Vitamin D levels in young children with femur fractures at Charlotte Maxeke Johannesburg Academic Hospital

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Dr Daniella Pinkus

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

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Declaration

I, Daniella Pinkus, declare that this Research Report is my own, unaided work. It is being submitted for the Degree of Master of Medicine in the branch of Orthopaedic Surgery at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

……………………

21st of December in Johannesburg

Dedication

To my husband, Dean Bernard, my daughter, Gia Bernard and my son Gadi Bernard.

Presentation arising from research project

Vitamin D levels in young children with femur fractures at Charlotte Maxeke Johannesburg Academic Hospital. CMJAH Research Symposium, Len Miller Theatre, Wits Medical School, 17 March 2022 (Podium Presentation).

Abstract

Introduction: Fractures are common in children with healthy bones. However, the literature has shown that some of these fractures may be related to underlying bone pathology such as vitamin D deficiency. The aim of this study was to determine if there is vitamin D insufficiency or deficiency in children five years of age and younger with femur fractures at Charlotte Maxeke Johannesburg Academic Hospital.

Methods: This study was a retrospective review of children with femur fractures admitted to the Charlotte Maxeke Johannesburg Academic Hospital paediatric orthopedic unit. The records of children admitted between 1 January 2017 and 31 December 2017 were retrieved. Demographic data were collected from clinical notes and electronic discharge summaries. Radiographs were assessed and blood results were retrieved.

Results: Forty-five (n = 45) patients were enrolled for this study. The study sample comprised of 30 (66.7%) males with a mean age of 2.9 ± 1.3 (SD) years and 15 (33.3%) females with a mean age of 1.9 ± 1.1 (SD) years. The overall mean age for the sample population was 2.56 years (SD = 1.3, CI = 2.21 – 2.95). Of these patients, 42 (93.3%) were Black, 2 (4.5%) were White and 1 (2.2%) was Coloured. Falls accounted for the only mechanism of injury (n = 45). Spiral fractures accounted for the greatest proportion of fractures, followed by transverse fractures. Most patients (n = 34, 75.6%) came from inner city areas whilst the minority were from outer city areas (n = 11, 23.4%). Most children (68.9%) with femur fractures had low levels of serum 25-hydroxyvitamin D compared to children (31.1%) who had sufficient levels of serum 25-hydroxyvitamin D.

Conclusion: This study showed that 68.9% of the children were vitamin D deficient or insufficient and 75.6 % were from inner city areas. This suggests that children aged five years and younger with femur fractures in our hospital may benefit from routine blood testing and vitamin D supplementation.

Acknowledgements

My heartfelt thanks go to my three supervisors.

Prof Anthony Robertson, thank you for your immediate enthusiasm when I mentioned that I would like to do an MMed in Paediatric Orthopaedics, for suggesting a topic with Dr Dina Simmons and for nominating yourself as my supervisor, despite you being so busy with being Head of Department and Head of Paediatric Orthopaedics at CMJAH. Thank you for always being available to meet despite your busy schedule. I appreciate all your encouragement, guidance and assistance. I was very privileged to be guided and learn from someone as experienced with research as you are. Thank you very much.

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My husband Dean, thank you for your help with editing my grammar, assisting with formatting, as well as your patience, support and encouragement that helped me to complete this research.

To my family, for your love, support, encouragement and enthusiasm for me to complete this research project.

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Nomenclature

AAP	American Academy of Paediatrics
ALP	Alkaline Phosphatase
CBD	Central business district
Ca^{2+}	Calcium
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
DEXA	Dual Energy X- ray Absorptiometry
HREC	Human Research Ethics Committee
IOM	Institute of Medicine
KDOQI	Kidney Disease Outcomes quality initiative
N	Sample size
Nmol/l	Nano Mole per litre
P	Probability
PACS	Picture archiving and communication system
PTH	Parathyroid Hormone
25OH vitamin D	25-hydroxyvitamin D
ZAR	South African Rand

CHAPTER 1

Introduction and literature review

1.1 Introduction

Rickets is a common disease worldwide which affects many children. It is described as a generalised metabolic bone disorder affecting growing bones, with a delay in or failure of bone mineralisation at the unfused growth plates. Failure and inhibition of normal apoptosis of the hypertrophied chondrocytes occurs. This results in widening of the epiphysis and a delay in appearance and irregularity of the provisional zone of calcification at the metaphyseal end of the epiphysis. (1)

An insufficient dietary intake or supply of calcium and phosphate are the two common causes of rickets in South Africa. The main causes can therefore be categorised into two major groups i.e. the inability to maintain normal calcium levels and the inability to maintain normal phosphate levels. Some examples of these causes can be categorised into the following groups: firstly vitamin D deficiency caused by inadequate sun exposure or low dietary intake, secondly congenital diseases that present with vitamin D deficiency, otherwise known as vitamin D dependent rickets, and lastly vitamin D metabolism abnormalities caused by chronic diseases such as renal failure, or due to chronic medications such as Phenytoin treatment. (2-4)

Children with rickets present clinically with bone pain and bone deformities. Part of the deformities may be widening and splaying of the metaphyseal region causing thickened ankles and wrists. Some children may have genu varum, genu valgum and enlargement of costochondral junctions otherwise known as rachitic rosary. (1, 5, 6)

The distinct pathognomonic radiological features of rickets are splaying, fraying and cupping of the metaphysis. Widening of the growth plates with loss of the provisional zone of calcification can also be seen. (1, 2)

In recent years clinicians have reported an emergence of rickets in inner-city areas. Despite adequate sunlight exposure, vitamin D deficiency is still prevalent. The main source of vitamin

