

https://dspace.org/

A cross-sectional observational study of the vitamin D status in children with chronic kidney disease at Charlotte Maxeke Johannesburg academic hospital

Raga, Shirika V

2016-11-04
http://hdl.handle.net/10539/21393

Downloaded from a DSpace repository. https://dspace.org/

A CROSS-SECTIONAL OBSERVATIONAL STUDY OF THE VITAMIN D STATUS IN CHILDREN WITH CHRONIC KIDNEY DISEASE AT CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

Sharika V. Raga

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfillment of the requirements for the degree in Masters of Medicine in Paediatrics (MMed)

Johannesburg, May 2015

DECLARATION

I, Sharika V. Raga declare that this research report is my own work. It is being submitted for the degree in Masters of Medicine in Paediatrics in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

……

The……..day of …….2015.

ABSTRACT

Introduction

Vitamin D has numerous important functions in the human body. There is limited data available regarding vitamin D status in children with Chronic Kidney Disease (CKD) in South Africa.

Objectives

To determine the vitamin D status, as well as factors that affect it, in children with CKD in Johannesburg, South Africa.

Methods

A cross-sectional observational study was performed on 69 patients who attended the Renal Outpatient Clinic, and required routine phlebotomy, at Charlotte Maxeke Johannesburg Academic Hospital, Division of Paediatric Nephrology, between 20/08/2013 and 20/05/2014.

Results

71% (n=49) of patients in our study sample were vitamin D sufficient. Significant factors that influenced vitamin D status included albumin levels and the presence of Nephrotic syndrome with relapse.

There was a statistically significant positive correlation between albumin and vitamin D (p=0.00). As albumin levels increased so did the vitamin D levels. Patients with Nephrotic Syndrome with relapse had significantly lower vitamin D levels compared to patients with Nephrotic Syndrome in remission (p=0.00).

Conclusion

The majority of children with Chronic Kidney Disease in Johannesburg, South Africa, are vitamin D sufficient. This is reassuring as it implies that there is no need for routine vitamin D supplementation in this sample of children. However patients, especially those with nephrotic syndrome with relapse, still need to be screened for vitamin D deficiency and insufficiency and supplemented if necessary.

ACKNOWLEDGEMENTS

I gratefully acknowledge and thank:

- My supervisor, Doctor Cecil Levy.
- Professor John Pettifor, for his advice and insight.
- Doctor Jaya George and the University of the Witwatersrand Chemistry Department.
- All the patients and their families whose data was used in this study.
- The Nephrology Unit and staff for their assistance.
- The Medical Advisory Committee and Charlotte Maxeke Johannesburg Hospital for allowing me to conduct the study.
- The department of Paediatrics and the Faculty of Health Sciences of the University of the Witwatersrand.

TABLE OF CONTENTS

	Page
DECLARATION	ii
ABSTRACT	iii
ACKNOWLEDGEMENTS	v
TABLE OF CONTENTS	vi
LIST OF FIGURES	viii
LIST OF TABLES	ix
ABBREVIATIONS	x
1.0 INTRODUCTION	
1.1 Literature review	1
1.2 Aim	5
1.3 Objectives	5
2.0 STUDY DESIGN AND METHODS	
2.1 Study Design	6
2.2 Sample Size	6
2.3 Study Methods	7
2.4 Biochemical Determinations and Definitions	8
2.5 Statistical Analysis	10
2.6 Limitations	11
3.0 RESULTS	
3.1 Patient Characteristics	12
3.2 25OHD trend	14
3.3 25OHD vs. Age	16
3.4 25OHD vs. Sex	17
3.5 25OHD vs. Race	18
3.6 25OHD vs. Financial Status	19
3.7 25OHD vs. Season	20
3.8 25OHD vs. Body Mass Index	21
3.9 25OHD vs. Glomerular Filtration Rate	22
3.10 25OHD vs. Duration of Illness	23
3.11 25OHD vs. Peritoneal Dialysis	24
3.12 25OHD vs. Albumin	25
3.13 25OHD vs. Nephrotic Syndrome with relapse	26
3.14 Albumin vs. Nephrotic Syndrome with relapse	26

Page

4.0 DISCUSSION 28

5.0 CONCLUSION 31

6.0 REFERENCES 33

7.0 APPENDICES

A: Consent/Assent form 35
B: Ethics approval from Human Research Ethics Committee 36

LIST OF FIGURES **Page**

Figure 1: Histogram – Vitamin D levels for the entire cohort of patients 15

Figure 2: Scatter plot - 25OHD vs age 17

Figure 3: Box and Whisker Plot-25OHD vs Race 19

Figure 4: Box and Whisker Plot-25OHD vs. Season 21

Figure 5: Scatter plot – 25OHD vs. BMI z Score 22

Figure 6: Scatter Plot-25OHD vs. GFR 23

Figure 7: Scatter plot-25OHD vs Duration of Illness 24

Figure 8: Scatter plot: 25OHD vs. Albumin 26

LIST OF TABLES **Page**

Table 1: Characteristics of patients 13

Table 2: Comparing mean 25OHD levels between males and females 18

Table 3: Comparing financial status with mean 25OHD levels 20

Table 4: Comparing mean 25OHD levels between patients on Peritoneal Dialysis and those who aren't on dialysis 25

Table 5: Comparing mean 25OHD levels in patients with Nephrotic Syndrome with relapse vs. remission 27

Table 6: Comparing mean Albumin level in patients with Nephrotic Syndrome with relapse and those with Nephrotic Syndrome in remission 27

ABBREVIATIONS

CKD	Chronic Kidney Disease
BMI	Body Mass Index
25OHD	25 hydroxy vitamin D
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
KDIGO	Kidney Disease Improving Global Outcomes
GFR	Glomerular Filtration Rate
EDTA	Ethylenediaminetetraacetic Acid
HPLC	High performance liquid chromatography
PDA	Photo Diodo Array
WHO	World Health Organization
CEO	Chief Executive Officer
HREC	Human Research Ethics Committee
VDBP	Vitamin D binding protein

1.0 INTRODUCTION

1.1 Literature Review

Vitamin D is produced from photosynthesis in the skin and dietary sources. Cholecalciferol (D_3) and ergocalciferol (D_2) are precursors that are hydroxylated to calcidiol (25-hydroxyvitamin D)(25OHD) in the liver. Calcidiol is converted to calcitriol (1-25-dihydroxyvitamin D), an active form of vitamin D, by the enzyme 1-alpha-hydroxylase, in the kidney. Calcitriol binds to vitamin D receptors that are found in many tissues in the body. Some of the major functions of vitamin D include calcium homeostasis, immune regulation and regulation of cell growth. [1]

Renal production of 1-25-dihydroxyvitamin D is tightly regulated by plasma parathyroid hormone levels and serum calcium and phosphate levels. Low extracellular calcium stimulates the parathyroid glands to secrete parathyroid hormone (PTH). An increase in PTH up-regulates 1-alpha-hydroxylase resulting in an increase in the synthesis of 1-25-dihydroxyvitamin D. [2]

Vitamin D binding protein (VDBP) plays an important role in renal vitamin D metabolism by transporting vitamin D metabolites in the blood. 80-90% of 25OHD and 1-25-dihydroxyvitamin D circulate bound to VDBP, 10-15% is bound to albumin and <1% is in their free form. In CKD, changes in the concentration of VDBP due to glomerular and/or tubular damage could impair vitamin D transport and metabolism. This may influence the bioavailability of 25OHD to target tissues. [5]

Predisposing factors for vitamin D deficiency include reduced skin synthesis as a result of sunscreen use or reduced exposure to sunlight among the housebound. Other factors include reduced bioavailability as a result of malabsorption or obesity, increased catabolism from drugs such as anticonvulsants, increased urinary loss in nephrotic syndrome and impaired vitamin D hydroxylation or activation. [3]

There is a high prevalence of vitamin D deficiency in children with Chronic Kidney Disease (CKD). They are more likely to have lower 25OHD levels due to reduced exposure to sunlight, a reduced intake of foods that contain vitamin D[4] as well as a reduced production of circulating 1-25-dihydroxyvitamin D by the kidney [5].
Lower levels of 25 hydroxy vitamin D (25OHD) are found in children with Nephrotic Syndrome, a specific kidney disease in childhood, especially during a relapse, due to the loss of 25OHD and vitamin D binding protein[6]. This has been found in numerous studies done previously on this topic[4, 7]. In addition, lower levels of 25OHD have also been found in patients on haemodialysis and on peritoneal dialysis [6,11,12].

