMJAH (p=0.25). There was also one patient referred on alfacalcidol and three referred on calcium carbonate tablets.

3.5.2. Treatment on confirming the diagnosis

Ninety four percent (n=67/71) of patients were treated with vitamin D of which four were also commenced on either a combination of calcium carbonate or alfacalcidol. The remaining four patients (6%) were not commenced on any treatment as the patients defaulted the first follow-up visit prior to initiating therapy.

3.5.3. Defaulted Treatment

Forty six percent (n=33/71) of patients defaulted follow-ups. Of the 33 patients, only two (6%) returned to the clinic. The patients returned after 5 and 20 weeks from their last appointment or visit.

3.6. Outcome of treatment:

Table 3.7 Outcome measures of treatment

Outcome measures of treatment	n	Both hospitals Median (IQ Range)	n	CHBAH Median (IQ Range)	n	CMJAH Median (IQ Range)
Resolution of biochemical markers to normal reference ranges (weeks)	20	12 (9.5-32)	12	12 (9.5-28)	8	18 (10.5-48)
Resolution of radiological scores (Thacher score = 0) in weeks	26	16 (12-24)	14	13.5 (12-20)	12	24 (14-30)
Weeks to discharge	34	31.5 (24-56)	21	32 (24-47)	13	28 (24-96)

3.6.1. Resolution of biochemical findings

Of the 38 patients that did not default therapy or follow-ups, 27 had repeat blood investigations. Of the 27 patients, 74% (n=20) had documented resolution of biochemical findings to normal as defined by a Calcium > 2.1 mmol/L and PO4 > 1.45 mmol/L, ± PTH < 6.9 pmol/L and ± ALP <345 IU. The overall median time to resolution of biochemical makers to normal was 12 weeks or 3 months. There was no significant difference in the weeks to resolution of biochemical markers to normal between the two hospitals.

3.6.2. Resolution of radiological findings (Thacher score = 0)

Only 26 follow-up x-rays were available for scoring by the researchers M.B and supervisor, K.T. X-rays were either missing or with the parents. The overall median duration to resolution of Thacher score to zero was 16 weeks or 4 months. Despite there being a 10.5 week difference between the hospitals in resolution of radiological scores, this was not significant.

3.6.3. Weeks to discharge

34 patients were documented as discharged, 21 from CHBAH and 13 from CMJAH. The overall median duration in weeks to discharge was 31.5 weeks or 7-8 months and there was no difference between the two hospitals.

Chapter 4: Discussion

4.1. Introduction

Vitamin D deficiency rickets is experiencing a resurgence worldwide and as a result garnered attention from the academic community with both developing and developed nations exploring the disease entity in the modern context. It is an easily identifiable disease with established risk factors, biochemical and radiological features and that has affordable and accessible screening and treatment which has warranted concern over the rising incidence of a disease that could, and should be, controlled. With the newly discovered non-skeletal physiological actions of vitamin D and the potential impact on the health of a population driving new research and exploration of vitamin D deficiency and insufficiency, vitamin D has become one of the most researched entities in the international community with thousands of publications originating every year in a growing trend (55).

Demographics vary with geographical location and impact the response of a population to disease. Every region of the world displays unique characteristics that define it and are required to be investigated and described (8). While there has been a flurry of research into vitamin D deficiency in all age groups and around the globe, Africa as a region is still poorly represented with a scarcity of data in all sub-sets of the population (8, 9) and is a population to which studies from other regions would relate poorly due to the variety of endemic factors limited to the African continent. This is one of the first studies to describe vitamin D deficiency rickets in the paediatric population of Southern Africa with the only prior study to date having been conducted in black children in the province of Kwa Zulu Natal in 1995 (58)

A few studies concentrating on the paediatric population have recently stemmed from an array of countries including northern regions represented by Canada and Glasgow (1, 11), Middle Eastern regions including Saudi Arabia (5) and Turkey (7) and southern regions represented by Australia (3), a collection that is also trans-continental. A striking absence is data from Southern American and African countries (9), with little to no recent data collection despite the other studies indicating an increasing trend in the prevalence of vitamin D deficiency rickets. The majority of research originating from Africa stems from Nigeria with the focus on nutritional rickets secondary to calcium deficiency or a combination of vitamin D and calcium deficiency, as opposed to rickets due to an isolated vitamin D deficiency and in which vitamin D deficiency is explored more as a contributory factor instead of the principal cause of rickets (56).

Limited studies have been published describing the afflicted population of Southern Africa with only one from the greater Johannesburg area, in urban children of 10 years of age (57), an area which has a significant populace. This study supported global findings regarding the effect of skin pigmentation with white children demonstrating a significantly higher 25(OH) D than their darker skinned counterparts as well as demonstrating a seasonal difference with higher 25(OH)D levels observed in the autumn and summer months (57). However, data suggested that vitamin D deficiency and insufficiency in 10 year old children in Johannesburg was a relatively uncommon occurrence with an incidence of only 7% deficiency and 19% insufficiency (57). A second study conducted in Kwa-Zulu Natal documented the incidence of Rickets in a total of 37 children aged 1-12 years. This study included a subset of vitamin D deficient patients (n=9/37) but the majority of data on this subset was reported as a group with other nutritional Rickets including calcipaenic Rickets (58). This study aimed to describe the patient subset presenting with clinical and biochemical features suggestive of vitamin D deficiency rickets attended to at the Metabolic Bone clinics in Johannesburg and collect and interpret data on risk factors, biochemical measurements and radiological scoring for identification, education, management and future research purposes.

4.2. Demographics

4.2.1. Ethnicity and gender

In global studies, ethnicity has been considered an important factor as immigrants and native populations comprise the majority of the study cohorts. Immigrant populations reflect geographical locale, with the highest proportion being from those areas adjacent to the area of study and include African, Asian, Indonesian, Indian and Hispanic sub-sets (1, 3, 11, 59). An important consideration is the darker pigmentation of these populations, both the immigrant and native populations occurring at a higher frequency, which is an independent risk factor for vitamin D deficiency. Immigrant populations reflect the vitamin D status of their country of origin as opposed to their adopted country of residence which contributes to a higher incidence as often immigrants come from countries with a poorer vitamin D status (3). Immigrants are often of lower socio-economic status and education, both factors that have been linked to an increased incidence (6, 7). Diet is another component of ethnicity affecting the variation in calcium and phytate content that occurs with the differences in food practices employed across the globe. Ethnicity can thus be interpreted to have many contributing factors which are difficult to isolate.

Ethnicity is difficult to establish in the African environment as it is often simplified to the detriment of important contributing factors. Due to the immigrant status of Johannesburg, patients hail from a multitude of countries and tribes that superficially may appear similar but have societal, behavioural and environmental practices that bear influence on risk factors. As immigrants do not often confide in their country of origin for a number of reasons including legal status and stigma, it remains too complicated to divide the population into more accurate groups in terms of country of origin. To explore the tribal implications would require a more in depth knowledge and assessment than is routinely available at time of presentation due to resource constraints. Therefore, ethnicity was limited to native African, Coloured, Indian or Caucasian in the context of our study.

The majority of patients were black (97.2%) and is influenced or biased by the general population and the populace of inclusion in the drainage area of the institutions. The predominance of black patients almost certainly also reflects the impact skin pigmentation has on production of vitamin D, with decreased dermal synthesis requiring 5 – 10 fold higher exposure to sunlight to produce quantities equal to that of a non-pigmented individual (30, 57). The impact of skin pigmentation on the incidence of vitamin D deficiency or insufficiency is well documented and is observed in darker skinned native populations and African Americans in studies from around the globe (11, 59) including studies from Canada reporting 89% of affected individual as intermediate or darker skinned (11) and America where 83% of affected individuals were documented as African American or black (59).

The majority of patients in this study were male (57.7%), a statistically insignificant finding that might suggest that this is an indication of the general populace as opposed to a predilection of the gender to the disease entity. Globally studies have indicated male gender dominance with some countries reporting a majority that may merit gender interpretation on the global scale to establish if there might be a statistically significant difference (3, 7, 11). The study from Sydney proposed that cultural influences form a selection bias in families choosing to breastfeed male infants over female infants which contribute to the differences observed in gender affliction through the effects of breastfeeding on the development of vitamin D deficiency (3).

4.2.2. Chronological age and anthropometric measurements

Age of inclusion varies greatly amongst studies including children of up to 18 years in some reports with a few studies concentrating on infants and children. Despite the differing inclusion criteria in the different studies, the results from this study were comparable. For the purposes of our study, the age of 5 years was chosen to represent what was experienced to be the most affected age group in the study population. The mean age at presentation of 18 months at CHBAH and 24 months at CMJAH differed by 6 months ($p<0.01$). The areas serviced by the two clinics are diverse in nature and the differing maternal and environmental risk factors may be responsible for the variation in age of presentation. The older age at presentation in the patients from CMJAH may be due to the greater number of patients residing in flats even after they start walking and are not sun-exposed compared to the patients from CHBAH who play outside and are sun-exposed. In addition, confounding societal factors may be contributing to the negative approach to health services by parents at CMJAH resulting in delayed presentation.

A more detailed assessment of risk factors and health seeking behaviour of patients and parents attending the two hospitals would be required before an explanation for the differing age at presentation could be determined. Despite the variable age ranges of global studies, means and medians in all were reported in the range of 14-24 months (1, 3, 5, 7, 11, 59) which compares with our mean age findings which support historic documentation of a susceptibility in this age group.

Vitamin D deficiency has scientifically been linked to a reduction in height and growth velocity through its effects on bone metabolism (60). Metabolism is affected by the three components of development: growth, body composition and biological events. The late foetal and early infant period are the most rapid growth phases during which calcium deposition occurs and thus disturbances of calcium metabolism would be expected to be the most prevalent during this time period. Although stunting is a recognised feature, few studies report epidemiological data on height and weight with the only recent study providing detailed anthropometric data stemming from Sydney (60) and limited data contributed from Saudi Arabia (5). Data from Sydney reported mean standard deviations (SD) of -1.01 for height and -0.85 for weight, statistically significant at a p value of <0.001. Subjects in our study had a more noticeable reduction in HAZ and WAZ from the general population with mean Z score of -2.83 and -1.33 respectively. The differences between these two studies might be contributed to by the differences in study populations. A delayed presentation in our context

that would place patients in a more advanced state of disease and the differences in nutritional status influenced by poorer socio-economic status in South Africa, may further worsen the growth status of our patients. Data for head circumference is only reported in one previous study from Saudi Arabia at a statistically significance difference (p=0.03) of 5.07cm greater than the average population suggesting a relative increase in head size. We did not assess head circumference in relation to the rest of our population and not all children had head circumference measurements recorded in the hospital files. The WAZ and BAZ scores were lower in the CHBAH group compared to the CMJAH group possibly due to the earlier insult on growth in these patients at CHBAH but the HAZ score was significantly low in both groups indicating severe stunting.

