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Association between vitamin D3 deficiency and Chronic Kidney Disease in Libyan patients

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by

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DEDICATION

TO MY

DEAR FAMILY

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ABBREVIATIONS

Alk. phosphatase	Alkaline phosphatase
CKD	Chronic Kidney Disease
CKD-MBD	Chronic Kidney Disease-Mineral Bone Disease
Cr c	Creatinine clearance
DM	Diabetes Mellitus
ESRD	End Stage Renal Disease
FGF	Fibroblast Growth Factor
GFR	Glomerular Filtration Rate
PTH	Parathyroid Hormone
SHPT	Secondary Hyperparathyroidism
VDR	Vitamin D Receptors
Vit	Vitamin

ABSTRACT

Vitamin D deficiency is common among patients with chronic kidney disease (CKD). The traditional supplementation of active vitamin D (1,25 dihydroxy-vitamin D) or alpha-hydroxylated vitamin D ((1α OH vit-D3or D2) to CKD patients, has been reported to control the level of secondary hyperparathyroidism due to negative feedback of active1,25 dihydroxy-vitamin D on parathyroid hormone (PTH) level, but not sufficient to replenish the body store of vitamin D (25-hydroxyvitamin D).
In our cross sectional study of different stages of CKD patients targeting to determine the prevalence of vitamin D deficiency in this particular group of patients and to identify risk factors either demographic or disease related. A study of vitamin D deficiency symptoms was undertaken to assess the prevalence and symptoms in these group of patients. Moreover, to evaluate the prevalence of vitamin D deficiency in treated and untreated patients.

Lab investigations were carried out in 50 female and 30 male CKD patients stages 3,4 and predialysis stage 5, at the nephrology departments in Benghazi Medical Center (BMC) and Al Majory polyclinics. in this study 29 healthy control included in which 12 female and 17male. The patients aged 62.04±14.403 years with creatinine (3.903±2.1) mg/dl and creatinine clearance (22.61±9.8) ml/min. Body weight was (75.5±16.887) kg. Calcium was (8.7293±0.996) mg/dl, PTH (353.73±313.441) pg/ml and serum albumin (3.909±0.575) g/dl. The mean of 25(OH)D was (18.4559±13.6) ng/ml for the patients and (19.035±11.496) ng/ml for the healthy controls.

Out of the 80 patients 13 were previously treated with vitamin D Ergocalciferol or Cholecalciferol therefore, we excluded them in the assessment of the prevalence of deficiency in patients but we included them to evaluate the effect of treatment.

In the present study we found that the level of 25(OH) vitamin D less than 20 ng/ml in 80.6% of untreated patients, hence classified as deficient while 19.4% had normal levels

more than 20 ng/ml. In our study the 29 healthy controls analyzed and found that 69% had vitamin D deficiency while 31% had normal levels. Furthermore, the prevalence of vitamin D deficiency in treated patients found 30.8%. Concerning the levels of 25(OH)vitamin D in different stages of CKD, no significant differences were detected regarding to stages. There is no significant differences detected for the 25(OH)vitamin D levels in males (15.3463±8.5025) ng/ml compared to females (16.4207±13.5596) ng/ml.

Considering the need for an adequate body store of vitamin D (25(OH)vitamin D) in CKD patients and the state of deficiency detected in our cases throughout this study. Thus we recommended that there should be a frequent vitamin D investigation and treatment, provided that an appropriate management of secondary hyperparathyroidism with one alpha (1,25 dihydroxy-vitamin D) or alpha-hydroxylated vitamin D to be carried out.

Chapter 1

Introduction

1. Introduction.

Normal vitamin D level is essential for human health. Vitamin D deficiency has been recognized as a risk factor for nearly all-causes of mortality in normal individuals and in chronic kidney disease (CKD) patients (1). Approximately 30 to50% of people are known to have low levels of vitamin D and therefore vitamin D deficiency has become global health problem over all the world (2).

Vitamin D deficiency or insufficiency is found to be common in patients with CKD, and vitamin D level appears to have an inverse correlation with kidney function. Growing evidence has indicated that vitamin D deficiency may contribute to deteriorating renal function as well as increased morbidity and mortality in patients with CKD (3, 4).

The prevalence of vitamin D deficiency around the world in the CKD population has been described to range between 70 and 80% (5).

New evidence has now showed that the role of vitamin D is no longer restricted to its classical function of maintaining calcium and phosphate homeostasis. However, vitamin D appears to play a more extensive role as a cell differentiating and antiproliferative factor with actions in a variety of tissues, including the renal, cardiovascular, and immune systems (6).These new information present convincing evidence for the necessity of vitamin D administration both in the 25 and 1,25 forms to supplement both the classical endocrine renal 1-alpha-hydroxylase vitamin D pathway as well as the autocrine intracellular 1-alpha-hydroxylase pathway through which vitamin D has now been shown to function. The effect of these new data will serve to shift the approach to vitamin D replacement in CKD patients into a new evidence where the use of vitamin D is no longer solely for the treatment of secondary hyperparathyroidism (7).

1.1.Vitamin D:

1.1.1.Introduction to vitamin D : Vitamin D is a fat-soluble steroid hormone derived from dietary intake such as milk, fortified foods, and fish (fresh or canned as sardine and salmon) (Table 1.1), as well as synthesis through the skin *via* exposure to sunlight. There are 2 major forms of vitamin D, Cholecalciferol (vitamin D-3) and Ergocalciferol (vitamin D-2) (Table 1.2). Vitamin D-2 is more rapidly catabolized than cholecalciferol. A number of epidemiologic studies suggested that the exposure to sunlight enhances the production of vitamin D_3 in the skin it is important in preventing many chronic diseases. since very few foods naturally contain vitamin D, sunlight supplies most of our vitamin D requirement (8).

Table(1.1):Dietary sources of vitamin D.

Food	Serving Size	International Units of Vitamin D	Percentage of the RDA
Cod Liver Oil	One tablespoon	1360	340
Swordfish	Three ounces	566	142
Salmon	Three ounces	477	112
Canned Tuna in Water	Three ounces	154	39
Orange Juice with Vitamin D	One cup	137	34
Milk with Vitamin D	One cup	115-124	29-31
Yogurt with Vitamin D	Six Ounces	80	20
Cooked Beef Liver	Three ounces	42	11
Eggs (Yolk)	One	41	10

Adapted from the National Institute of Health Vitamin D Fact Sheet (2011): http://ods.od.nih.gov/factsheets/vitamind-HealthProfessional/ Retrieved April 2012

Table(1.2): Vitamin D nomenclature.

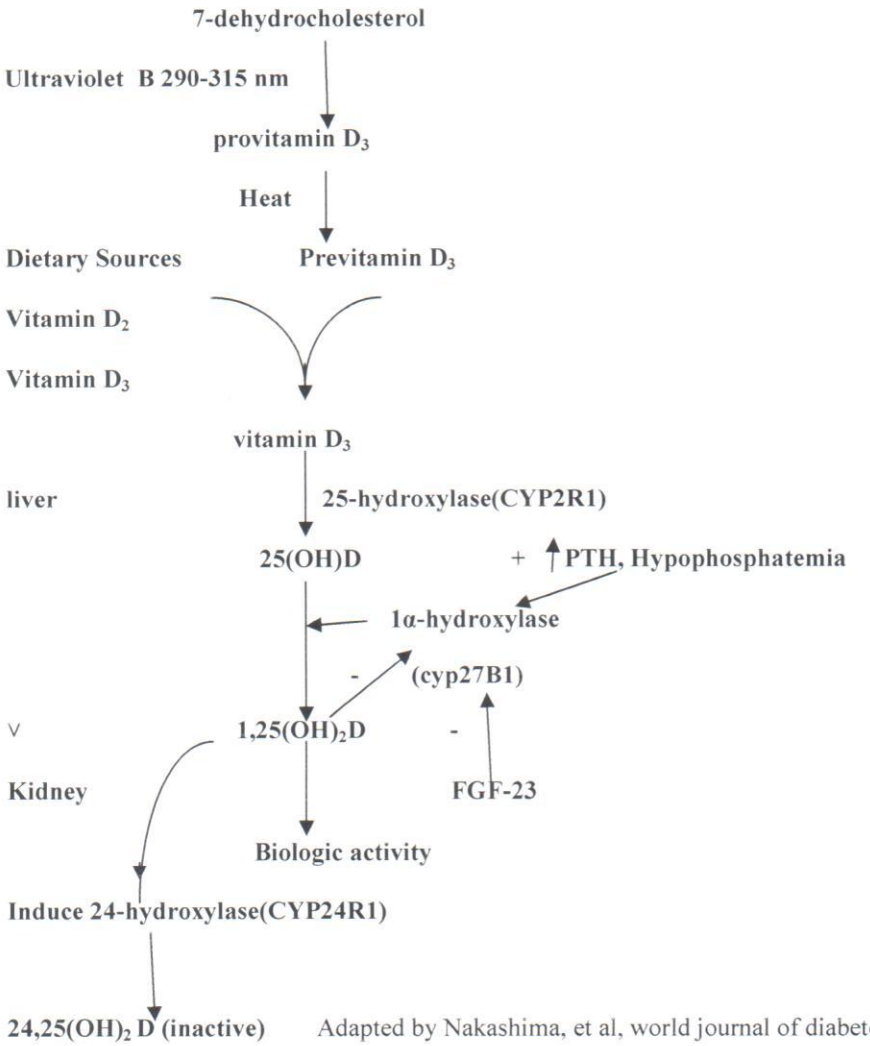
Collective terminology	Vitamin D_2 and metabolites	Vitamin D_3 and metabolites
Parent compound Vitamin D(from diet or UVB Light)	Vitamin D_2 (plant sources) Ergocalciferol	Vitamin D_3 (animal sources) Cholecalciferol
Product of 1αhydroxylation 25-hydroxyvitamin D	25-Hydroxyvitamin D_2 25(OH)D_2 Ercalcidiol	25-Hydroxyvitamin D_3 25(OH)D_3 Calcidiol
Product of 2nd hydroxylation 1,25-dihydroxyvitamin D (active) 1,25$(OH)_2$ D Vitamin D Hormone	1,25 Dihydroxyvitamin D_2 1,25(OH)D_2 Ercalcitriol	1,25 Dihydroxyvitamin D_3 1,25$(OH)_2 D_3$ Calcitriol

Adapted from: kidney disease improving global outcomes(KDIGO)CKD-MBD Work group. kidney int. 2009:76:S1-S130

1.1.2. Vitamin D synthesis and catabolism: During exposure to sunlight, the ultraviolet B (UVB) radiation (290–315 nm) is absorbed by 7-dehydrocholesterol in the skin to form previtamin D_3. Previtamin D_3 is unstable and rapidly converts by a temperature-dependent process to vitamin D_3 (Figure1.1). Once vitamin D3 formed it is transferred from skin cells into the extracellular space then into the dermal capillary bed by the vitamin D–binding protein (DBP) (9). Vitamin D which formed in the skin or from diet is either stored in adipose tissue or converted to 25(OH)D in the liver (2).

After release from DBP to the liver vitamin D undergoes the first hydroxylation on C-25 by the vitamin D-25-hydroxylases (25-OHase; also known as CYP27A1, CYP3A4, CYP2R1, CYP2J3) to 25-hydroxyvitamin D [25(OH)D], 25(OH)D is bound to DBP, and this complex binds to megalin on the plasma membrane of the renal tubule cell and is transported into the cell. Once inside, 25(OH)D is released from the DBP complex and is converted in the mitochondria by the 25-hydroxyvitamin D-1α-hydroxylase [1-OHase; also known as CYP27B1] to form 1,25-dihydroxyvitamin D [1,25$(OH)_2$D]. calcitriol (1,25$(OH)_2$D) is the biologically active form of vitamin D responsible for maintaining calcium and phosphorus homeostasis (9).