There is limited data available regarding the vitamin D status in children with CKD in South Africa. In a review published in the Indian Journal of Paediatrics, vitamin D deficiency was highly prevalent in children with Chronic Kidney Disease[8]. Included in the review was a study by Menon *et al.* where 56.1% of patients had vitamin D insufficiency and 21.1% were deficient of vitamin D[9]. Another study by

Kalkwarf *et al.* found that nearly half of children included in the study with stage 2-5 kidney disease were vitamin D deficient[10].

Similar results were found in a study based in Miami, where more than half of patients across all stages of CKD did not have sufficient vitamin D levels (32% had vitamin D insufficiency and 28% were deficient in vitamin D)[11]. Factors that may contribute to these findings include the fact that chronically ill children may not spend as much time outdoors thus minimizing the amount of sunlight they're exposed to. Other factors that may influence vitamin D status include sex, age, season, and Body Mass Index (BMI) [5,13,14,15,26,27,28].

A study by Denburg *et al*. found that the concentration of all vitamin D metabolites were lower with advanced CKD[5]. Winter season and older age were associated with lower concentrations of free and bioavailable 25OHD[5]. Black race was associated with lower total 25OHD, but not free or bioavailable 25OHD [5]. Another study published in the Journal of Paediatrics also found lower mean 25OHD levels in winter compared to summer[9]. In the same study, rates of vitamin D insufficiency and deficiency were similar in male and female patients but significantly higher in older and overweight patients [11]. Obesity alters the release of vitamin D into the circulation and decreases its bioavailability because of deposition in adipose tissue compartments[12].

In a study published in the United States of America, mean total vitamin D intake was found to be less in the low-income group compared to the high-income category, although results were not significant[11]. In addition, whites had a significantly higher total intake of vitamin D than blacks and Hispanics [13]. Closer to home, it was found that children living in the majority of developing countries were found to have a calcium intake one third to a half of those children living in developed countries[14]. It is important to note, however, that these studies were not done on children with CKD. Further studies are required to investigate the calcium intake and socioeconomic status with regards to vitamin D status in children with CKD.

Our motivation for conducting this study was to look at the vitamin D status in children with a spectrum of renal disease in Johannesburg, South Africa, in order to determine if there might be a need for vitamin D supplementation in this sample of patients.

1.2 Aim

To determine the vitamin D status in children with renal disease attending the Renal Outpatient Clinic at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), Division of Paediatric Nephrology, between 20/08/2013 and 20/05/2014.

1.3 Objectives

To determine the effect of the following factors on Vitamin D status:

i) Age

ii) Sex

iii) Race

iv) Financial status

v) Season

vi) Body Mass Index (BMI)

vii) Estimated Glomerular Filtration Rate (eGFR)

viii) Duration of illness

ix) Peritoneal Dialysis

x) Serum albumin level

xi) Nephrotic Syndrome with relapse

2.0 **STUDY DESIGN AND METHODS**

2.1 Study Design

A cross-sectional observational study was performed on all patients who attended the Renal Outpatient Clinic at the Division of Paediatric Nephrology, CMJAH, between 20/08/2013 and 20/05/2014, and who required routine phlebotomy.

Inclusion/Exclusion Criteria

All patients described above were included in the study, namely, those who routinely required phlebotomy at the visit. This cohort consisted of patients with CKD and others with renal disease attending the clinic. Patients who did not require routine phlebotomy were excluded from the study because the ethics application specifically stated that we would not subject our patients to the pain of an additional needlestick for the purposes of the study. Patients were included provided that consent/assent was obtained.

The ages ranged from 8 months to 24 years. These accounted for 90% of the total number of patients attending the Renal Clinic.

2.2 Sample Size

The minimum number (n) of children required for the study was calculated using the formula: [15]

$n = z^2 p(1-p)/d^2$

'z' is the standard normal deviate corresponding to the 95% confidence interval = 1.96

'p' is the estimated prevalence of vitamin D deficiency in children with renal disease from a previous study = 0.07 [16]

'd' is the absolute sampling error that can be tolerated = 0.05

Therefore, the minimum sample size is 'n' = 100

The number of participants in our study (74) was short of the minimum requirement of 100. This was due to time constraints for the data collection period as well as a lack of funding.

2.3 Study Methods

All patients who met the criteria for inclusion in the study had an extra ethylenediaminetetraacetic acid (EDTA) blood tube taken for 25 hydroxy vitamin D (25OHD) analysis. We opted to measure 25OHD as opposed to 1-25-dihydroxyvitamin D because we wanted to determine the vitamin D status, not how it is metabolized. We did not subject any child to an additional needle stick.

The blood samples were taken, within 4 hours, to the Chemistry Laboratory at the University of the Witwatersrand (on the same property as the hospital), where they were spun. The spun samples were stored in a freezer at -80 degrees Celsius until analysis. Samples were run on a high performance liquid chromatography (HPLC) with photo diodo array (PDA) using a commercially available kit method for vitamin D analysis (RECIPE ClinRep HPLC Kit, Munich, Germany). Plasma samples were

extracted using liquid-liquid extraction techniques as described on the package insert. 50µl of supernatant was then injected onto the HPLC system for detection. Control checks were in place to ensure standardization of results.

An HPLC photodiode detector was used for the analysis. This separated the 25OHD into vitamin D_2 and D_3, which were then added together to get a total. Matrix matches internal controls, which are commercially available, were run on every 10 samples. This was at two levels, which span the analytical range of the assay. The assay CV was 8% at the low range and 7.4% at the high range. The inter-assay CV ranged from 4.1-10.8% and intra-assay CV from 0.3-9.6%. External quality assurance was performed using DEQAS, which was run every month. Internal quality controls were run on every 10 samples and extracted with the samples per batch prepared.

All results were reported in the unit nmol/L.

Vitamin D binding protein was not measured as our laboratory did not have the facilities to do so, and we did not have enough funding to outsource it.

2.4 Biochemical Determinations and Definitions

Vitamin D status: Vitamin D status was defined according to serum 25OHD levels, in accordance with Institute of Medicine guidelines[17], namely;

- Vitamin D deficient <30nmol/L
- Vitamin D insufficient 30-50nmol/L
- Vitamin D replete >50nmol/L

The Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guidelines were used to classify the CKD stage of our patients[18].

Financial Status: Patients' families were divided into two groups namely those that were exempted from paying a hospital fee ("Non Paying") and those that had to pay as per hospital criteria ("Paying").

Season: The seasons were divided into two groups. Group One included winter and spring, and Group Two included summer and autumn.

BMI: BMI was calculated using the weight (kg) divided by height (m) squared. Anthropometric z scores were calculated using the World Health Organization (WHO) Anthro and AnthroPlus software programmes (Department of Nutrition, WHO, Geneva, Switzerland).

Glomerular Filtration Rate: GFR was estimated from the patient's height and creatinine using the modified Schwartz formula[19].

Duration of illness: This was defined as the interval between the date of the first presentation of the patient to the clinic and the date of phlebotomy.

Nephrotic Syndrome relapse: Patients were defined as having Nephrotic Syndrome relapse if they were known to have a diagnosis of Nephrotic Syndrome and had, on urine dipstix performed up to six months prior to the blood sample

being taken, greater than or equal to 2+ proteinuria detected. This was an arbitrary definition recommended by the Division of Paediatric Nephrology at CMJAH for the purposes of the study, as there is no universal agreement on the criteria for dipstick diagnosis of relapsed Nephrotic Syndrome.

Informed consent was obtained from the legal guardians of patients included in the study and assent was obtained from children old enough to understand the study (Appendix A).

Permission to conduct the study at CMJAH was obtained from the Chief Executive Officer (CEO) of the hospital. Ethics approval to undertake the study was granted by Human Research Ethics Committee (HREC), University of Witwatersrand (M130227) (Appendix B).

Funding: The vitamin D Kits were purchased using existing funds belonging to the Division of Paediatric Nephrology at CMJAH.

2.5 Statistical Analysis

Study data was collected and managed using *Microsoft Excel* and IBM *SPSS Statistics*, *Version 22,* hosted by University of the Witwatersrand.
Descriptive statistical analysis was performed by the primary investigator, and checked by a senior colleague experienced in this field.