4.2.3. Areas of Residence

South Africa bears some defining characteristics in terms of populations served due to the availability of specialty services being limited to major, centralised hospitals. This impacts on study dynamics as the patients seen at the clinics encompass an abnormally broad geographic base. The gross majority of patients originated from Gauteng, which is to be expected in view of the areas the hospitals originally service. A small proportion of patients derived from the North West which may be explained by the referral protocol of lower level care institutions adjacent to Gauteng and the specialist services provided in the tertiary level care centres in their province of origin.

The areas were further divided into settlement categories of inner city, township and outer city. This is an important distinction as inner city and township dwelling imparts an inherent increase in exposure to risk factors contributing to vitamin D deficiency due to the lifestyle adopted in these areas that includes limitation in sunlight exposure, increased air pollution and absence of external public community areas. Sixty eight percent of patients resided in the inner city and this finding is comparable with Canada in which two thirds of their study participants was defined as city dwellers. A statistically significant portion of patients attended to at CMJAH were from the inner city compared to the patients at CHBAH ($p<0.05$) whilst patients attending the CHBAH resided in the township areas in comparison to CMJAH ($p<0.05$) which once again reflects the area demographics as CHBAH provides health services to areas with a number of informal settlements and the hospital is located adjacent to Soweto.

Dwelling type imparts significance in terms of economic and environmental impact on risk factor exposure. The most obvious being a limitation in sun exposure associated with flat occupation. When assessing dwelling type, the majority of CMJAH patients resided in flats compared to a statistically smaller proportion from CHBAH ($p<0.05$)

Overcrowding, smaller houses, lower income and lower levels of education (56-59) are factors that have previously been associated with an increased incidence of vitamin D deficiency rickets and are factors more frequent in inner city and township habitation (6, 7, 61). These factors can be inferred in our populace but cannot be accurately correlated as this was not part of the data collected and often is not accurately recorded in hospital files.

4.3. Referral

4.3.1. Referral Origin

Due to the specialty based nature of the Metabolic Bone clinic, prior assessment and referral from an original health care contact is required on which to base a provisional diagnosis that would benefit from the services provided at the clinics. The majority of patients seen at CMJAH were referred from orthopaedic surgeons (49%); and may reflect internal referral protocol or be influenced by pattern of presentation. This is a far higher proportion than Glasgow where a mere 15% of patients were of orthopaedic origin (1). When taking into consideration the large proportion of patients presenting with genu varum deformities (75%) of the lower limbs, this referral pattern may reflect those patients with lower limb deformities seen by orthopaedic surgeons requiring surgical intervention. Although there is limited data from prior studies documenting the point of referral of patients, there is a recent study from orthopaedic surgeons in Britain (62) identifying 75 patients with associated vitamin D deficiency or insufficiency in the presence of lower limb deformities, which may suggest that orthopaedic surgeons are an important contributor to their diagnosis and introduction to care. Approximately a third (33.3% at CHBAH and 31% at CMJAH) of patients seen at each hospital were referred from paediatricians which is unsurprising as most often this is the first contact with specialised care. Glasgow reported referral origins of 18% from the Outpatient department, 15% from Accident and Emergency and 38% from primary care physicians, a role which would be fulfilled by our primary care clinics. Although only a small number in this study were referred from local clinics, it is likely that most patients were originally

assessed at a local clinic and referred to an array of specialists prior to their final transfer to the Metabolic bone clinic.

4.3.2. Reasons for referral

Vitamin D deficiency and insufficiency has a multitude of clinical presentations that have only recently begun to be explored and described. The most overt and well known of the spectrum are the bony malformations that are relatively easily identified clinical features. The majority of patients were referred with a provisional diagnosis of rickets (53.5%) and this diagnosis is most often based on the clinical detection of bony abnormalities. The clinic has a specific scope of practice and the referral must be related to the patient subset served so it is unsurprising that the largest proportion already had a refined diagnosis. CHBAH, which had a younger subset of patients, had a noticeable greater number of referrals for hypocalcaemic seizures. Hypocalcaemic seizures are a presentation of rickets that occurs predominantly in infants which may contribute to the younger age of presentation at CHBAH compared to CMJAH. The remaining majority of indications for referral encompassed limb deformity and gait abnormalities, which might reflect the origin of referral trend being that from orthopaedic surgeons or the shared burden of diagnosis between orthopaedic surgeons and the Metabolic bone clinic for the patients that are identified with these abnormalities at the local clinic or district hospital level. Global studies have no detailed data regarding reason for referral with the majority using inclusionary criteria of an already confirmed diagnosis of rickets at the paediatrician or sub-specialist level (5, 7, 11, 59, 63).

4.4. Predisposing factors

A number of studies looking at the prevalence of specific risk factors in vitamin D deficiency have been published but most of the recent studies investigating vitamin D deficiency rickets in the context of a specific population concentrated mainly on those risk factors most commonly associated with vitamin D deficiency. The two dominant predisposing factors in our study were breastfeeding and lack of sun exposure, both of which are well established risk factors with breast milk being deplete in vitamin D (44, 46) and sun exposure being responsible for the conversion of vitamin D into its active metabolite (23).

Breastfeeding has been suggested to be the most important factor in the development of vitamin D deficiency with prolonged breastfeeding being well associated with an increased incidence throughout the world, a finding that has been repeatedly supported in many studies

(1, 2, 5, 7, 11, 43). This is primarily due to the low vitamin D content of breast milk, which contains 1.5-3% of maternal serum vitamin D levels at an estimated range of 20-60IU/L (46). Unmetabolised vitamin D is the major contributor in breast milk with only very small quantities of the biologically active metabolites 25 hydroxyvitamin D and 1,25 dihydroxyvitamin D transferring into breast milk (44). The low content in breastmilk is exacerbated by the prevalence of vitamin D deficiency in at risk populations within the child bearing age such as the African American population of the USA which reports that 41% of potential mothers are deficient by the end of winter (64). The half-life of 25 (OH)D is 2-3 weeks and after the neonatal period, the 25(OH)D pool derived from placental transfer will have been exhausted. The neonate has a reduced capacity to buffer the vitamin D insufficiency of breast milk and the adaptation to deficits begins (44). Compounding this natural deficit is the vitamin D deficiency trend of pregnant and lactating mothers that has been reported to be as high as 97% in African-Americans (55). Seventy seven percent of this total study population were breastfed which is similar to the Saudi Arabia study (80%) (5) whilst a lower percentage of breastfeeding was reported in Turkey (59%) (7) and a much higher percent in Canada (94%) (11). Duration of breastfeeding holds additional relevance over and above the established impact of low levels of vitamin D in breastmilk and furthermore, the clinical signs and symptoms of vitamin D deficiency are the overall manifestation of the disease process that progresses over a long period of time with on-going deficits. Our mean duration of breastfeeding of 12 months compared well to that of 13.8 months from the Canadian study (11) perhaps representing the end of the disease spectrum and degree of vitamin D deficiency required to present clinically and would be applicable to future interpretation of dietary history in assessing vitamin D status.

It is scientifically supported, through the established metabolism of vitamin D, that sunlight is the primary contributor to the production of vitamin D in the human body with 90% of total body requirements met by dermal synthesis (10, 23). Sunlight exposure has two important considerations: geographical location of latitude and seasonal variation associated with tilting of the earth's axis, both of which influence the angle of entry and hence the effectiveness of UVB radiation (38, 65). Johannesburg is of a more temperate climate due to location at latitude of 26^{O}S with a daily average of 8.76 hours of sunshine/year. A study performed in 10 year old urban children of Johannesburg reported no seasonal variation in 25 OH D levels in black children implying that seasonal variation is non-contributory in our setting or in Johannesburg (57). In vitro study of vitamin D synthesis has confirmed that latitude may be influential with decreased production documented in Cape Town but in Johannesburg in vitro

production was adequate (38). Seasonal and geographical variations are unlikely to be contributing in Johannesburg and thus inferring that lack of sun exposure is behavioural. Sun exposure was limited in 56% of patients at CHBAH and 78% of patients at CMJAH with a total of 66.2% having limited sunlight. The difference (though not statistically significant) is likely to be related to the areas in which patients reside; with the inner city and flat-dwelling population of CMJAH reporting less sun exposure due to the limitations of their environment. Urban centres from international studies have reported an increase in rickets relative to rural or undeveloped areas with air pollution and overcrowding being identified as contributory factors but other factors not having been well elucidated (7, 40, 61, 66). This supports our hypothesis that sun exposure is an important factor in contributing to vitamin D deficiency rickets but the role of breastfeeding which was reported in the majority of the patients, needs to be acknowledged. Studies from Canada, Sydney and Saudi Arabia also report significant sunlight restriction with comparative values of 89%, 89% and 90% respectively, indicating that the presence of sunlight does not ensure exposure (4, 5, 11). With the recent increased awareness of the damaging effects of UVB radiation in the development of cutaneous carcinoma, lack of sunlight exposure is becoming increasingly important as a cause of vitamin D deficiency as populations actively avoid it (35, 67).

Two factors assessed independently but related to sun exposure were crèche attendance and purdah. Two thirds of the group attending crèche had a crèche located in a flat, an environmental limitation of sun exposure. The practice of purdah limits skin exposure to sun. Our hospitals do serve a small percent of foreign and national Muslims that are known to practice purdah. Unfortunately, purdah was not well documented with only a single confirmed case practising and another single case confirmed not to be practising purdah. The explanation for absence of documentation of practising purdah lies in the assumption that it is only customary in the minority of the population and it is assumed that the patient does not ascribe to it unless specifically stated otherwise. As a result this risk factor cannot be accurately assessed in the context of this study. In areas where one would expect purdah to have a significant impact, there is also limited data. Saudi Arabia did not explore it independently of sun exposure but Turkey indirectly may have assessed the practise of purdah, in terms of detailed descriptions of infant and maternal clothing covering (6, 7).