Figure 1.1: Mechanism of vitamin D synthesis.



Adapted by Nakashima, et al, world journal of diabetes. 2016;7(5):89-100.

CYP27B1 (1α-hydroxylase) gene expression in the kidney which is affected by various factors as Parathyroid hormone (PTH), hypocalcaemia, hypophosphatemia, and calcitonin cause the activation of CYP27B1 to increase 1,25-$(OH)_2$D levels. On the other hand, 1,25-$(OH)_2$D and fibroblast growth factor-23 (FGF-23) inhibit CYP27B1 and can decrease 1,25-$(OH)_2$D levels (10).

For that In the case of high FGF23 concentrations, tissues and organs may be unable to convert serum 25(OH)D into $1,25(OH)_2D$ locally(11)(Figure1).

The final step in the vitamin D metabolic pathway is its inactivation, a process which catalyzed by 24-hydroxylase, resulting in catabolism of $1,25(OH)_2D$ and 25(OH)D into 1,24,25 trihydroxyvitamin D [$1,24,25(OH)_3D$], and ultimately into water-soluble calcitroic acid and the inactive blood metabolite $24,25(OH)_2D$ (12, 13).

1.1.3.Vitamin D and Calcium Homeostasis:

Calcitriol ($1,25(OH)_2D_3$) with PTH represents the first-line defense against low nutritional calcium supply by increasing both intestinal absorption and renal calcium reabsorption. Enhanced bone resorption and simultaneous inhibition of mineral deposition can thereby avoid a futile cycle of calcium resorption from bone and its immediate reuse for mineralization of bone (14).

In the intestine $1,25(OH)_2D$ (the active form vitamin D) induces the expression of an epithelial calcium channel, calcium-binding protein (calbindin), and a variety of other proteins to help the transport of calcium from the diet into the circulation. Active vitamin D ($1,25(OH)_2D$) interacts with its vitamin D receptors in the osteoblast and stimulates the expression of receptor activator of NFκβ ligand (RANKL) similar to parathyroid hormone (PTH). Thus $1,25(OH)_2D$ maintains calcium homeostasis by increasing the efficiency of intestinal calcium absorption and mobilizing calcium stores from the skeleton (15).

1.1.4. Effect Of Vitamin D On Bone:

The effect of vitamin D action would be to defend systemic calcium homeostasis by making calcium available for the extracellular fluid pool from the intestine if possible but from any internal source if required. In case of very low external calcium supply, high levels of $1,25(OH)_2D_3$ would use the bone calcium reservoir for serum calcium homeostasis at the (temporary) expense of bone mass and strength. this seems a logical strategy as later access to nutritional calcium could then allow the rebuilding of the skeleton. Thus

$1,25(OH)_2D_3$ acting through the VDR may favor replication and maintenance of immature cells of the osteoblast . These roles may be critical for bone resorption and 'preventing' futile bone mineralization under conditions of calcium stress. On the other hand, during a 'recovery' phase, $1,25(OH)_2D_3$ acting through the VDR which act in more mature cells may encourage terminal differentiation of mineralizing cells. Consistent with studies of action of $1,25(OH)_2D_3$ on osteoblastic cells at different stages of their maturation, this may provide a drive to re-mineralization of the skeleton after surviving the calcium stress situation. This divergence between sacrifice of bone for calcium homoeostasis and rebuilding of the skeleton at other time is the important action of vitamin D (14, 16).

1.1.5. Non-classical Role of Vitamin D:

Although other tissues discovered to have 1α-hydroxylase enzymatic activity, and since VDR is expressed in a variety of organs, such as the heart, liver, blood vessels, and the central nervous system, this extra renal 1α-hydroxylase enzymatic activity is controlled in different ways than that in renal tubular cells (2, 4).

The discovery that the vitamin D receptor (VDR) as well as 1α-hydroxylase (which converts 25(OH)D to $1,25(OH)_2D$ locally) are found in most tissues in the body give a hint of non-classical functions of vitamin D and consequences of its deficiency. This locally produced $1,25(OH)_2D$ binds to the VDR in an autocrine/paracrine manner and has been found to have several important roles (11)(Figure 1.2).

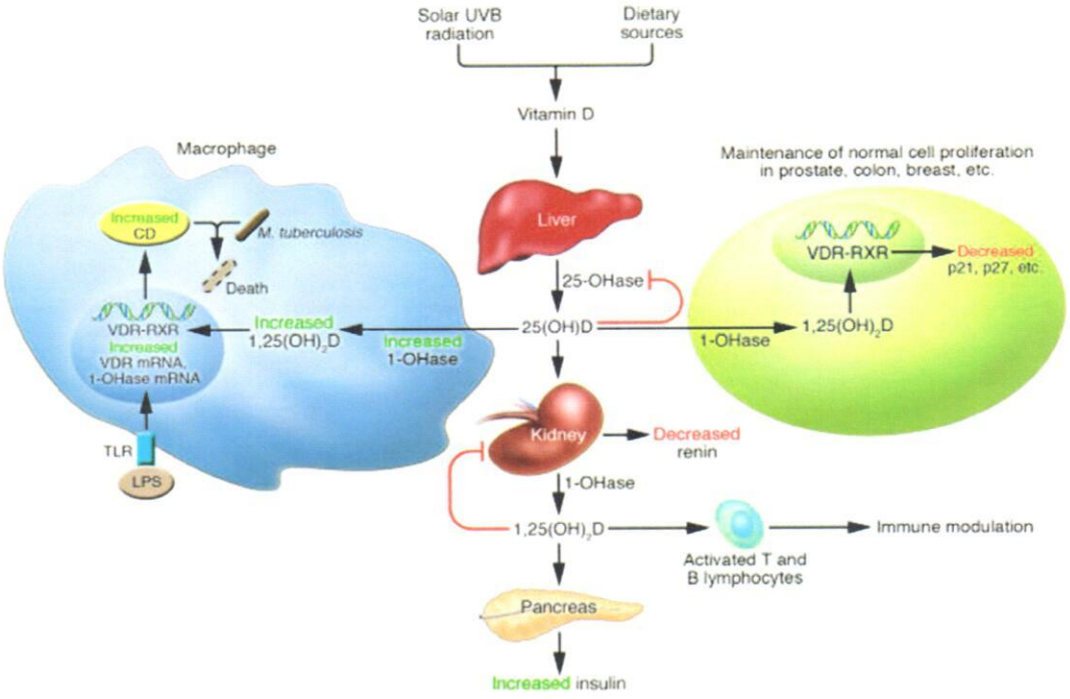


Figure (1.2). Noncalcemic functions of $1,25(OH)_2D$.

Adapted by Holick MF, the American journal of clinical nutrition,2004;79(3):362-71.

Calcitriol ($1,25(OH)_2D$) interacts with its nuclear vitamin D receptor (VDR), which in turn bonds with the retinoic acid-X-receptor. This complex is recognized by specific gene sequences known as the vitamin D responsive elements (VDRE) to unlock genetic information that is responsible for its biologic actions which is also known as non calcemic function of vitamin D (15).

By binding with its intracellular vitamin D receptor (VDR) in these tissues, calcitriol ($1,25(OH)_2D_3$) can regulate cellular proliferation and differentiation, inflammation, in the immune system and the endocrine system, including RAS, insulin resistance and lipid metabolism (17).

The kidney appears to be a major target organ for both the classical and non-classical actions of vitamin D, with the vitamin D receptor being appropriately highly expressed in this site. The non-classical effects of vitamin D may play a relevant role in the mortality and morbidity of patients with CKD, specifically affecting the possible progression of their renal disease (18).

1.2.1.Vitamin D Deficiency: The United States Institute of Medicine defines vitamin D deficiency as 25(OH)D levels less than 20 ng/ml and greater than 20 ng/ml is sufficient upon evidence related to bone health(2). Numerous of studies reported that people with 25(OH)D levels less than 20 ng/ml is at increase the risk of fracture (19).

Patients with 25 (OH) vitamin D levels between 20 and 30 ng/ml are referred to as vitamin D insufficient (20).

1.2.2. Measurement of vitamin D: Because $1,25(OH)_2D$ has a short half-life (approximately 15 h), Its levels are not considered to be a good indicator for vitamin D levels. Since 25(OH)D is more stable in the blood than $1,25(OH)_2D$, and blood concentrations of 25(OH)D are 500 to 1000 times higher than $1,25(OH)_2D$ concentrations, therefore, to evaluate vitamin D deficiency and insufficiency, serum 25(OH)D concentrations are considered as a good biomarker (19).

1.2.3.Causes Of Vitamin D Deficiency: Factors such as low sunlight exposure, age-related decreases in cutaneous synthesis, and diets low in vitamin D contribute to the high prevalence of vitamin D inadequacy(21). in addition vitamin D deficiency is prevalent in infants who are solely breastfed and who do not receive vitamin D supplementation, and in adults of all ages who have increased skin pigmentation or wear sun protection regularly or limit their outdoor activities (8).

1.2.4.Diseases Associated With Vitamin D Deficiency:

Vitamin D deficiency causes poor mineralization of the collagen matrix in young children's bones leading to growth retardation and bone deformities known as rickets. In adults, vitamin D deficiency induces secondary hyperparathyroidism, which causes loss of matrix and minerals, thus increasing the risk of osteoporosis and fractures. The poor mineralization of newly laid down bone matrix in adult bone results in the painful bone disease of osteomalacia. In addition vitamin D deficiency causes muscle weakness, increasing the risk of falling down and fractures (15).

Low vitamin D levels are also associated with hypertension, cancer, and cardiovascular disease(2), multiple sclerosis, and rheumatoid arthritis (15).

Vitamin D influences the renin-angiotensin system, inflammation, and mineral bone disease, which may be associated with the cause and progression of CKD. There is increasing evidence that vitamin D deficiency may be a risk factor for DM and CKD (2).

There is some evidence suggesting that vitamin D status is associated with poor clinical outcomes in patients with CKD. Low 25(OH)D levels are associated with all-cause mortality and cardiovascular disease in patients with CKD. The risk for end stage renal disease is higher in patients with low vitamin D status. Among patients undergoing hemodialysis and peritoneal dialysis, low 25(OH)D levels are associated with cardiovascular disease (2).

1.3.CHRONIC KIDNEY DISEASE

1.3.1.Definition: defined as progressive and irreversible loss of kidney function (22).

Patients with chronic kidney failure--defined as a glomerular filtration rate persistently below 15 mL/min/ 1·73 m^2, and found to have an unacceptably high mortality rate compared to healthy persons. In developing countries, mortality results primarily from an

absence of access to renal replacement therapy as (hemodialysis and peritoneal dialysis) or transplants for maintenance of life (23).

1.3.2.Stages of CKD: According to the National Kidney Foundation criteria, CKD has been classified into five stages with stage 1 being the earliest or mildest CKD state and stage 5 being the most severe CKD stage (20).

Table (1.3): Stages of CKD.

stages	description	criteria
Stage1	Kidney damage with normal or increase GFR	GFR≥90 ml/min. but with urine findings(albuminuria, hematuria or structural kidney abnormality
Stage2	Kidney damage with mild reduction in GFR	GFR 60-89 ml/min
Stage3	Moderate reduction in GFR	GFR 30-59 ml/min
Stage4	Severe reduction in GFR	GFR 15-29 ml/min
Stage5	Kidney failure	GFR<15 ml/min or requirement of renal replacement therapy

Adapted by Nigwekar su,et al, Bonekey Rep 2014 feb 5;3,498.