Relationships between continuous variables were expressed as scatter plots, and theoretically expressed by Pearson's correlation. Independent sample t tests were used to compare intergroup differences in the mean level of vitamin D when there were two variables. The one-way ANOVA was used to compare data in cases where there were more than two variables.

A p-value of <0.05 was considered to be statistically significant.

2.6 Limitations

i) The vitamin D status of the background population was unknown.

ii) The number of participants in our study was short of the minimum requirement of 100.

iii) In the group on dialysis, only patients with Nephrotic syndrome on dialysis were included, hence the numbers were much smaller than other studies looking at children on dialysis.

iv) The study included patients on vitamin D replacement.

v) The diagnosis of Nephrotic Syndrome was arbitrary.

vi) The patients' vitamin D status was not always assessed at the time the patients had Nephrotic Syndrome with relapse.

vii) The intake of natural vitamin D sources was not documented

viii) Patients with malabsorption and liver disease were not specifically excluded from the study although there were no patients in the sample who had a diagnosis of these two conditions.

3.0 RESULTS

3.1 Patient Characteristics

The characteristics of patients in terms of their age, sex, race, BMI Z Score; if they were receiving peritoneal dialysis; the season blood samples were taken; whether they were paying for medical services as a marker of socioeconomic status; their CKD staging; duration of illness and whether they were receiving vitamin D supplementation or not is shown in Table 1 below.

Table 1-Characteristics of patients

Variable	**Value**
Patients (n)	69
Age (years)	10.7 ± 4.2*
Sex n (%) Male Female	 31 (45) 38 (55)
Race n (%) Black Asian Coloured White	 56 (81) 3 (5) 5 (7) 5 (7)
Peritoneal Dialysis n (%) Yes No	 12 (17) 57 (83)
Season of samples n Winter/Spring Summer/Autumn	 50 19
Paid for medical services n (%) Yes No	 30 (43) 39 (57)
CKD Staging n (%) 1 2 3 4 5	 33 (48) 11 (16) 8 (12) 8 (12) 9 (13)
Duration of Illness (months)	69 ± 59*
Vitamin D supplementation n (%)	21 (30)
BMI Z Score	-0.09 ± 0.08*

* Mean ± SD

A total of 74 patients met the inclusion criteria and were initially included in the study. Of these, four subjects were excluded due to technical factors that prevented accurate 25OHD levels from being obtained from their samples. One subject was excluded because a blood sample for 25OHD was not taken.

3.2 25OHD trend in our patient population

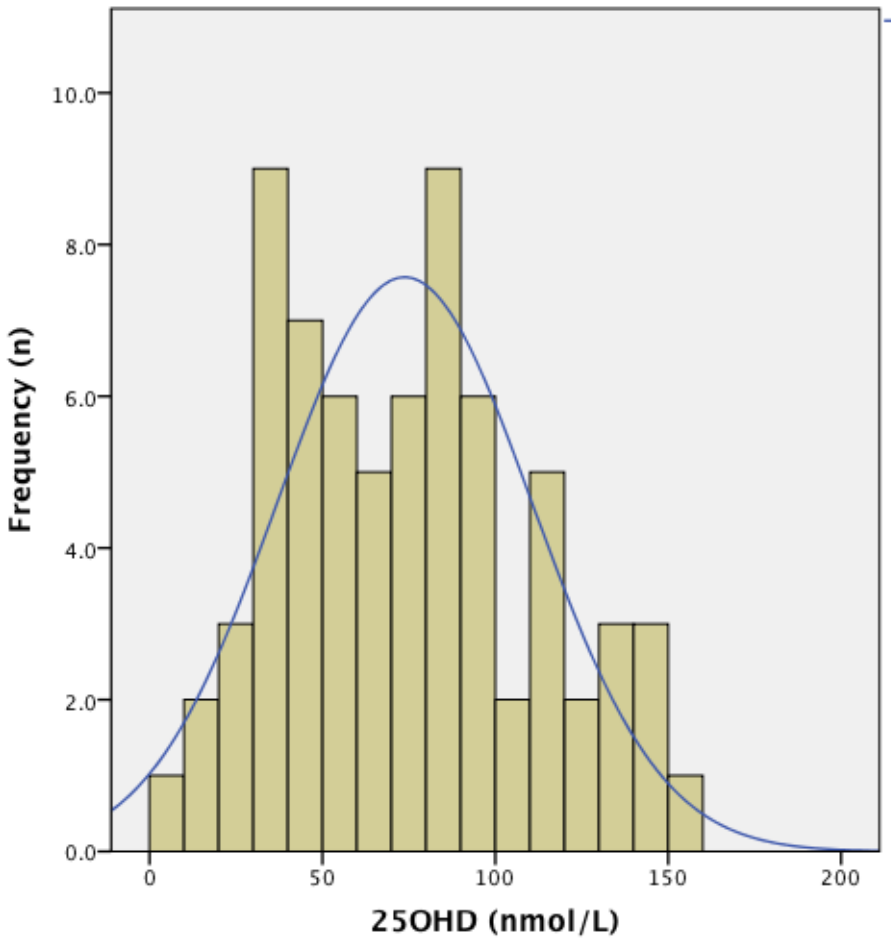


Figure 1: Histogram – Vitamin D levels for the entire sample of patients

The trend of 25OHD levels in the sample of patients, as depicted by the histogram above, indicates that there was a skewed distribution of vitamin D levels to the left. The overall median 25OHD concentration was 74 nmol/L (IQR 44-95 nmol/L). Six (9%) patients were vitamin D deficient, 14 (20%) insufficient and the remaining 49 (71%) replete.

There were 21 (30%) patients on vitamin D supplementation. When these patients were excluded from the analysis, the median vitamin D level decreased to 63 nmol/L (IQR 44-93 nmol/L). This level is still within normal limits. Patients on supplementation were not excluded from all analyses.

As noted, there are two peaks in the histogram. The first peak represents patients who are vitamin D insufficient, and the second peak those who are have sufficient vitamin D levels.