Another risk factor that could not be accurately assessed was skin pigmentation. Skin pigmentation reduces the capacity for dermal synthesis up to 50 fold and is a risk factor that has been repeatedly established as important in the development of vitamin D deficiency (36). One of the inherent flaws in assessing skin pigmentation is the difficulty in objective

quantification with studies often using subjective descriptions. The gross majority of patients attended to at CMJAH and CHBAH are black and there is hardly any indication to document skin pigmentation formally in the hospital files unless there is a deviation from the expected norm and relevance to the presentation. It is therefore assumed that unless stated otherwise, the patients are black. As a result, there was no documentation regarding skin colour in 96% which corresponds with the fact that majority of the patients (97%) were black with the remaining 4% being documented not to be dark skinned. This would constitute as a limitation of the study in reviewing this particular risk factor and is mainly due to the retrospective collection of data. From global studies, two reported on skin pigmentation with the USA study reporting a majority (83%) of African-American/black patients being dark-skinned (59) and Canada reporting 89% of dark skinned individuals from their native population (11).

Diet is a difficult factor to accurately assess in terms of both calcium and vitamin D content. A diet sufficient in calcium is negated in the absence of vitamin D and the ability to absorb the calcium, reduced to 10-15% (68). Assessing vitamin D in the diet is difficult when the diet does not contain fortified food and vitamin D supplementation. The majority of vitamin D is produced by the skin with a limited dietary contribution. However, with modern society introducing greater limitations to sun exposure with concerns over skin carcinoma, dietary vitamin D is becoming an important consideration. The most significant contribution to dietary vitamin D is fortified food which remains loosely regulated and required daily allowance (RDA) may not meet the adequate requirements in the presence of counteracting substances. A quarter of patients reported a diet insufficient in calcium but almost half (45.1%) reported adequate calcium intake. If our patients with sufficient calcium intake had an isolated vitamin D deficiency, the hindrance in calcium absorption might render them equal to a dietary calcium insufficiency. Superimposed calcium insufficiency may also present in a more pronounced clinical picture of vitamin D deficiency but this was not explored in these patients. Although a diet may be sufficient in calcium, absorption is paramount and a factor that was not explored in this study was the presence of phytates and oxalates which impair calcium absorption and are often used in low-income environments as supplementary food (69). These supplementary foods often comprise what are considered to be staple foods (69). The role phytates and oxylates have on calcium absorption has only recently been explored and at the time of diagnosis for the earlier patients, was not well established. Their presence would result in a relative insufficiency and contribute to dietary inadequacy in calcium content. As staple food is often targeted for fortification, it is important to regulate addition of vitamins to ensure adequate amounts are provided. In the presence of

phytates and oxylates, it is uncertain if sufficient vitamin D supplementation is provided in the most commonly used staple diet items of South Africa.

Recently multiple studies have begun to hypothesise a link between vitamin D and multiple aspects of health and disease that are commonly associated with the effects that vitamin D has on the immune system(55). If vitamin D influences a wide variety of health facets it could be postulated that patients with vitamin D deficiency or insufficiency might present with a number of clinical scenarios resulting in admission. A fifth of patients in this study had prior admissions of which almost half of these patients were diagnosed with bronchopneumonia and tuberculosis (TB). New studies are exploring a possible link between vitamin D deficiency and pneumonia, having discovered vitamin D deficiency in patients admitted for severe acute lower respiratory tract infections. A number of studies support this hypothesis with one particular study from Nigeria describing a 13 fold increase of rickets in patients under 5 years presenting with pneumonia (70), one from Karachi identifying pneumonia as a significant presentation of vitamin D deficiency (71) and a Tehran study from as early as 1985 identifying 43% of rachitic children afflicted by pneumonia (72). Similar to the association to bronchopneumonia, lower levels of vitamin D have also been discovered in patients with TB with Mycobacterium Tuberculosis being isolated as an organism to which vitamin D dependent immunity responds (55). With the contribution that vitamin D is hypothesised to have in terms of innate immunity and microbial defence, it seems probable that deficiency may be related to TB infection. Further studies will be required before these hypotheses are proven but the admission pattern is interesting in view of these suppositions. One factor that has been proven to be contributory in the development of lower respiratory tract infections in the context of vitamin D deficiency is the bone abnormalities which give rise to softened ribs resulting in decreased efficacy of breathing and coughing required to clear infection. A history of prior admission could also be responsible for identifying vitamin D deficiency rickets and referral to a specialist level. This is almost certain in the third of admissions presenting with hypocalcaemic seizures and the tenth of patients presenting with femur fractures in which vitamin D deficiency rickets was considered as the underlying diagnosis in pursuit of identifying a cause for the fractures.

4.5. Features on presentation

4.5.1. Clinical features of rickets on presentation

The clinical features most commonly associated with rickets are bony abnormalities which are subsequently the most commonly reported features. One element that needs to be taken into account in interpretation of bony abnormalities is the effect of vitamin D on growth velocity. Stunting as a feature of the clinical presentation of vitamin D deficiency masks the manifestation of bone abnormalities which requires growing bones. Subsequently, patients with significant stunting could potentially manifest fewer bony features or abnormalities. The majority of patients presented with widened wrists and rachitic rosary. These clinical features were not assessed as a qualitative measurement in prior studies but rather as a constellation of clinical features commonly seen in rickets. This correlates well with a study performed in Nigeria concentrating on clinical presentation of rickets in which widened wrists and rachitic rosary were identified as the most commonly encountered clinical features (50), a finding which was reinforced in two recent studies, one of which was repeated in Nigeria conducted by different investigators and one in Eastern Turkey (7), with the same results. Thickened wrists and a rachitic rosary were also noted as presenting features in all patients with rickets in the cohort investigated in Kwa Zulu Natal, South Africa, which further supports these findings in a more local context (58). Wharton found the wrist bracelet of widened epiphyses and rachitic rosary to have a specificity of 81% and 64% respectively in the context of diagnosing vitamin D deficiency rickets and wrist widening to be the most common presentation in infancy(73). This would infer that finding a large proportion of patients with these features would bear significance in our context. Widened wrist epiphyses were found in a greater number of patients in CMJAH in comparison to CHBAH ($p<0.05$). This may be due to the differences in age at presentation with CMJAH having an older cohort, greater severity of the disease and more prolonged exposure to the disease. There are no studies documenting the progression of specific bone deformities with age, in patients with vitamin D deficiency, so there is no reference point to compare the different age groups in terms of clinical presenting features of rickets.

The next most common clinical finding was frontal bossing, another feature not well reported in prior studies. A recent Nigerian study reported frontal bossing in only a 10^{th} of the cohort, an incidence even below that of craniotabes and differing markedly from our finding in which it presented in almost half of the study population.

Abnormalities of the lower limbs in prior studies were often loosely described as bony deformities or bowed leg and the definition of which was not fully explored. Bowed legs were present in a range of 10% to 40% (22.5% in Sydney (3), 40% in Glasgow (1), 10% in Saudi Arabia (4). A few studies report skeletal and bony abnormalities on presentation but do not quantify or describe them in detail (59, 63). In this study, three quarter of the patients presented with genu varum deformities, 9% with genu valgum and 1% with wind-swept deformities which is a greater prevalence of lower limb deformities compared to other countries. This may be due to more severe and long standing rickets being seen at these specialist clinics.

Milestones are difficult to interpret as delay is influenced by a number of factors that affect development but is still identified as a feature of rickets. Hypotonia which results from vitamin D deficiency is likely to contribute to delay in milestones but is not reported as often. The motor milestone delay of vitamin D deficiency rickets is likely to originate from a combination of hypotonia, bone pain, fractures and irritation. Delayed motor milestones were present in approximately a third of patients which was greater than global studies which reported values ranging from 2% in Glasgow to 11.5% in Canada (1, 11). Hypotonia was reported in only 4% of patients which is markedly different from the 78% reported in the vitamin D deficiency rickets cohort from Kwa-Zulu Natal (58). The disparity between the international and local incidence of milestone delay as well as that between the incidence of hypotonia in rural and urban South Africa may be attributed to the wide variety of contributory factors including socio-economic and nutritional status.

Hypocalcaemic seizures occur most commonly and usually as the only clinical manifestation in infants and are hypothesised to be related to either a refractory response to PTH or to the increased metabolic demand for calcium in the period of accelerated growth up to 6 months of age (74). Hypocalcaemia in this age group is compounded by the natural nadir encountered in infancy as calcium levels adjust from foetal to adult values and there are a number of contributing factors including reduced 25 (OH)D pools, due to limited placental transfer, and the low vitamin D breast milk content in vitamin D deficient mothers (44). Hypocalcaemia seizures accounted for only 6% of the presentations and were reported predominantly in infants < 7 months of age, less than global reports ranging from 12-41% (1, 4, 7, 11, 74). Despite the documentation of hypocalcaemic seizures in the adolescent group (74), likely secondary to increased metabolic demands for calcium, the Canadian study which included adolescents reported that the gross majority (85%) of patients presenting with hypocalcaemic seizures were less than one year of age (11). Additionally, Turkey's cohort comprised of

children exclusively below 3 years of age and reported an incidence of 40% (7). These findings infer that in a population group including infants, a significant portion should present with hypocalcaemic seizures. The inconsistency of our results may be accountable to missed diagnoses as vitamin D deficiency rickets is not commonly recognised as a cause of hypocalcaemic seizures and the prevalence of vitamin D deficiency in our infant population in Soweto is low (4.6%)(75). Contrary to the impaired response to the PTH hypothesis, only 4 out of 8 infants that were 6 months or younger had PTH levels above the median. It would be expected that these levels should be higher or elevated in the event of hypocalcaemia as a refractory response.

4.5.2. Biochemical findings on initial presentation

Biochemical markers are an objective measure of disease activity and the only means by which to definitively prove vitamin D deficiency and insufficiency. The biochemical markers also fluctuate in the course of the disease helping to establish in which stage the patient presents with vitamin D deficiency rickets and how advanced the process is at presentation (51). As a result of the adaptation process that occurs in the presence of vitamin D deficiency, a reduction in calcium causes an increase in PTH and a reduction in phosphate is expected secondary to decreased renal absorption and there is an elevation in ALP, in response to osteoclastic destruction of bone. Biochemical findings in this study were in accordance with anticipated values of low calcium and phosphate, and elevated ALP and PTH levels.