Glomerular filtration rate (GFR), either directly measured by computing urinary clearance of filtration marker or estimated by cockcroft-gault equation by using serum marker such as creatinine and considering age and body weight using Cockcroft-Gault equation: {CrC = [(140-age) x weight]/(72 x Cr)}, multiplied by 0.85 for women is the most commonly used parameter to assess kidney function (20).

1.3.3.Causes Of Vitamin D Deficiency In CKD:

There are many of theories describing the pathogenesis of vitamin D deficiency in CKD. One of these theories involving Megalin, which is present in endocytic receptors in proximal tubule cells, it is involved in the reabsorption of DBP from glomerular ultra filtrates, and mediates the subsequent intracellular conversion of 25(OH)D to its active form. As kidney function declines, megalin expression in the proximal tubule decreases. Megalin function is attenuated with reduced kidney function, because of damages from low

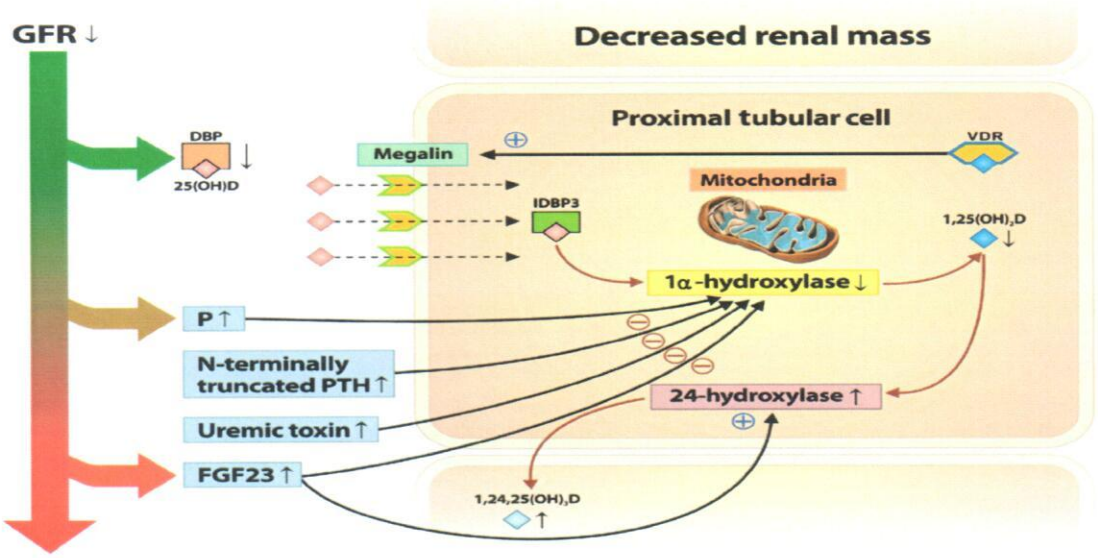
molecular weight proteinuria. The other expected cause is the activity of CYP27B1(1α-hydroxylase) known to be associated with decreasing kidney function. As FGF-23 reduces expression of co transporters NaPi-IIa and NaPi-IIc, of the brush border in the proximal tubules, these mechanisms inhibit phosphorus absorption and CYP27B1 activity (2) (Figure1.3).

In addition to the decline of $1,25(OH)_2D$ levels(Calcitriol), 25(OH)D(Calcidiol) levels also decrease in patients with CKD. There are several possible mechanisms that may explain the decreases in 25(OH)D. The complex of 25(OH)D and DBP leaks with proteinuria. In addition the Uptake of 25(OH)D decrease due to down-regulation of megalin levels (2).

Table(1.4):Reasons of altered vitamin D metabolism in CKD.

Calcidiol deficiency 25(OH)D	Reduced sun exposure, reduced skin synthesis, reduced ingestion of foods rich in vitamin D, loss of DBP with proteinuria
Calcitriol deficiency $1,25(OH)_2D$	Reduced calcidiol availability, reduced renal 1-α hydroxylase availability, down regulation of renal 1-α hydroxylase from hyperphosphatemia and FGF-23, reduced endocytotic uptake by megalin, increased degradation of calcitriol by PTH and FGF-23
Calcitriol resistance $1,25(OH)_2D$	Loss of VDR in parathyroid glands, impaired binding of active vitamin D to VDR and impaired binding of vitamin D–VDR complex to the VDR element

Adapted by Nigwekar su,et al, Bonekey Rep 2014 feb 5;3,498.

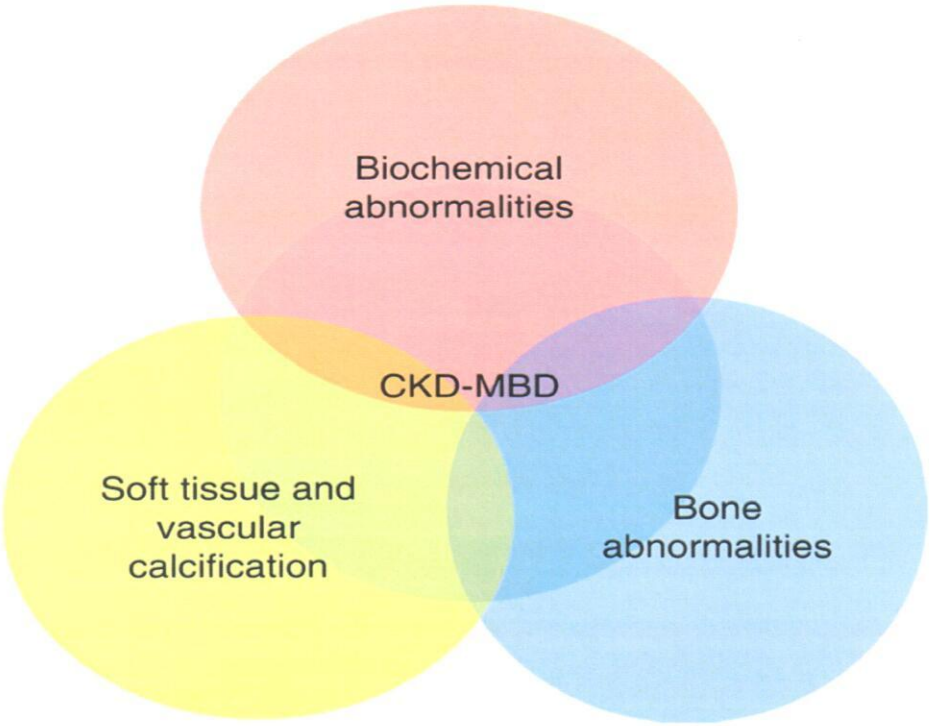


Figure(1.3):Altered vitamin D metabolism in the course of kidney disease progression.
In chronic kidney disease progression, decreased renal mass limits the amount of 1α-hydroxylase in renal proximal tubular cells. In addition, decreases in glomerular filtration rate (GFR) and low megalin content contribute to impaired 25(OH)D uptake and protein reabsorption. Moreover, increased levels of serum level of phosphate (P), fibroblast growth factor 23 (FGF23), N-terminally truncated parathyroid hormone (PTH) fragments, and uremic toxins, along with kidney function decline may contribute to the suppressed activation of 1α-hydroxylase, resulting in decreased levels of 1,25(OH)2D. Also, increased FGF23 up regulates the expression of 24-hydroxylase, resulting in the catabolism of 1,25(OH)2D to the inactive form of vitamin D, 1,24,25(OH)3D. DBP, vitamin D binding protein; VDR, vitamin D receptor, IDBP3, intracellular vitamin D binding protein. adapted by kim cs,et al, korean journal of internal medicine 2008;29(4):416-27.

1.3.4.CKD-MBD Pathogenesis And Role Of Vitamin D:

Mineral and bone disorders are common in CKD and are now collectively referred to as CKD– mineral and bone disorder (MBD), Alteration in vitamin D metabolism is one of the key features of CKD–MBD and this alteration accompanied by soft tissue and vascular calcification is one of the most common and important consequences of CKD development and progression(20)(Figure 1.4).

Figure(1.4):Chronic kidney disease–mineral and bone disorder.



Adapted by Nigwekar su,et al, Bonekey Rep 2014 feb 5;3,498.

Essential points for diagnosis of CKD–MBD:

(1) biochemical abnormalities in calcium, phosphorous, parathyroid hormone (PTH), vitamin D and fibroblast growth factor-23 (FGF-23).

(2) changes in bone morphology such as bone volume, bone turnover and bone mineralization .

(3) calcification of soft tissue and blood vessels.

These factors are inter-related and their major target organs include parathyroid gland, kidneys, bone and intestinal tract. The classic biochemical abnormalities in CKD–MBD are hypocalcaemia, hyperphosphatemia, hyperparathyroidism, hypovitaminosis D and elevated FGF-23; however, significant variations especially in serum calcium are common (24).

1.3.5. Secondary Hyperparathyroidism:

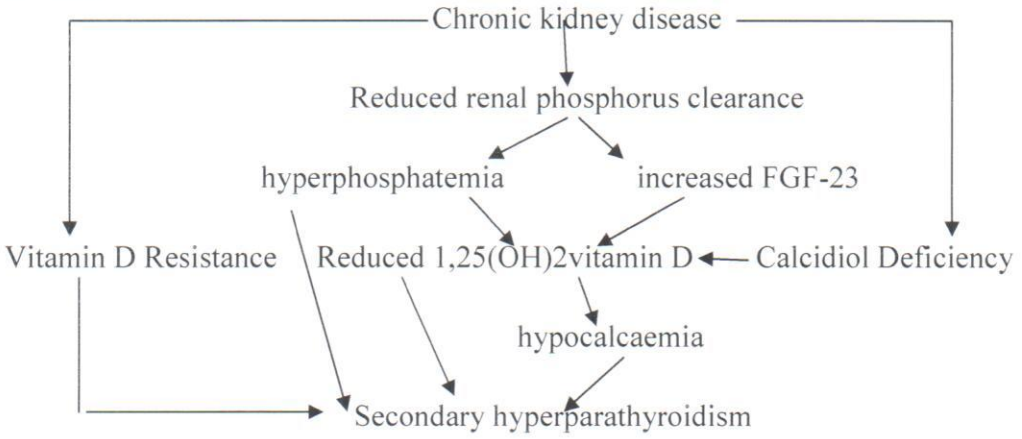
Secondary hyperparathyroidism (SHPT) is a challenge frequently encountered in the management of patients with chronic kidney disease (CKD).

Down regulation of the parathyroid vitamin D and calcium-sensing receptors represent critical steps that lead to abnormalities in mineral metabolism: high phosphate, low calcium, and vitamin D deficiency. These imbalances result in parathyroid hyperplasia and contribute to vascular calcification (24).

Pathogenesis of SHPT:

Continuous stimulation of the parathyroid glands by a combination of elevated extracellular phosphate concentration, decreased extracellular ionized calcium concentration, and markedly reduced serum calcitriol leads to increased PTH synthesis and release. At the same time, elevated FGF-23 expression down regulates residual renal 25(OH)-1-hydroxylase, which exacerbates the effective deficiency of calcitriol, acting as an additional driver to SHPT (24).Figure (1.5).

Figure(1.5):Role of vitamin D in the development of secondary hyperparathyroidism in CKD.



Adapted by Nigwekar su,et al, Bonekey Rep 2014 feb 5;3,498.

The treatment of secondary hyperparathyroidism in chronic uremia focuses on avoiding hyperphosphatemia by the use of oral phosphate binders, which bind phosphate in the intestine and a concomitant substitution by a 1 alpha-hydroxylated vitamin D analog in order to compensate for the reduced renal hydroxylation. The most commonly used active vitamin D drug was either the natural 1,25(OH)2D3, or the 1 alpha-hydroxylated analog, 1alpha(OH)D3 which after 25-hydroxylation in the liver is converted to 1,25(OH)2D3.(25).