3.3 25OHD and Age

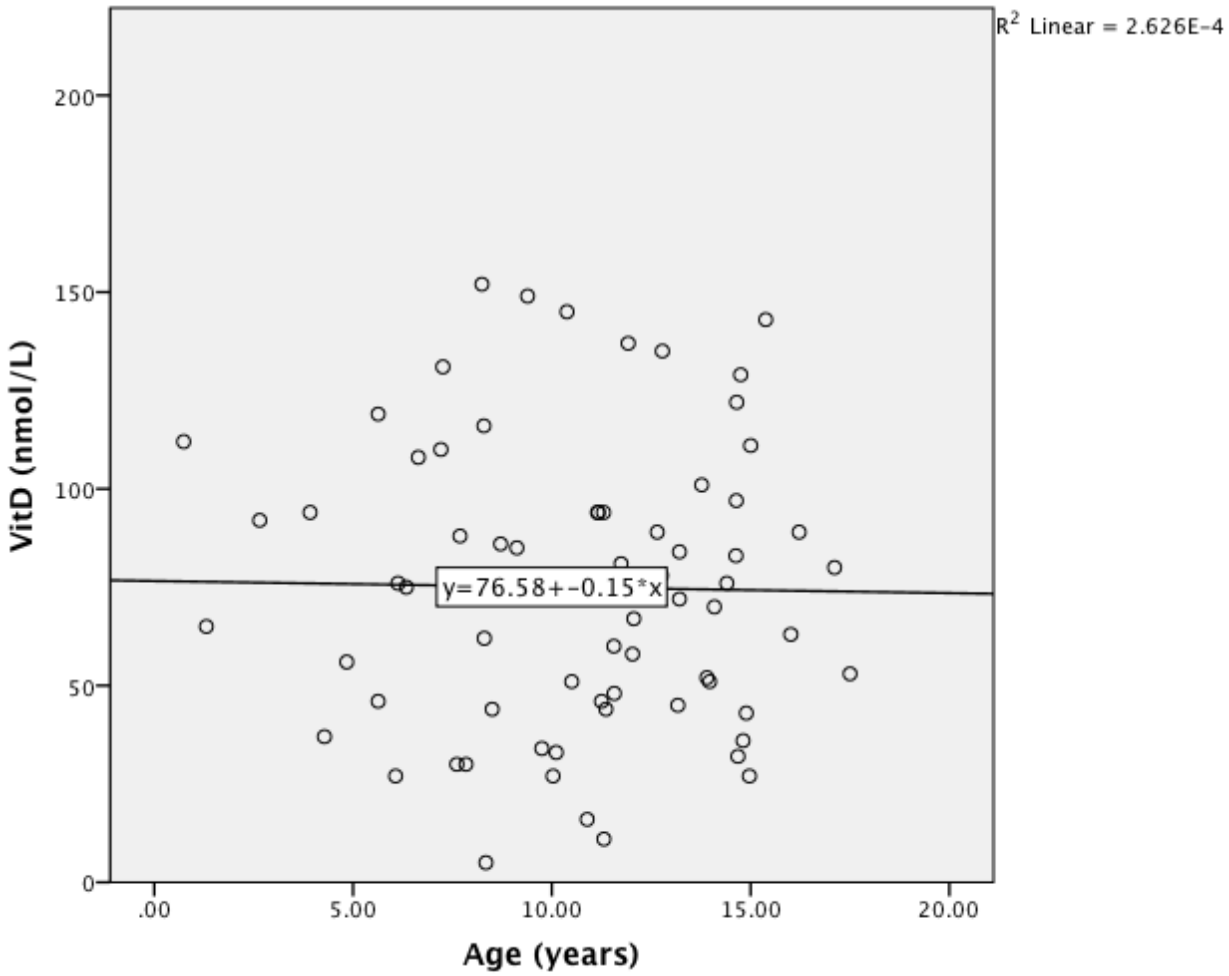


Figure 2: Scatter plot - 25OHD vs age

There was no significant correlation between 25OHD and age (r= -0.07, p=0.90).

3.4 25OHD and Sex

Table 2: Comparing mean 25OHD levels between males and females

	Sex	N	Mean	Std. Deviation	p value	95% Confidence Interval of Mean Difference
25OHD	Male	40	79.05	34.53	0.22	-3.2; 34.0
	Female	29	67.86	39.50		

There was no significant difference between mean 25OHD levels and the sexes (p=0.22).

3.5 25OHD and Race

Figure 3 shows the mean 25OHD levels in the different ethnic groups.

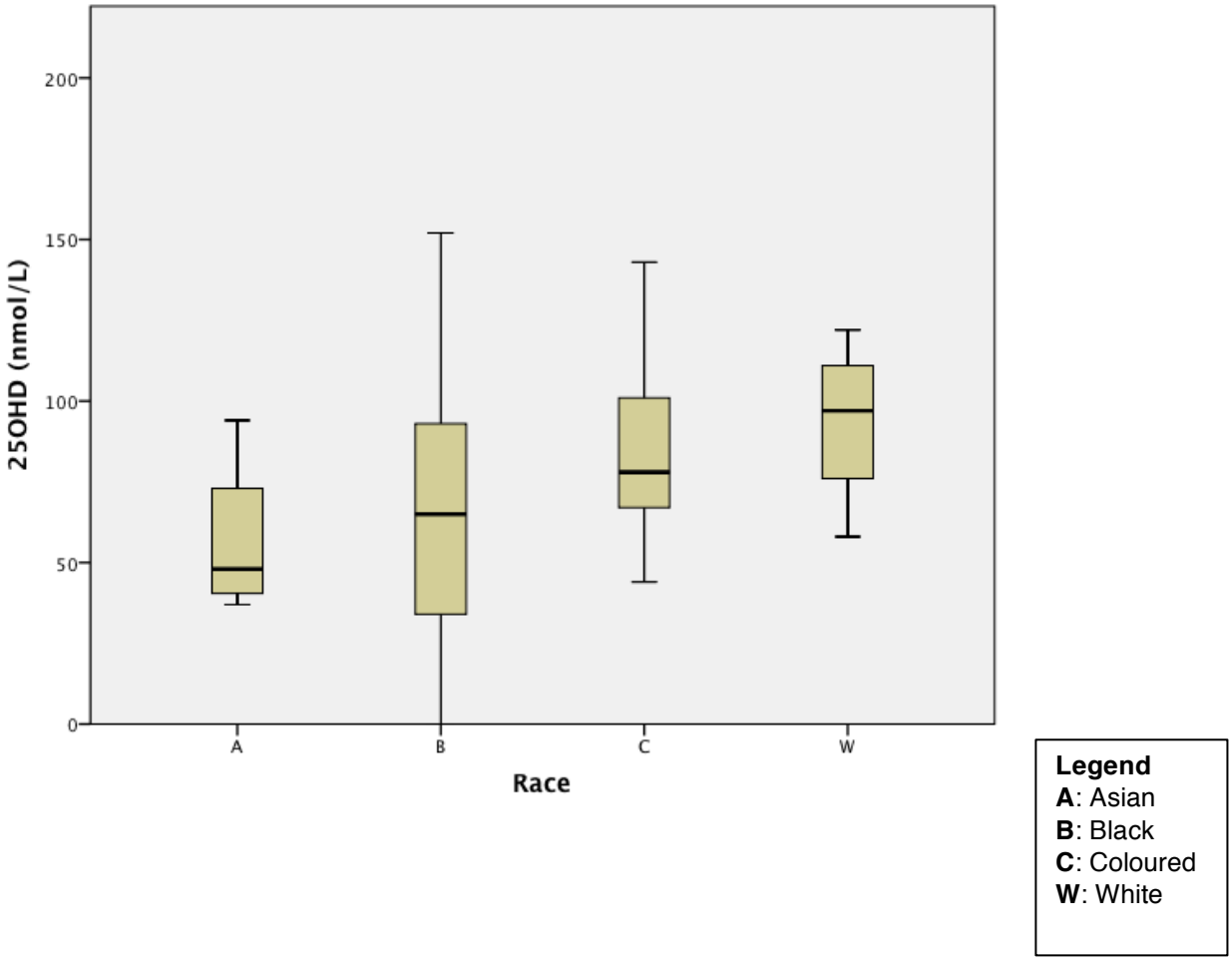


Figure 3: Box and Whisker Plot-25OHD vs Race

The majority of the patients in our cohort were black. The greater variation in vitamin D levels in the black race group when compared to the other race groups is most likely explained by the fact that there were more patients in the black group than in the other race groups. There was also a trend that the 25OHD levels in the black race group were lower compared to the other races. White patients had the highest 25OHD levels. Overall there was no significant difference in 25OHD levels between ethnic groups (p=0. 360).

3.6 25OHD and Financial status

Table 3: Comparing financial status with mean 25OHD levels

	Financial Category	N	Mean	Std. Deviation	p value	95% Confidence Interval of Mean Difference
25OHD	Non Paying	39	73.97	35.14	0.92	-18.8; 17.1
	Paying	30	74.83	39.55		

There was no significant difference between mean 25OHD levels and financial status (p=0.92).