Serum calcium levels drop in response to decreased intestinal absorption but are buffered by the PTH mediated increase in renal absorption and release from bone (51). A reduction in serum calcium is an indication of advanced disease as indicated by the inability of bone to maintain the required levels. The calcium levels were marginally low at a median of 2.15mmol/L (CHBAH) and 2.19mmol/L (CMJAH). Forty eight percent of patients in this study had a calcium <2.1mmol/L which is similar to findings from studies in Sydney (52%) and the USA (55%). Saudi Arabia reporting hypocalcaemia in more than 30% (5) of patients but did not quantify the exact cut-off level (4). The Turkey cohort, described a range of values and reported on the lower measurements at the bottom of the range which would suggest that their study group had more extreme hypocalcaemia (7), usually an indication of more advanced disease. The milder deficits in our study would suggest that the adaptive processes had not yet depleted bone reserves in the effort to maintain normal values.

Expected normal values for serum phosphate are higher in children than in adults and are age dependent with reference values dropping as the age of the child advances. To accurately interpret phosphate, the age of the child is required to assess deficits. This imparts a difficulty in determining hypophosphataemia in a study population comprising of children of differing ages with different reference values. For the purposes of interpretation in our study, a level above 1.45mmol/L was used to describe normal values. Median phosphate levels were below normal at 1.15mmol//L with 80.3% of the study population falling below the normal reference level. This was a greater proportion of patients with hypophosphataemia compared to 64% reported in patients in the USA study, although hypophosphataemia was not clearly defined in this study (59). Sydney reported means of 1.32mmol/L in patients above 6 months of age (3) which is below normal but relatively higher than medians obtained in other studies (5, 7).

Alkaline phosphatase (ALP) is not specific to bone in origin but is used as a marker of bone destruction in the context of vitamin D deficiency and will still be applicable in this context as the majority of ALP will be derived from bone. Using bone specific ALP allows investigators to isolate the origin and is now available and in practice making results more accurate. Unfortunately at the time of diagnosis for a proportion of study subjects, bone specific ALP was not yet available. Similar to phosphate, the reference values change with age in a triphasic pattern of distribution, dual peaks being reached in the age group less than 6 months of age and at puberty. Values above 350U/L are generally accepted as abnormal (4, 76, 77). In interpreting the ALP of our patients less than 7 months of age, five out of eight (62%) patients had markedly elevated levels (>800U/L) and the remaining patients had values ranging from 373-562U/L. In addition, 33 patients above this age range had ALP >800U/L suggesting that the elevations observed in this study was not related to age specific peaks. This would infer that we could attribute an elevation in ALP in this context to be due to pathological changes although age could be influential. The median ALP values of 980U/L (CHBAH) and 1043U/L (CMJAH) were slightly lower than those of Sydney (1237U/L) and Turkey (1341U/L) which also expressed a wider range with more extreme values at the lower and upper ranges. Ninety one percent of patients in this study had an ALP > 500U/L, higher than the 82% reported in Sydney (3) and 75% in Saudi Arabia (5) but less than the 99% reported in the USA (59), although the definition for elevated ALP for USA study and Sydney was not described and could be lower than the threshold in other studies (1, 4, 5). Taking into account global reports, it would appear that elevated ALP is present in the majority of patients and would be more sensitive but not specific in assisting in biochemical confirmation of vitamin D deficiency rickets.

PTH secretion is up-regulated in response to low serum calcium levels. It is responsible for the normocalcaemia and hypophosphataemia observed in the biochemical changes of vitamin D deficiency rickets and is expected to be elevated in the context of vitamin D deficiency. The median values of 22.2pmol/L (CHBAH) and 44.6pmol/L (CMJAH) were well above normal reference values whose upper limits are at 6.9pmol/L. It is interesting to note that the higher median PTH of CMJAH is associated with lower median 25 (OH)D values obtained in the CMJAH subjects. Eighty two percent of patients in this study had elevated PTH levels on referral, similar to the 80% in the study done at Sydney (3) but below the USA reports of 94% (59). The median of 22.2pmol/L from patients at CHBAH closely reflects that from the Saudi Arabia study of 23.59pmol/L (5). The Turkish study range included a lower limit value well within normal reference range (3.6pmol/L in comparison to lower limits of this study, 17.7pmol/L (CHBAH) and 18.6pmol/L(CMJAH)) and the upper limits was between the levels of this study at the different institutions (47pmol/L in comparison to 33pmol/L(CHBAH) and 66.8pmol/L(CMJAH))(7). Taking into account these global studies it would appear that, similar to ALP, elevated PTH is present in the majority of patients. Further studies may be able to relate the degree of hyperparathyroidism secondary to the degree of vitamin D deficiency. In one study, the elevation of PTH in response to altered vitamin D levels was observed to occur only when vitamin D levels reach the deficient range (56). This would infer that all younger infants presenting with elevated PTH should have deficient vitamin D levels. Of our infants less than 6 months of age, three out of eight of the patients either had no PTH or no 25 (OH) D on referral for interpretation in this context. As expected, a negative correlation was established between PTH and phosphate levels and between PTH and calcium levels although the origin differs in that the presence of hypocalcaemia gives rise to the elevation in PTH whilst the elevated levels of PTH are responsible for the lower phosphate levels.

25 (OH) D is the definitive biochemical marker of vitamin D insufficiency or deficiency with the exact definitions of each being contentious in present literature and the scientific community. The differing opinion is due to the current hypotheses under investigation regarding the non-skeletal benefits of vitamin D and the levels at which vitamin D exerts these effects. In the context of bone metabolism, a level less than 25-30nmol/L has been associated with bone remodelling (69). For our purposes, insufficiency was described as 30nmol/L – 50nmol/L and deficiency < 30nmol/L. 25(OH)D is the gold standard due to the absence of any physiological regulation resulting in measured levels being directly proportional to vitamin D availability (68). Although the specimens for the investigation do

not require specific preparation or transport, the test is specialised and often centralised at larger facilities. Due to logistical and transportation errors, specimens may be lost or misprocessed, accounting for the fewer data entries available for interpretation (42). Multiple assays exist for the measurement of 25 (OH) D with a high inter-assay variability of 25%. National laboratory services standardise assays but over time as improved methods are developed, it is likely that the laboratories employ the newer assays to improve accuracy and this may impact the values obtained over a period. The intra-assay variability of 10% was unlikely to adversely affect results as the cut-off values used to describe insufficiency and deficiency are broad and would require a significant difference before impact. The median and IQ range at CHBAH fell within the insufficient category at 43nmol/L (31nmol/L-48nmol/L) which is above levels described in prior studies that report medians well within the deficient range (1, 3-5, 7, 59). Of all the patients, 16/70 patients were on vitamin D on referral and 9/16 (56%) had no 25(OH)D measurements on referral. Of the remaining 7 (44%), only 2/7(29%) had a normal value and 5/7 (71%) were in the insufficient range. The CMJAH median of 27.5nmol/L fell within the deficient range but was still above the medians reported from international studies of 15nmol/L from Sydney and 4.35nmol/L-21.75nmol/L in Turkey. The 25 (OH) D levels were significantly lower ($p<0.03$) in the CMJAH than CHBAH cohort. There was no significant difference in the number of patients on treatment at referral to CHBAH and CMJAH ($p=0.25$) and is unlikely to have resulted in the discrepancy. The associated relative elevation in PTH at CMJAH along with lower 25 (OH) D levels might suggest biochemically more significant disease in this group. The higher medians obtained in our study may suggest any number of factors that influence 25 (OH) D values including the demographic variations and laboratory elements that needs to be further investigated.

4.5.3. Radiological findings on presentation

The Thacher score uses a selection of radiological findings to validate bone findings secondary to rickets. It has been reviewed in practice and found to be effective as a method of assessing both the presence and severity of rickets (53, 78). 56/71 patients had radiographs (XR) available for review by the researchers. Median Thacher scores were 8 (4-10) at CHBAH and 8 (6-10) at CMJAH with 37.5% (21/56) of patients presenting with an initial score of 10. This suggests radiologically evident rickets with significant changes in the majority of patients. These scores closely reflect those from an Indian study that assessed XR findings in 176 untreated patients with nutritional rickets in which the baseline score was a mean of 6.8± 3.2 and 43% of patients had a score of 10 (78).

In a comparison of biochemical markers to Thacher scores, a negative correlation was discovered between phosphate and a positive correlation between ALP and the Thacher scores. ALP originates from bone and in the event of more extensive bone destruction as visualised on XRs, ALP would be higher in those patients displaying more severe bone disease. In an investigation of comparison of biochemical markers to radiological severity in 1985, ALP was originally identified as the most closely related biochemical indicator to XR features of vitamin D deficiency rickets and its severity (76).

The bone remodelling that occurs in rickets, reflected in the widening of the growth plate and irregularity of the epiphyseal surfaces, results from the failure of apoptosis of hypertrophied chondroblasts at the microscopic level. Apoptosis of chondroblasts allows for the development of blood vessels and cartilage required for normal bone turnover. The absence of apoptosis occurs as a result of lower phosphate levels and hence lower levels would be expected to yield more severe XR changes which reflect the bone remodelling process (79).

There was no correlation between calcium serum levels and Thacher scores possibly due to the action of PTH on calcium which causes an increase in calcium levels as the disease process progresses. Studies from Sydney and Glasgow reported an increased proportion of XR confirmed rickets in hypocalcaemic patients but did not describe if there was a significant correlation between the calcium levels and radiological findings (1, 3).

The most recent Canadian study suggested that a lower age may be associated with no XR changes with a median of 0.68 years being associated with no changes (11). In this study, forty percent (2/5) of patients less than or equal to 7 months of age had XR scores of >9 and 60% (3/5) had scores <4. The numbers are too small to make any relevant comparisons.

4.6. Referral

4.6.1. Treatment on referral

Treatment has many facets that are often not all fully addressed. Replacement is essential to facilitate healing but dietary considerations and sun exposure are important with respect to education, viability and maintenance. Often sun exposure is the more affordable and feasible option in comparison to diet modification required to increase calcium and vitamin D content in the context of the lower socio-economic groups encountered in the clinics. The only

measurable component of treatment is replacement therapy although diet modification and sun exposure play important roles in obtaining resolution.