Paricalcitol: Paricalcitol (19-nor-1alpha-25-dihydroxyvitamin D2), a new vitamin D analog developed for the treatment of secondary hyperparathyroidism, It is an analog of 1,25-dihydroxyergocalciferol, the active form of vitamin D_2 (ergocalciferol).

Alfacalcidol: an active vitamin D3 metabolite, and therefore does not require the second hydroxylation step in the kidney, and has significant effects on the immune system, including regulatory T cells. It is the most commonly prescribed vitamin D metabolite for patients with end stage renal disease.

1.4.Vitamin D_2 versus Vitamin D_3 Supplements:

Both D_2 (Ergocalciferol) and D_3 (cholecalciferol) are available as dietary supplements. The relative efficacy of D_2 versus D_3 in humans continues to be debated, although both appear to be effective for preventing or treating disease, provided that an adequate total 25(OH)D blood level is obtained. The efficacy of both may relate to differences in serum half-life and is clinically relevant for dosing and monitoring frequency. A single dose of 50,000 IU of D_2 or D_3 produces a similar increase in the serum 25(OH)D concentration, but the longer half-life of D_3 suggests that less frequent dosing may be needed. A daily dosing study of 1000 IU of D_2 vs D_3 showed no difference in any resulting vitamin D level [$25(OH)D_2$, $25(OH)D_3$, or total 25(OH)D]. However, a recent study comparing 1600 IU of D_2 once daily vs 1600 IU of D_3 once daily versus 50,000 IU of D_2 once monthly versus 50,000 IU of D_3 once monthly suggested that D_3 is superior in that it showed slightly higher levels of $25(OH)D_3$ at the end of 1 year. An important limitation of this study was that the mean total 25(OH)D level at the beginning of the study was already in the reference range (33 ng/mL),

and those with hypovitaminosis D may respond differently. Therefore, it has been recommend that the use of D_3, particularly if dosing is infrequent (i.e. less than once weekly). One situation in which D_2 may be preferred is a vegetarian or vegan diet. It is recommended that both D_2 and D_3 be taken with a meal containing fat to ensure maximum absorption (26).

1.5.Aim of study:

Since patients with CKD have been reported to suffer from vitamin D deficiency and which in turn may progress to renal failure, we undertook this study aiming to:

- Determining the prevalence of vitamin D deficiency in CKD patients and its severity.
- Studying the relation between renal impairment stages and vitamin D deficiency.
- Identifying the causes of deficiency either demographic or disease related.
- Assessing the effect of treatment using vitamin D (Ergocalciferol or Cholecalciferol) and 1 alpha(1,25-(OH)$_2$ vitamin D or alpha-hydroxylated vitamin D) respectively on vitamin D and PTH levels.
- Proposing updated guidelines and recommendations for high risk groups to minimize morbidity and mortality in this high risk group.

Chapter 2

Subjects and Methods

2. Subjects and methods

2.1. Subjects:

Serum levels of 25(OH)D were analyzed in 80 patients (50 females and 30 males) at grade 3,4 and pre-dialytic grade 5 CKD. They were aged (62.04 ±14.403)years, being clinically stable and followed-up at Almajory polyclinic in Benghazi and Benghazi Medical Center (BMC) during the period from April to June 2016.

A total of 29 (12 females and 17 males) apparently healthy individuals were selected for this study. They were Age and sex- matched healthy individuals recruited from blood donor in blood bank and doctors in BMC hospital to serve as controls.

Informed consent was obtained from all subjects before the study. Face-to- face interview were based on a questionnaire (Appendix I) that included variables such as age, sex, weight, physical activity, musculoskeletal pain and weakness , history of fracture, drug history specially vitamin D and 1-alpha treatments, dietary history specially diet rich with vitamin D, history and time of sun exposure.

Blood pressure was measured using mercury sphygmomanometer with subjects in a seated position.

All patients presented with stable metabolic conditions. Patients presenting any disease that could affect their vitamin D level rather than kidney cause as liver disease or malabsorpion disease were excluded.

A cross-sectional analysis of serum levels of 25(OH)D and the correlation with anthropometrical [body weight], and lab data [urea, creatinine, alkaline phosphates, serum calcium, phosphorus, albumin, parathyroid hormone (PTH), fasting blood sugar and HA1C] were performed.

CKD was defined as the estimated creatinine clearance (CrC) < 90 ml/min and signs of renal lesion, estimated by the Cockcroft-Gault equation: CrC = [(140-age) x weight]/(72 x

Cr)}, multiplied by 0.85 for women. however this research is dealing with patients in grade 3,4 and predialysis grade5, therefore estimated creatinine clearance selected equal or below 60 ml/min.

Serum levels of vitamin D were considered as adequate when the concentration of 25(OH)D was higher than 30 ng/ml, levels between 20-30 ng/ml were considered as insufficient, and values that were equal or lower than 20 ng/ml defined the diagnosis of vitamin D deficiency and values less than 10 ng/ml were considered as sever vitamin D deficiency.

The control group consisted of healthy subjects who were not suffering from any acute infection, metabolic or psychological disorder, or chronic diseases such as diabetes mellitus (DM), hypertension (HTN), ischemic heart disease (IHD). Moreover, they were non-smokers and overweight individuals were avoided.

2.2. Methods:

2.2.1. Blood collection:

Venous blood samples were drawn from all the participants after informed consent.

Blood samples were collected in plain tubes. Samples were liquated into two tubes, one used for biochemical investigations and the other one stored at $-20\ C^{\circ}$ until the vitamin D assay were performed.

2.2.2. Instruments:

The biochemical parameters CBC, RFT, Calcium, Phosphorus, and Albumin were estimated using the standard procedures with available commercial kits in a fully automated system COBAS INTEGRA 400 plus (ROCH, Germany).

PTH was measured using COBAS e 411 (ROCH, Germany).

2.2.3.Estimation of serum albumin: Colorimetric method

Test principle: The method1 is based on the specific binding of bromocresol green (BCG), an anionic dye, and the protein at acid pH with the resulting shift in the absorption wavelength of the complex. The intensity of the color formed is proportional to the concentration of albumin in the sample.

$$\text{BCG} + \text{Albumin} \xrightarrow{\text{PH4.3}} \text{BCG-albumin complex}$$

The color intensity of the dye formed is directly proportional to the albumin concentration. It is determined by measuring the increase in absorbance at 630 ± 20 nm.

2.2.4.Estimation of serum calcium: Colorimetric method

Test principle: The method is based on the specific binding of arsenazo III and calcium at acid pH with the resulting shift in the absorption wavelength of the complex. The intensity

of the cromophore formed is proportional to the concentration of total calcium in the sample.

Arsenazo III + Calcium $\xrightarrow{\text{PH 6.5}}$ Arsenazo III-Calcium complex

The color intensity of the dye formed is directly proportional to the calcium concentration. It is determined by measuring the increase in absorbance at 650 ± 20 nm.

2.2.5.Estimation of Inorganic phosphate : INORGANIC Colorimetric method

Test principle: Inorganic phosphate reacts with molybdic acid forming a phosphomolybdic complex. Its subsequent reduction in alkaline medium originates a blue molybdenum color which intensity is proportional to the amount of phosphorus present in the sample.

$PO_4^{3-} + H^+ + (NH_4)_6 Mo_7O_{24} \xrightarrow{p1}$ Phosphomolybdic Complex

Phosphomolybdic Complex $\xrightarrow{\text{PH>10}}$ Molybdenum blue

The colour intensity of the dye formed is directly proportional to the phosphorus concentration. It is determined by measuring the increase in absorbance at 740 ± 10 nm .

2.2.6.Estimation of serum creatinine: Kinetic colourimetric method

Test principle: This procedure is based upon a modification of the original picrate reaction (Jaffe). Creatinine under alkaline conditions reacts with picrate ions forming a reddish complex. The formation rate of the complex measured through the increase of absorbance in a prefixed interval of time is proportional to the concentration of creatinine in the sample.

Creatinine + Picric acid $\xrightarrow{\text{PH>12 / 37°C}}$ Red addition complex

Photometer or colorimeter with a thermostatted cell compartment, able of reading at 510 ± 10 nm.

2.2.7.Estimation of vitamin D level:

Vitamin D level done by measuring 25 hydroxy vit D by using ELISA test Microplate Washer and Reader (LINEAR, Spain).

All reagents was brought to room temperature (18°C to 25°C) approx. 30 minutes before use. The reagents mixed thoroughly before use.

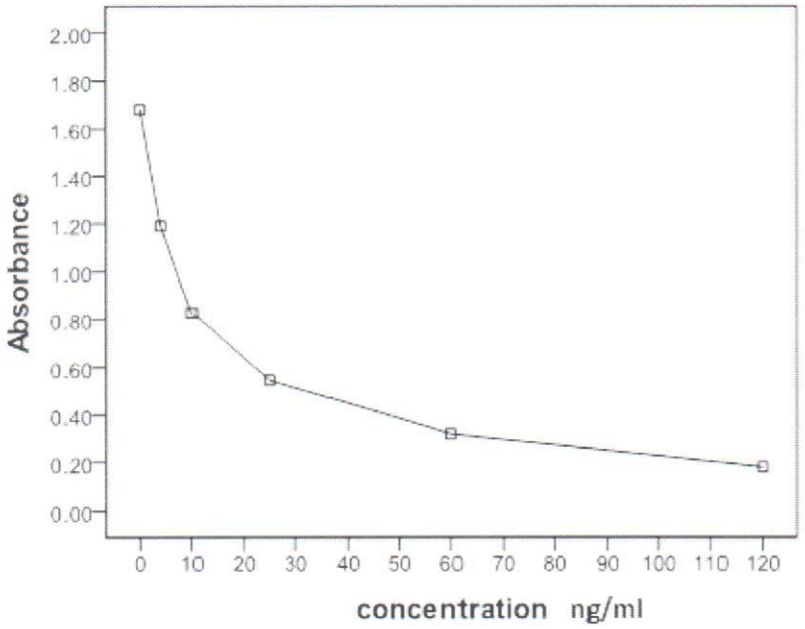
The biotin is a 100x concentrate. Mixed thoroughly before diluting. The required volume removed with a clean pipette tip and diluted in sample buffer (1 part biotin plus 99 parts sample buffer). Example: 1 ml biotin concentrate plus 99 ml sample buffer.

The serum of patients and controls were diluted with biotin-labeled 25-OH vitamin D and kept for 10 minutes at room temperature. After that added to microplate wells coated with monoclonal anti 25-OH vitamin D antibodies. Then 200 µl of sample diluted in biotin/sample buffer into each of the microplate wells. Incubated for 2 hours at room temperature (18°C to 25°C). This recognized by Streptavidin-Peroxidase Conjugate 100 µl of enzyme conjugate (streptavidin-peroxidase) Pipetted into each of the microplate wells and incubated for 30 minutes at room temperature (18°C to 25°C). All unbound materials were then washed away by Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubated for 15 minutes at room temperature (18°C to 25°C) and protected from direct sunlight.

Then 100 µl of stop solution Pipetted into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Photometric measurement of the color intensity measured at wavelength 450 nm and a reference wavelength between 620 nm and 650 nm within 30 minutes of adding the stop solution. Prior to measuring, the microplate were shake well to ensure a homogeneous distribution of the solution

Standard curve:



Calculation: Calculations of vitamin D concentrations were performed automatically by the microplate reader.

2.3.Statistical analysis:

The data were analyzed using the statistical package for the social sciences (SPSS version 18).