D for most people is endogenous synthesis which is dependent on exposure to ultraviolet light from sunlight. Dwelling type imparts significance in terms of economic and environmental impact on risk factor exposure. The most obvious being a limitation in sun exposure with flat/apartment occupation. An increased incidence of vitamin D deficiency rickets has been shown to be associated with overcrowding, smaller houses, lower income, tall buildings, flats occupation and lower levels of education. These factors are more common in inner city and township habitation. Gauteng is divided into settlement categories of inner city, township and outer city. It is important to make this distinction as inner city and township residence are at an increased exposure to risk factors contributing to vitamin D deficiency due to the lifestyle adopted in these areas, including a limitation to sunlight exposure, increased air pollution and absence of external public community areas. (7)

Vitamin D is important for calcium homeostasis and bone development/remodelling. Normal bone mineralisation is promoted if vitamin D levels and its metabolites are sufficient. This is achieved by the process of calcium absorption from the gut and delivery to the bone to promote bone mineralisation. Vitamin D deficiency results in reduced serum calcium which triggers parathyroid hormone (PTH) secretion to release calcium and phosphorous from bone in an attempt to maintain normal serum calcium levels. Ultimately, this leads to rickets in children. (8, 9)

The main route for vitamin D synthesis is through the sun. Solar ultraviolet radiation (UVR) in the UV-B range (290-315 nm) converts 7-dehydrocholesterol in the epidermis to previtamin D3 which thermally isomerises to vitamin D3. This is followed by hydroxylation, first to 25-hydroxyvitamin D in the liver, considered biologically the inactive form and then to the active form 1.25 hydroxyvitamin D in the kidney. Vitamin D is the biologically active form responsible for regulating calcium and bone metabolism, by enhancing intestinal calcium absorption and mobilizing calcium from the skeleton. (10)

Vitamin D deficiency is reported worldwide and a trend that has been attributed to lifestyle changes related to rapid urbanisation. The prevalence of vitamin D deficiency has been looked at in temperate regions but very few have been done in Africa. It has been shown that individuals of African ancestry compared to other ethnicities are more commonly vitamin D deficient. Lowest concentrations of vitamin D were observed in Northern African countries and South Africa. Globally the prevalence of vitamin D deficiency varies. It has been reported in the USA to be 23-30%, Middle East 30-90%, Australia 20% and China 56 %. The large variations could be accounted for known determinants of vitamin D status, such as fortification of foods with vitamin D in North American countries and some parts of Europe. (11-13)

Vitamin D deficiency has long been recognised as a cause of increased fracture risk associated with rickets in infants and osteomalacia in adults. This nutritional deficiency is more commonly found in dark skinned infants, decreased sun exposure or exclusive breast fed infants without additional vitamin supplements and poor dietary intake of vitamin D and calcium.(8)

In children with healthy bones, long bone fractures attributed to falls and injuries are common. Once they have sustained their first fracture, 40% of these children will sustain another fracture in the future. (11, 14) There is uncertainty as to why some healthy children sustain fractures from minor trauma and others do not. Most fractures are attributed to normal childhood development. (14)

Despite poor evidence in the paediatric population, recent literature has shown that fractures in children may be related to underlying bone pathology. This may be attributed to poor bone mineralisation associated with genetic and environmental factors including inadequate nutrition, and an inactive lifestyle. One of the proposed causes of bone pathology in children is vitamin D deficiency. (11)

The association between vitamin D levels and fracture risk has not been studied extensively in children and adolescents; however, the relationship between vitamin D levels and fracture risk has been analysed thoroughly in infants. Vitamin D deficiency has been shown to increase the fracture risk in infants with rickets. There is no specific method to predict the demographic, medical or social factors that increase a child's risk to fracture. (11, 14)

Vitamin D deficiency is measured by assessing the chemical 25-hydroxyvitamin D (25OH vitamin D) as it has a long half-life of two to three weeks, a strong circulating concentration and is resilient to fluctuations in Parathyroid Hormone (PTH). A challenge in this field is the lack of consensus on what serum concentration level is likely to reflect a clinically significant deficiency and hence require treatment. Figures as low as 25nmol/l versus 100nmol/l of 25OH vitamin D have been advocated. (4, 6, 15)

The controversy arises around whether to accept higher or lower levels of 25OH vitamin D as normal. If lower levels are accepted as normal, an under diagnosis of 25OH vitamin D deficiency may result. (4)

Table 1.1 below summarises normal and abnormal serum vitamin D concentrations according to the various classifications. The American Academy of Paediatrics and Institute of Medicine (AAP & IOM), the Endocrine Society, National Kidney Foundation and Kidney Disease Outcomes Quality Initiative (KDOQI) have their own guidelines as to what is considered a normal and abnormal 25OH vitamin D level. (4, 16, 17)

Table 1.2: Reference ranges for 25OH vitamin D levels according to various classification systems.

	AAP & IOM 2008 nmol/l	Endocrine Society nmol/l	KDOQI nmol/l	Adult New England Journal of Medicine 2007 nmol/l
Severe Deficiency	<12	-	<12	-
Mild to Moderate Deficiency	12-37.4	<50	12-37.4	<50
Insufficiency	40-50	52.4-75	40-75	50-75
Sufficiency	52-250	77-150	>75	77-150
Excess	252-372	-	-	-
Intoxication	>374	-	-	>374

It has been shown that the use of the APP and KDOQI vitamin D Guidelines, which both accept lower levels of 25OH vitamin D as normal, has caused an underestimation and missed diagnoses of vitamin D deficiency/insufficiency in the paediatric population. A missed diagnosis hinders the treatment of vitamin D deficiency with oral supplementation. (18)

Currently the Haematology Laboratory at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) uses the Endocrine Society Guidelines as a reference for normal and abnormal values of 25OH vitamin D. Higher levels of 25OH vitamin D is considered normal when using the aforementioned guidelines. This minimises the risk of underdiagnosing vitamin D deficiency/insufficiency; and therefore, this study made use of the Endocrine Society's Guidelines for 25OH vitamin D levels.

1.2 Literature review

The risk of fractures in children with low 25OH vitamin D levels has been reported in the literature. Several authors have investigated the biochemical profile of 25OH vitamin D deficiency or insufficiency in children with fractures. The findings obtained from these studies are discussed below.