There are two major options for treatment of vitamin D deficiency – daily oral replacement therapy or single dose stoss therapy. The advantages to stoss therapy are the guaranteed compliance and reduced risk of incorrect dosing but it not readily available and the absolute dosage remains contentious. In our practice, only daily oral replacement is available in an almost uninterrupted supply. Twenty three percent of patients received calciferol on referral in the range of 500-5000IU. The most appropriate course of action in the event of suspected rickets would be to confirm the diagnosis and commence treatment. As the majority of patients were referred with suspected rickets, the percentage on treatment was disproportionately low and most likely due to the reluctance of physicians to implement therapy in the presence of an unconfirmed diagnosis. It might be prudent to commence treatment at doses higher than daily requirements when the diagnosis is suspected, especially in the South African context where waiting times for specialist clinics can be extensive and the delay in treatment might have a significant impact. Education regarding vitamin D replacement may change this referral treatment pattern in improving physician confidence in prescription of safe therapeutic doses of vitamin D. The recommended treatment for vitamin D deficiency rickets in children according to the British Children's formulary is shown in Table 4 below (54).

Table 4: Dosage of calciferol for treating vitamin D deficiency

Age	Calciferol dosage
0-6 months	3000IU/d for 8 weeks
6 months – 12 years	6000IU/d for 8 weeks
>12 years	9000IU/d for 8 weeks

An additional 71% of patients were commenced on treatment prescribed at the Metabolic bone clinic. Ninety percent of patients responded to isolated calciferol treatment dosages whilst 4 out of 71 required additional therapy of either alfacalcidol or calcium carbonate as these patients were symptomatic from severe hypocalcaemia.

4.6.2. Defaulting Treatment

A number of socio-economic and cultural factors contribute to the poor compliance encountered in our setting. The relationship between patient and health care professional is one in which the patient has a need that they wish to be fulfilled. A large contributory factor to defaulting is that the need of the patient is fulfilled at the first consultation or that the patient determines that the system is unable to address their need. When taking into consideration the impetus to seek medical attention, parents consider the risk that the symptoms reflect a serious disease process and the ability of the disease to restrict activities of daily living. If these issues are both addressed and in the face of obstacles to seeking routine medical care, the parents often determine that the disease no longer poses a threat and do not feel compelled to return. Financial constraints are also a defining factor limiting access to health facilities. Almost half of the patients defaulted follow up in this study, a significant proportion with only two patients returning after defaulting their initial follow up appointment. The reasons for defaulting follow up were not investigated as this was a retrospective study.

4.7. Outcomes

4.7.1. Resolution of rickets

The recovery of bone abnormalities seen on radiographs and when combined with serial biochemical measurements is an accurate assessment of response to treatment and evidence confirming healed rickets.

4.7.2. Resolution of biochemical measurements

Due to the significant number of defaulters, the number of follow up patients was small. Thirty eight percent of patients had follow up biochemical investigations of which 74% exhibited complete normalisation of biochemical findings. The CHBAH patients had resolution of biochemical markers to normal reference ranges within a median of 12 weeks, 6 weeks earlier than the median of 18 weeks at CMJAH but not statistically significant. The most striking differences between the patients at the two hospitals were the older age of presentation and lower 25(OH)D values at CMJAH. It may be possible that the older patients at CMJAH reflect a more advanced disease process with a more marked deficiency that took a slightly longer time to resolve.

4.7.3. Resolution of XRs

Used as one of the diagnostic criteria for rickets, XRs in conjunction with biochemical indices are an objective measure of the resolution of rickets. Repeat XRs were only available in 26 out of 56 (46%) of the patients who had XRs on presentation due to the high default rate. Median duration to resolution of XR features described as healing rickets or a Thacher score of zero was 16 weeks (4 months). This finding correlates fairly well with the conclusion of Chaterjee et al that 100% of patients will display complete resolution by 6 months after initiation of treatment at mean resolution duration of 126 days (43% of patients had resolved by 3 months (78)).

Resolution of radiological features at CHBAH at a median of 13.5 weeks was 10.5 weeks less than the median of 24 weeks at CMJAH (not statistically significant). This is in association with an increased duration of resolution for biochemical markers at CMJAH. There are identifiable factors associated with delayed radiological resolution present in the CMJAH cohort. Older age has been related to more advanced activity on XRs (76), but this was in an older study population comprised of children aged 6 months to 12 years with a mean age of presentation of 3 years and 4 months and it is uncertain if it renders itself to application in our study. Inadequate sunlight exposure at presentation and breastfeeding for more than 6 months has been linked to both severity and delay in recovery (78), both of these factors were present in this study. Both hospitals reported a mean duration of breastfeeding of greater than 6 months with a difference of only two months in the mean which would render this factor less significant in explaining the difference in the recovery periods. Lack of sunlight exposure differed with 77% at CMJAH versus 56% at CHBAH although this was not statistically significant and could be considered to be contributory. A marginally higher percentage of lack of sun exposure of patients at CMJAH, is a reasonable hypothesis for the increased time required for healing of vitamin D deficiency compared to patients at CHBAH despite the presence of similar Thacher scores at initial presentation in both the hospitals.

4.7.3. Weeks to discharge

The time taken from initial consultation to discharge reflects a multitude of factors that were not explored within the scope of this study. The largest influence on this parameter was the high default rate of 46% with only 6% returning to clinic. Taking into account the high default rate of 46%, the majority of patients were discharged from the Metabolic bone clinic with 34/38 [71 total – 33 defaulters] (89%)] of patients documented as discharged. The difference

of 4 weeks in discharge between CHBAH and CMJAH (32 vs 28 weeks) patients may reflect the increased time required for resolution of the more severe radiological and biochemical rickets observed at CMJAH. No previous studies have reported on the time period from initial presentation to discharge to make a meaningful comparison with this study.

Chapter 5: Limitations

5.1. Data restrictions

Due to the retrospective nature of the study, data collection was limited to information already documented which on occasion was incomplete. With respect to variables obtained on verbal history, these may have been inaccurate. Retrospective collection of data also limited the scope of variables that may have already been omitted to those already identified and recorded in the files and the data collection sheet.

5.2. Calcium deficiency as a contributory factor

Calcium deficiency is a recognised contributor to the development of rickets in the developing world and cannot be differentiated from rickets as a result of an isolated vitamin D deficiency on the amount of clinical, radiological and biochemical data we had available for interpretation. As diets in Africa have been identified to be deficient in calcium, this would be a potentially important variable in our demographics. Due to the retrospective nature of the study, information regarding the calcium content in the diet of our cohort was either incomplete or not documented for interpretation and may have impacted upon the outcomes of our study. However, from our analyses of dietary calcium intake, CHBAH had 29% (7/24) of the patients reporting inadequate dietary calcium intake as opposed to 44% (12/27) in the CMJAH which was not significantly different ($p=0.4$).

Chapter 6: Conclusion

There is a definite paucity of data regarding vitamin D status and the clinical sequelae of insufficiency and deficiency from the Sub-Saharan region of Africa including South Africa (Figure 5a and 5b) (9).

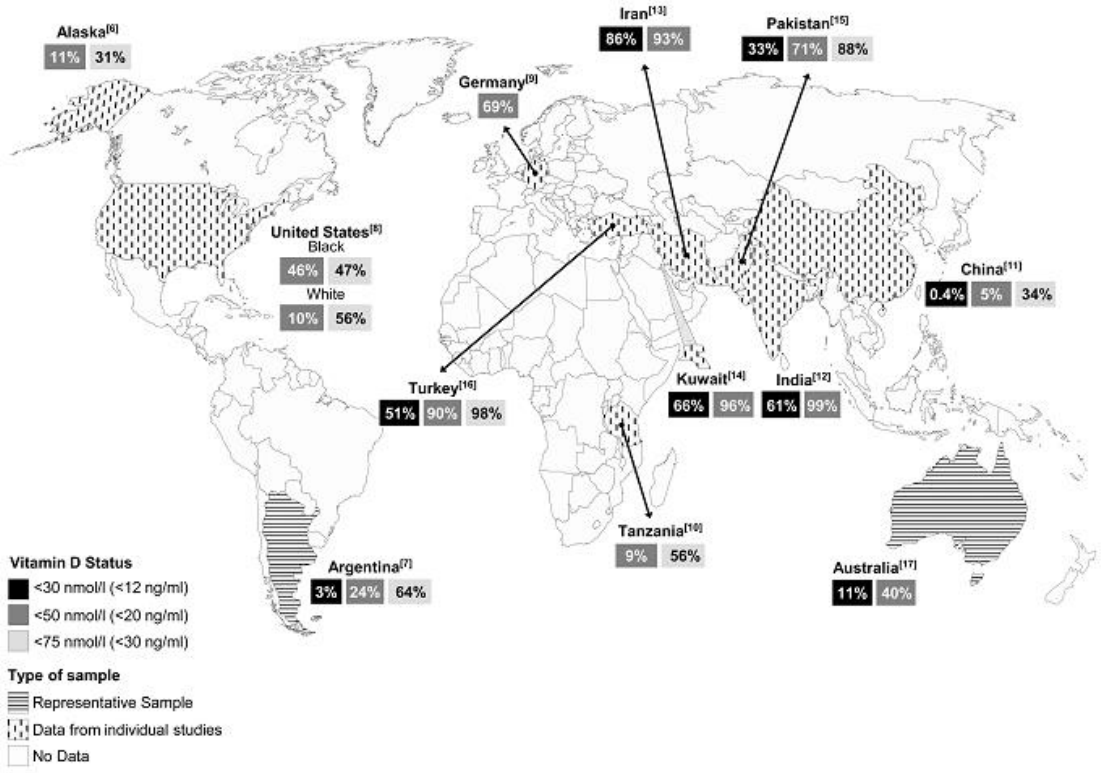


Figure 5a. Prevalence of low vitamin D status of infants worldwide (Sourced from Palacios C, Gonzalez L. Is vitamin D deficiency a major global public health problem? J Steroid Biochem Mol Biol. 2014)(9)