Descriptive characteristics of the study participants were calculated as mean ± standard deviation (SD).

Chi-squared test was used to compare categorical variables. T-test was used to compare continuous variables and correlation test was used to determine differences in subject characteristics.

Pearson's correlation coefficient determination was done to evaluate the degree of association between vitamin D and biochemical parameters.

P value (two- tailed) < 0.05 was considered as statistically significant.

Chapter 3

Results

3. Results

Total number of 80 patients were selected in this study (50 females and 30 males). The number of healthy controls included in our study was 29 (12 females and 17 males). The mean age and standard deviation (SD) of the patients was 62.04 ± 14.403years (ranging from 18-90 years). The mean age and SD of the healthy control subjects was 36.28 ± 10.194years (ranging from 17-58 years).

Mean weight of the patients was 75.5 ± 16.887kg. Serum creatinine was 3.903± 2.1mg/dl, and the estimated creatinine clearance was 22.61 ± 9.8 ml/min.

3.1. Vitamin D level among patients:

Out of the 80 analyzed patients 13 of them had history of receiving vitamin D therapy either Ergocalciferol or Cholecalciferol previously either Ergocalciferol or cholecalciferol and the remaining 67 patients untreated with vitamin D. We compared between treated and untreated patients and found significant difference of vitamin D level (p=0.001) (see table 3,1).

Table 3.1: Comparison between CKD patients who treated and untreated with vitamin D.

Vit D level in ng/ml	Treated with vitamin D Ergocalciferol or cholecalciferol	Untreated with vitamin D Ergocalciferol or cholecalciferol	P value
≤ 20	4 30.8%	54 80.6%	0.001***
>20	9 69.2%	13 19.4%	
Number of patients	13	67	

*** =very strong relation≤0.001.

This means that 80.6% of patients who didn't receive vitamin D treatment had vitamin D deficiency in contrast to treated patient with only 30.8% had deficiency .

Twenty five(25)patients=37.3% of the 67 untreated patients had sever vitamin D deficiency with serum level less than 10 ng/ml and 29 patients=43.3% had vitamin level between 10-20 ng/ml which also deficient and 7 patients=10.4% consider as insufficient with vitamin D level 20-30 ng/ml and the remaining 6 patients =8.9% had sufficient vitamin D level >30 ng/ml (Figure 3.1).

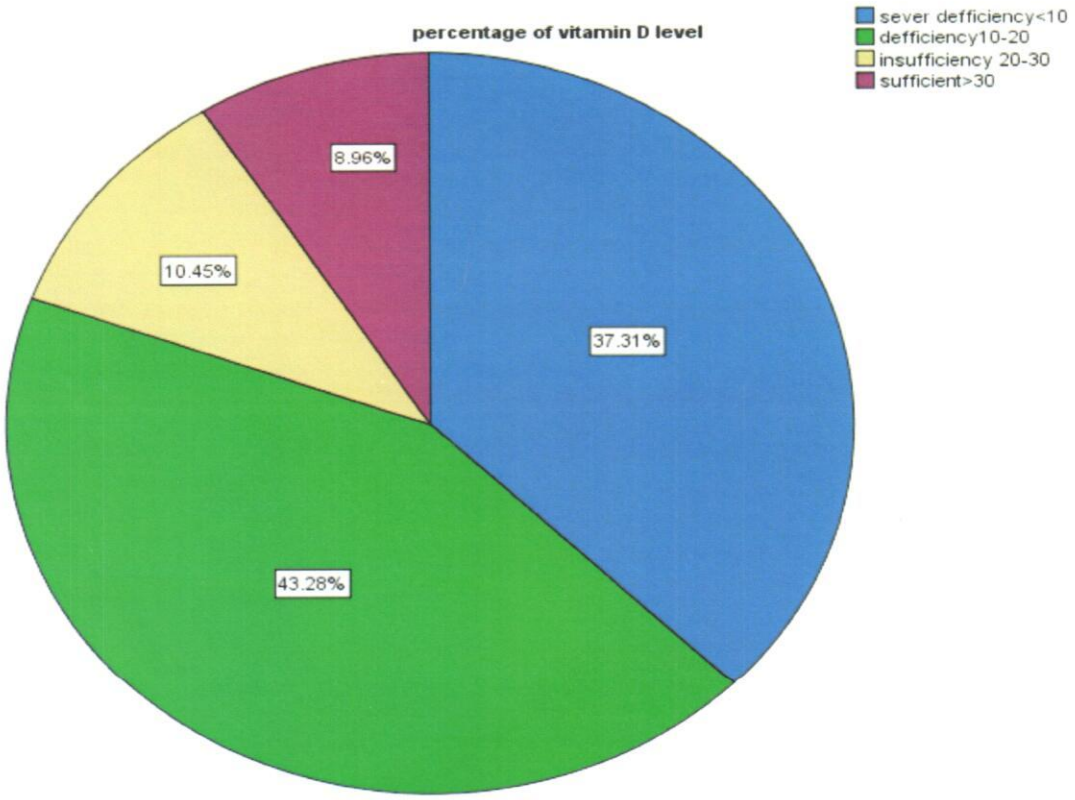


Figure 3.1: Severity of vitamin D deficiency in untreated patients.

Table 3.2: Mean ± SD of vitamin D in treated and untreated patients.

	Receive vitamin D Ergocalciferol or cholecalciferol	N	Mean	St. Deviation	P value
Vit D level in ng/ml	Treated	13	31.2592	16.25018	0.000***
	Untreated	67	15.9717	11.64993	

*** =very strong relation≤0.001.

3.2. Difference between untreated patients and controls:

Number of patients 67 patients, 28 of them male=41.8% and 39 female =58.2%

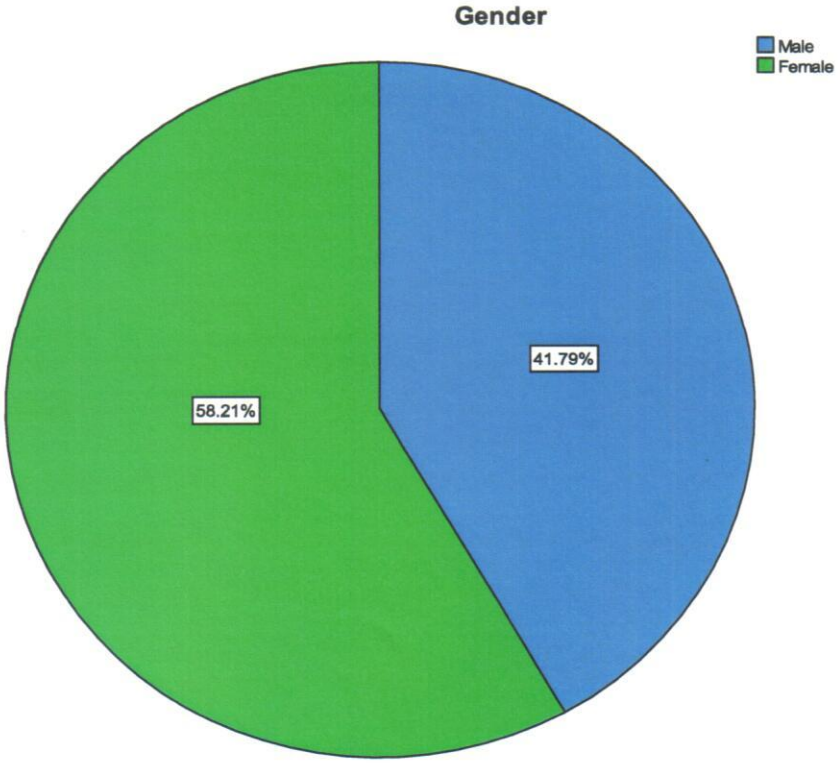


Figure 3.2: Percentage of male and female in patients.

The mean of vitamin D level and standard deviation (SD) of the (67) patients selected for this study was 15.97±11.649ng/ml compared to the mean of (29) controls was 19.035±11.496ng/ml .

Table 3.3: Percent of vitamin D deficiency in patients and controls.

Vit D level in ng/ml	Patients	Controls	P value
≤ 20	54 80.6%	20 69%	0.213
>20	13 19.4%	9 31%	

This means that 80.6% of patients had vitamin D deficiency and 19.4% had normal levels compared to controls in which 69% had vitamin D deficiency and 31% had normal levels.

Table3.4: Severity of vitamin D deficiency in patients and controls.

Vit D level in ng/ml		sever deficiency <10	Deficiency 10-20	Insufficiency 20-30	Sufficient >30	Total no.	P value
Subjects	Patients	25 37.3%	29 43.3%	7 10.4%	6 8.9%	67	0.054
	Controls	3 10.3%	17 58.6%	6 20.7%	3 10.3%	29	
Total		28	46	13	9	96	

with more analysis to control:

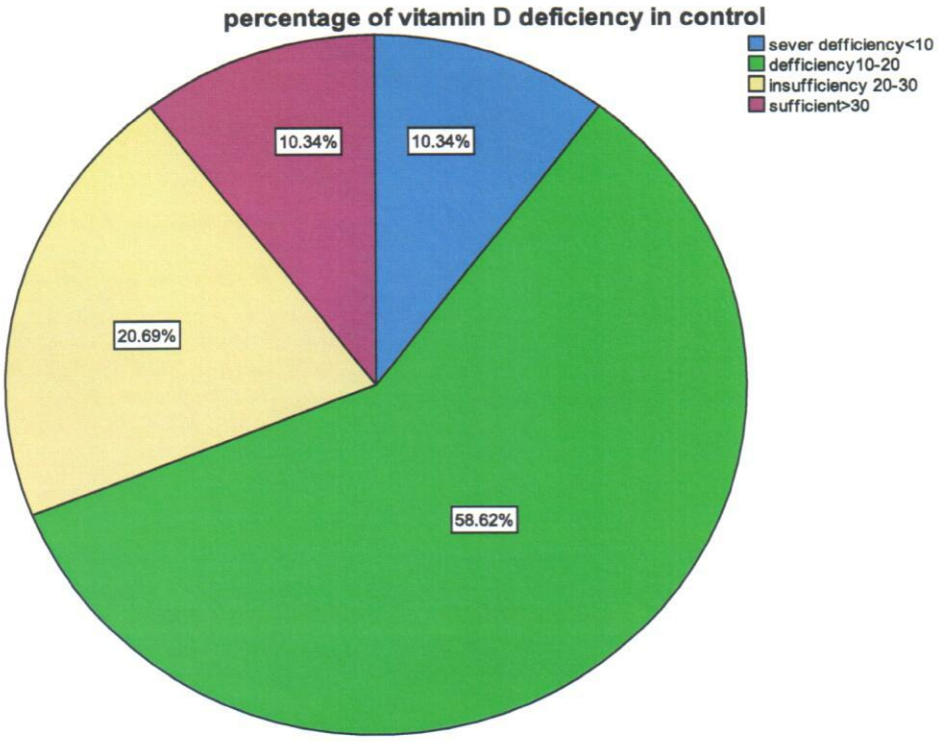


Figure 3.3: Severity of vitamin D deficiency in healthy controls.

That mean 3 patients=10.3% had sever deficiency with serum level of vitamin D less than 10 ng/ml and17 patients=58.6% had vitamin D level between 10-20 ng/ml which were deficient, 6 patients=20.7% had vitamin D level between 20-30 ng/ml and the remaining 3 patients=10.3% had vitamin D levels >30 ng/ml.

From this study it appears that significant difference between patients and control in percent of vitamin D deficiency (p=0.054).

3.3. Relation between the gender and vitamin D level:

Table 3.5: Effect of gender on the mean of vitamin D in patients.

Gender	Number of patients	Mean of vit D	St, deviation	P value
Male	28	15.3463	8.5025	0.713
Female	39	16.4207	13.5596	

This means that there is no difference between males and females because nearly equal. The mean of vitamin D in both gender in males was15.3463±8.5025ng/ml and in females was 16.4207±13.5596ng/ml .Our results showed that there is no relation between the gender and vitamin D level (p=0.713)

The following Table (3.6) shows the relation between the gender and vitamin D deficiency.

Table3.6: Relation between gender and vitamin D deficiency in patients.

	Vit D level in ng/ml	Male	Female	Total number	P value
Percent of total Percent in gender	≤ 20	22 32.8% 78.6%	32 47.8% 82.1%	54 80.6%	0.762
Percent of total Percent in gender	>20	6 9% 21.4%	7 10.4% 17.9%	13 19.4%	
Total number Total percent in gender		28 100%	39 100%	67	

Our results indicate that number of females more than males 39:28 and most of them are vitamin D deficient =47.8% of the total . Regarding female we found (82.1%) are deficient and (17.9%) are normal. However, in male we found (78.6%) are deficient and (21.4%) are normal. From these comparison it seems that there is no significant change between the two gender (p=0.762).

3.4.Relation between one alpha treatment and vitamin D level:

Table 3.7: Effect of one alpha treatment on the mean of vitamin D.

	One alpha	No.	Mean of vit D	St. deviation	P value
Vit D level in ng/ml	Treated	40	17.7519	13.42715	0.129
	Untreated	27	13.3343	7.87340	

No.=number of patients

This means that there is no relation between one alpha treatment and the mean of vitamin D (p=0.129)

Table 3.8: Relation between one alpha treatment and vitamin D deficiency .

Vit D level in ng/ml	Treated with one alpha	Untreated with one alpha	P value
≤ 20	31 77.5%	23 85.2%	0.538
>20	9 22.5%	4 14.8%	
Total count Percent of total	40 100% 59.7%	27 100% 40.3%	

This means that there were (40)patients=59.7% of total were taking one alpha and (31) patients of them (77.5%) had vitamin D deficiency and (9) patients (22.5%) had not vitamin D deficiency .

And (27) patients (40.3%) of total didn't take one alpha treatment, (23 of them=85.2%) had vitamin D deficiency and (4) patients (14.8%) had not vitamin D deficiency.

This means that there is no significant relation between one alpha treatment and vitamin D deficiency (p=0.538).

3.5. Relation between vitamin D levels and stages of renal failure.

(Tables 3.9 and 3.10) (Figures 3.4 and 3.5).

Table 3.9: Effect of stages of renal failure on mean of vitamin D.

Stages of renal failure	Number of patients	Mean of vit D (25 hydroxy vit D) ng/ml	St. deviation	P value
Stage3	14	14.0251	7.32643	0.939
Stage4	39	16.4106	12.60891	
Stage5	14	16.6957	12.86159	

It appears that no relation between the stages of renal failure and mean of vitamin D (p=0.939)

Table 3.10: Relation between stages of renal failure and vitamin D deficiency

Vit D level in ng/ml		Stages			Total	P value
		Stage 3	Stage 4	Stage 5		
≤20	Count	11	32	11	54	0.939
	Percent within vit D deficiency	20.4%	59.3%	20.4%	100.0%	
>20	Count	3	7	3	13	
	Percent within vit D deficiency	23.1%	53.8%	23.1%	100.0%	
	Count	14	39	14	67	
	Percent within vit D deficiency	20.9%	58.2%	20.9%	100.0%	

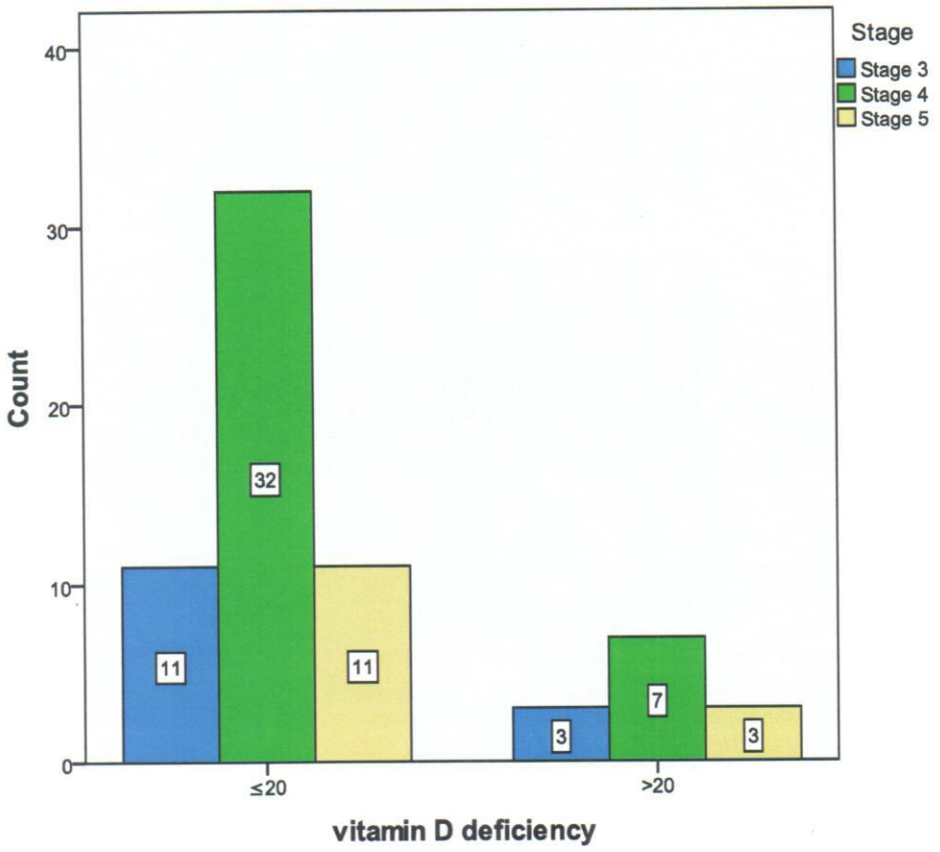


Figure 3.4 : Relation between vitamin D (25 hydroxy vit D) levels and stages of renal failure.

This indicates that there is no relation between vitamin D level and the stages of renal failure because most of patients with vitamin D deficiency were in grade 4, (32) patients=59.3% as well as not deficient patients (7) of them =53.8% also found in grade 4. Moreover, nearly equal percentage was noticed in grade 3 and 5 (p=0.939).

Therefore, these results of our patients confirmed that there is no relation between vitamin D level and stages of renal failure.

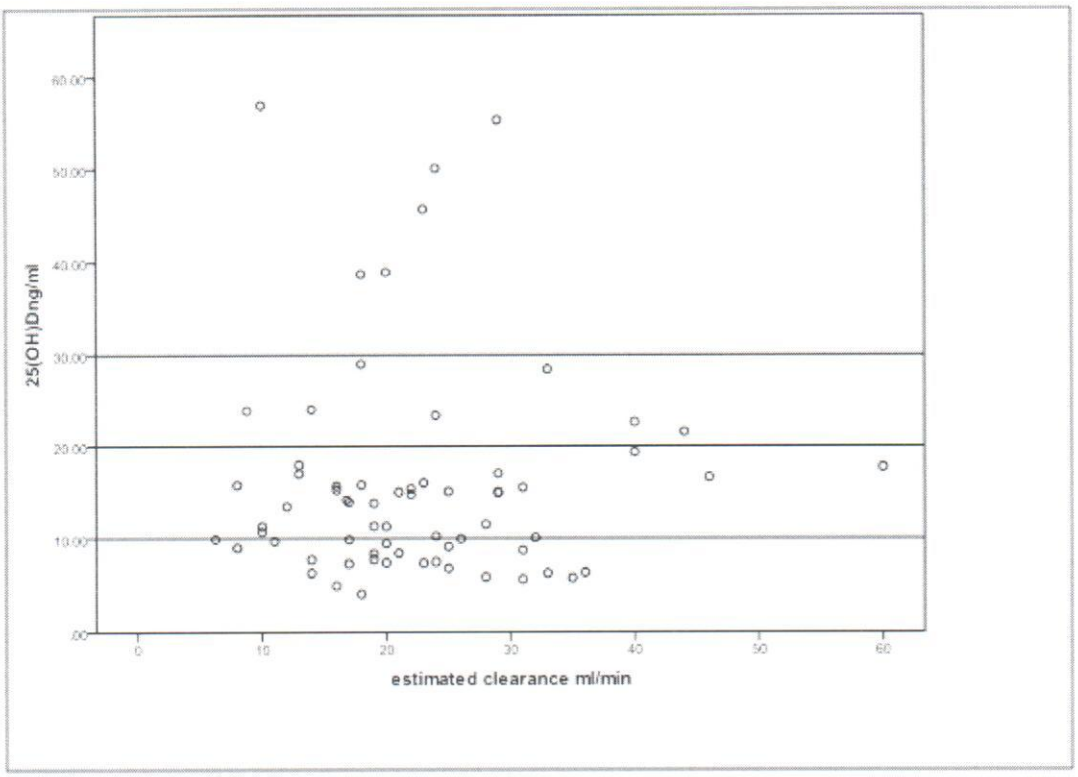


Figure 3.5 Relation between Estimated Glomerular Filtration rate and vitamin D level.

Our study showed that there was no correlation between serum levels of 25(OH)D and the estimated creatinine clearance of patients

3.6. Relation between vitamin D treatment and symptoms of deficiency:

Symptoms of vitamin D deficiency such as musculoskeletal pain and weakness and history of fracture (Tables 3.11 and 3.12)

Table 3.11: Effect of vitamin D treatment on musculoskeletal pain .

Vitamin D treatment	With musculoskeletal pain	Without musculoskeletal pain	Total number	P value
Treated	6 46.2%	7 53.8%	13 100%	0.313
Untreated	41 61.2%	26 38.8%	67 100%	

Our result showed that most of the untreated patients (41)=61.2% had musculoskeletal pain and the remaining (26)=38.8% had no musculoskeletal pain. In contrast treated patients (53.8%) had no pain, but with non-significant negative relation (p=0.313).

Table 3.12: Relation between vitamin D treatment and history of fracture.

Vitamin D	H/O fracture	No H/O fracture	Total number	P value
Treated	2 15.4% 20%	11 84.6%	13 100%	0.731
Untreated	8 11.9% 80%	59 88.1%	67 100%	

This mean that 80% of fractured patients were not treated with vitamin D .

Table 3.13: Relation between vitamin D deficiency and history of fracture, (p=0.719).

			Vit D deficiency		
			≤20	>20	Total
Fracture	H/O fracture	Count	8	2	10
		% within fracture	80.0%	20.0%	100.0%
		% within vit D deficiency	13.8%	9.1%	12.5%
	no H/O fracture	Count	50	20	70
		% within fracture	71.4%	28.6%	100.0%
		% within vit D deficiency	86.2%	90.9%	87.5%
Total		Count	58	22	80
		% within fracture	72.5%	27.5%	100.0%
		% within vit D deficiency	100.0%	100.0%	100.0%

This means 80% of the patients which had history of fracture were vitamin D deficient .

3.7. Effect of vitamin D treatment on biochemical parameters .

Table 3.14: Relation between vitamin D treatment and mean and st. deviation of albumin, calcium, phosphorus, alkaline phosphatase, PTH, creatinine, EGFR, and vitamin D level.

	Vitamin D Treatment	N	Mean	St. Deviation	P value
Albumin	Treated	6	3.9183	.98773	0.967
	Untreated	35	3.9077	.49565	
Calcium	Treated	11	9.0473	.99989	0.251
	Untreated	58	8.6690	.99271	
Phosphorus	Treated	10	4.7830	.66717	-0.783
	Untreated	55	4.9127	1.44820	
Alkaline phosphatase	Treated	4	205.1000	145.95310	0.314
	Untreated	16	137.7000	109.56289	
PTH	Treated	8	118.5625	107.73327	-0.020*
	Untreated	47	393.7700	319.86648	
Creatinine	Treated	13	3.8077	2.20849	
	Not-Receive	67	3.9225	2.14211	-0.861
EGFR	Treated	13	22.85	9.780	
	Untreated	67	22.56	9.970	0.926
Vit D level in ng/ml	Treated	13	31.2592	16.25018	
	Untreated	67	15.9717	11.64993	0.000***

N=number of patients.

*=significant relation p≤0.05.

***=very strong relation p≤0.001.

This means that there is no effect of vitamin D treatment on levels of albumin, calcium, phosphorus ,alkaline phosphatase, creatinine, and EGFR , but with good inverse relation

with PTH (p=-0.020) and that in parallel with the negative feedback of vitamin D on PTH level and the role of vitamin D in preventing secondary hyperparathyroidism. .

We noticed that there is very strong relation between vitamin D treatment and level of 25(OH)vit D (p=0.000).

3.8. Effect of vitamin D treatment on PTH level.

Table 3.15: Effect of vitamin D (Cholecalciferol or Ergocalciferol) treatment on PTH level.

	Vitamin D treatment	N	Mean	St. error	P value
PTH	Treated	8	118.5625	38.06	-0.020*
	Untreated	47	393.7700	46.69	

*=significant relation p≤0.05.

It appeared that inverse relation of vitamin D treated patients with PTH level (p=-0.020) and that go with the negative feedback of vitamin D on PTH level and the role of vitamin D in preventing secondary hyperparathyroidism .

3.9. Effect of 1-alpha treatment on PTH level

Table 3.16: Effect of 1-alpha (1,25 OH vitamin D or 1 alpha-hydroxylated analog) treatment on PTH level.

	1-alpha treatment	N	Mean	St. error	P value
PTH	Treated	29	436.4793	62.55	0.250
	Untreated	18	324.9606	67.61	

This indicates that there is no relation between 1-alpha treatment and the mean of PTH ($p=0.250$).

And this attributed to the insufficient dose as well as in proper effect of 1-alpha even at secondary hyperparathyroidism.

Chapter 4

Discussion

4. Discussion

In the present case-control study of 80 patients with nondialytic CKD and 29 healthy control subjects, we found a high prevalence of vitamin D deficiency/insufficiency in patients with pre-dialytic CKD. This condition was noticed in 80.6% of the studied patients and 69% of the controls.

Multiple observations have shown that CKD is associated with high incidence of vitamin D (25(OH)D) deficiency. In a study performed in São Paulo in Brazil, 2012 on 125 patients it was found that 92 patients (72.6%) had suboptimal levels of 25(OH)D < 30 ng/ml, and 65 patients (52%) had vitamin D insufficiency (15–29 ng/ml), whereas 27 (21.5%) had vitamin D deficiency (5–14 ng/ml) and only one patient had severe vitamin D deficiency (< 5 ng/ml)(27).

Another observational studies have shown low levels of both 25(OH)D and $1,25(OH)_2D$ in patients with CKD and end stage renal disease (ESRD) as in a cross-sectional analysis of 825 consecutive patients baseline vitamin D levels from within a prospective cohort of incident US hemodialysis patients in US by Wolf M et al, 2007. Out of these patients, 78% were considered as vitamin D deficient and 18% severely deficient(5).

In another study carried out on nine hundred and eight (908) patients with 25-hydroxyvitamin D levels were identified from the Accelerated Mortality on Renal Replacement (ArMORR) cohort of incident U.S. dialysis patients found 79% of the population were vitamin D deficient (25-hydroxyvitamin D <30 ng/ml)(28).

However, it is important to observe that the high frequency of vitamin D deficiency in our patients with CKD was 80.6% and this indicates no large difference from that of healthy controls in which 69% presented with serum levels of 25(OH)D < 20 ng/ml (p=0.213).

such results of vitamin deficiency need more researches on general population to clarify the causes of deficiency which can either dietary or inadequate sun exposure, or another un diagnosed causes.

Despite the non significant difference in vitamin D deficiency between patients and controls, we noticed the difference in the severity of deficiency between both of them in which 37.3% of patients had sever vitamin D deficiency (i.e,25(OH)vit D less than 10 ng/ml) and 10.4% insufficiency(i.e. between 20-30ng/ml). On the other hand, the healthy controls had only 10.3% sever deficiency and 22.7% insufficiency (p value=0.054).

Our results demonstrated that there is no relation between the stages of renal impairment and vitamin D deficiency and this in agreement with Sao Paulo study,2012(27).

There was no effect of gender on vitamin D level as the mean of vitamin D in male was (15.3463±8.5025) ng/ml and in female was (16.4207±13.5596)ng/ml (p=0.713). In contrast to a study performed in São Paulo, 2012 on 125 patients, the serum levels of 25(OH)D were higher for males (38.1 ± 20.6 ng/ml) *versus* (22.4 ± 9.7 ng/ml) for females, p < 0.0001) (29).

In addition in our study there is no relation between age and vitamin D deficiency (p=0.552).

Therapies with ergocalciferol or cholecalciferol in patients with CKD with 25(OH)vitamin D deficiency has been little described in literature, thus showing it is associated with high serum levels of this vitamin for most patients because there are big and significant different in vitamin D levels between untreated and treated groups as we found the Mean ± SD of vitamin D in treated 31.2592±16.25018 ng/ml and in untreated 15.9717±11.64993 ng/ml ($p=0.000^{***}$). Furthermore, the percent of vitamin D deficiency 80.6% in untreated patients and only 30.8% in treated patients (p=0.001)

Our results are in agreement with those reported by Al Aly, et al. 2007 that showed that patients with CKD stages 3and4 with serum 25(OH)vitamin D level<30 ng/ml treated by 50.000I.U ergocalciferol one weekly for 12 weeks and once monthly for a total 6 months a significant increase in vitamin D level from 16.6±0.7 to 27.2±1.8 ng/ml ($p<0.05$)(30).

This persistence of vitamin D deficiency in 30.8% despite of treatment may be due either to inadequacy of treatment with vit D (ergocalciferol or cholecalciferol) as the change in serum concentration of 25(OH)vitD depend on multiple factors such as: (1)dose administered,(2)starting serum concentration of 25(OH)vitamin D, (3)Body mass index(BMI) as increase the weight of patient increase the required dose of vitamin D, (4)age of patient,(5)serum albumin concentration. Or due to resistance to treatment as in malabsorpion syndrome or mutation of vitamin D receptors as in type II vitamin D resistance rickets as some of its types cause decrease the affinity of vitamin D to its receptors lead to the need of large doses of vitamin D, while the normal level in untreated groups may refer to compensation through diet and/or sun exposure.

Furthermore, Ziyad Al Aly, et al,2007 found significant decrease in plasma PTH level from 231±26 to 192±25pg/ml after ergocalciferol treatment($p <0.05$)(30).

Their results are in agreement with the present study which revealed significant difference in PTH level between vit D treated and un treated patients, with PTH level (118.56±107.73pg/dl) in treated patients and (393.77±319 pg/dl) in un treated patients

(p value=0.02).

In our study it was found that there is no significant differences between 1 alpha treated and un treated patients regarding PTH level and this can be explained by the use of insufficient dose or improper effect of 1-alpha even at secondary hyperparathyroidism, which mean that vitamin D (ergocalciferol or cholecalciferol) treatment is better than one alpha treatment in control of secondary hyperparathyroidism.

However, our results are in disagreement with studies carried out in the USA on 168 hemodialysis patients which conclude that a single intravenous high dose of 10 mg of 1alpha(OH)D3 or 1,25(OH)2D3 significantly suppressed plasma PTH. The acute suppressive effect of 1,25(OH)2D3 was three times greater than that of 1alpha(OH)D3(25).

Furthermore our results are in disagreement with another American study conducted in 2012 in which showed that Paricalcitol in a dose 1 to 2 μg/day for 16 weeks is more effective than Ergocalciferol 50,000 units titrated to achieve serum levels ≥30 ng/ml at decreasing PTH levels. this study done on 80 patients with CKD stages 3 or 4,with 25(OH)D level <30 ng/ml and SHPT in a single medical center (31).

Interestingly, in the same American study revealed that serum 25(OH)vit D increased significantly after 16 weeks in only ergocalciferol group but not in the Paricalcitol group and that result was in agreement with our study, which reveal no significant effect of 1-alpha treatment on vitamin D level .

This Indicates that 1-alpha treatment not enough to correct vitamin D level and restore the body store of 25(OH)VIT D.

For that physicians must start to manage the vitamin D deficiency and insufficiency by vitamin D (Ergocalciferol or Cholecalciferol) supplementation throughout all the stages of CKD to cover the classical (endocrine) action and the nonclassical (autocrine and paracrine) actions while continuing to provide the active form calcitriol or alpha hydroxylated analogs to stages 3-5 to control PTH and prevent secondary hyperparathyroidism(SHPT).

The effect of vitamin D treatment on kidney function could not be assayed because of short duration of our study on CKD patients and they needed follow up before and after treatment. Our observation of already treated patients on kidney function require another study to confirm the effect of vitamin D treatment on renal function and compare to

previous research which conclude that vitamin D is potentially interesting treatment modality for renoprotection in patients with chronic kidney disease(32).

However, in this study we showed that there was no relation between vitamin D levels and the levels of albumin, calcium, phosphorus ,alkaline phosphatase, creatinine, and EGFR and these results are in agreement with other studies, which were conducted to evaluate the association between 25 (OH) vitamin D levels and biochemical abnormalities in calcium, phosphorous and PTH in CKD. Therefore, it appears that there is no association between serum 25 (OH) vitamin D levels and elevated serum PTH or lower serum calcium (20).

Some studies and a number of observations reported that vitamin D deficiency in 80% to 90% of children and adults is associated with pain, myalgia and weakness(33).

But in our study we found that 63.8% of vitamin D deficiency is associated with pain and weakness and 36.2% without musculoskeletal symptoms in contrast to non deficient patients 45.4% with symptoms and 54.5% without symptoms.

Moreover, recent randomized blinded-placebo-controlled trial showed no benefit of vitamin D supplementation for such symptoms(34). This is in agreement with our results in which no significant differences in symptoms in treated and un treated patient ($p=0.313$) .

Studies of the association between 25(OH) vitamin D levels, bone morphology, bone mineral density and bone fractures in CKD and/or ESRD are limited in number but very informative. Ambrus *et al.* 2014 retrospectively examined the association between fracture and vitamin D status in 130 patients on maintenance hemodialysis. In Ambrus et al. study patients with fractures had significantly lower 25(OH) vitamin D levels compared with patients without fractures, and lower vitamin D levels were independently associated with increased fracture risk (20). In our study we found that 80% of the patients which had history of fractures and these patients suffered from vitamin D deficiency.

conclusions:

Patients with CKD have an exceptionally high rate of severe vitamin D deficiency so must consider annually vitamin D assay by measure 25(OH)VIT D in moderate and sever CKD patients and treat the patients with vitamin D deficiency.

Recent guidelines have publishing vitamin D supplementation recommendations for the healthy population , and others have addressed recommendations for patients with specific disease, such as CKD.

In the past, $1,25(OH)_2D$, the active form of vitamin D, was thought to be exclusively acting in calcium homeostasis, and simply replacing it with calcitriol or one of its analogs was thought to be sufficient in CKD patients who had deficient $1,25(OH)_2D$ levels. Understanding the ability of vitamin D to be activated by many other extra renal cells and explanation of its role in gene expression involved in immunity, cell differentiation, proliferation, and apoptosis have led to recommendations that vitamin D (Cholecalciferol or Ergocalciferol) supplementation should be taken in patients with CKD to achieve adequate level of vitamin D after control PTH with one alpha (calcitriol or alpha hydroxylated analogs) .

Physicians must depend on vitamin D level to start treatment of vitamin D deficiency not on symptoms of deficiency.

Chapter 5

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Appendix I

Appendix I

(Questionnaire)

- **CASE protocol of vitamin D deficiency in CKD:**
- **NAME:.....AGE:.....years.**
- **Gender: M ( ) F ( )**
- **Weight:.....kg.**
- **Height:.....m.**
- **Blood Pressure: sys BP: ..... mmHg. Dias BP: ..... mmHg.**
- **Duration of CKD.....**
- **Stage of CKD (eGFR).....ml/min.**
- **DM. 1.yes( ) 2.no( )**
- **HTN 1.yes( ) 2.no( )**
- **Heart disease 1.yes( ) 2.no( )**
- **Other chronic disease.....**
- **Take 1-Alpha. 1.yes( ) 2.no( ).**
- **duration.....dose..... µg/day**
- **other drug history.....**
- **Receive vit d ttt. 1.yes( ) 2.no( ).**
- **Sign and symptoms of vit d deficiency;**
- **Musculoskeletal Pain. 1.yes( ) 2.no( ).**
- **History Of Fracture 1.yes( ) 2.no( ).**
- **Sun Exposure.1. over 30 minutes of sun exposure daily ( ) 2.once weekly( ) 3. < once weekly( ) 4.no sun exposure( )**
- **Diary History. 1.no diary at all( ) 2. 1aunce/day( ) 3. 2 aunce/day( ). 4.>2aunce/day ( )**
  fish.....tuna.....sardine.....
- **Investigations:**
- **cbc.Hb;.....wbc;.....plt.....**
- **fasting .b.sugar..... mg/dl..HbA1c..... %..**

- **RFT. urea( ) cr ( ) Na( ) k( ) U.A( )**
- **LFT. Alt ( ) Ast ( )**
- **s,albumin.....**
- **s.calcium.....s.phosphorus.....**
- **alk ,phosphates.....**
- **PTH.....**
- **vit d3 level.(25 .OH,vit D3).....**

<u>NOTE</u>; liver disease and malnutrition to be excluded

Arabic

Summary

فيتامين د هو فيتامين ذائب في الدهن موجود بشكل طبيعي في عدد قليل من الأطعمة، وهو فيتامين ينشّطه الجسم ليقوم بنشاط هرمونيّ (الكالسيفيرول)، ولا يعتبر تناوله من الغذاء أساسيّاً كباقي الفيتامينات، ولكن لا بدّ من الحرص على الحصول عليه بالتعرض الكافي لأشعة الشمس.

وظائف فيتامين د في الجسم: يعمل فيتامين د بشكل رئيسي كهرمون ستيرويدي يطلق عليه ثنائي هيدروكسيل الكولي كالسيفيرول أو الكالسيتريول، وهو يعمل عن طريق تفاعله مع مستقبلات فيتامين د في الخلايا مؤثراً في عمليّة نسخ الجينات.
من أبرز وظائف فيتامين د في الجسم دوره في توازن الكالسيوم والفسفور، إذ إنّه يحفز تكوين البروتين الرابط للكالسيوم في جدار الأمعاء والذي يمتصّه، وهو يحفّز أيضاً قنوات الكالسيوم لامتصاصه كما أنّه يساهم في امتصاص الفسفور، ويعيد امتصاص كل من الكالسيوم والفسفور في الكليتين، بالإضافة إلى دوره مع هرمون الغدة الجار درقية في تحفيز خروج الكالسيوم من العظام وطرح الفسفور في البول في حال انخفض مستوى الكالسيوم في الدم، وبهذه الميكانيكيات يلعب فيتامين د دوره الأساسيّ في المحافظة على تركيز الكالسيوم والفسفور في الدم ليسمح للعظام بترسيبهما، كما أنّ الحصول على كميات كافية من فيتامين د والكالسيوم يحافظ على مستوى الكالسيوم في الدم، ويمنع من ارتفاع مستوى هرمون الغدة الجار درقيّة الذي يحفز خروج الكالسيوم من العظام.

يلعب هرمون الكالسيتريول دوراً هامّاً في النمو الطبيعيّ للخلايا وتمايزها وتكاثرها في العديد من أنسجة الجسم، مثل الجلد والعضلات وجهاز المناعة والغدة الجار درقيّة والدماغ والجهاز العصبي والأعضاء التناسلية والغضاريف والبنكرياس والثدي والقولون، وهو يمنع أيضاً النمو غير الطبيعيّ للخلايا مخفّضاً بذلك من خطر الإصابة بالسرطان.

يلعب فيتامين د دوراً هاماً في العمليّات الأيضية في العضلات مؤثراً في قوتها وانقباضها، ويسبب نقصه ضعفاً في العضلات، وخاصة عضلة القلب.

وجدت العديد من الدراسات الحديثة دوراً للفيتامين د في تنظيم استجابات جهاز المناعة، حيث إنّ الخلل في استجابات جهاز المناعة تحدث بعض أمراض المناعة الذاتية، مثل مرض السكري من النوع الأول والتصلب اللويحي وأمراض الأمعاء الالتهابيّة وأمراض الروماتيزم الناتجة عن اختلال المناعة الذاتية.

فيتامين د وعلاقته بمرض الكلى المزمن (CKD):

يعتبر مرض الكلى المزمن مشكله صحيه عامه وكثيرة الحدوث وقد تؤثر على كثير من اعضاء الجسم كالقلب والاوعيه الدموية .

كما أنه يؤدى إلى نقص شديد فى مستوى فيتامين د في الجسم بسبب عدم قدرة الجسم على تحويل(25(OH)D) إلى الشكل النشط (1,25 dihydroxy-vitamin D) .

وهناك أدله جديده قد تحسن من فهمنا لوظيفة فيتامين د ومنها الوظائف الكلاسيكيه endocrineg action والتى تختص بتنظيم مستوى الكالسيوم والفوسفور في الجسم وصحة العظام،والغير كلاسيكيه autocrine action ،حيث اتضح دور فيتامين د كوسيط في عديد من المسارات داخل وخارج الكلى كدوره فى جهاز المناعه والتعبير الجينى وتمايز الخلايا .

ونتيجة لإكتشاف عملية التنشيط التى تحدث خارج الكلى في عديد من أعضاء الجسم وجد أن أعطاء مرضي القصور الكلوى جرعات بسيطه من فيتامين د النشط (1,25 dihydroxy-vitamin D) أو احد نظائره (alpha-hydroxylated vitamin D) لتعويض النقص في التنشيط
(hydroxylation) غير كافي لتعويض النقص في مستوى فيتامين د حيث وجد تأثيره على الغدة الجار دراقيه فقط ولا يؤثر على المخزون (25(OH)D) من فيتامين د وبذلك وجد أن أعطاء الكولي كالسيفيرول د3 والايرقوكالسيفيرول د2 كعلاج وخاصة للأشخاص الأكثر عرضة للنقص مهم جدا لتعويض هذا النقص في المخزون.

الهدف من البحث هو قياس مستوى فيتامين د فى الجسم لمرضى القصور الكلوى وتحديد نسبة انتشاره في هذه الفئه المعينه من المرضى ومقارنته مع الأشخاص الطبيعيين وكذالك مقارنة مستوى فيتامين د بين المرضى الذين اخذو فيتامين د كعلاج والمرضى الذين لم يأخذو العلاج فى السابق.

الطريقة: قياس مستوى (25(OH)D) فى الدم لمرضى القصور الكلوى من مرحلة 3,4,5ماقبل الغسيل predialysis لمرضى العياده الخارجيه لمستشفى الهوارى المتواجدين في عيادة الماجوري وقسم الكلي بمركز بنغازى الطبى .

تم الحصول على المعلومات السريرية والتاريخ الطبي من خلال استعراض الملفات الطبية للمرضى والمقابلات مع المرضى و الحالات الضابطة . تم سحب عينات من الدم الوريدي من جميع المشاركين بعد أخذ الاذن منهم وتعبأه النموذج الخاص بالاسئله ثم فصلنا العينه الى جزئين لاجراء التحاليل الروتينيه والكالسيوم والفوسفور والغده الجار دراقيه بالاجهزه المتوفره فى المستشفى وتم وضع السيرم في الفريز عند درجه حراره (-20 درجه مئويه) لحين الانتهاء من تجميع العينات كامله واجراء تحليل فيتامين د بقياس 25 هيدروكسي فيتامين د(25(OH)D) في السيروم، فقد تم قياس تركيزه بأستخدام (ELISA)، وتحليل البيانات بواسطه SPSS النسخه 18 واعتبر ان قيم p اصغر من 0.05 تعتبر ذات دلاله أحصائيه .

في هذه الدراسة درسنا (80) من المرضى (الذين تتراوح أعمارهم بين (14.403±62.04) عاما. وقد كانو(50)من الإناث و (30) من الذكور، مع متوسط الكرياتينين creatinine (2.1±3.903) ملغم / ديسيلتر وتصفية الكرياتينين creatinine clearance(9.8±22.61) مل / دقيقة. وزن الجسم كان Body weight(75.5±16.887) kg كجم، والكالسيوم Calcium ملغم / دل mg/dL (0.996±8.7293) ، الغدة الجار دراقيه (PTH) (313 ± 353.73)، 441 pg/mL جزء من الغرام / مل، وألبومين المصل g/dl(0.575±3.909) serum albumin غ / دل، ومتوسط فيتامين د كان ng/mL (13.6±18.4559) The mean 25(OH)D was. و(29) من العينة الطبيعية (control) لتكون بمثابة الضوابط ، حيث كانت (12) الإناث و (17) ذكور، وكان متوسط فيتامين د (11.496 ± 19.035) 25(OH) D نانوغرام / مل.

من 80 مريضا وجدنا أن (13) منهم سبق لهم العلاج فيتامين د كما الجرعة القصوى ولذالك تم استبعادهم في تحديد نسبه النقص للفيتامين ودراسة الأسباب أم الباقي (67) مريضا فوجد أن 80.6% منهم يعانون من نقص فيتامين د و19.4% كان المستوى طبيعي وبالمقارنة مع العينة الطبيعية (control) الضوابط 69% منهم لديهم نقص فيتامين د و31% كان المستوى طبيعي .

وبمقارنة مرضى القصور الكلوي كل مرحله (stage) مع الأخرى وجد انه لا يوجد فرق في مستوى فيتامين د بين المراحل التي شملتها الدراسة وهى الثالث والرابع والخامس ما قبل الغسيل الكلوي.

وكذالك وجد المستوى تقريبا متساوي بين الذكور والإناث حيث وجد متوسط فيتامين د The mean 25(OH)D was في الذكور 15.3463±8.5025 وفى الإناث 16.4207±13.5596.

ونتيجة لدراستنا وللدراسات السابقة التي وجدت مستويات متدنية لفيتامين د في مرضي القصور الكلوي وجب إجراء التحليل سنويا وتعويض النقص بإعطاء جرعات كافيه من فيتامين د(25(OH)D) ليعوض المخزون بعد علاج الغدة الجار دراقية أو التحكم في مستوى PTH بواسطة one alpha .