In a retrospective study by Chapman et al., the authors assessed the x-rays of infants and toddlers, aged between 12-24 months, with rickets and determined the stage at which rickets was radiographically evident. Rickets was diagnosed using radiographs, with evidence of fraying and cupping of the metaphysis, and laboratory results indicating elevated alkaline phosphatase (ALP) levels (1.5 times above normal). The authors also investigated the typical age at which the children sustained fractures and categorised these fractures accordingly. Amongst the 45 children identified with rickets, 17.5% of them sustained fractures; however, the fractures did not resemble high risk non-accidental trauma fractures. (19)

X-ray features of fracture patterns in patients with non-accidental injuries differed to those in patients with rickets. The ability to distinguish between fracture patterns helps with identifying the cause of the fracture. Furthermore, the presence of rickets can be treated, and abused children can be identified and protected thereby making treatment more effective. (19)

Most of the patients diagnosed with rickets were nutrient deficient. The fractures were consistent with a failure of load bearing. The study supports the assertion that low vitamin D levels are a cause of fractures in mobile infants with rickets. (19)

In a prospective study by Ryan et al., the authors determined the prevalence of vitamin D insufficiency and abnormal bone density in African American children with forearm fractures aged between 5-9 years (N=17). Bone pathology was evaluated using 25OH vitamin D levels and Bone Mineral Density was evaluated using Dual Energy X-ray Absorptiometry scan (DEXA).

Of the seventeen patients included in the study, 59% of them were vitamin D insufficient. The DEXA scans showed that their bony mineralisation was normal for their age. These authors showed that vitamin D insufficiency plays an unrecognisable role in African American children with forearm fractures. (11) The results obtained from the study provided new information on the association between low vitamin D levels and fractures in children. (11)

In 2015, Paterson performed a systematic review based on 26 published reports which included 35 patients younger than two years of age with rickets. It was found that 18/35 children (51.4%) sustained multiple fractures, and most of the fractures were asymptomatic. Only a few patients showed clinical signs of a fracture. The remaining patients had fractures that were spontaneous and diagnosed radiographically. This study highlighted the importance of recognising that fractures may occur spontaneously in children with vitamin D deficiency rickets. (15)

In a prospective case control study by Thompson et al., the authors investigated the role of vitamin D in fracture risk in urban children aged between 2 and 14 years. One hundred and twenty (120) children were included in the study of which 60 children sustained fractures, and the remaining 60 children were non-fractured (control). A significant difference in 25OH vitamin D levels between the two groups was found ($p=0.023$), and there was a higher fracture incidence associated with 25OH vitamin D insufficiency. (14)

The paediatric population is at an increased risk for acquiring a fracture when vitamin D levels are low. The above study is unique in that it is the first to analyse the relationship between serum 25OH vitamin D and fractures across the diverse races (including all ethnicities). Furthermore, the authors looked at different levels of daily sun exposure; however, there was no relationship between 25OH vitamin D levels and fractures, and no association between daily sun exposure and 25OH vitamin D. A limitation of this study was that the study and the control groups were not perfectly matched. (14)

Minkowitz et al. published a retrospective study that focused on comparing 25OH vitamin D levels in patients with (N= 369) and without fractures (N=662). 25OH vitamin D levels were obtained from the Bone Health Survey and medical record data. All the patients included in the study were 18 years of age and younger, and the population comprised all races. (20)

The authors found that vitamin D levels were not associated with paediatric fractures; however, children with lower levels of vitamin D were reported to be at a higher risk of sustaining more severe fractures. (20) The authors demonstrated that African American, dark skinned children had lower 25OH vitamin D levels compared to the other children. Their findings supplement those reported in previous literature. (20)

In 2020, Hosseinzadeh et al. performed a prospective non-randomised study, which included one hundred children with low energy forearm fractures, prospectively enrolled from one hospital. (8) The authors assessed the vitamin D incidence and risk factors for these children with forearm fractures. They showed that vitamin D deficiency and insufficiency is common in children with forearm fractures, especially in obese children with fractures where surgery is indicated, thus emphasising that vitamin D deficiency may be a contributing risk factor for forearm fractures. (8)

Based on the findings reported in the current literature, it has become a routine in ward 275 at CMJAH to draw blood to test for rickets in children with long bone fractures. The study focused specifically on 25OH vitamin D levels in children five years and younger with femur fractures, as the current literature highlights a link between vitamin D status and fractures in children. The purpose of this study was to review the results and see if this ongoing practice will be justified .Furthermore, should they be vitamin D deficient, to assess if there is a need to start the children on vitamin D supplementation, to prevent further fractures.

1.3 Study Aim and Objectives

The aim of this study was to determine if there is an association between serum 25OH vitamin D levels and femur fractures in children five years of age and younger at CMJAH.

The objectives of this study were:

- To determine the incidence of 25OH vitamin D deficiency in children five years of age and younger with femur fractures at CMJAH.
- To relate vitamin D status to the demographics of our population group.
- To determine if there is a need to continue doing routine bone health blood sampling and determine if there is a need to provide vitamin D prophylaxis.

CHAPTER 2

Methodology

2.1 Research Question

Is there an association between vitamin D levels and femur fractures in children five years of age and younger at CMJAH?

2.2 Research Design

This study was a retrospective review of 45 children who sustained a femur fracture and were admitted to the paediatric orthopaedic ward at CMJAH between 01 January 2017 and 31 December 2017. All the children were clinically and radiologically diagnosed with femur fractures by orthopaedic registrars under the supervision of paediatric orthopaedic consultants.

Patient Inclusion criteria:

- Children five years of age and younger
- Children with a femur fracture

Patient Exclusion criteria:

- High energy injuries such as high velocity motor vehicle accidents or falls from a height
- Localised bone pathology such as bone cysts, rickets or osteogenesis imperfecta
- Non-accidental injuries
- Patients whose 25OH vitamin D blood specimens were rejected by the Biochemistry laboratory

2.3 Data Collection

Ethical approval was obtained from the Human Research Ethics Committee (HREC) (Medical) at the University of Witwatersrand (protocol number: M180509, see Appendix A) prior to data collection. Furthermore, written permission to conduct the study and access the patients' blood results was obtained from the CMJAH CEO (see Appendix B).