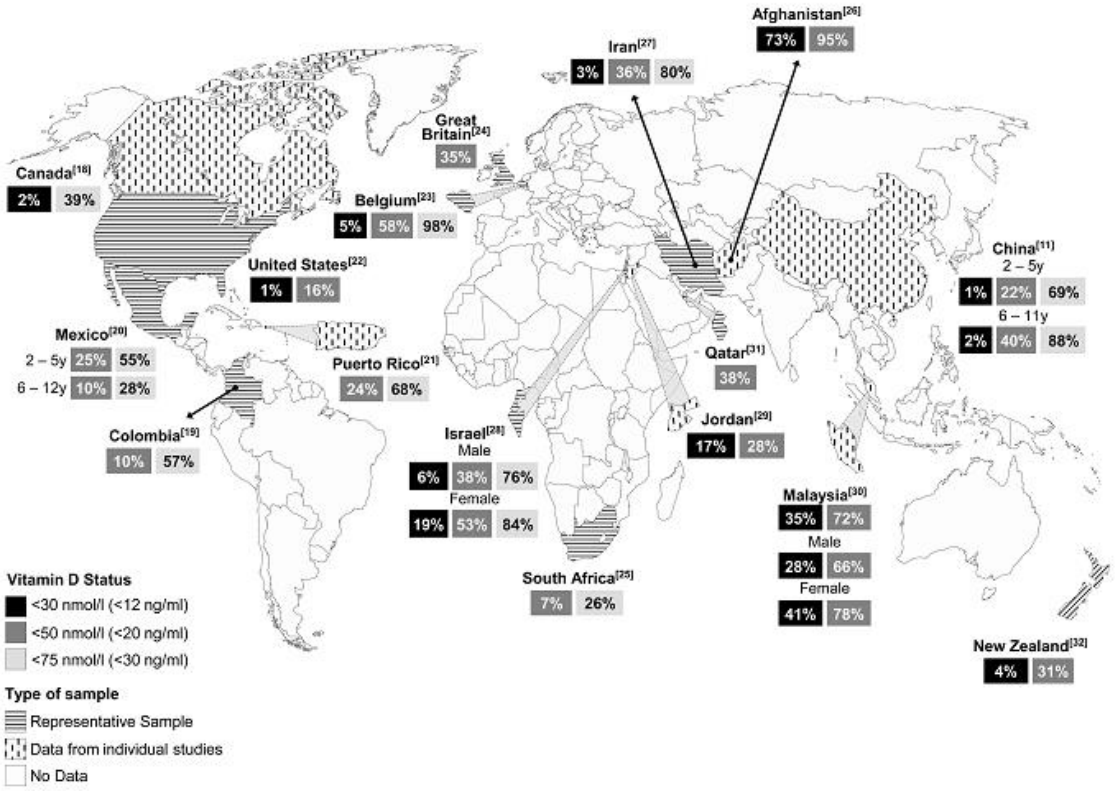


Figure 5b: Prevalence of low vitamin status in children worldwide (Sourced from Palacios C, Gonzalez L. Is vitamin D deficiency a major global public health problem? J Steroid Biochem Mol Biol. 2014)(9)

The results from our study support the observations reported in other studies examining vitamin D deficiency rickets from around the globe.

Our data was derived from a specific population subset that represents the end of a spectrum of a much larger issue that ranges from subclinical vitamin D insufficiency to overt clinical deficiency and therefore is not representative of the entire population. Taking into account maternal deficiency and insufficiency and rickets that remain undiagnosed, the impact that vitamin D deficiency rickets has on our overall burden of illnesses that we face in South Africa is likely to be significant and would benefit from further research.

Within our selected study group, one cohort displayed a more significant burden of disease. The CMJAH cohort reflected a statistically significant older demographic age at presentation (>2yrs) with more severe biochemical features of vitamin D deficiency. There are a multitude of socio-demographic factors that could contribute to this aberration in age presentation that were not explored in the context of this study and, due to the retrospective nature of the study as well as a small sample size with missing data, a regression model could not be applied to validate this finding. Important factors that may contribute include the status of families with

respect to size, income and education; the large immigrant population of the inner city and the differences in dwelling types. This cohort represents inner city occupancy with flat dwellings dominating the residences associated with overcrowding. Ninety percent of CMJAH children resided in flat dwellings in comparison with 30% of their CHBAH counterparts. This supports findings from global studies that have linked inner city dwelling, riddled with the risk factors of overcrowding, pollution and limited sun exposure, to an increased incidence of vitamin D deficiency rickets. Lack of sun exposure may be a particularly important contributor in the CMJAH cohort due to the hazardous environment of the inner city and safety concerns of the parents rendering children of all ages unexposed to sunlight and are kept indoors.

Pertinent to both groups examined, two risk factors were identified: lack of sun exposure and breastfeeding, both of which are synonymous with being important factors identified in international studies. The isolation of these two factors suggests that the South African population is subject to the same risk factors found in populations around the world. Independently finding the same risk factors validates the importance of these factors and contributes to the global information pool that can assist in targeted prevention of vitamin D deficiency rickets.

Chapter 7: Recommendations

Vitamin D deficiency is a global epidemic and South Africa has not been spared. By isolating risk factors, we can identify areas and at risk groups to target through public awareness, education, supplementation and replacement therapy.

National strategies should include education regarding the importance of sun exposure and the need for supplementation in the context of breastfeeding whilst at risk populations can benefit from more targeted intervention. These strategies could be employed as a component of the ante-natal care rendered to potential mothers or on a larger scale as national awareness campaigns. The role of vitamin D in food fortification could also be explored in the context of a population whom access cereals most readily.

Vitamin D supplementation should be recommended for all breastfeeding infants, mixed feeding infants on both breast milk and formula and populations in which sun exposure is limited. As no determinants were identified as to what puts a child at risk of limited sun exposure, the role of national supplementation should be entertained, perhaps as a component of the national immunisations schedule. Supplementation would likely be optimised if distributed on a national basis but economic viability would need to be explored as part of the implementation. Complementary feed introduction in the first year of life is often poor in dairy products and fortified cereals (6), a combination of which renders the diet low in vitamin D sources which combined with limited sun exposure places this population subset at risk. Suggested supplementation would be in accordance with IOM guidelines: 400IU daily for all breastfed infants of at risk populations and 600IU daily for children over the age of 1 year.

An important and often neglected aspect of supplementation should target the child bearing female population. Infant vitamin D status is affected by multiple maternal factors included foetal transfer of vitamin D and maternal levels during lactation. By targeting this population, maternal values can be optimised which should correlate with an improvement in infant vitamin D status. Access to childbearing females could be performed at multiple levels including high school students and women accessing healthcare during pregnancy in the ante-natal sessions. Supplementation of the pregnant or lactating mother to achieve levels of 500IU to 800IU in breastmilk requires 6400IU per day (80). As long term safety with this level of replacement has not been established, 2000IU/day has been recommended with the potential to increase delivery with direct supplementation of the infant (44).

For those already rendered deficient, adequate replacement therapy is a necessity. Identifying deficient individuals in the absence of clinical manifestations remains difficult. Education of health practitioners is essential in recognising the need for replacement and the place for specialised care and intervention and can be targeted through education institutions. Replacement therapy has been examined both as a daily and stoss regime (23, 54). In populations in which compliancy may be problematic, stoss therapy may be advantageous. As observed in our population, defaulting continuing care, likely related to access to health care and ability to follow up, may render stoss based replacement a more viable option. An affordable replacement strategy might be a 50 000IU tablet of vitamin D2 once a week for 8 weeks followed by once every 2 to 4 weeks or , 200 000IU of vitamin D3 or 600 000IU of vitamin D intramuscularly every 3 months (81).

Our recommendations are based on findings in our study cohort and support international recommendations. Prospective studies would be required to investigate and support any recommendations for intervention or prevention which was outside the scope of this study. By conducting more research into the populations affected and refining preventative and replacement strategies, South Africa could identify and target children prone to and affected by Vitamin D deficiency rickets and join the global community in eradicating this preventable disease.

References

1. Ahmed SF, Franey C, McDevitt H, Somerville L, Butler S, Galloway P, et al. Recent trends and clinical features of childhood vitamin D deficiency presenting to a children's hospital in Glasgow. Arch Dis Child. 2011;96(7):694-696.
2. Munns CF, Simm PJ, Rodda CP, Garnett SP, Zacharin MR, Ward LM, et al. Incidence of vitamin D deficiency rickets among Australian children: an Australian Paediatric Surveillance Unit study. Med J Aust. 2012;196(7):466-468.
3. Robinson PD, Hogler W, Craig ME, Verge CF, Walker JL, Piper AC, et al. The re-emerging burden of rickets: a decade of experience from Sydney. Arch Dis Child. 2006;91(7):564-568.
4. Al-Atawi MS, Al-Alwan IA, Al-Mutair AN, Tamim HM, Al-Jurayyan NA. Epidemiology of nutritional rickets in children. Saudi J Kidney Dis Transpl. 2009;20(2):260-265.
5. Al-Mustafa ZH, Al-Madan M, Al-Majid HJ, Al-Muslem S, Al-Ateeq S, Al-Ali AK. Vitamin D deficiency and rickets in the Eastern Province of Saudi Arabia. Ann Trop Paediatr. 2007;27(1):63-67.
6. Hatun S, Ozkan B, Bereket A. Vitamin D deficiency and prevention: Turkish experience. Acta Paediatr. 2011;100(9):1195-1199.
7. Ozkan B, Doneray H, Karacan M, Vancelik S, Yildirim ZK, Ozkan A, et al. Prevalence of vitamin D deficiency rickets in the eastern part of Turkey. Eur J Pediatr. 2009;168(1):95-100.
8. Mithal A, Wahl DA, Bonjour JP, Burckhardt P, Dawson-Hughes B, Eisman JA, et al. Global vitamin D status and determinants of hypovitaminosis D. Osteoporos Int. 2009;20(11):1807-1820.
9. Palacios C, Gonzalez L. Is vitamin D deficiency a major global public health problem? J Steroid Biochem Mol Biol. 2014;144 Pt A:138-145.
10. Pettifor JM. Vitamin D &/or calcium deficiency rickets in infants & children: a global perspective. Indian J Med Res. 2008;127(3):245-249.
11. Ward LM, Gaboury I, Ladhani M, Zlotkin S. Vitamin D-deficiency rickets among children in Canada. CMAJ. 2007;177(2):161-166.
12. Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, et al. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet. 2010;376(9736):180-188.