3.7 25OHD and Season

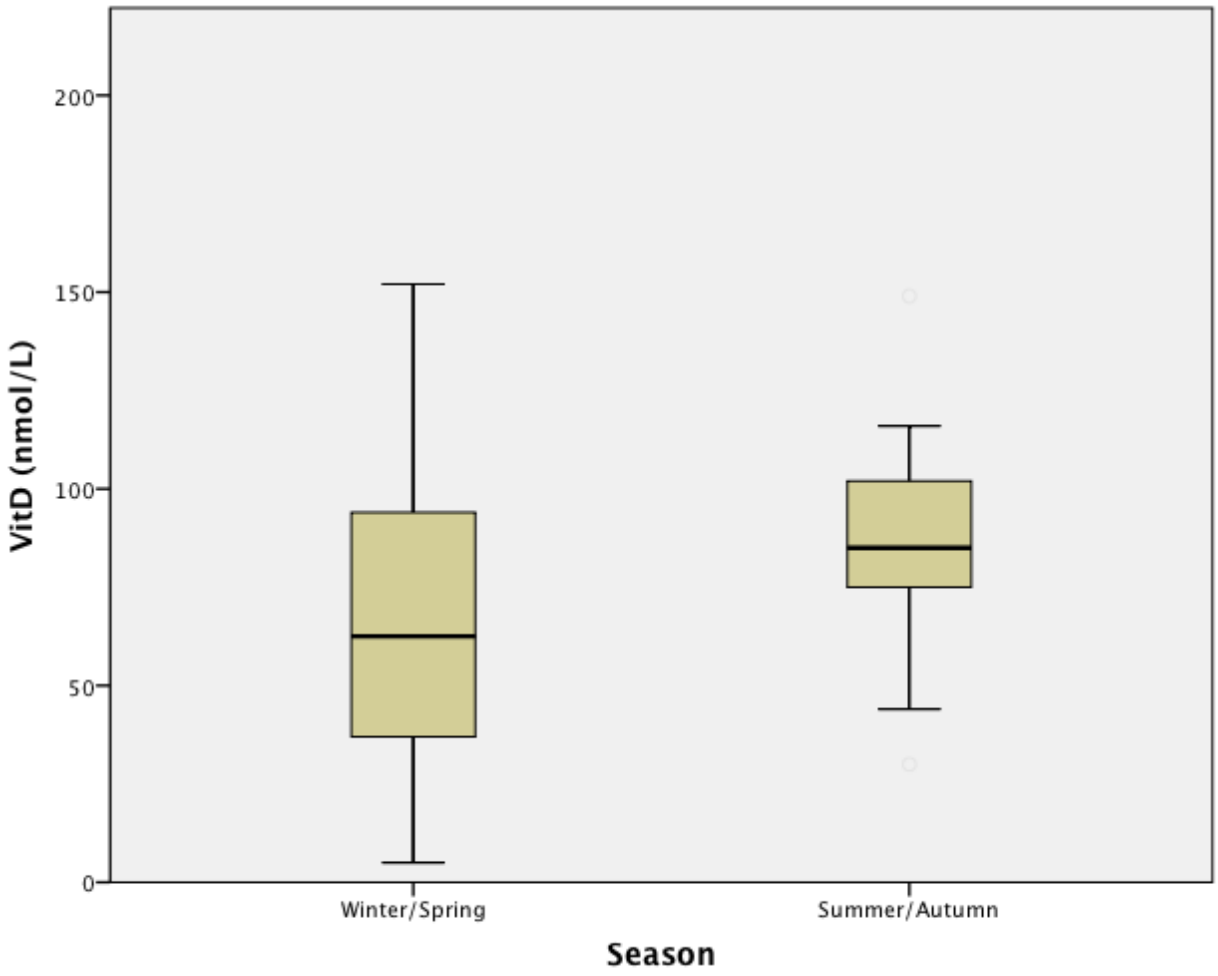


Figure 4: Box and Whisker Plot-25OHD vs. Season

Mean 25OHD levels tended to be lower in winter and spring compared to summer and autumn although this was not statistically significant (p=0.14). There was more variation in winter, which could be attributed to many more blood samples taken during this period.

3.8 25 OHD and BMI

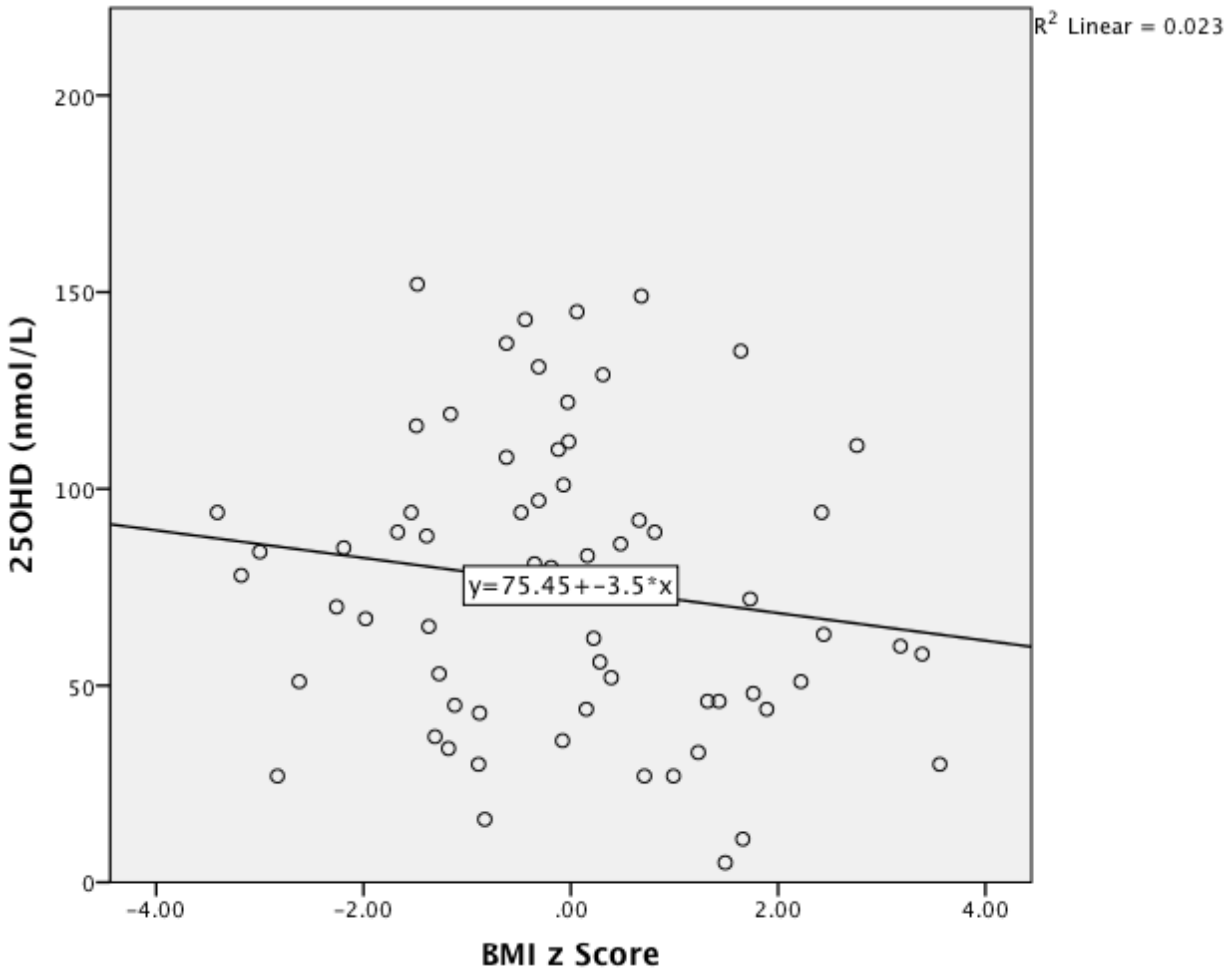


Figure 5: Scatter plot – 25OHD vs. BMI Z Score

There was no correlation between 25OHD and BMI Z Score (r= -0.15, p=0.22).