The following demographic data and injury characteristics of each patient were collected from the admission slips and electronic discharge summaries and captured on an Excel spreadsheet using Microsoft Excel 2016 (refer to Appendix C):

- Age
- Gender
- Race
- Location (inner/outer city)
- Mechanism of injury
- Fracture type (pattern)
- Side of fracture
- Previous fracture
- Radiological features of rickets

Defining the location as inner *versus* outer city: The administration of the City of Johannesburg Metropolitan Municipality was initially divided into eleven regions prior to the ending of the Apartheid system. Subsequently in 2006, the regions were consolidated and have now been divided into seven regions. The regions are named A to G as illustrated by the map shown in Appendix D. Region F refers to the inner city and is a diverse region as it ranges from degraded residential areas to commercial areas. Region F is centred on the central business district (CBD), and comprises the following suburbs: Yeoville, Bellevue, Troyeville, Jeppestown, Berea, Pageview and Fordsburg. For the purpose of this study, any areas that fell within region F were captured as 'inner city' and any areas that fell outside of this region were captured as 'outer city' (refer to the data collection sheet shown in Appendix C). (21, 22)

Onetime routine sampling of serum calcium, phosphate, PTH, ALP and 25OH vitamin D levels were measured as part of the ward's protocol to exclude non-accidental injuries, and for diagnostic purposes, and these blood results were captured on an Excel spreadsheet (refer to Appendix C).

The data for each patient with a femur fracture was retrieved from ward 275's admission book, clinical notes, and electronic discharge summaries. Furthermore, patients' radiographs were assessed using the Picture Archiving and Communication System (PACS), and blood results were checked using the Lab track login system with the patient's hospital number. Each patient was assigned a case number to maintain patient confidentiality and anonymity.

2.4 Data Analysis

Statistical analysis was conducted using IBM SPSS statistical software, version 23. Continuous variables with a normal distribution were reported in terms of mean and standard deviation Comparisons of the means of continuous variables between vitamin D deficient, insufficient and sufficient groups were performed by using the analysis of variance (ANOVA) test. Categorical data were analysed using the Chi-squared test. A p value < 0.05 was considered statistically significant. The Wilcoxon test is used to compare mean differences between two independent groups.

CHAPTER 3

Results

Forty-five (n = 45) patients were enrolled in this study. Initially the sample size was 61 patients but unfortunately seven patients were excluded as the mechanisms of injury were other than falls. A further nine patients were excluded as a result of the specimens being lost in transit to the laboratory or being rejected by the laboratory itself due to the specimens being insufficient or clotting.

The study sample comprised of 30 (66.7%) males with a mean age of 2.9 ± 1.3 (SD) years and 15 (33.3%) female patients with a mean age of 1.9 ± 1.1 (SD) years. The overall mean age for the sample population was 2.56 (SD = 1.3, CI = (2.21 – 2.95)) years. Among the 45 enrolled patients, 42 (93.3%) were Black, 2 (4.4 %) were White and 1 (2.2%) was Coloured. There were no Indian study participants. Furthermore, 75.6% of the patients (n=34) who experienced a fracture were from the inner city and 24.4% (n=11) were from the outer city. Low energy falls accounted for all the mechanisms of injury (n=45) in the study population. Children involved in motor vehicle accidents, pedestrian vehicles accidents, high velocity falls from a height and gunshots were excluded from the study population.

Table 3.1: Patient demographics and injury characteristics per 25 OH vitamin D category.

Variables	**All (n=45)**	**25OH vitamin D deficient (n=12)**	**25OH vitamin D insufficient (n=19)**	**25OH vitamin D sufficient (n=14)**	***P*-value**
Age (Mean ± SD) (Years)	2.56 ± 1.29	2.83 ± 1.75	3.0 ± 0.94	1.88 ± 1.07	*0.04
Gender (M/F)	30/15	6/6	14/5	10/4	0.36
Race (Black/White/Coloured)	42/2/1	12/0/0	17/1/1	13/1/0	0.99
Location (Inner city/Outer city)	34/11	10/2	16/3	8/6	0.15
Fracture Pattern (Buckle/Intertrochanteric/Oblique/ Spiral/Subtrochanteric/ Transverse)	1/1/5/25/2/11	1/1/0/6/1/3	0/0/2/12/0/5	0/0/3/7/1/3	1.00
Fracture side (R/L)	22/23	3/9	11/8	8/6	0.15
Previous Fracture (Y/N)	0/45	0/12	0/19	0/14	
X-ray features of rickets (Y/N)	0/45	0/12	0/19	0/14	

* Statistically significant

When fracture types are considered, spiral fractures accounted for the greatest proportion of fractures (n=25), followed by transverse fractures (n=11). Other fractures included oblique (n=5), subtrochanteric (n=2), buckle (n=1) and intertrochanteric (n=1) fractures. The percentage of each fracture type is shown in Figure 3.1 below.

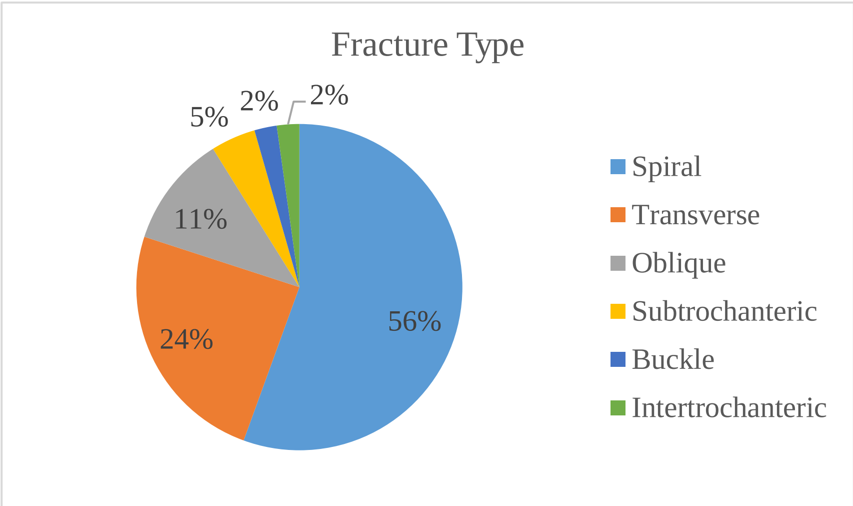

Figure 3.1: A pie chart showing the percentage distribution of each fracture type.

3.2 Vitamin D Status

Patients were grouped into three 25OH vitamin D categories based on the Endocrine Society Guidelines. There were 12 vitamin D deficient patients, 19 vitamin D insufficient patients and 14 vitamin D sufficient patients (see Figure 3.2). Thus, 26.6% were vitamin D deficient and 42.2% were vitamin D insufficient. When combining the vitamin D deficient and insufficient groups this accounted for 68.9% of the children.

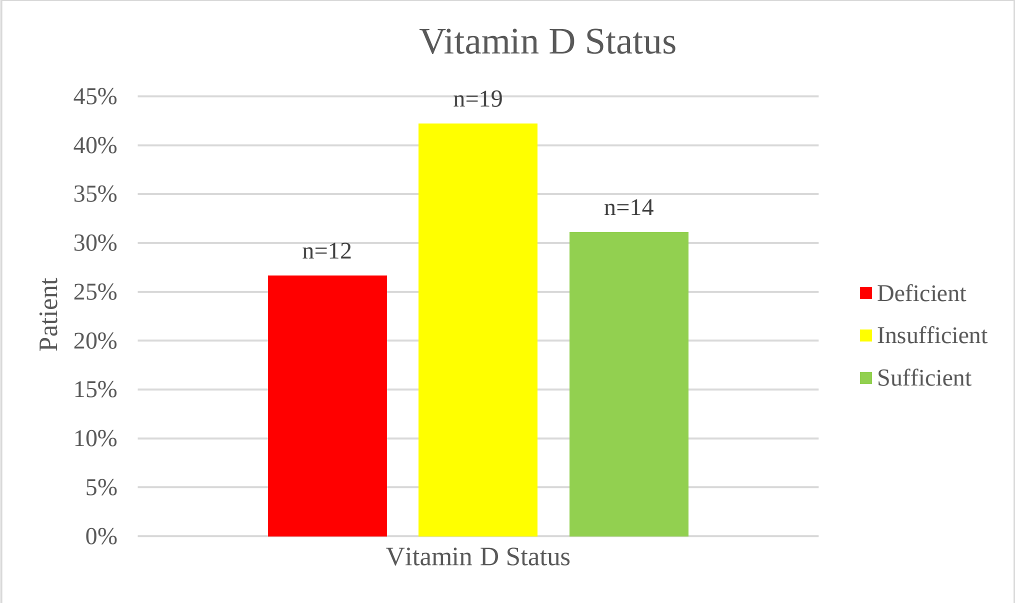

Figure 3.2: Patients 25 OH Vitamin D status.

A one-way ANOVA test was used to compare the mean Ca^{2+}, PO_4 and PTH concentration levels among the three previously mentioned groups based on their vitamin D status. The Wilcoxon test was used to compare the means of ALP between the vitamin D deficient and sufficient groups, and between the vitamin D deficient and insufficient groups however, no difference was found. Patients with vitamin D deficiency were not likely to have elevated PTH (p = 0.52) or ALP (p = 0.32) levels compared to patients with vitamin D insufficiency and sufficiency, respectively.

As shown in Table 3.1, statistical analyses indicate that the ages of the children were evenly distributed amongst the 25OH vitamin D categories (p = 0.04) showing the means of the ages ranged evenly between one and five years of age. There are no significant differences or correlations in any of the other variables namely, gender, race, location, MOI, fracture type, fracture side, Ca^{2+}, PO_4 , PTH and ALP among these groups ($p > 0.05$).

Table 3.2: Patients 25 OH vitamin D status.

Variables	All (n=45)	25OH vitamin D deficient (n=12)	25OH vitamin D insufficient (n=19)	25OH vitamin D sufficient (n=14)
	Mean ± SD			
25OH vitamin D (nmol/L)	64.0 ± 23.1	37.4 ± 10.2	59.2 ± 7.5	93.3 ± 13.6

Table 3.3: Patient biochemical parameters per 25 OH vitamin D category.

Variables	All (n=45)	25OH vitamin D deficient (n=12)	25OH vitamin D insufficient (n=19)	25OH vitamin D sufficient (n=14)	***P*-value**
	Mean ± SD				
CA^{2+}(mmol/L)	2.17 ± 0.70	1.97 ± 0.92	2.24 ± 0.56	2.26 ± 0.70	0.21
PO_4 (mmol/L)	1.61 ± 0.45	1.58 ± 0.59	1.70 ± 0.28	1.61 ± 0.52	0.69
PTH (pmol/L)	2.22 ± 1.87	2.38 ± 1.75	2.92 ± 1.50	2.51 ± 2.43	0.58
ALP (U/L)	244.02 ± 249.17	307.75 ± 407.51	201.89 ± 104.22	246.57 ± 219.62	0.35

In terms of exploring the relationship of calcium levels between 25OH vitamin D deficient and sufficient children, the following was noted: calcium levels were higher in children with sufficient 25OH vitamin D than in those who were deficient ($p = 0.03$). However, a relationship with PO_4, PTH and ALP between the two groups was not found ($p = 0.34$, $p = 0.58$ and $p = 0.36$, respectively).

A comparison was performed between patients in each 25OH vitamin D category (deficient, insufficient and sufficient). The comparison was mainly based on the patient covariates namely, Ca^{2+}, PO_4, PTH and ALP among the different categories. This was to see if there was any relationship between the three categories based on each covariate.

There was no statistical significant difference between vitamin D deficient and insufficient children against the patient level covariates Ca^{2+}, PO_4 , PTH and ALP as their *p* - values were > 0.05 for Ca^{2+} ($p = 0.13$), PO_4($p = 0.76$), PTH ($p = 0.79$), and ALP ($p = 0.43$).

Vitamin D was an independent variable of the biochemical markers when comparing these two groups. Extrapolated from this data, Vitamin D concentration has no effect on the biochemical parameters. Moreover, vitamin D deficient and insufficient patients were grouped together as they are both considered vitamin D deficient. There was a statistically significant difference between the grouped vitamin D deficient and sufficient children against the patient level covariates (Ca^{2+}, PO_4, PTH and ALP) as their *p* - values were all < 0.05 for Ca^{2+} ($p < 0.001$), PO_4 ($p < 0.001$), PTH ($p = 0.03$) and ALP (p=0.02).

The results in table 3.4 shows that 72.2% of the inner city patients were vitamin D deficient/Insufficient compared to 55.6% of the outer city patients. A proportional difference test, however revealed the proportions do not differ significantly (p = 0.334) this is most likely due to a small sample size. With comparing the vitamin D levels in the inner city vs outer city patients, from an observed perspective the odds ratio of 2.08 is reported. It is 2.08 times more likely for inner city patients to be vitamin D deficient/insufficient compared to the outer city patients.

Table 3.4: A table showing comparison between vitamin D levels in Inner city versus Outer city.

Vitamin D groups	**Inner City n (%)**	**Outer City n (%)**	**Total n (%)**	**P- value**	**Odds Ratio**
Deficient & Insufficient	26 (72.2%)	5 (55.6%)	31 (68.9%)	0.334	2.08
Sufficient	10 (27.8%)	4 (44.4%)	14 (31.1%)		

Table 3.5: A table comparing vitamin D deficient/insufficient patients versus sufficient in terms of age.

Vitamin D status	n	Mean age (years)	Std Error mean
Deficient/insufficient	31	2.94+-1.237	0.222
Sufficient	14	2.02+-1.076	0.288

Table 3.6: A table illustrating an Independent Sample T-test for vitamin D deficient/insufficient patients versus sufficient in terms of mean age.

T- value	Degrees of freedom	Two-sided p-value	Cohen's d-value
2.394	43	0.021	0.77

*equal variances assumed

An independent sample t-test indicates a significant difference in the mean age of patients categorised as vitamin D deficient or insufficient (M=2.94; SD 1.237) compared to sufficient patients (M=2.02; SD=1.076) (p=0.021). The magnitude of the differences in the mean (mean difference =0.92) is considered large (Cohen's d=0.77)

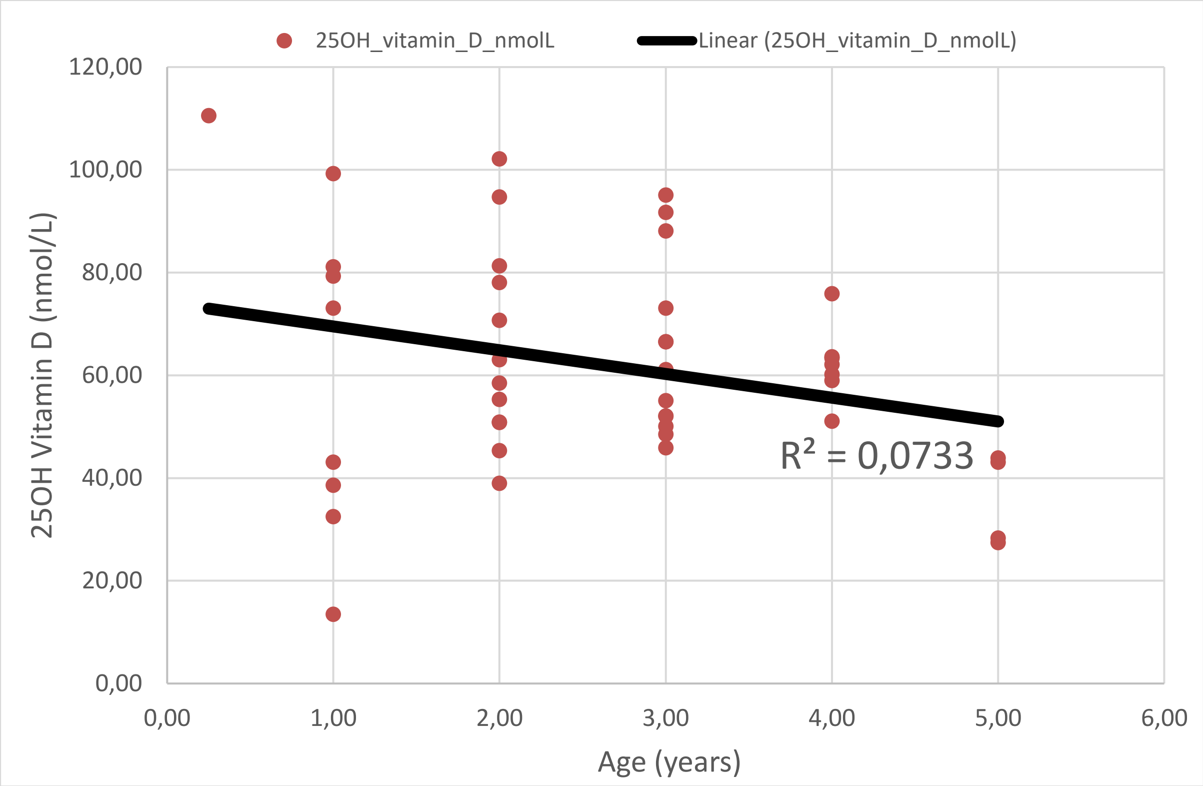

Figure 3.3: A scatter plot graph showing the correlation between age & vitamin D levels.

The above scatter plot graph shows a correlation between age and vitamin D levels i.e. vitamin D concentrations decreased as age increased.

CHAPTER 4

Discussion

Vitamin D is known to be important for bone health however, it has been unclear whether vitamin D is associated with fracture risk in childhood. (1) The key nutrient that helps maintain normal bone health and promotes skeletal mineralisation is vitamin D. Vitamin D deficiency causes a body storage of calcium and phosphorous depletion. (1, 10) Poor bone mineralisation has been proposed to predispose children to pathological fractures. (11)

In recent literature there is conflict regarding the role of vitamin D deficiency and the risk of fractures. Some studies have shown that patients with fractures have lower vitamin D levels compared to control subjects and others have shown that there is no association. (20, 23)

In our study of 45 children with femur fractures, it was established that 42.2% were vitamin D insufficient and 26.67% were deficient; therefore, a combined total of 68.9% were vitamin D deficient and insufficient, and 31.1% were sufficient. Interestingly, none of the vitamin D deficient children had florid rickets radiologically, they had isolated low vitamin D levels, and the clinical and radiological findings were not in keeping with rickets amongst these children.

A statistically significant association between concurrent 25OH vitamin D concentration and femur fracture risk was found. The results corroborate the findings reported in a study by Minkowitz et al. with 369 fractured patients and 662 non-fractured control patients aged 2 to 18 years. The incidence in their fracture group was similar to ours i.e. 45% were vitamin D insufficient and 20% were vitamin D deficient, thus showing that a poor vitamin D status could be associated with fracture risk. (20)

In another recent prospective study conducted by Hosseinzadeh et al., the authors attempted to relate vitamin D status and fracture risk. They assessed vitamin D levels and other risk factors in one hundred children, aged 3 to 17 years old, with forearm fractures at a single hospital. They reported a vitamin D insufficiency rate of 49%, and a deficiency rate of 21% which is similar to our findings. (8) Therefore, there appears to be an association between low vitamin D levels and fracture risk.

In our results (see Table 3.3), vitamin D was an independent variable of all the biochemical parameters investigated. This is not surprising as calcium gets extracted from bone to maintain a serum calcium balance. We also found a statistically significant difference amongst the mean age groups as shown in Table 3.1 showing age of the patient was evenly distributed from one to five years of age and yet in the older age group there was an increased vitamin D deficiency found.

There may be a correlation between age and vitamin D levels, despite the p - value not being significant which may be attributed to a small sample size. The results showed as the children increased in age, their vitamin D levels decreased. This could be due to a change in the children's diet as they age and transition from obtaining vitamin D from their mother's breast milk versus solid foods and milk which may not contain adequate amounts of vitamin. Another explanation is that they are most likely getting limited sunlight exposure once they are placed in crèches with tall overcrowded buildings. In contrast to the findings reported in the literature, nutritional vitamin D deficiency has been shown to be more likely in infants, with limited sun exposure and a history of breast feeding due to inadequate vitamin D intake from the mother's diet. (11, 24) However, this finding could be due to a lack of investigation in vitamin D deficiency and its effects in children and adolescents as it has mostly been investigated in infants (11).

Forty-two out of the forty-five patients in our study were of black race. All the vitamin D deficient/insufficient children had dark skin pigmentation. The literature has shown that dark skin pigmentation is a risk factor for vitamin D deficiency. (11) However, in this study we cannot conclude this being an added contributing factor to the vitamin D deficiency, as the majority of the population were black and thus not a true representation of the population group. This could be further investigated in the South African context.

Despite adequate sunlight availability in the climate in South Africa, vitamin D deficiency was surprisingly still prevalent in this population group. This may be due to less sunlight exposure of these children. The results showed that the majority (75.6%) of the children were found to be from the inner city region. Dwelling type imparts significance in terms of economic and environmental impact on risk factor exposure. Limited sun exposure has been shown to be associated with overcrowding, smaller houses, lower income, high buildings, flat occupation, and low levels of education. These factors are more common in the inner city and town habitation which is the majority of where our population is from predisposing them to vitamin D deficiency/insufficiency. (7, 21, 25)

In our results (see Table 3.4), 26 (72.2%) of the children who were vitamin D deficient/insufficient were from the inner city areas compared to five (55.6%) who were from the outer city areas. Children from the inner city areas are 2.08 times more likely to be vitamin D deficient/insufficient compared to the outer city children. This supports our objective that the Vitamin D status of our population can be related to its demographics. These results are in keeping with the literature related to inner city and vitamin D deficiency/insufficiency. (3, 7, 26)

Norval et al. (2015) published a systematic review looking at the 25OH vitamin D levels of the South African population groups of varied skin colours and ethnicities. The study assessed whether 25OH vitamin D plays an important role in protection against diseases that are currently causing burdens on the health service in South Africa. It included 134 normal healthy children. These children's vitamin D 25OH statuses were assessed and analysed. (27) Unfortunately, we were unable to use this study as a control as the mean values of the 25OH vitamin D levels between ages one and seven years were only provided, and not the individual 25OH vitamin D levels of each child at a specific age.

In another study by Richter et al., the authors used the Bone Health sub-cohort of the Birth to Twenty project, and looked at the prevalence of vitamin D deficiency in children and adults in the greater Johannesburg metropolitan area. (28) Once again, we were unable to use it as a control study as their population age group did not match our population age group and the vitamin D levels were not provided. (29)

Majority of the children in the study were vitamin D deficient/insufficient. This could justify the continuation of doing the routine blood sampling in the orthopaedic paediatric ward. The high vitamin D deficiency rate in these children is clearly an ongoing finding and can be suggested to be associated with femur fractures. Necessary treatment can then be started once the deficiency/insufficiency is detected.

It has been recommended for prevention of vitamin D deficiency, to have adequate sun exposure of at least three hours a week. Breast milk does not contain sufficient vitamin D to prevent deficiency, therefore adequate exposure of the infant to sunlight is of the utmost importance or will need to provide the infant with vitamin D until weaning. (29) The American Academy of Paediatrics (AAP) recommends newborns and infants who are exclusively breastfed or not on an adequate volume of commercial milk formula should be given Vitamin D daily. (30) Mothers who are vitamin D deficient or at higher risk for Vitamin D deficiency are advised to take a prophylactic vitamin D dose once daily to achieve an adequate vitamin D diet. (4, 16, 17, 30)

Educating parents on the importance of vitamin D intake in children can effectively help reduce the prevalence of vitamin D deficiency. (30) It is vital to help improve parent's perceptions and understanding of their vitamin D information and how they can increase their children's Vitamin D uptake. It is important to emphasise to the parents the three main ways to ensure adequate vitamin D levels. The first being to ensure their child's skin is exposed to sufficient sunlight weekly. Secondly, to ensure Vitamin D is available in their child's diet and lastly via vitamin D supplementation .(4, 17)

Our study findings will add new information to the South African and International literature. This study looked at children of the age groups older than neonates and younger than five years with femur fractures and their corresponding 25OH vitamin D levels. There is currently a gap in the literature of this age group, and of a particular fracture site. As far as we are aware, our study is the first to define factors associated with vitamin D deficiency in children with isolated femur fractures aged five years and younger.

The results of this study supports the current literature by highlighting the association that has been found between 25OH vitamin D deficiency and fractures in children. This re-emphasises the relevance and importance of this study in our population group, which assessed the incidence of 25OH vitamin D deficiency/insufficiency in children with femur fractures at CMJAH.

4.1 Limitations

The primary limitation of this study is that it is retrospective. The study lacked a matched control group of South African children was unavailable. The study is limited by a small sample size.

4.2 Recommendations

Based on the results obtained from this study, the following recommendations are made:

- The results have shown it would be beneficial to continue routine bone health blood sampling in ward 275, especially since the incident rate is high in this population group. This would allow the vitamin D deficiency to be detected and thereafter treated.

- Due to a six-week delay of the vitamin D blood results, it would be beneficial to provide prophylactic vitamin D to children under five years of age with femur fractures. The prophylaxis can be adjusted once the results are available and indicate a normal level.

- As the study sample size was small and lacked a control group, there is a need for a prospective control study to show the impact of vitamin D deficiency amongst the South African paediatric population.

- Based on the high vitamin D deficiency/insufficiency incident rate detected in this study, educating the parents amongst the inner city population in terms of adequate sun exposure, appropriate nutrition and possible vitamin D supplementation would be of great benefit. This could be achieved through community projects, providing talks at schools and handing out informative flyers.

CHAPTER 5

Conclusion

Our study has shown that vitamin D deficiency and insufficiency are common in children with low energy femur fractures. This finding is consistent with the current literature showing a strong association between vitamin D deficiency and fractures.

Vitamin D deficiency was found to be more common in children from the inner city areas. Despite a high incident rate of vitamin D deficiency amongst these patients, none of them had rickets clinically, radiologically or biochemically.

A correlation between age and vitamin D levels was also found. As the children got older they were more likely to be vitamin D deficient or insufficient. This finding is contradictory to the current literature and needs to be further investigated.

Furthermore, due to the high vitamin D deficiency prevalence rates in our population, it is justified to continue to perform the routine blood sampling for these children and assess if empirical vitamin D treatment is needed.

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Appendices

Appendix A: Ethics Clearance Certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Daniella Pinkus

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M180509

NAME: (Principal Investigator)	Dr Daniella Pinkus
DEPARTMENT:	Orthopaedic Surgery Charlotte Maxeke Johannesburg Academic Hospital Paediatric Orthopaedic Unit Ward 275
PROJECT TITLE:	Vitamin D levels in young children with femur fractures at Charlotte Maxeke Johannesburg Academic Hospital
DATE CONSIDERED:	25/05/2018
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr D Simmons; Prof A Robertson & Dr B Milner
APPROVED BY:	Professor CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	06/08/2018

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

DECLARATION OF INVESTIGATORS

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

Principal Investigator Signature

Date 31/08/18

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix B: Hospital CEO Clearance

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

Enquiries:
Ms. N. Mzila
Office of the Clinical Director
Email:Nolwazi.Mzila@gauteng.gov.za
Tell: (011): 488-4812
07 February 2018

Dear Dr. Pinkus

STUDY TITTLE: A Retrospective Review Study in Paediatric Orthopaedic Ward 275 at Charlotte Maxeke Johannesburg Academic Hospital

Permission to conduct the above mentioned study is provisional 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.

Supported / ~~not supported~~

Dr. M.I. Mofokeng
Clinical Director
DATE: 08/02/2018

Approved / not approved

Ms. G. Bogoshi
Chief Executive Officer
DATE: 08.02.2018

Appendix C: Data Collection Sheet

Patient ID	Age	Gender (M/F)	Race (Black/White/ Indian/Colour ed)	Area	Location (Innercity/ Outercity)	MOI	Fracture Type	Fracture side (R/L)	Previous fracture (Y/N)	X-Ray Feature of Rickets (Y/N)
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										

Appendix D: A map of the seven regions of Johannesburg (25)

Region A	Diepsloot, Kya Sands, Dainfern, Midrand, Lanseria, Fourways
Region B	Randburg, Rosebank, Emmarentia, Greenside, Melville, Mayfair, Northcliff, Rosebank, Parktown, Parktown North
Region C	Roodepoort, Constantia Kloof, Northgate, Florida, Bram Fischerville
Region D	Doornkop, Soweto, Dobsonville, Protea Glen
Region E	Alexandra, Wynberg, Sandton, Orange Grove, Houghton
Region F	Inner City, Johannesburg South
Region G	Orange Farm, Weilers Farm, Ennerdale, Lenasia, Eldorado Park, Protea South