13. Holick MF. Resurrection of vitamin D deficiency and rickets. J Clin Invest. 2006;116(8):2062-2072.
14. Holick MF, Chen TC, Lu Z, Sauter E. Vitamin D and skin physiology: a D-lightful story. J Bone Miner Res. 2007;22 Suppl 2:V28-33.
15. Holick MF, MacLaughlin JA, Clark MB, Holick SA, Potts JT, Jr., Anderson RR, et al. Photosynthesis of previtamin D3 in human skin and the physiologic consequences. Science. 1980;210(4466):203-205.
16. Henry HL, Norman AW. Vitamin D: metabolism and biological actions. Annu Rev Nutr. 1984;4:493-520.
17. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357(3):266-281.
18. DeLuca HF. Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr. 2004;80(6 Suppl):1689s-1696s.
19. Armas LA, Hollis BW, Heaney RP. Vitamin D2 is much less effective than vitamin D3 in humans. J Clin Endocrinol Metab. 2004;89(11):5387-5391.
20. Holick MF. Vitamin D: A millenium perspective. J Cell Biochem. 2003;88(2):296-307.
21. Mughal MZ. Rickets. Curr Osteoporos Rep. 2011;9(4):291-299.
22. Christakos S, Ajibade DV, Dhawan P, Fechner AJ, Mady LJ. Vitamin D: metabolism. Rheum Dis Clin North Am. 2012;38(1):1-11, vii.
23. Holick MF. The D-lightful vitamin D for child health. JPEN. 2012;36(1 Suppl):9s-19s.
24. Yoshida T, Stern PH. How vitamin D works on bone. Endocrinol Metab Clin North Am. 2012;41(3):557-569.
25. Suda T, Takahashi N, Udagawa N, Jimi E, Gillespie MT, Martin TJ. Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. Endocr Rev. 1999;20(3):345-357.
26. Murayama A, Takeyama K, Kitanaka S, Kodera Y, Kawaguchi Y, Hosoya T, et al. Positive and negative regulations of the renal 25-hydroxyvitamin D3 1alpha-hydroxylase gene by parathyroid hormone, calcitonin, and 1alpha,25(OH)2D3 in intact animals. Endocrinology. 1999;140(5):2224-2231.
27. Henry HL, Dutta C, Cunningham N, Blanchard R, Penny R, Tang C, et al. The cellular and molecular regulation of 1,25(OH)2D3 production. J Steroid Biochem Mol Biol. 1992;41(3-8):401-407.

28. Brenza HL, Kimmel-Jehan C, Jehan F, Shinki T, Wakino S, Anazawa H, et al. Parathyroid hormone activation of the 25-hydroxyvitamin D3-1alpha-hydroxylase gene promoter. Proc Natl Acad Sci U S A. 1998;95(4):1387-1391.
29. Henry HL. Regulation of vitamin D metabolism. Best Pract Res Clin Endocrinol Metab. 2011;25(4):531-541.
30. Shimada T, Kakitani M, Yamazaki Y, Hasegawa H, Takeuchi Y, Fujita T, et al. Targeted ablation of Fgf23 demonstrates an essential physiological role of FGF23 in phosphate and vitamin D metabolism. J Clin Invest. 2004;113(4):561-568.
31. Segawa H, Yamanaka S, Ohno Y, Onitsuka A, Shiozawa K, Aranami F, et al. Correlation between hyperphosphatemia and type II Na-Pi cotransporter activity in klotho mice. Am J Physiol Renal Physiol. 2007;292(2):F769-779.
32. van der Meer IM, Karamali NS, Boeke AJ, Lips P, Middelkoop BJ, Verhoeven I, et al. High prevalence of vitamin D deficiency in pregnant non-Western women in The Hague, Netherlands. Am J Clin Nutr. 2006;84(2):350-353; quiz 468-359.
33. Das G, Crocombe S, McGrath M, Berry JL, Mughal MZ. Hypovitaminosis D among healthy adolescent girls attending an inner city school. Arch Dis Child. 2006;91(7):569-572.
34. Fischer PR, Thacher TD, Pettifor JM. Pediatric vitamin D and calcium nutrition in developing countries. Rev Endocr Metab Disord. 2008;9(3):181-192.
35. Matsuoka LY, Ide L, Wortsman J, MacLaughlin JA, Holick MF. Sunscreens suppress cutaneous vitamin D3 synthesis. J Clin Endocrinol Metab. 1987;64(6):1165-1168.
36. Clemens TL, Adams JS, Henderson SL, Holick MF. Increased skin pigment reduces the capacity of skin to synthesise vitamin D3. Lancet. 1982;1(8263):74-76.
37. Holick MF. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. Am J Clin Nutr. 2004;79(3):362-371.
38. Pettifor JM, Moodley GP, Hough FS, Koch H, Chen T, Lu Z, et al. The effect of season and latitude on in vitro vitamin D formation by sunlight in South Africa. S Afr Med J. 1996;86(10):1270-1272.
39. Ward LM. Vitamin D deficiency in the 21st century: a persistent problem among Canadian infants and mothers. CMAJ. 2005;172(6):769-770.
40. Agarwal KS, Mughal MZ, Upadhyay P, Berry JL, Mawer EB, Puliyel JM. The impact of atmospheric pollution on vitamin D status of infants and toddlers in Delhi, India. Arch Dis Child. 2002;87(2):111-113.
41. Mughal MZ, Salama H, Greenaway T, Laing I, Mawer EB. Lesson of the week: florid rickets associated with prolonged breast feeding without vitamin D supplementation. BMJ. 1999;318(7175):39-40.

42. Pugliese MT, Blumberg DL, Hludzinski J, Kay S. Nutritional rickets in suburbia. J Am Coll Nutr. 1998;17(6):637-641.
43. Ziegler EE, Hollis BW, Nelson SE, Jeter JM. Vitamin D deficiency in breastfed infants in Iowa. Pediatrics. 2006;118(2):603-610.
44. Thandrayen K, Pettifor JM. Maternal vitamin D status: implications for the development of infantile nutritional rickets. Endocrinol Metab Clin North Am. 2010;39(2):303-320.
45. Mulligan ML, Felton SK, Riek AE, Bernal-Mizrachi C. Implications of vitamin D deficiency in pregnancy and lactation. Am J Obstet Gynecol. 2010;202(5):429.e421-429.
46. Hollis BW, Roos BA, Draper HH, Lambert PW. Vitamin D and its metabolites in human and bovine milk. J Nutr. 1981;111(7):1240-1248.
47. Reeve LE, Chesney RW, DeLuca HF. Vitamin D of human milk: identification of biologically active forms. Am J Clin Nutr. 1982;36(1):122-126.
48. Awumey EM, Mitra DA, Hollis BW, Kumar R, Bell NH. Vitamin D metabolism is altered in Asian Indians in the southern United States: a clinical research center study. J Clin Endocrinol Metab. 1998;83(1):169-173.
49. Thacher TD, Clarke BL. Vitamin D insufficiency. Mayo Clin Proc. 2011;86(1):50-60.
50. Thacher TD, Fischer PR, Pettifor JM. The usefulness of clinical features to identify active rickets. Ann Trop Paediatr. 2002;22(3):229-237.
51. Shore RM, Chesney RW. Rickets: Part II. Pediatr Radiol. 2013;43(2):152-172.
52. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab. 2011;96(1):53-58.
53. Thacher TD, Fischer PR, Pettifor JM, Lawson JO, Manaster BJ, Reading JC. Radiographic scoring method for the assessment of the severity of nutritional rickets. J Trop Pediatr. 2000;46(3):132-139.
54. Gordon CM, Williams AL, Feldman HA, May J, Sinclair L, Vasquez A, et al. Treatment of hypovitaminosis D in infants and toddlers. J Clin Endocrinol Metab. 2008;93(7):2716-2721.
55. Adams JS, Hewison M. Update in vitamin D. J Clin Endocrinol Metab. 2010;95(2):471-478.
56. Pettifor JM. Nutritional rickets: deficiency of vitamin D, calcium, or both? Am J Clin Nutr. 2004;80(6 Suppl):1725s-1729s.
57. Poopedi MA, Norris SA, Pettifor JM. Factors influencing the vitamin D status of 10-year-old urban South African children. Public Health Nutr. 2011;14(2):334-339.

58. Bhimma R, Pettifor JM, Coovadia HM, Moodley M, Adhikari M. Rickets in black children beyond infancy in Natal. S Afr Med J. 1995;85(7):668-672.
59. Weisberg P, Scanlon KS, Li R, Cogswell ME. Nutritional rickets among children in the United States: review of cases reported between 1986 and 2003. Am J Clin Nutr. 2004;80(6 Suppl):1697s-1705s.
60. Soliman A, Kalra S. Adaptation to vitamin D deficiency: Age specific clinical presentations. Indian J Endocrinol Metab. 2013;17(5):775-779.
61. Harinarayan CV, Ramalakshmi T, Prasad UV, Sudhakar D. Vitamin D status in Andhra Pradesh : a population based study. Indian J Med Res. 2008;127(3):211-218.
62. Davies JH, Reed JM, Blake E, Priesemann M, Jackson AA, Clarke NM. Epidemiology of vitamin D deficiency in children presenting to a pediatric orthopaedic service in the UK. J Pediatr Orthop. 2011;31(7):798-802.
63. Blok BH, Grant CC, McNeil AR, Reid IR. Characteristics of children with florid vitamin D deficient rickets in the Auckland region in 1998. N Z Med J. 2000;113(1117):374-376.
64. Nesby-O'Dell S, Scanlon KS, Cogswell ME, Gillespie C, Hollis BW, Looker AC, et al. Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988-1994. Am J Clin Nutr. 2002;76(1):187-192.
65. Webb AR, Kline L, Holick MF. Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. J Clin Endocrinol Metab. 1988;67(2):373-378.
66. Gannage-Yared MH, Chemali R, Yaacoub N, Halaby G. Hypovitaminosis D in a sunny country: relation to lifestyle and bone markers. J Bone Miner Res. 2000;15(9):1856-1862.
67. Looker AC, Pfeiffer CM, Lacher DA, Schleicher RL, Picciano MF, Yetley EA. Serum 25-hydroxyvitamin D status of the US population: 1988-1994 compared with 2000-2004. Am J Clin Nutr. 2008;88(6):1519-1527.
68. Shore RM, Chesney RW. Rickets: Part I. Pediatr Radiol. 2013;43(2):140-151.
69. Pettifor JM. Calcium and vitamin D metabolism in children in developing countries. Ann Nutr Metab. 2014;64 Suppl 2:15-22.
70. Muhe L, Lulseged S, Mason KE, Simoes EA. Case-control study of the role of nutritional rickets in the risk of developing pneumonia in Ethiopian children. Lancet. 1997;349(9068):1801-1804.