3.9 25OHD and GFR

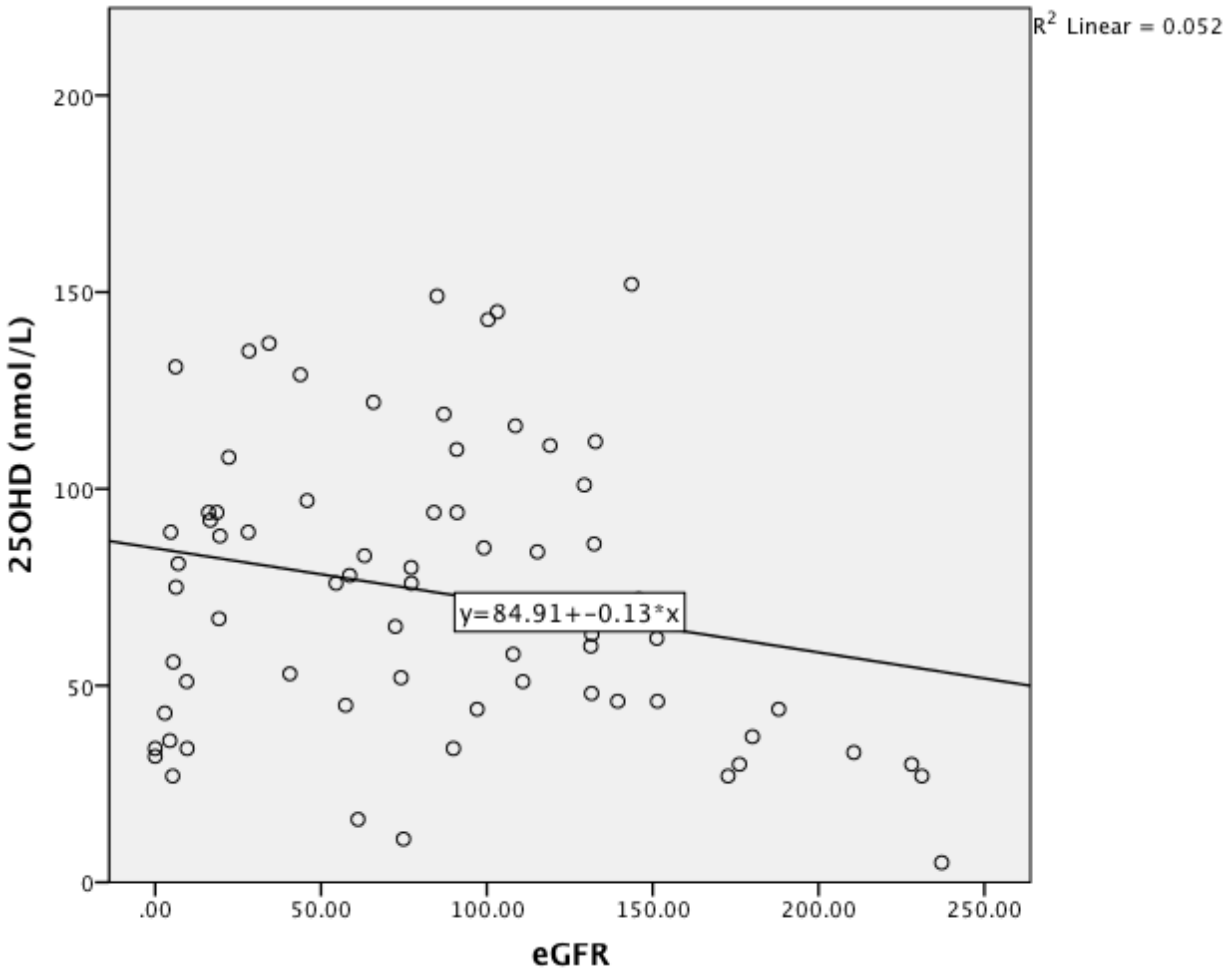


Figure 6: Scatter Plot-25OHD vs. eGFR

Although there was a trend that patients with worse renal function had higher levels of 25OHD, this was not found to be statistically significant (r= -0.23, p=0.06).

3.10 25OHD and Duration of Illness

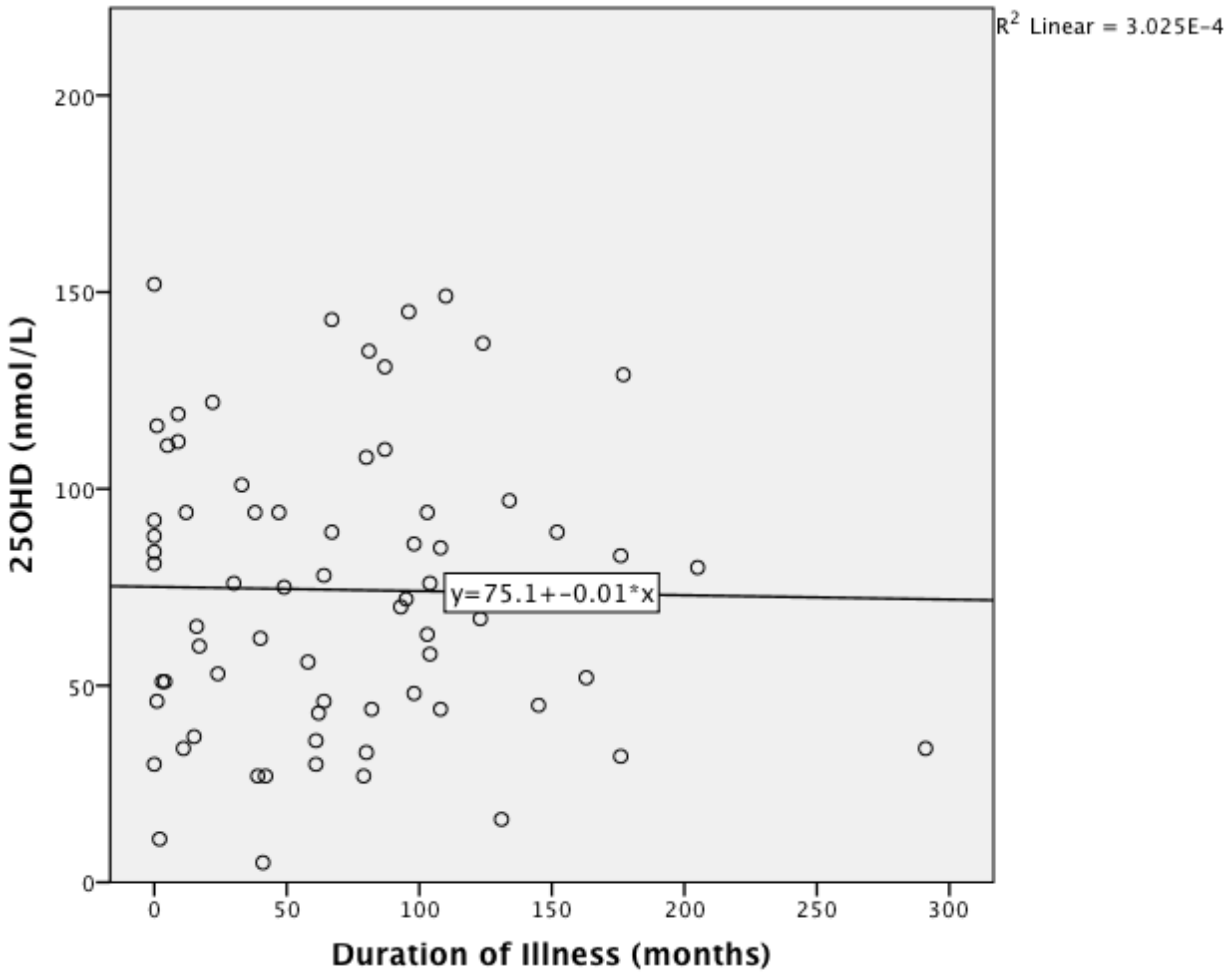


Figure 7: Scatter plot-25OHD vs Duration of Illness

There was no relationship between duration of illness and 25OHD (r= -0.02, p=0.09).

3.11 25OHD and Peritoneal Dialysis

Table 4: Comparing mean 25OHD levels between patients on Peritoneal Dialysis and those who aren't on dialysis

	Peritoneal Dialysis	N	Mean	Std. Deviation	p value	95% Confidence Interval of Mean Difference
25OHD	Yes	12	66.92	37.93	0.45	-32.4; 14.4
	No	57	75.91	36.76		

There were 12 (17.4%) patients on dialysis and although mean 25OHD levels in this group were marginally lower than those who weren't on dialysis (p=0.45) this was not statistically significant.