71. Haider N, Nagi AG, Khan KM. Frequency of nutritional rickets in children admitted with severe pneumonia. J Pak Med Assoc. 2010;60(9):729-732.
72. Salimpour R. Rickets in Tehran. Study of 200 cases. Arch Dis Child. 1975;50(1):63-66.
73. Wharton B, Bishop N. Rickets. Lancet. 2003;362(9393):1389-1400.
74. Ladhani S, Srinivasan L, Buchanan C, Allgrove J. Presentation of vitamin D deficiency. Arch Dis Child. 2004;89(8):781-784.
75. Ranchod H, Pettifor JM, Thandrayen K. Vitamin D status of mothers and their newborns in Soweto. 14th Congress of NOFSA; Durban: JEMDSA; 2010. p. 40.
76. Abdul-Motaal A, Gettinby G, McIntosh WB, Sutherland GR, Dunnigan MG. Relationships between radiological and biochemical evidence of rickets in Asian schoolchildren. Postgrad Med J. 1985;61(714):307-312.
77. Carpenter TO, Herreros F, Zhang JH, Ellis BK, Simpson C, Torrealba-Fox E, et al. Demographic, dietary, and biochemical determinants of vitamin D status in inner-city children. Am J Clin Nutr. 2012;95(1):137-146.
78. Chatterjee D, Gupta V, Sharma V, Sinha B, Samanta S. A reliable and cost effective approach for radiographic monitoring in nutritional rickets. Br J Radiol. 2014;87(1036):20130648.
79. Pettifor JM, Prentice A. The role of vitamin D in paediatric bone health. Best Pract Res Clin Endocrinol Metab. 2011;25(4):573-584.
80. Wagner CL, Hulsey TC, Fanning D, Ebeling M, Hollis BW. High-dose vitamin D3 supplementation in a cohort of breastfeeding mothers and their infants: a 6-month follow-up pilot study. Breastfeed Med. 2006;1(2):59-70.
81. Shah BR, Finberg L. Single-day therapy for nutritional vitamin D-deficiency rickets: a preferred method. J Pediatr. 1994;125(3):487-490.

APPENDIX A: APPENDIX A: ETHICS CLEARANCE CERTIFICATE BY THE HUMAN RESEARCH COMMITTEE OF THE UNIVERSITY OF THE WITWATERSRAND

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Marisa Beretta

CLEARANCE CERTIFICATE	**M120727**
PROJECT	A Retrospective Audit of the Presentation and Management of Vitamin D Deficiency Rickets in Johannesburg
INVESTIGATORS	Dr Marisa Beretta
DEPARTMENT	Paediatrics Chris Hani Baragwanath Academic Hospital
DATE CONSIDERED	27/07/2012
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 13/02/2015 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Dr K Thandrayen

DECLARATION OF INVESTIGATOR(S)
To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**
PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

APPENDIX B: LETTER OF APPROVAL OF PROTOCOL

Faculty of Health Sciences
Medical School, 7 York Road, Parktown, 2193
Fax: (011) 717-2119
Tel: (011) 717-2076

Reference: Ms Salamina Segole
E-mail: salamina.segole@wits.ac.za
14 November 2012
Person No: 0102462T
PAG

Dr MR Beretta
Po Box 1297
Randpark Ridge
2156
South Africa

Dear Dr Beretta

Master of Medicine in the specialty of Paediatrics: Approval of Title

We have pleasure in advising that your proposal entitled "*The clinical and demographic presentation of vitamin D deficiency rickets in Johannesburg, South Africa*" has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

APPENDIX C: DATA COLLECTION SHEET

Data Collection Sheet for Vitamin D Deficiency Rickets Review Study

Study No:_____

Demographics

Male◯ Female◯

CHBAH ◯ CMJAH ◯

Area of residence on hospital sticker:

Gauteng ☐ Area:_____Postal Code_____

North West ☐

Limpopo ☐

Mpumalanga ☐

Western Cape ☐

Eastern Cape ☐

Northern Cape ☐

Kwazulu Natal ☐

Northern Province ☐

Other Country_____

Country of Origin: Recorded_____ or Not recorded ◯

Type of residence: Flat◯ Shack◯ RDP housing◯

Formal housing◯ Not recorded◯

Inner-city Yes◯ No◯ Not recorded◯

Child is residing in Johannesburg? Yes◯ No◯ Not recorded◯If No, where?_____

Year and month of referral:_____

Age at referral:_____ months

Health service referral: Yes◯ No◯ Not recorded ◯ If yes, from:

Orthopaedics◯

Local clinic◯

General paediatrics◯

General practitioner◯

Peripheral hospital inside Gauteng area ◯

Peripheral hospital outside Gauteng area◯

Reason for referral:_____

Clinical Presentation

Height: _____ cm

Weight: _____ kg

Head circumference: _____cm

Genu varus ◯

Genu vulgus ◯

Wind swept deformities ◯

Widened epiphyses◯

Rachitic rosary◯

Harrison's sulcus◯

Growth retardation◯

Frontal bossing◯

Delayed anterior fontanel closure (>18 months)◯

Craniotabes◯

Delayed dentition (>12 months no eruption of teeth) ◯

Pain / Irritability◯

Hypotonia◯

Hypocalcaemic seizures◯

Delayed motor milestones◯

If yes: milestone/s and by how many months: 1._____

2._____

3._____

History of bone fracture ◯ Not recorded◯

Confirmed fracture on xrays◯

Tetany ◯

Stridor ◯

Laryngospasm ◯

Scoliosis / Kyphosis / Kyphoscoliosis ◯ If yes: Fixed ◯ Functional ◯

Biochemical Markers

Biochemical Marker	On Referral	On follow-up (1)	On follow-up (2)	On follow-up (3)	On Discharge
Date:					
Calcium (ionised) mmol/					
Calcium (corrected) mmol/l					
Phosphate mmol/l					
PTH pmol/l					
25 OH D nmol/l					

Predisposing Factors

Dark skin pigmentation◯ Not recorded◯

No sunlight exposure on history ◯ Not recorded◯

Cultural / religious practices of "purdah" performed by mother ◯ or the infant ◯ or both ◯ Not recorded ◯

Child is attending a crèche◯ ,at home◯ Not recorded◯ .

The crèche is in a Flat◯ House◯ or Other◯ Not recorded◯

Dietary insufficiency of calcium on history◯ Not recorded◯

Was the child breastfed: Yes◯ No◯ Not recorded◯

If yes, duration: Years _____ Months _____

Is the child currently breastfed? Yes◯ No◯ Not recorded◯

Formula feeds: Yes◯ No◯ Not recorded◯

If yes, how much per day? _____

Does the child drink milk? Yes◯ No◯ Not recorded◯

If Yes, how much?_____

Prior admissions: Yes◯ No ◯ Not recorded ◯

If yes, for what? _____

Radiological Scoring System (Thacher – attached appendix)

Score = wrists:____ Knees: _____ Total: _____

Were the X-rays done before treatment started? Yes◯ No◯

If not, how long after starting treatment? ____months

Treatment

Treatment on referral: Yes◯ No◯

If yes, what treatment: Vitamin D ◯ Dose:_____

One alpha ◯ Dose:_____

Titralac ◯ Dose:_____

Other: _____Dose: _____

1. First line medication prescribed:
   Drug:_____
   Dosage: _____
2. Second line medication prescribed:
   Drug: _____
   Dosage: _____
3. Third line medication prescribed:
   Drug: _____
   Dosage: _____

Defaulted treatment:

Failed to return for follow up on their assigned date: Yes◯ No◯

If yes, did patient return for follow-up? Yes◯ No◯

If yes: After how long? _____weeks or _____months

Non-compliant on prescribed medication Yes◯ No◯

If yes, for how long? _____years _____ months

Was the rickets healed Yes◯ No◯ If not, was treatment recommenced?

Yes◯ No◯

Follow up

Time to normalisation of abnormal biochemical markers (Calcium > 2.1 mmol/l and PO4 > 1.45 mmol/l, ± PTH < 6.9 pmol/l and ± ALP <325 IU): _____weeks or not repeated

Time to radiological resolution (normal x-rays of wrists and knees) :_____weeks

Time to discharge from Metabolic Bone clinic: _____weeks or_____months

APPENDIX D: LETTER OF PERMISSION TO CONDUCT RESEARCH FROM THE CEO OF CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

MEDICAL ADVISORY COMMITTEE
CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

PERMISSION TO CONDUCT RESEARCH

Date: 12 July 2012

TITLE OF PROJECT: A retrospective audit of the presentation and management of vitamin D deficiency rickets in Johannesburg

UNIVERSITY: Witwatersrand

Principal Investigator: Dr M Baretta

Department: Paediatrics

Supervisor (If relevant): Dr K Thandrayen

Permission Head Department (where research conducted): Yes

Date of start of proposed study: August 2012
Date of completion of data collection: Sept 2012

The Medical Advisory Committee recommends that the said research be conducted at Chris Hani Baragwanath Hospital. The CEO /management of Chris Hani Baragwanath Hospital is accordingly informed and the study is subject to:-

- Permission having been granted by the Committee for Research on Human Subjects of the University of the Witwatersrand.
- the Hospital will not incur extra costs as a result of the research being conducted on its patients within the hospital
- the MAC will be informed of any serious adverse events as soon as they occur
- permission is granted for the duration of the Ethics Committee approval.

.....
Recommended
(On behalf of the MAC)
Date: 12 July 2012

.....
Approved/Not Approved
Hospital Management
Date: 11/08/12

APPENDIX E: LETTER OF PERMISSION TO CONDUCT RESEARCH FROM THE CEO OF CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

Enquiries:
Office of the Chief Executive Officer
Charlotte Maxeke Johannesburg Hospital
Tell: 011 488 3792
Fax: 011 488 3753
Email: lindiwe.mngomezulu@gauteng.gov.za
Date: 14th March 2013

Dr. M. Beretta
Registrar – Paediatrics Department
University of the Witwatersrand

Dear Dr. Beretta

RE: "A retrospective audit of the presentation and management of vitamin D deficiency rickets in Johannesburg"

Please note that permission to conduct the above mentioned study is provisionally approved. Your study can only commence once ethics approval is obtained. Please forward a copy of your ethics clearance certificate as soon as the study is approved by the ethics committee for the CEO's office to give you the final approval to conduct the study.

Recommended/~~not recommended~~

Dr. M. Mofokeng
Clinical Director
Date: 14/3/2013

Approved/not approved

Ms. G. Bogoshi
Chief Executive Officer
Date: 16.03.2013

1

APPENDIX F: TURNITIN REPORT

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ORIGINALITY REPORT

4%	3%	3%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Vitamin D, 2010. Publication	2%
2	mobile.wiredspace.wits.ac.za Internet Source	1%
3	orbi.uliege.be Internet Source	1%
4	www.pediatricsdigest.mobi Internet Source	1%
5	www.scielo.org.za Internet Source	1%

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		