3.12 25OHD and Albumin

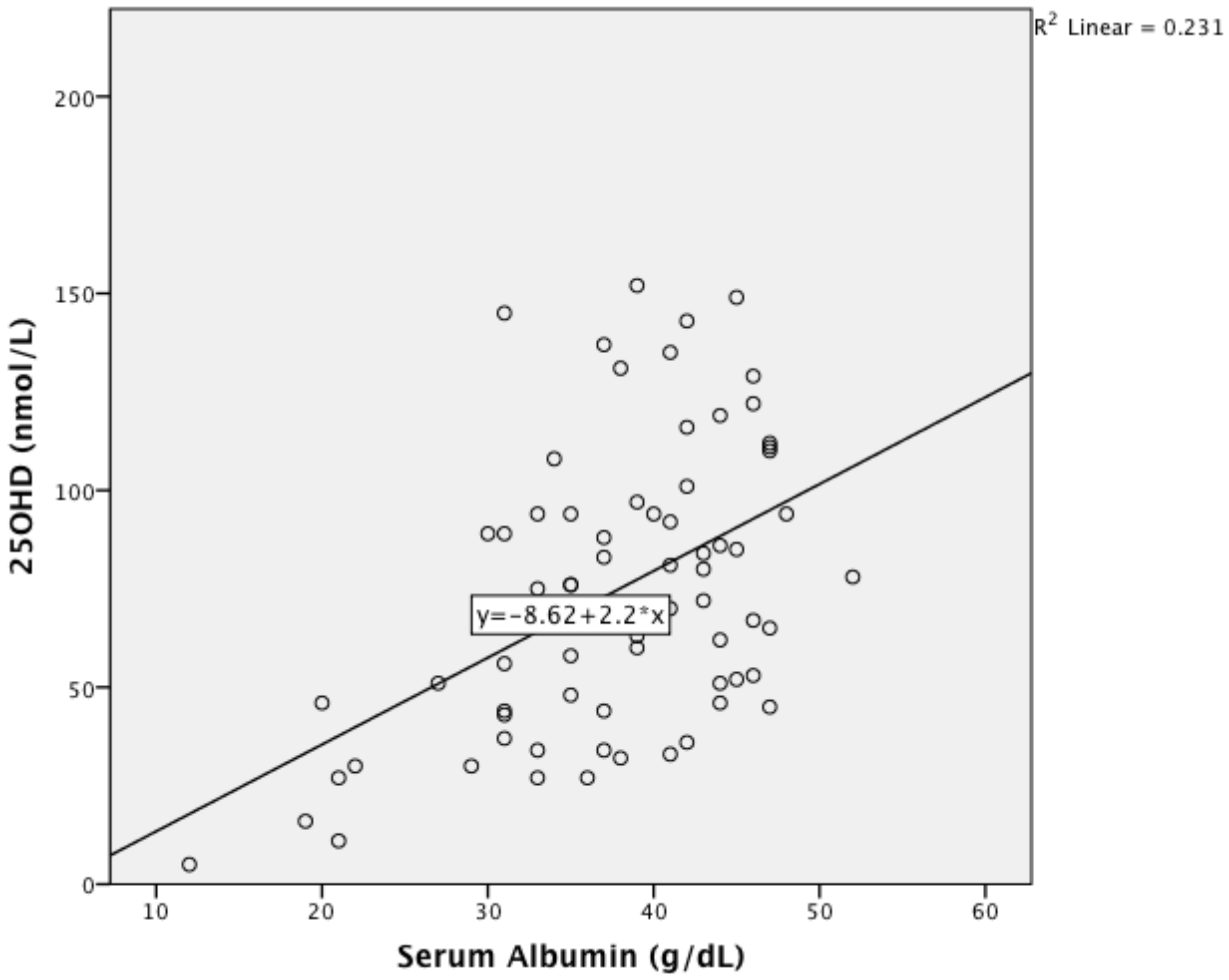


Figure 8- Scatter plot: 25OHD vs. Serum Albumin

Serum 25OHD levels showed a significantly positive correlation with serum albumin (r=0.48, p=o.oo).

3.13 25OHD and Nephrotic Syndrome Relapse vs. Remission

Table 5 – Comparing mean 25OHD levels in patients with Nephrotic Syndrome with relapse vs. remission

	Nephrotic Syndrome	N	Mean	Std. Deviation	p value	95% Confidence Interval of Mean Difference
25OHD	Relapse	19	49.90	32.40	.000	-50.0; -13.0
	Remission	50	83.64	34.32		

There was a significant difference in mean 25OHD levels between the children who had been defined as having Nephrotic Syndrome with relapse and those who were in remission. Mean 25OHD levels in patients who had Nephrotic Syndrome with relapse were insufficient, and those in remission were replete.

3.14 Serum Albumin and Nephrotic Syndrome with Relapse

Table 6 – Comparing mean Serum Albumin levels in patients with Nephrotic Syndrome with relapse vs. remission:

	Nephrotic Syndrome	N	Mean	Std. Deviation	p value	95% Confidence Interval of Mean Difference
Albumin	Relapse	19	31.80	10.14	.000	-12.0; -4.2
	Remission	50	39.87	5.80		

There was a significant difference in mean serum albumin levels in patients with Nephrotic Syndrome with relapse compared to those in remission. Mean serum

albumin levels in patients who had relapsed were lower and those in remission were normal giving us confidence that our definition of Nephrotic Syndrome with relapse was correct for the study.

4.0 DISCUSSION

There is limited data available regarding the vitamin D status of children in South Africa, and even less so in chronically ill children. Vitamin D sufficiency, defined as >50nmol/l, was found in 71% of patients in our study population. Similar results were found in a study of healthy 10-year-old urban children living in Johannesburg, where 74% were vitamin D sufficient[16].

A study in Brazil demonstrated that vitamin D deficiency was rare in a sample of chronic kidney disease patients inhabiting a subtropical region of Brazil, where ultraviolet radiation is elevated throughout the year[20]. Only 0.6% were vitamin D deficient, however, 39.6% of patients in the study had vitamin D insufficiency[20]. Vitamin D deficiency was rare in our study with only 9% of patients with levels <30nmol/l. It is important to note however that different values were used to define vitamin D insufficiency in the study in Brazil, which had a higher threshold of labeling patients as vitamin D insufficient. This may have accounted for the larger numbers of vitamin D insufficiency seen in that group.

An Asian study found 25OHD insufficiency and deficiency was more common in children with CKD and was indirectly related to the level of kidney function[21]. Our study found no significant relationship between 25OHD and level of renal dysfunction. The Asian study had a large sample, namely 2895 CKD patients, compared to our 70 patients, which may account for differing results.

In two separate studies done previously, there was an overall negative correlation of 25OHD levels with age[6, 7]. This is a contrast to our study, where there was no relationship between 25OHD levels and age. However, both these studies only looked at children with Nephrotic Syndrome, whereas we looked at all children attending the Paediatric Renal Outpatient Clinic. The age range of patients in one study was between 2 and 15 years of age[6]. The author attributed their findings to the increasing demand of a growing skeleton to achieve peak bone mass[6]. The age range of children in our study was much wider and perhaps factors other than the increasing demand of a growing skeleton may play a role.

In an Argentinian study, low 25OHD levels were more frequently seen in female patients and in patients with an increased BMI[22]. Similar results were found in a study done in Saudi Arabia, where there was a significant inverse relationship between circulating vitamin D levels and fat mass[23]. This could partly be explained by the fact that females have more fat as a percentage body mass, which results in reduced vitamin D bioavailability[24]. Mean 25OHD levels were lower in female compared to male children in our sample, although the results were not statistically significant. There was also a trend in our population that children with larger BMI Z scores tended to have lower 25OHD levels ($r= -0.15$). However, once again, the results were not statistically significant ($p=0.22$).

There wasn't any difference found between 25OHD levels and families who had to pay a fee and those who did not, as a marker of the relationship between vitamin D status and socioeconomic status. A study conducted in Delhi, India, found a

third of the children to be vitamin D deficient, with the majority of the children in the study coming from low socioeconomic backgrounds. These findings may have been attributed to the fact that dietary calcium intake was significantly lower in the low socioeconomic group as other parameters such as sunlight was the same between the groups. [25] The findings in our study may have differed because of the small sample size. In addition, our marker of socioeconomic status, whether patients paid a fee on not, may not have been entirely reflective for economic status, as they may have been exempted from paying a fee for another reason.

It is important to note that some studies looking at the relationship between BMI Z Scores and socioeconomic status with vitamin D status were done in healthy children and may not be a meaningful comparison to our study. Further studies are needed to investigate these risk factors for low 25OHD levels in children with CKD.

In patients with Nephrotic Syndrome, vitamin D binding protein, which has a molecular weight of less than that of albumin, is lost in the urine together with 25OHD[6]. In keeping with this finding, 25OHD and albumin levels were found to be significantly lower in patients with Nephrotic Syndrome with relapse compared to those in remission in our sample. In the 19 (27.5%) children in our subgroup who had Nephrotic Syndrome with relapse the mean 25OHD levels 49.9 +/- 32.4nmol/L, which was borderline insufficient.

In a previous study looking at the vitamin D status in patients on chronic dialysis, there was a high prevalence of deficiency and insufficiency in children on chronic

dialysis[26]. In our study there was a trend for patients on peritoneal dialysis to have lower 25OHD levels, although these results were not statistically significant. There were very few patients in our study who were on dialysis (12 subjects) and that may contribute to the results not being statistically significant. Another factor that may contribute to differing results[26] is that the 25OHD levels in the published study were taken during winter, which may have accounted for their results being lower.

This study has a number of limitations. There was no control group of normal individuals and there were fewer subjects than that of the calculated sample size required. In the group on dialysis, only patients with Nephrotic syndrome on dialysis were included, hence the numbers were much smaller than other studies looking at children on dialysis.

5.0 CONCLUSION

In conclusion, in contrast to other studies on this topic, it would appear from our results that the majority of children with renal disease in Johannesburg, South Africa are vitamin D sufficient. This is reassuring as it implies that there is no need for routine vitamin D supplementation in this sample of children. However, at risk patients (dark-skinned or Nephrotic syndrome with relapse) may still need to be

screened for vitamin D deficiency and insufficiency and supplemented if necessary.

Just less than a third of patients in our cohort were receiving vitamin D supplementation. When this group was excluded from the analysis the mean 25OHD levels were still sufficient.

Mean albumin levels were significantly lower in children with Nephrotic Syndrome who had relapsed compared to children who were in remission confirming that our definition of Nephrotic Syndrome with relapse accurately reflected the clinical picture. There was a significant difference in 25OHD levels in children with Nephrotic Syndrome with relapse compared to those in remission in keeping with previous studies as mentioned above.

Our recommendations would be to do future studies with a larger number of patients especially those with Nephrotic Syndrome in relapse and in those on dialysis.

6.0 REFERENCES

1. Hull S. Vitamin D Guidance. Barts and The London School of Medicine and Dentistry. 2011.
2. Fukumoto S. Physiological regulation and disorders of phosphate metabolism--pivotal role of fibroblast growth factor 23. Internal medicine. 2008;47(5):337-43.
3. Pearce SH, Cheetham TD. Diagnosis and management of vitamin D deficiency. Bmj. 2010;340:b5664.
4. Hari P, Gupta N, Hari S, Gulati A, Mahajan P, Bagga A. Vitamin D insufficiency and effect of cholecalciferol in children with chronic kidney disease. Pediatric nephrology. 2010;25(12):2483-8.
5. Denburg MR, Kalkwarf HJ, de Boer IH, Hewison M, Shults J, Zemel BS, et al. Vitamin D bioavailability and catabolism in pediatric chronic kidney disease. Pediatric nephrology. 2013;28(9):1843-53.
6. Banerjee S, Basu S, Sengupta J. Vitamin D in nephrotic syndrome remission: a case-control study. Pediatric nephrology. 2013;28(10):1983-9.
7. Weng FL, Shults J, Herskovitz RM, Zemel BS, Leonard MB. Vitamin D insufficiency in steroid-sensitive nephrotic syndrome in remission. Pediatric nephrology. 2005;20(1):56-63.
8. Chau YY, Kumar J. Vitamin D in chronic kidney disease. Indian journal of pediatrics. 2012;79(8):1062-8.
9. Menon S, Valentini RP, Hidalgo G, Peschansky L, Mattoo TK. Vitamin D insufficiency and hyperparathyroidism in children with chronic kidney disease. Pediatric nephrology. 2008;23(10):1831-6.
10. Kalkwarf HJ, Denburg MR, Strife CF, Zemel BS, Foerster DL, Wetzsteon RJ, et al. Vitamin D deficiency is common in children and adolescents with chronic kidney disease. Kidney international. 2012;81(7):690-7.
11. Seeherunvong W, Abitbol CL, Chandar J, Zilleruelo G, Freundlich M. Vitamin D insufficiency and deficiency in children with early chronic kidney disease. The Journal of pediatrics. 2009;154(6):906-11 e1.
12. Arabi A, El Rassi R, El-Hajj Fuleihan G. Hypovitaminosis D in developing countries-prevalence, risk factors and outcomes. Nature reviews Endocrinology. 2010;6(10):550-61.
13. Moore CE, Radcliffe JD, Liu Y. Vitamin D intakes of children differ by race/ethnicity, sex, age, and income in the United States, 2007 to 2010. Nutrition research. 2014;34(6):499-506.
14. Pettifor JM. Calcium and vitamin D metabolism in children in developing countries. Annals of nutrition & metabolism. 2014;64 Suppl 2:15-22.
15. organization Wh. Planning a health survey1986 1986.
16. Poopedi MA, Norris SA, Pettifor JM. Factors influencing the vitamin D status of 10-year-old urban South African children. Public health nutrition. 2011;14(2):334-9.
17. 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. The Journal of clinical endocrinology and metabolism. 2011;96(1):53-8.

18. Foundation TNK. Kidney Disease Improving Global Outcomes Clinical Practice Guidelines 2013 [cited 2015 06 February]. Available from: http://kdigo.org/home/guidelines/.
19. Schwartz GJ, Munoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, et al. New Equations to Estimate GFR in Children with CKD. Journal of the American Society of Nephrology : JASN. 2009;20(3):629-37.
20. Cuppari L, Carvalho AB, Draibe SA. Vitamin D status of chronic kidney disease patients living in a sunny country. Journal of renal nutrition : the official journal of the Council on Renal Nutrition of the National Kidney Foundation. 2008;18(5):408-14.
21. Satirapoj B, Limwannata P, Chaiprasert A, Supasyndh O, Choovichian P. Vitamin D insufficiency and deficiency with stages of chronic kidney disease in an Asian population. BMC nephrology. 2013;14:206.
22. Del Valle E, Negri AL, Aguirre C, Fradinger E, Zanchetta JR. Prevalence of 25(OH) vitamin D insufficiency and deficiency in chronic kidney disease stage 5 patients on hemodialysis. Hemodialysis international International Symposium on Home Hemodialysis. 2007;11(3):315-21.
23. Al-Musharaf S, Al-Othman A, Al-Daghri NM, Krishnaswamy S, Yusuf DS, Alkharfy KM, et al. Vitamin D deficiency and calcium intake in reference to increased body mass index in children and adolescents. European journal of pediatrics. 2012;171(7):1081-6.
24. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. The American journal of clinical nutrition. 2000;72(3):690-3.
25. Marwaha RK, Tandon N, Reddy DR, Aggarwal R, Singh R, Sawhney RC, et al. Vitamin D and bone mineral density status of healthy schoolchildren in northern India. The American journal of clinical nutrition. 2005;82(2):477-82.
26. Cho HY, Hyun HS, Kang HG, Ha IS, Cheong HI. Prevalence of 25(OH) vitamin D insufficiency and deficiency in pediatric patients on chronic dialysis. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2013;33(4):398-404.

7.0 APPENDICES

Appendix A: Consent/Assent Form

Dear Patient, Parent/Guardian

Good day,

My name is Dr. Sharika Raga and I am currently studying to become a paediatrician. As part of my training, I need to do a research project. I will be doing a study about vitamin D levels in children who stay in hospital for a long time and children who have kidney disease. You are welcome to be a part of it.

Vitamin D is important in the human body, especially with the growth of bones. Sunlight helps Vitamin D to work. The kidneys also help make vitamin D.
When you are admitted to hospital, especially for a long time, you may not get enough sunlight. This may make it difficult for the vitamin D to work properly.

We are doing a study to see if vitamin D levels are less when you stay in hospital or if you have kidney disease and visit our clinic.

In order for us to measure vitamin D, we have to take some blood from you. When we take blood for your routine blood tests (when you are admitted or when you visit our clinic) we will take an extra tube of blood (2ml). If you stay in hospital for 4 weeks or longer, we will take some more blood before you go home. This blood sample will be taken along with your routine blood tests. There will no extra needle sticks as we will only take the extra blood if we were going to do a blood test anyway.

If you do not want us to take the extra blood tube, or if you agree and then change your mind, we will still provide the same treatment to you.

All the results we get from the extra test will be kept strictly confidential and you will not be identified in the study.

If there are any problems with the blood results we will let you know and your doctors will treat you accordingly.

We are hoping that this study will help us help you and other children too.

Thank you for taking the time to consider our request. If you are willing to be a part of this study, please could you kindly sign below.

Dr S Raga and Dr CS Levy
Contact Number: 072 934 3040

Patient Name_____

Signature_____ Date_____

Appendix B: Ethics Clearance Certificate

R14/49 Dr Sharika V Raga

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M130227

NAME: (Principal Investigator)	Dr Sharika V Raga
DEPARTMENT:	Paediatrics and Child Health Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	A Prospective Cross Sectional Observation Study of the Vitamin D Status in Children with Chronic Kidney Disease at Charlotte Maxeke Johannesburg Acacademic Hospital (Changed Title 24/10/2014)
DATE CONSIDERED:	22/02/2013
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Cecil Levy
APPROVED BY:	Professor A Woodiwiss, Co-Chairperson, HREC (Medical)
DATE OF APPROVAL:	29/04/2013

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 Secretary in Room 10004, 10th floor, Senate House, University.
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**.

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES