An-Najah National University

Faculty of Graduate Studies

ASSOCIATION BETWEEN VITAMIN D-BINDING PROTEIN AND VITAMIN D3 LEVEL IN HEMODIALYSIS PATIENTS

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Dedication

I proudly dedicate this work to:

My greatest Parents Mr. & Mrs. Hamadneh

My wonderful Brother & Sisters

My lovely friends

Everyone who taught me even a letter from my childhood until now

Everyone who supported me in my academic and practical career

I appreciate your support for developing me to be where I am now

I really respect you

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Declaration

I, the undersigned, declare that I submitted the thesis entitled:

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I declare that the work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

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Signature: Ayat Taysir

Date: 7/3/2024

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Abstract

Vitamin D deficiency is a common problem among patients with end-stage renal failure who undergo dialysis, which requires extensive research and interventions due to its widespread repercussions in the body.

Understanding the physiology of vitamin D, as well as identifying factors contributing to its deficiency, has been the focus of research. In addition, vitamin D binding protein (VDBP) and its potential role in influencing vitamin D levels have been explored, with the hope of finding therapeutic interventions.

For the time being, there is a limited research about the relationship between VDBP and other vital signs, especially among dialysis patients in Palestine. This study aimed to treat this gap by investigating the relationship between VDBP and vitamin D levels in dialysis patients.

A prospective study, conducted among hemodialysis patients at Al-Najah hospital, Nablus, Palestine, over 10 months. The patients were treated with different doses of Alfacalcidol and Cholecalciferol for a total of 17 weeks. VDBP measured by specific sandwich utilizing enzyme-linked immunosorbent assay (ELIZA) technique by R&D used polyclonal rabbit anti-VDBP antibodies, by ELIZA measured also is vitamin D defined before and after course of tretment both of them ,technique.

The results indicated that after a course of vitamin D treatment, VDBP levels decreased significantly while vitamin D levels increased significantly, with no correlation between them. These results were consistent with previous research that showed no significant relationship between VDBP and vitamin D levels in different population groups.

Demographic variables such as age and gender did not show a conclusive association with VDBP levels among dialysis patients, which is in contrast to results from other studies. In addition, there was no significant relationship between VDBP levels and the results of other laboratory tests such as albumin, calcium, phosphorus, and parathyroid hormone (PTH).

Overall, this study underscores the importance of vitamin D treatment in dialysis patients and highlights the need for further research to fully understand the role of VDBP in vitamin D metabolism and its implications for clinical management.

Keywords: Vitamin D; Vitamin D binding protein; End stage renal disease; Hemodialysis patients.

Chapter One
Introduction

1.1 Background

Vitamin D is a member of fat soluble vitamins group that can be generated from plant sources which are called Ergocalciferol (vitamin D2) and animal sources named Cholecalciferol (vitamin D3), both are inactive [1].

It is not a single substance, but rather a collection of approximately 50 metabolites generated from cholesterol through a complicated cascade of enzymatic and non-enzymatic processes [2].

Chemically, they are secosteroids, defined by a broken bond in one of the steroid rings. Individual metabolites vary significantly in their plasma concentrations and biological activity [2].

End-stage renal disease is one of the common diseases with high rates of morbidity and mortality, it is the advanced stage of chronic kidney disease that develops as a gradual decline of kidney function, then, progresses to terminal disease [3].

Among the numerous factors implicated, vitamin D binding protein (VDBP) has emerged as a focal point of scientific studies because of its critical role in vitamin D metabolism and immune modulation [4].

The association between VDBP and CKD demonstrates a complicated web of molecular and biochemical features that hold considerable promise for enhancing diagnostic knowledge and CKD treatment options [4].

Patients with CKD stages 3 and 4 frequently have a vitamin D deficit, which is a sign of a bad prognosis. Similarly, 60–80% of pre-dialysis children with CKD have low levels of 25 (OH) D [5].

Referring to the recommendations of the Kidney Disease Improving Global Outcomes (KDIGO) foundation, the biomarkers that can be used to diagnose chronic kidney disease (CKD) are level of the protein in the urine and glomerular filtration rate (GFR) [6].

Chronic kidney disease is known as the existence of both criteria, filtration rate in the glomerulus [GFR] lower than 60 mL per min which is calculated by Creatinine Clearance (Cockcroft-Gault Equation [7] and albumin higher than 30 mg per one gram of creatinine along with abnormalities in the structure or function of kidney for more than three consecutive months.

A GFR lower than 15 mL/min indicates end-stage renal disease [3].
Chronic kidney disease may present with no symptoms at the beginning due to the existence of early homeostatic mechanisms, the progressive nephrons destruction is compensated by the residual normal nephrons which develop hyper-filtration and reimburse hypertrophy, that is how the kidney maintains the GFR which is also, discovered to be a critical point in developing renal dysfunction [8].

This is a reason that mild renal impairment patients may show normal creatinine values at the initial period, and the disease can be undiscovered for some time [8].

The etiology of kidney disease can be cardiovascular causes such as hypertension, hypotension and vascular disease, from renal origin like glomerular disease (primary or secondary), cystic kidney diseases, tubule-interstitial disease, recurrent kidney stone disease and unrecovered acute kidney injury.

Others such urinary tract infections, obstruction or dysfunction, congenital (birth) defects of the kidney or bladder and certain medications including anti-retroviral drugs, non-steroidal anti-inflammatory drugs (NSAIDs) and inhibitors of calcineurin [9].

The clinical history of patients with end stage renal disease (ESRD) relates to the cause of the disease.

1.2 ESRD and Vitamin D deficiency

ESRD like other diseases has many critical complications which should be monitored and prevented as possible, some of these are related to vitamin D deficiency such as mineral and bone disorders (consequence to vitamin D deficiency and hyperparathyroidism) and hyperphosphatemia [10].

Passing to the complication of kidney disease and its consequences that include disruptions in mineral and vitamin D metabolism developed in bone abnormalities, and

secondary hyperparathyroidism prognoses to parathyroid nodular gland hyperplasia due to constant over-stimulation [10].

Parathyroid gland consequently, becomes with lower sensitivity to vitamin D and calcium signals due to the loss of interactive receptors, in severe cases resistant to medical treatment leads to para-thyroidectomy [10].

The clinical syndrome called CKD-mineral bone disorder (CKD-MBD), this syndrome increases the risk of vascular calcification due to high serum levels of phosphate leading to disposition of calcium phosphate salts on vascular walls, thus leads to cardiovascular diseases [11]

Calcium is an extracellular electrolyte and regulation of blood calcium is subjected to typical endocrine feedback, in hypercalcemic conditions, calcium in its role binds and triggers calcium-sensing receptors (CaSR) exist on parathyroid cells in the extracellular fluid (ECF) causing increase in calcium intracellularly, that in turn making reduction in release of parathyroid hormone (PTH) [12].

The contradictory sequence of occurrences happens in hypocalcemia, which lowers calcium inside the cells and increases the production of PTH and its secretion.

PTH and its role:

- Cause rapid increases in renal reabsorption of calcium and, through hours to days, ameliorates osteoclast resorption of bone and releases calcium and phosphate from the skeleton,
- It also, stimulates the liberation of fibroblast growth factor 23 (FGF23) from mature osteoblasts and osteocytes.
- Enhances the conversion of D (25[OH]D) to 1,25(OH)2D inside the kidney, which in turn will increase intestinal absorption of calcium. Continuing vulnerability to elevated PTH due to prolonged hypocalcemia may also result in release of phosphorus and calcium from bone.

Fibroblast growth factors (FGF) are polypeptide growth factors with diverse biological activities [13], FGF23, is a bone-derived hormone secreted mainly by osteocytes and

osteoblasts in bone, increase or decrease of its level leads to various hereditary diseases [14].

regulates vitamin D metabolism in addition to acting on phosphaturia, FGF23 primarily targets the kidney, suppressing transcription of 1α-hydroxylase, which activates vitamin D hormone (1,25(OH)2D), and activating transcription of 24-hydroxylase, which degrades vitamin D in the proximal renal tubules [14].

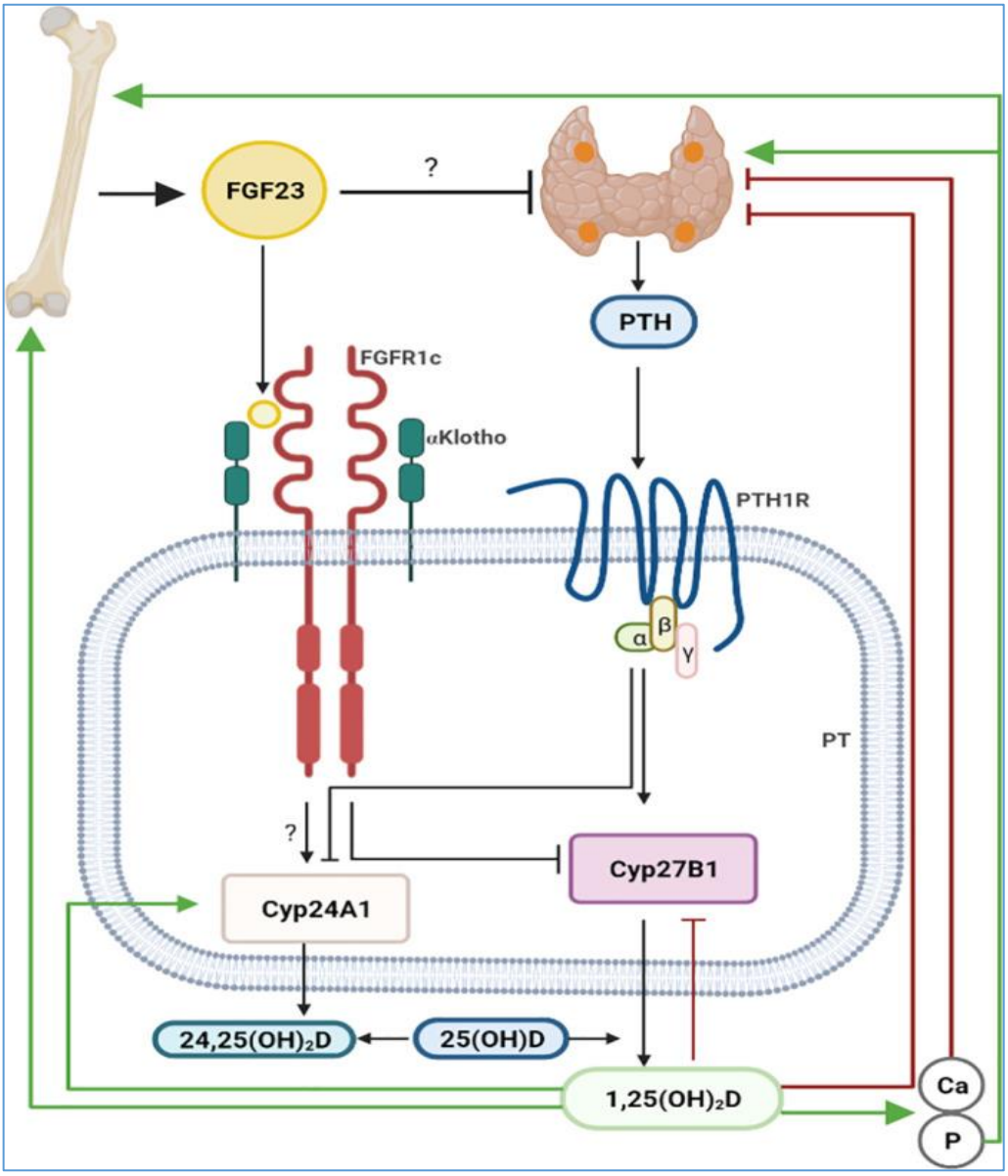
The circulating concentration of 1,25(OH)2D acts as a positive regulator of FGF23 secretion in bone, resulting in a feedback loop between the kidney and the bone [14].

FGF23 has a crucial role in regulating vitamin D metabolism as in its absence, renal 1α-hydroxylase overproduces 1,25(OH)2D in humans [14].

Determination of FGF23 in human plasma or serum can be done using of enzyme-linked immunosorbent assay (ELISA) commercially available kits [13].

Figure 1

Schematic representation of FGF23 and vitamin D metabolism in the kidney



Sources: Latic, N. and R.G. Erben, *FGF23 and Vitamin D Metabolism.* JBMR Plus, 2021. 5(12): p. e10558.

By the mentioned effects, normal ECF calcium can be restored and cause inhibition in PTH production and 1,25(OH)2D level.

Understanding the status of vitamin D in ESRD patients, vitamin D in low levels were found in several kinds of research that were conducted to study that topic in these patients.

it's worth discussing some determined reasons for the deficiency such as hyperpigmentation of skin due to melanin accumulation, which impairs the cutaneous

synthesis of vitamin d despite sunlight exposure, loss of appetite due to uremia [15], and impairment of the final step of active vitamin D production.

In addition, the dominant cause for deficiency of 1,25-dihydroxyvitamin D3 is weakened uptake of 25-hydroxyvitamin D3 by injured kidneys nephrons [16].

ESRD is mostly reached after CKD stages which causes deficiencies in both inactive vitamin D as well as active vitamin D for several reasons.

In addition to the impaired synthesis in skin and advised food restrictions limiting the supply of cholecalciferol and ergocalciferol which considered the vitamin D prototypes, the chronic disease of kidney leads to an impaired biosynthesis pathway of vitamin d by suppressing the activity of 1α-hydroxylase enzyme (CYP27B1), that catalyzes the triggering process of 25-hydroxyvitamin D [17].

Furthermore, losing of vitamin D binding proteins and 1,25-dihydroxyvitamin D caused by proteinuria and uremia, respectively, that are manifestations associated to CKD [18].

Additionally, recent research indicates that both age and metabolism might influence CYP27A activity as aged humans have lower expression of CYP2R1 than their younger counterparts, making them less susceptible to vitamin D supplements. Because vitamin D can be sequestered in adipose tissue, it is not surprising that nutrition and body composition influence CYP2R activity [2].

1.3 Production and metabolism of vitamin D and its action

D2 is formed by ergosterol exposure to Ultraviolet B (UVB) radiation. Ergosterol is found in plants, yeast, and many fungus types like mushroom[19].

Its chemical formula has a double bond connecting between C22-C23 and at C24 [19].

In side chain there is a methyl group in which make D2 different from D3 and lower its affinity for VDBP, that cause fast clearance of circulation D2, also, alter its transformation to 25-hydroxyvitamin D, and affect its catabolism by the 24-hydroxylase (CYP24A1) [19].

Both 25(OH)D3 and also 1,25 (OH) 2D3 levels are metabolized via 24-hydroxylase metabolizing enzyme CYP24A1 which appears to be the primary inactivating enzyme

for vitamin D and doing a major activity in controlling their levels in the body by catabolizing them to 24,25 di-hydroxyvitamin D3 or 1,24,25 tri-hydroxyvitamin D3, letting for additional catabolism toward calcitonic acid, then, eventual elimination by the urine [20, 21].

7-dehydrocholesterol is a precursor has cholesterol like being, present in the epidermal skin cells. It can be remodeled to pre-vitamin D after being exposed to UVB radiation (spectrum 280–320 nm) in sunlight [22].

The resultant pro-vitamin D undergoes 25-hydroxylation in the liver to 25(OH)D called (Calcidiol), via the action of a diversity of enzymes have 25-hydroxylase role which the dominant one is CYP2R1[22].

Calcidiol is considered the significant metabolite of vitamin D in the circulation and is currently utilized as a vitamin D status indicative, it has a half-life of 2 to 3 weeks [1].

The clinical recommendations continuously recommend measuring 25(OH)D to determine vitamin D status [23].

However, 1,25(OH)2D is indicated solely in a very few clinical conditions, such as severe chronic kidney disease, hereditary phosphate-losing disorders, oncogenic osteomalacia, pseudovitamin D-deficiency rickets, vitamin D-resistant rickets, chronic granuloma-forming disorders such as sarcoidosis, and some lymphomas [23].

Calcidiol is transformed by a specific enzyme to its greatest dynamic form, 1,25(OH)2D (calcitriol) by renal 1-alpha-hydroxylation via the mitochondrial enzyme CYP27B1[22], the half-life of calcitriol is 4 to 6 hours [1].

The step of calcidiol conversion to calcitriol occurs at most in the kidney nephrons, but also, such step can happen in other extra-renal tissues [24, 25].

Bone cells, melanocyte, epidermal keratinocyte, parathyroid glands, testes, placenta, decidua and macrophages[24, 25] have been found by a recent studies to have active 1α-hydroxylase due to the presence of extra renal CYP27B1 [20, 26],

These tissues appear to possess significant paracrine and also autocrine effects, which considered especially important in cases of impacted lowered renal mass and patients with injured production 1,25(OH)2D in the kidney [25].

In addition to CYP27B1, the presence of CYP24A1 in the extra-renal calciotropic tissues which maintain calcium homeostasis as parathyroid gland increase the ability for regional tissue specific vitamin dactivation, activity and also metabolism [20].

Vitamin D metabolized to several metabolites, the one with higher levels is the circulating 25(OH)D than those of the others vitamin D metabolite in the blood, and major concentration of the 25(OH)D in human body is exist in the blood [27].

However, the most physiologically functioning type of vitamin D is 1,25(OH)2D3, or calcitriol, other vitamin D metabolites are known to linked with a number of biological activities [20].

Vitamin D metabolism is regulated by parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23) [28, 29].

PTH is secreted from parathyroid glands in response to low serum calcium levels; it promotes bone turnover and increases 1,25(OH)2D levels by inducing renal expression of the implicated cytochrome (CYP27B1) [28, 29].

FGF23, is on the other hand, produced via osteoblasts and osteoclasts as a response to high phosphate and calcitriol blood levels and inhibits calcitriol production by acting on proximal renal tubules in the kidney by inhibiting CYP27B1 and initiating transcription of 24-hydroxylase (CYP24A1), the main enzyme responsible for vitamin D destruction[28, 29].

Vitamin D status in the body can be indicated by measuring of the primary biomarker, serum total 25-OH D levels since it represents both outputs from dietary sources and cutaneous biosynthesis by ultraviolet radiation [19].

Moreover, the liver hydroxylation is neither regulated nor rate limited [19].
In addition to the advantage of long lasting in the circulation and its measurement is more accurate and reliable, compared to the active form 1,25(OH)2D along with that it is not being under tight homeostatic control, > 75 nmol/L (30 ng/mL) can be considered a normal result [19].

Table 1

Vitamin d and its metabolites

Names of vitamin D Metabolite	Other names	Synthesis and Location	Clinical effect	Measurement manner
Vitamin D2	Ergocalciferol, Calciferol, Viosterol	Synthesized via yeast and fungi in the existence of UV light	Gives information about vitamin D2 ingestion	Measured in research studies not in routine basis via Liquid Chromatography with tandem mass spectrometry (LC-MS-MS) and radioimmunoassay (RIA)
25-Hydroxy-vitamin D2 [25(OH)D2]	25-Hydroxy-ergocalciferol Ercalcidiol, 25-Hydroxy-ergocalciferol 25-Hydroxy-calciferol	Produced inside the liver, major circulating form of vitamin D2	Usefully measured in combination with D3	Selective quantitation can be by HPLC and LC-MS-MS
Total 25-Hydroxy-vitamin D [total 25(OH)D]		Sum of 25(OH)D2 and 25(OH)D3.	The best indicator of vitamin D stores.	Immunoassays can quantitate total 25(OH)D but cannot differentiate D2 from D3
1α,25-Dihydroxy-vitamin D2 [1α,25(OH)2D2]	1-Alpha, 25-Dihydroxy-ergocalciferol	Produced in the kidney as active metabolite and in extra-renal tissues by endocrine, autocrine and paracrine actions		
24R,25-Dihydroxy-vitamin D2 [24R,25(OH)2D2]	24,25-dihydroxy-ergocalciferol	Produced in the kidney and can be subjected to 1α-hydroxylase		
Vitamin D3	Cholecalciferol, Calciol	Made from dietetic sources or produced into the skin when exposed to ultraviolet light	Gives findings about synthesized and/or ingested vitamin D3	Measured for purposes of research studies not in routine basis by LC-MS-MS and RIA
25-Hydroxy-vitamin D3 [25(OH)D3]	25-Hydroxy-cholecalciferol Calcidiol Calcifediol	Produced inside the liver, major circulating form of vitamin D3	Usefully measured in combination with D2	Selective quantitation can be by HPLC and LC-MS-MS
1α,25-Dihydroxy-vitamin D3 [1α,25(OH)2D3]	Calcitriol, 1-Alpha,25-Dihydroxy-vitamin D3, 1-Alpha, 25-Dihydroxy-cholecalciferol, Dihydroxy-vitamin D3	Produced in the kidney as active metabolite and in extra-renal tissues by endocrine, autocrine and paracrine actions	Measurement is indicated in: -calcium disorders -calcipenic rickets/osteomalacia -distinguish FGF23 from non-FGF23 phosphopenic rickets	Decreased in kidney disease and measured by LC-MS-MS and immunoassays
24R,25-Dihydroxy-vitamin D3 [24R,25(OH)2D3]	24,25-dihydroxy-vitamin D3, Secalciferol, 24,25-dihydroxy-cholecalciferol	Produced in the kidney and can be subjected to 1α-hydroxylase	Measured to examine the 24-Hydroxylase inactivating mutations	Decreased in kidney disease and measured by LC-MS-MS and immunoassays
C3-Epimers of vitamin D metabolites	3-Epi-25-Hydroxy-vitamin D3, 25-Hydroxy-3-epi-vitamin D3, [C3-epi-25(OH)D3]	C3-epi-25(OH)D3 is the most prevalent epimer of 25(OH)D	Present in definite quantities in neonates and should be considered in pediatric samples, may also found in adults but quantification is not recommended in adults, it may interfere with measurement of 25(OH)D	Can be separated and quantified only by LC-MS-MS

Sources: Makris, K., et al., Recommendations on the measurement and the clinical use of vitamin D metabolites and vitamin D binding protein - A position paper from the IFCC Committee on bone metabolism. Clin Chim Acta, 2021. 517: p. 171-197.

In some kidney's pathological situations that cause proteinuria, measurement of free 25(OH)D is favored than total 25(OH)D levels in diagnosing Vitamin D deficiency and determining the treatment [31].

Most of 1,25(OH)2D effects are carried via vitamin D receptor (VDR) which consider a transcription factor found in most cells [26].

It collaborates with other various transcription factors like retinoid X receptor that once attached to 1,25(OH)2D, it controls the process of gene transcription for either positive or negative effects according to the other cofactors which it interacts or binds [26].

Vitamin D exerts several important physiological and biological activities, besides its role in regulating calcium and phosphate homeostasis which is previously known as the sole role of vitamin D [32].

It has important immunomodulatory effects [32], endocrinal regulatory effects on the Renin–Angiotensin–Aldosterone System [33], and erythropoiesis [31].

Also, it reduces the occurrence of many conditions including many cardiovascular events as hypertension[34], left ventricular hypertrophy [35], vascular dysfunction by regulating nitric oxide bioavailability and endothelial function [36], arterial stiffening [34], worsened metrics of diabetes [16], and hyperlipidemia [37].

Moreover, it decrease bone diseases [38], psychiatric ailments [39] and all-cause mortality based on more recent reports [16, 31].

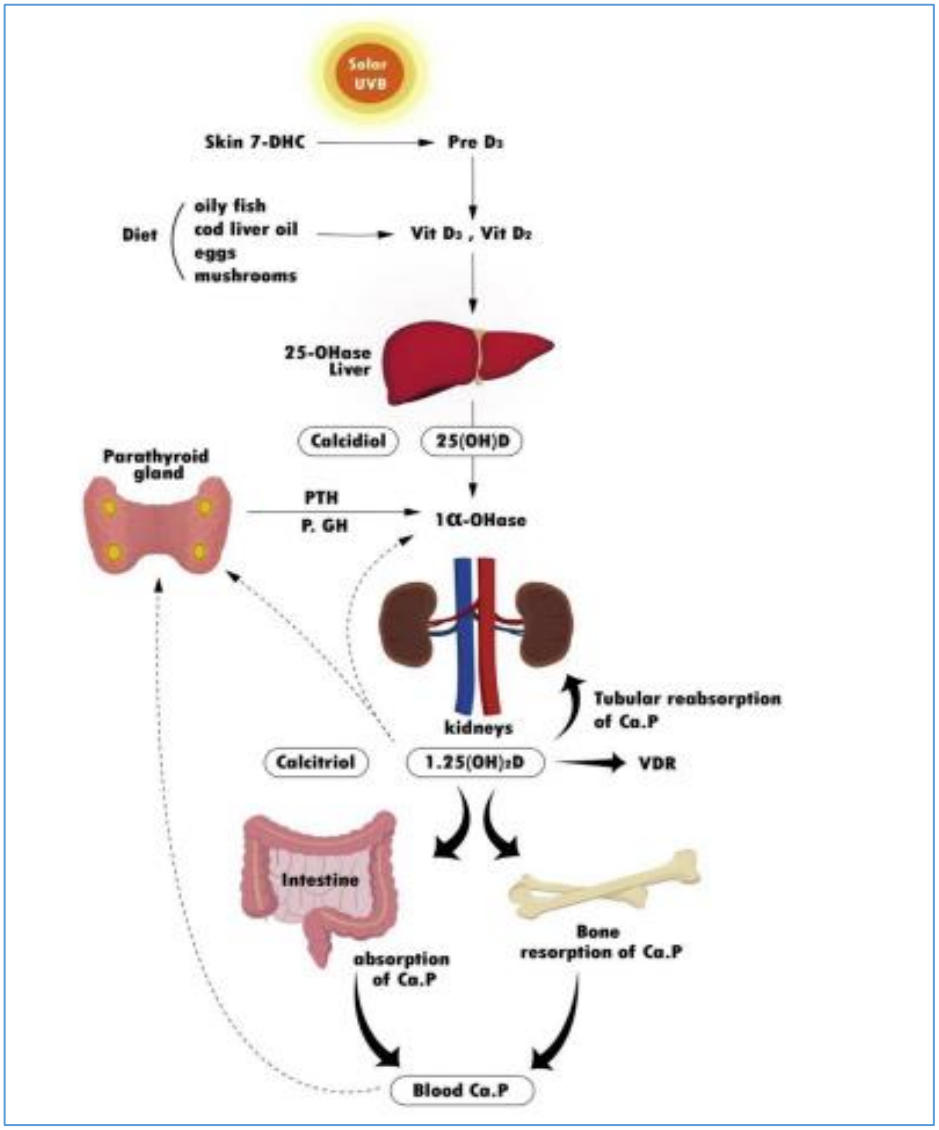
Vitamin D [25(OH)D] is considered a steroidal hormone having broad regulatory actions and binds to the receptor intracellularly. It controls the expression of a wide variety of genes elaborated in the differentiation of many cell lines, its activation, and proliferation [40].

The activated vitamin D can modulate the differentiation of Th17 cells, inhibit the expression of IL-2 and INF-, and contribute to the balance of Th17/T regulatory cells [41].

Its deficiency has been attributed in epidemiological studies to a number of illnesses, including autoimmune disorders, cancer and cardiovascular diseases [41].

Figure 2

The metabolism and bioactivity of Vitamin D. Flow diagram of vitamin D's metabolism



Solid arrows demonstrate the direct effects of its products and dotted lines indicate the negative-feedback of plasma calcium or 1,25(OH)2D (Ca: calcium; 7-DHC: 7-dehydrocholesterol; GH: growth hormone; 1aOHase: 1-alpha-hydroxylase; 25-OHase: 25-hydroxylase; P: phosphate, PTH: parathyroid hormone; VDR: vitamin D receptor; Vit: vitamin) [1].

1.4 Vitamin D Binding Protein

Vitamin D and every one of its metabolites are attached to a definite vitamin D binding protein (VDBP) [42].

The mentioned protein was realized by its worldwide polymorphism and known as Group-specific Component (GC), it is from genetic aspect considered the albuminoid family's eldest member which include α-fetoprotein, albumin and afamin, that each of them is involved in transport of hormones and fatty acids [42].

The gene for DBP/GC is found on human chromosome 4q11-q13 [42]. It is expressed in various type of tissues in the body, its expressed in large concentration in the liver as this organ is the producer for Vitamin D binding protein and the other proteins of the same family, unlike other tissues which express the gene in low concentration [43].

VDBP is widely recognized for its single nucleotide polymorphisms (SNP), the most prevalent of which are rs7041 and rs4588, both found in exon 11 of the VDBP gene. SNPs are the most common genetic variable in genomes [40].

SNPs are the most common genetic variable in genomes. SNPs may affect protein stability, folding, flexibility, and aggregation; functional sites, reaction kinetics, and dependence on environmental parameters like pH, salt concentration, and temperature; protein expression and subcellular localization; and protein-small molecule, protein-protein, protein-DNA, and protein-membrane interactions [40].

Many studies have found connections between SNPs and protein concentrations, as well as substance protein movement via VDBP in this particular example, which affect the response of people for vitamin d supplementation [44].

VDBP is found also in lower concentrations in the majority of body fluids (serum, urine, saliva, cerebral fluid, breast milk, seminal liquid, or ascites liquid) [43].

Normal VDBP serum concentrations range between 350 and 500 g/L and vary throughout the day (lower in the morning and higher later in the day) [43].

VDBP regulation is not enhanced by vitamin D itself, it is promoted by estrogen hormone, glucocorticoid hormones and by the effects of the inflammatory cytokines [45].

The quantity of VDBP produced every day can be estimated about 700–900 mg/d for an adult person (10 mg/kg/d)[46], it has a short half-life in plasma that is 2.5 days [47]

25(OH)D and its activated form 1,25(OH)2D are circulating as bound form, the majority are bound to vitamin D-binding protein (VDBP), a small amount is bound to albumin, or, as free unbound form [26].

The binding of vitamin D-binding protein has a 1000-fold apparent affinity for vitamin D metabolites as albumin, and consequently binds as much as 90 of vitamin D metabolites in plasma [30, 48].

Vitamin D also found to bind chylomicrons which is lipoprotein particles through the initial inflow of vitamin D generated by dietary and oral supplementation routes, but with lower levels and affinity [49].

A different theory holds that a major function of vitamin D-binding protein is binding, solubilization and transports vitamin D metabolites to target cells, such as those in the renal proximal tubule, and enhances their endocytosis via contact with megalin, which is a multifunctional receptor [43, 48].

Almost five percent of total plasma VDBP is combined to vitamin D while the lasting twenty five percent found in different organs such as the vital organs kidneys, heart, brain and lungs [45].

In addition to the spleen, genital organ tests, and in uterus [45] and serves many other physiological roles including bone development regulation, actin sequestration, binding of fatty acids, immune modulation and inflammatory responses [47, 50].

Actin scavenging is a critical action of VDBP that has far less attention than binding to vitamin D metabolites [45].

Damaged cells released large amounts of actin after trauma [51], liver trauma [45, 52], sepsis [53], burn injuries[54], acute lung injury [55], surgeries [56]

and preeclampsia [57] and form polymerized filamentous F-actin that, when combined with coagulation activated factor V (Va), can cause disseminated intravascular coagulation and multi-organ failure unless dealt with [22].

Gelsolin and VDBP make up the system that scavenges actin. F actin is depolymerized by gelsolin to become G (globular) actin. Due to VDBP's strong affinity for G-actin, it is prevented from re-polymerizing and removed from the circulation [55].

In addition to albumin as the main fatty acid transporter, VDBP can function as a contributing fatty acid transporter by binding primarily monounsaturated, saturated, and a small amount of polyunsaturated fatty acids [58].

Poly- and monounsaturated fatty acids reduce the affinity of both 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D for VDBP, while saturated fatty acids do not have that effect [58].

These fatty acids cause VDBP to undergo unique structural modifications, which may account for the differing competition strengths between 25-hydroxyvitamin D and VDBP [22].

Since fatty acids have low solubility in aqueous solutions like interstitial fluid and the plasma of blood, binding proteins are needed in order to raise their levels in extracellular fluid, and vascular and interstitial components [22].

Familiar genetically polymorphisms create three major genetic variants of the VDBP (Gc1F, Gc2, and Gc1S), that each of them has different affinity for 25(OH)D [59].

VDBP is a protein with highly polymorphic property that can result with at least 120 isoforms separated by electrophoresis [22].

Based on previous reports, these isoforms of VDBP differ in the affinity for 25(OH)D, other reports resulting that in some clinical condition including patients with liver disease and in pregnancy, there is a change in that affinity [60].

The binding for all of the metabolites of vitamin D occurs on an exclusive binding site on VDBP with a higher affinity for 25OHD and 1,25(OH)2D, by that, circulating

25OHD create a large pool, which prevents developing accelerated vitamin D deficiency [42].

In comparison, the half-lives of VDBP and 25OHD are 1.7 days and about 15 days, respectively, which mean that it is markedly shorter for VDBP than 25OHD [46].

The elimination site of DBP is not fully discovered, it appears that it is cleared partially by filtration in the glomerulus, followed by reabsorption in the tubules by a carrier cargo receptor mechanism called megalin, then, intracellular degradation [46].

Compared to the complex of the binding protein of vitamin D with 25-OH vitamin D that it is undergoing filtration in the glomerulus, then, the cargo receptor megalin at the brush border of tubular epithelial cells is together with cubilin are necessary to reabsorb VDBP or the VDBP-25OHD complex, herewith, preventing the urinary loss of 25OHDand its binding protein [42].

VDBP-metabolite complex can enter other cells than the kidney as the gland of parathyroid by a megalin/cubilin mechanism [42].

Electrophoresis is a laboratory method designed to be used for separation of DNA, RNA, or protein molecules according to their size and electrical charge [61].

Serum proteins are mainly distinguished by their electrophoretic mobility and vary accordingly as α, β, or γ globulins, VDBP protein discovered as α-globulin that is the major carrier protein in human and mammals in general [30, 42, 49].

The reality that the GC protein was analogues to VDBP was verified by describing the purification of VDBP from human serum, monitored by prior addition of [3H]25OHD [42].

The products of the mentioned protein are primarily produced and therefore secreted by hepatocytes. The VDBP gene is also found in kidney and testis, moreover, it is expressed in endocrine pancreatic cells, and fat cells [42].

Renal impairment exposes the patient to be at high risk of Vitamin D deficiency caused by several reasons:

- Alterations in vitamin d sources such as the limitations of nutrients containing Vitamin D consumption to avoid the resultant imbalance in phosphorus absorption and the interrupted sunlight exposure [22].
- Loss of its significant carrier, the vitamin D-binding protein due to proteinuria since the megalin/cubulin system's maximum transport capacity is reached [22],
- The impaired function of the cubilin–megalin–amnionless receptor complex in the renal proximal tubule [31]
- Loss of activity of 1-a-hydroxylase in injured epithelial cells of tubules [47].

VDBP in serum and urine in CKD and hemodialysis (HD) patients can be measured using enzyme immunoassay, an ELIZA technique by R&D used polyclonal antibodies to measure serum VDBP concentration [30, 46].

1.5 Megalin and Cubilin

Megalin acts as an endocytic receptor found in abundance in epithelial cells of renal proximal tubules, microvilli, glomerular podocytes, dense apical tubules and endocytic vessels as well as other calciotropic extrarenal cells that express the enzymes metabolizing vitamin D, such as bone tissue and parathyroid cells [62, 63].

The receptor participates in the absorption of vitamin D-binding protein (DBP) bound to 25(OH)D3, allowing intracellular conversion of 25(OH)D3 precursor to active 1,25(OH)2D3 [62, 63].

The importance of renal reabsorption of 25(OH)D3 and 1,25(OH)2D3 mediated via megalin has been clearly demonstrated by experiments, and it is shown that extrarenal megalin has important roles in modulating vitamin D homeostasis in fatty tissues, bones, muscles, colon, mammary cells, placenta and mesenchymal stem cells [62].

Megalin of parathyroid gland might regulate calcium signaling, raising the prospect of megalin-mediated communication between calcium as well as vitamin D regulation through the parathyroid [20].

Megalin expression seems to be downregulated among various chronic kidney disease (CKD) [20].

The prevalent expression of megalin corresponds to its supposed role as a multi-ligand scavenging receptor, as many physiologically substrates have been identified as megalin ligands, involving hemoglobin, albumin, retinol-binding protein, sterols, insulin, and most importantly for this review, vitamin D-binding protein (VDBP) [64].

Human megalin extracellular domain has ligand-binding properties [64].

Cubilin is DBP-binding endocytic receptor present in the proximal tubule, it collaborates and interacts with megalin. Cubilin and megalin co-express and localize in the endocytic apparatus of the absorptive epithelium in the colon, kidney, yolk sac, and gallbladder, among other tissues [64].

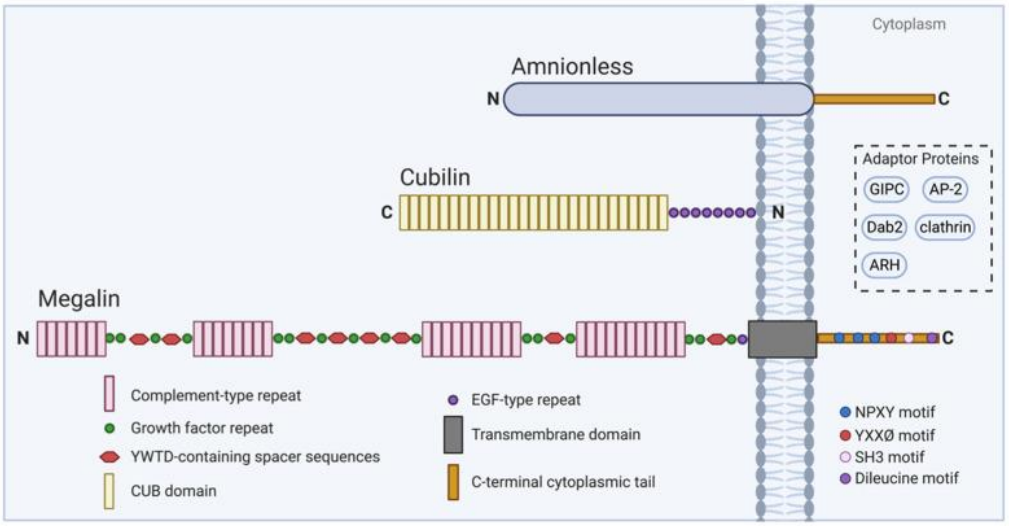
Cubilin's structure shows that it has an essential functional interaction with megalin, as it lacks a transmembrane domain and a cytoplasmic tail but is attached to the membrane by a complex with the proteins found in membrane called amnionless (AMN) and with megalin [63, 65] as described in figure 1 below.

In-vitro and in-vivo investigations have revealed that cubilin binding to megalin is Ca^{2+}-dependent, with coupling significantly decrease the existence of EDTA which is Ca^{2+}-chelating agent [63, 66].

Functional cubilin was precipitated by specific antibody from renal brush border membranes in complex with AMN and megalin, and suppressing of either megalin or AMN resulted in an 85-90% decline in cubilin expression and a 2-fold reduce in its half-life, indicating that cubilin interaction with both megalin and AMN is required for intracellular stability [63, 66].

Figure 3

Megalin and cubilin structure and related molecules are shown schematically



Sources: Khan, S.S., et al., Megalin and Vitamin D Metabolism-Implications in Non-Renal Tissues and Kidney Disease. Nutrients, 2022. 14(18).

Megalin mutant mice exhibit lower cubilin existence and substance incorporation, and antibodies targeting megalin inhibit cubilin attachment with the membrane and enhance degradation by 50-60%, suggesting that megalin and cubilin have a functional interaction [20, 67].

According to a case study about patient had cubilin deficiency, cubilin malfunction does not affect endocytosis mediated by megalin but rather exacerbates the loss of common ligands such as VDBP [20, 67].

As concluded by previous studies, endocytosis was needed to maintain systemic VDBP concentrations since urine VDBP was found in megalin-knockout animals and not in controls [68].

Any binding or absorption of endogenous VDBP in the kidney collapsed in the absence of renal megalin, highlighting megalin as the major renal VDBP receptor. VDBP-bound 25(OH)D3, megalin, and potentially play important and linked roles [68].

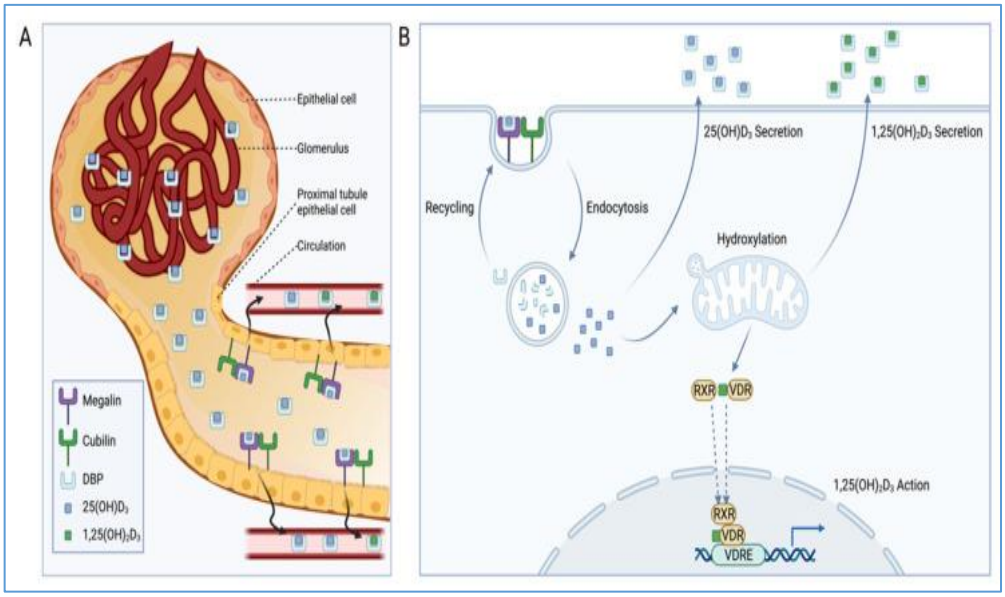
TGF-β and angiotensin II, key mediators in glomerulosclerosis and interstitial fibrosis, have been shown to negatively impact receptor-mediated endocytosis, leading to higher urinary VDBP excretion rates [69].

The loss of macroprotein receptors, as well as the negative control of their endocytosis, reduces urinary VDBP reabsorption from the renal tubules, increasing excretion [69].

Furthermore, endocytosis process was necessary for regulating systemic 25(OH)D3 concentrations since plasma 25(OH)D3 levels were lowered by 80% and associated with by severe bone disease [68] as displayed in figure 3.

Figure 4

Megalin and cubilin play role in renal vitamin D homeostasis



Sources: Khan, S.S., et al., Megalin and Vitamin D Metabolism-Implications in Non-Renal Tissues and Kidney Disease. Nutrients, 2022. 14(18).

(A) Through a megalin and cubilin-mediated mechanism, epithelial cells in proximal tubular absorb VDBP-bound vitamin D metabolites from the glomerular ultrafiltrate.

(B) DBP is degraded and reclaimed after endocytosis within cells expressing megalin, 25-hydroxyvitamin D3 (25(OH)D3) can subjected to 1-hydroxylation to the active 1,25(OH)2D3 for following vitamin D receptor (VDR) agonism via binding retinoid X receptor and VDR response elements. To maintain circulation vitamin D homeostasis, endocytosed 25(OH)D3 or generated 1,25(OH)2D3 can be secreted.

1.6 Problem Statement

Low levels of vitamin D and its related pathogenesis as low bone density and fracture risk become one of the most suffering sources for hemodialysis patients.

The causes of vitamin D deficiency are partially known in these patients and most of them treated with 1,25 (OH)2D tablets.

However, some of chronically HD patients have low levels of vitamin D although they being treated and the causes are not completely clear which is a burden on their physician.

Moreover, a little data related to VDBP levels in HD patients is available in literature review especially in Palestine and surrounding countries to being a clinical reference for physician in kidney department.

1.7 Study significance

Vitamin D level is one of the major challenges that face the kidney specialist physician, how to assess, correct a low level, maintain a normal value, what are the factors that affect vitamin D level in hemodialysis patients and what are the corrective actions in cases of recurrent deficiency of vitamin D in hemodialysis patients.

This correlation is still not studied neither in Palestine nor the surrounding countries. Although, these findings can indicate a corrective action or utilize further studies and researches in order to finds a pharmacological solution for the chronic low level of vitamin D in these patients.

1.8 Study objectives

1.8.1 The general aims

- Evaluate the effects of vitamin d treatment on vitamin d, VDBP, albumin, calcium, phosphorus and parathyroid hormone levels in HD patients

1.8.2 The specific aims

- Find a correlation between vitamin D level and its binding protein level in hemodialysis patients.
- Conclude a corrective action for low level of vitamin D in these patients according to the result of the study.

1.9 Research questions and hypothesis

1.9.1 Research questions

1. What are the results of serum vitamin D among HD patients before and after vitamin d treatment?
2. What are the results of VDBP among HD patient before and after vitamin d treatment?
3. How the results of VDBP affect vitamin D level in HD patients?
4. Are low levels of the VDBP a cause of vitamin D deficiency in the body?
5. Are there treatment interventions can be indicated after the results of the study?
6. Are there significant effects of vitamin d treatment on albumin, calcium, phosphorus and parathyroid hormone levels in HD patients?

1.9.2 Hypothesis

1.9.2.1 Alternative non-directional hypothesis

1. There is a relationship between vitamin d levels and VDBP levels in hemodialysis patients.
2. There is an effect for vitamin d treatment on vitamin d, VDBP, albumin, calcium, phosphorus and parathyroid hormone levels in hemodialysis patients?

1.9.2.2 Null hypothesis

- There is no relationship between vitamin d levels and VDBP levels in hemodialysis patients.
- There are no effects for vitamin d treatment on vitamin d, VDBP, albumin, calcium, phosphorus and parathyroid hormone levels in hemodialysis patients?

1.10 Literature review

1.10.1 Vitamin D deficiency in hemodialysis patients

Vitamin D deficiency has been correlated with an elevated probability of cardiovascular morbidity, immune dysfunction and increased risk of developing immune related disease as rheumatoid arthritis, respiratory infection,COVID-19 and sepsis [1, 70] as well as poor survival in CKD patients and previous systematic research found that vitamin D supplementation enhance clinical and biochemical endpoints [71].

Chronic kidney disease – mineral and bone disorder (CKD-MBD) is a systemic condition of mineral metabolism caused by phosphorus retention and high levels of FGF23 and PTH, with a negative impact on skeletal integrity [72].

Alteration in the main CKD-MBD biomarkers (calcium, phosphorus, vitamin D, and PTH) are related with abnormalities in bone turnover, mineralization, as well as volume, extra-skeletal calcifications, in addition to atherosclerosis [72].

Secondary hyperparathyroidism accompanied by low vitamin D levels has been linked to an increase of all-cause and cardiovascular (CVD) mortality in a several pathways [73, 74].

Furthermore, it triggers the Renin Angiotensin System (RAS) system activation, exacerbate hypertension, and complicates anemia by aggravating calcium and phosphorus metabolism abnormalities and hence vascular calcification [73, 74].

1.10.2 Vitamin D supplementation in hemodialysis patients

Several studies have found that dialysis patients may require greater doses of vitamin D than the general population to maintain acceptable serum levels of 25(OH)D [75-77].

Dosages exceeding 100,000 IU indicated to ensure adequate replacement, also, to increase 1,25(OH)2D, and decrease PTH [75-77].

The administration of a high dose of vitamin D3 to patients on dialysis over a lengthy period of time (9 months) was proven to be safe without causing any evident toxicity [76, 77].

It supports previous studies' results, especially those concerning calcium and phosphorus levels stability within normal ranges. During the studies period, the authors did not detect any episodes of hypercalcemia [76, 77].

Several previous studies report that cholecalciferol supplementation in dialysis patients increase both levels of 25(OH)D and 1,25 (OH)2D, indicating that extra-renal activity has a significant role in hemodialysis patient [21].

However, currently there is no conclusive evidence to prefer one formulation over another of nutritional vitamin D in CKD [60], also, no clear evidence supporting the benefits from combining nutritional vitamin d and activated vitamin d [21].

According to the majority of recently published studies, dietary forms of vitamin D have weak PTH-lowering efficacy, and giving the preference to the activated vitamin d than dietary vitamin D supplementation for hyperparathyroidism treatment, for dialysis patients [78, 79].

A study by Kandula et al , dietary vitamin D raises 25(OH)D levels without affecting calcium or phosphorus levels, but lowers serum PTH levels, particularly in dialysis patients [80].

Jean et al. reported a beneficial impact of pre-dialysis 25(OH)D supplementation in preventing secondary hyperparathyroidism (SHPT) [81]

Proteinuria was reduced in diabetic CKD patients using angiotensin-converting enzyme inhibitors by supplementing with calcidiol, since, it has podocyte preservation and anti-inflammatory effects [82].

In addition, vitamin d regulates the renin–angiotensin–aldosterone system [83].

Many researchers discovered that cholecalciferol increased CYP27B1 and VDR expression in monocytes while decreasing serum IL-6 and C-reactive protein levels [84, 85].

Mann and others found no significant impact of vitamin D supplementation on mortality in a meta-analysis study [86].

Rugang Li and his colleagues showed evidences that vitamin D may decrease all-cause mortality in elderly with CKD via cohort study using a representative sample [87].

They found an L-shaped relation between serum 25(OH)D concentrations and all-cause and CVD mortality, with the threshold at 90 nmol/L of 25(OH)D concentrations which may be a target for therapies to minimize the risk of death [87], due to various plausible mechanisms for the relation between decreased 25(OH)D levels and an increased mortality risk [87-89].

Low vitamin D levels have been related to a several fatal conditions, including hypertension, coronary artery calcification, dyslipidemia, diabetes and thickening of the carotid intima-media [87-89].

1.10.3 VDBP binding of vitamin D

A study conducted by Rene F. Chun described who VDBP do the primary function which is to preserve vitamin D for the organs as it considered an essential element and make it accessible for the usage of tissues [49].

Vitamin D could also access tissues while still linked to VDBP via active-receptor-mediated absorption, allowing vitamin D to influence gene expression [49].

Even though DBP has more efficient high affinity depot for circulating vitamin D metabolites than albumin, but, its tight affinity indicates that the bound vitamin may be less accessible for passage into target tissues until DBP becomes endocytosed and destroyed [48].

While low affinity of vitamin d metabolites for albumin, makes albumin-bound vitamin D to be more diffusible, and consequently more "bioavailable" to the tissues surrounding it [48].

The DBP has a high affinity for vitamin D metabolites and a larger molar concentration than its ligands, as a result, the free concentration of all its metabolites is significantly lower than other nuclear receptor ligands [90].

The free concentrations of 25OHD and 1,25(OH)2D were determined using metabolite concentrations, DBP concentrations, affinity estimation, and the law of mass action [90].

1.10.4 Affinity of DBP for Vitamin D Metabolites

In DBP, all vitamin D metabolites bind to a single site, whereas albumin contains many low affinity binding sites [91].

25OHD binds with strong affinity, however the absolute value varies significantly between species and depends on the buffer media, pH, and temperature [91].

DBP has a significantly higher affinity (approximately 10 times) in barbital buffer at pH 8.6 compared to buffers at pH 7.4. DBP has the highest affinity for 25OHD-lactones, followed by an equal affinity for 25OHD, 24R,25(OH)2D, or 25S,26(OH)2D [91].

The affinity for 1,25(OH)2D is approximately 10-100 lower than that of 25OHD [91].

The lowest affinity is for the vitamin D itself. The structure of the cleft on human DBP aligns with these affinities [22].

Natural side chain variations in vitamin D2 and its metabolites can impact DBP binding. For humans and most mammals, the affinity difference between 25OHD2 and 25OHD3 is only about 20% [46].

Compared to other transport proteins, DBP has a high affinity and concentration, resulting in low free concentrations of 25OHD and 1,25(OH)2D[90].

The concentrations of free 25OHD and 1,25(OH)2D are approximately 10 and 1 pmol/l, respectively [17].

The free 25OHD concentration is less than 0.1% of total 25OHD, while free 1,25(OH)2D is approximately 1% of total concentration [90].

The molar ratio of total 25OHD over total 1,25(OH)2D is around 500 due to affinity differences. However, free 25OHD is only 10 times higher than free 1,25(OH)2D [90].

1.10.5 VDBP physiological functions

Binding of VDBP and vitamin D consequently has many important physiological roles, it maintains plasma vitamin D levels through reabsorption in the kidneys, protects vitamin D from biodegradation leading to enlargement of biological half-life of vitamin D and limiting its access to target tissues [46].

VDBP involved in preserving total and free 25(OH)D concentrations, it limits at least in part some doings of vitamin D, through executing it inaccessible to make effect on its target cells [92],

Based on previous huge researches, only the free 25(OH)D can pass to the cells and do its biological effect according to the free hormone hypothesis [22].

While protein-bound are inactive[93], VDBP acting as a buffer for the rising concentration of free vitamin D metabolites, the substantial molar excess of VDBP may be crucial in the prevention of vitamin D toxicity [94].

In Daniel and Janice study that used mouse of VDBP knocked out and others with megalin knocked out in order to define the physiological role of VDBP, VDBP knocked out mouse provides a clear illustration of the physiologic function of VDBP.

Since the albumin levels are normal, it is likely that all of the vitamin D metabolites are free and/or accessible in these animals [22].

Mice missing VDBP, in contrast to megalin knocked out mice, do not display signs of vitamin D insufficiency unless fed a diet deficient in vitamin D, despite having very low serum levels of 25(OH)D and 1,25(OH)2D and accelerated loss of these metabolites in certain mice [22].

The amazing findings of a study conducted on mice undertaken to show that VDBP is unquestionably necessary for the use of vitamin D produced by UV light in the skin epidermis were published by Elizabeth G. Duchow and her colleagues [95].

Most convincingly, UVB exposure in the lack of VDBP found with no effect on vitamin D deficiency or hypocalcemia. Whereas, UVB treatment for mice with vitamin D

deficiency in the presence of VDBP restored blood calcium and 25(OH)D to normal [95].

The fact that the VDBP knockout mice exhibit normal phenotypes and have measurable 25(OH)D and 1,25(OH)2D levels when fed diets rich in vitamin D suggests that VDBP is not necessary for vitamin D absorption from the gut [95, 96].

In fact, it has been consistently shown that chylomicrons in the gut allow for the absorption and transportation of vitamin D. Also indicating that VDBP is not necessary for their release from the liver or kidney in the presence of 25(OH)D and 1,25(OH2D) [95, 96].

1.10.6 Physiological factors affect VDBP

"Genetically VDBP Variations are formerly known as GC1F, GC1S, and GC2, were initially described over 50 years ago and could be related to changes in DBP affinity for binding or serum concentration [93].

The protein variations are now known to be caused by polymorphisms in the VDBP gene GC [93].

The single-nucleotide polymorphisms rs7041 and rs4588 distinguish the phenotypic differences in the DBP amino acid sequence. Blacks and Asians are more probable to have GC1F DBP, which has the greatest affinity for 25(OH)D and is linked to low DBP levels [93].

Asian population and Blacks are more inclined to have GC1F DBP, which exhibits the greatest affinity for 25(OH)D as well as it is linked to low DBP levels. while whites are more likelihood to have GC1S DBP [97, 98].

GC2, which has a reduced affinity for 25(OH)D and is linked to increased DBP levels, is more common in whites and less common in blacks [97, 98].

Since the prevalence of GC1F is high in blacks, they have bioavailable 25(OH)D concentrations comparable to the whites, and homozygosity of the CG1F allele was found in 53% of African Americans versus just 6% of Caucasians and 13% of Hispanics [97, 98].

These allele combinations tend to affect VDBP concentration, affinity for binding 25(OH)D, and 25(OH) levels [99].

Regarding the endocrinal effect on VDBP, androgens have no effects, that's mean, either increase or decrease in androgen level does not affect serum VDBP [46].

While estrogen affect serum VDBP positively, it increases in the cases of using estrogen or estrogen-progesterone contraceptives or during pregnancy as the serum VDBP concentration at delivery is roughly twice that found in cord serum [46], also, in pre-menopausal women whom have higher serum VDBP levels than postmenopausal women [100].

In cord serum and human fetus, the concentration of VDBP is lower than in adult serum [101].

1.10.7 Pathological conditions affect VDBP

Going through the hormonal and chronic diseases that may affect VDBP, it is found that patients with malnutrition, liver cirrhosis and peritoneal dialysis have low serum VDBP [46].

Moreover, serum 25(OH)D and VDBP decreased in acute inflammatory conditions and following surgeries, so, serum 25(OH)D may be an unreliable indicator of vitamin D status in these cases [102].

VDBP concentrations in type 1 diabetes patients' serum are slightly lower[103], although better diabetes control can recover these level [46].

Many endocrine diseases include hyperthyroidism, hyperparathyroidism, Addison's disease, or growth hormone deficiency, have been studied and found not to cause changes in serum VDBP concentration [46].

Acromegaly is disease result from an excess Growth hormone (GH) and insulin like growth factor (IGF-1) and they role in the regulation of VDBP is not currently known [104].

Altinova and others in their study that tried to show the levels of free vitamin d and VDBP in acromegaly, they found that free 25(OH)D is decreased in acromegaly active

patients which may result from the increase in serum VDBP level that also found in this study or may be related to the higher BMI in the active acromegaly group, whereas total 25(OH)D is significantly not different [104].

When comparing between patients who were treated with somatostatin and those who were not treated, there is no differences in the levels of circulating VDBP and total and free 25(OH)D [104].

Linda Björkhem-Bergman and others conducted a study to understand the effect of high dose vitamin D supplementation on VDBP levels, they found that less than 5% of VDBP binding sites are occupied by 25(OH)D and the relation is not significant [50], that is why the VDBP levels have just a minor influence on the levels of free 25-(OH)D, it is mean, only extreme VDBP depletion could result in 25(OH)D deficiency [47].

Patients with genetically inherited or acquired megalin or cubilin deficiency were shown to have low VDBP concentrations, these two proteins serve as cargo receptors to reabsorb proteins of serum [22].

Also, it's worth to mention that in patients with many types of kidney diseases, low amount of VDBP are detected due to renal loss of VDBP, nephrotic syndrome patients lose large amounts of VDBP more than for albumin since VDBP has lower molecular weight [46].

According to previous study conducted by Lai et al., liver cirrhosis patients with low albumin had lower VDBP and total 25(OH)D levels and found that total 25(OH)D is not accurate biomarker for vitamin D status in cirrhosis patients [105].

Urinary VDBP is an indicator of tubulointerstitial damage; it exists apart of proteinuria and appears in the early stages of the inflammatory response and tubulointerstitial fibrosis [69].

Tubulointerstitial injury is a significant cause of diabetic nephropathy. Furthermore, high urine VDBP excretion predicts intra- and extrarenal vitamin D insufficiency [69].

Despite VDBP changes may affect vitamin D level as urinary loss of VDBP leads to urinary loss of 25OHD which results in vitamin D deficiency, changes in vitamin D level or vitamin D resistance appear to have no effect on VDBP concentration [46].

The specific involvement of DBP in the pathophysiology of these disorders is not fully understood, it can be summarized as GC/DBP serves two primary functions [105]:

1. Transporting all vitamin D metabolites through a single binding cleft in the A domain of the protein. The strong selectivity for vitamin D metabolites and high protein concentration result in extremely low free concentrations.
2. DPB-Actin complex prevents actin polymerization in serum following tissue injury.
3. Other GC/DBP functions require additional validation.

1.10.8 VDBP polymorphism and its measurement method

There are various genetic variants in the GC gene that encode DBP that may affect its important function of vitamin D binding affinity and capacity for carrying and transportation. GC1S, GC1F, and GC2 are the three prevalent variances of DBP (that also called as GC globulin) [106].

Each variant is distinguished by a unique fusion of two single nucleotide polymorphism, which results in two amino acid transforms and distinct glycosylation ways [106].

Several authors investigated separately the electrophoretic motion of vitamin D in serum either by scaling the anti-rachitic activity, or, via applying radiolabeled vitamin D or (25OHD), immunoassays is the dominant technique used today [107].

Numerous follow-up investigations utilizing polyclonal DBP were generated for DBP from humans, rats, chicks, rabbits, mice, guinea pigs, and dogs.and assays based on mass spectrometry indicated conclusively that the tests used monoclonal DBP to measure DBP in populations with heterogeneous genetics should be withheld or re-explained [48].

The reporter of the Powe et al. publication also collaborated that the DBP results from monoclonal DBP assay investigations were incorrect [48].

The level of DBP in normal human serum is in the µmolar range (about 6 µmol/l or 300 ml/l) [108].

But varies laboratories have found mean concentrations ranging from 200 and 600 mg/L. Most of these discrepancies are likely due to lack of standardization of DBP assays and references [108].

Commonly using of polyclonal antibodies in assays, minimizing the impact of protein polymorphism on final measurements [46].

Chapter Two
Methodology

2.1 Study Design

A prospective study was conducted among hemodialysis patients in which participants were conveniently hired to participate according to inclusion and exclusion criteria.

The study was carried out at hemodialysis unit in Al-Najah hospital, Nablus, Palestine, over 10 months extended from May 2022 to February 2023.

2.2 Ethical considerations

All of the steps implemented in this study were assigned and referred to the standards related to ethics of the Institutional Review Board (IRB) according to Helsinki declaration.

The patients were introduced and got aware to this research, its goals or purposes and its beneficial outcomes.

It is confirmed that the patients were safe and there was no risk or any harm affected them, and the rights of patients in withdrawing during the study were served and considered.

The participation was optional and voluntary, patient's privacy was assured and their information confidentiality also was guaranteed.

The qualified nursing staff who were working in the kidney department of the hospital treated or dealt respectfully with all participants. Everything related to the trial or the study was clear and understood by all patients.

2.3 Study Sample

Patients with end stage renal disease undergoing hemodialysis with different disease etiology, hemodialysis duration and medical history.

2.4 Sample Size

By using Raosoft to calculate the sample size, the study needs 166 patients considering the total patients in dialysis unit is 290 patients with confidence level 95% and margin of error 5%.

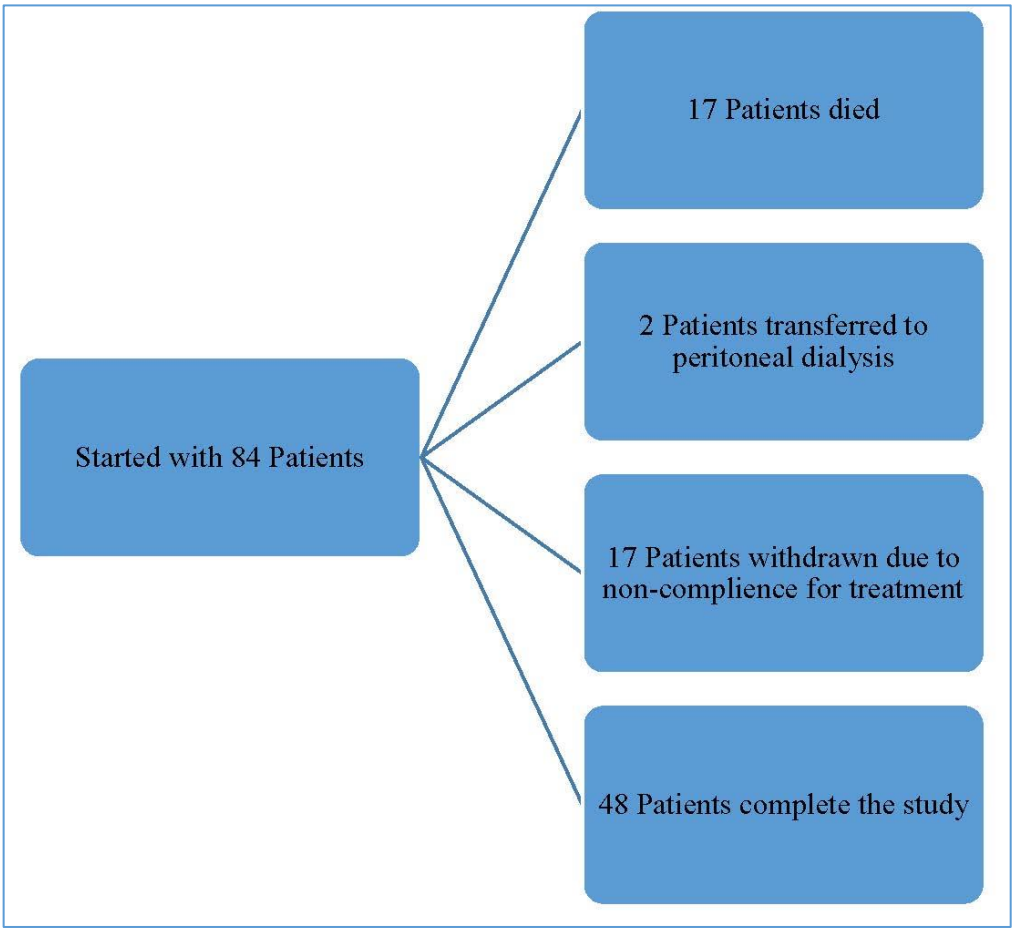
However, the study was initiated with 84 patients according to VDBP Kit size at the first stage which is before starting vitamin D treatment course.

Through the study duration, 36 patients were withdrawn based on withdrawal criteria (17 patients was died, 2 patients transferred to peritoneal dialysis and 17 patients were withdrawn for non-compliance reasons related to vitamin D treatment as failure to complete the treatment course or not taking it from the beginning).

The study was completed with a total of 48 patients who were undergoing the second stage which follows the treatment course.

Figure 5

Chart of Sample size of the study



2.5 Inclusion criteria

Both men and women, age above 18 years, end-stage renal disease undergoing hemodialysis treatment for more than 20 months who were capable to give informed consent.

2.6 Withdrawal criteria

Death, renal transplantation, non-compliance, refusing or inability to be objected to treatment, clinical complications affecting oral ingestion of tablet, patient transferal to another dialysis department and withdrawal of informed consent.

2.7 Exclusion criteria

Age below 18 years and dialysis less than 20 months.

2.8 collection Sample

A non-fasting blood samples were collected at the start of the dialysis sessions by nurses from the hemodialysis unit during different working shifts using clot activator plain tube.

The collected samples were centrifuged at 5000 rpm for 5 min at 4 °C, and the serum part was picked up and stored at - 80°C until the sample collection was completed, and all of the collected samples were used at the same time to examine VDBP using its Kit.

Referring to the used VDBP Kit guidebook, serum and plasma samples can be used for the test and the samples can be stored for 9 months at - 20°C.

Results of routine lab tests as calcium, phosphorous, PTH, albumin and 25(OH)D in addition to the medical and hemodialysis history were collected from patient files, the results used that of the approximate period to perform the VDBP assay.

2.9 Method

The study was conducted over two stages, at May 2022 as a stage before treatment, VDBP measured and reported as long as with reporting a results of several related biomarkers as 25-OH D, PTH, Calcium, Phosphorus and Albumin, the last results found in the pts files were taken.

The results in October 2021 was taken for vitamin D and parathyroid hormone levels, and results in May 2022 for Calcium, Phosphorus and Albumin.

The treatment was started in May 2022, there was a total of 17 weeks of treatment, the treatment algorithm was as the following:

- Patients with a baseline vitamin D level of less than 10 have been given 100,000 international units per week
- Patients with a vitamin D level baseline greater than 10 have been given 50,000 units per week.

In September 2022, vitamin D levels were measured and determined to be high, so, vitamin D treatment was discontinued at that point.

Patients remained without any treatment from September 2022 to February 2023, the levels of vitamin D measured in December 2022 and in February 2023, and found to be:

1. The mean of baseline levels of vitamin D was 13.4 and after 17 weeks of treatment, it increased to 330.
2. The means of levels of repeated labs after stopping vitamin D treatment were 85.7 in December 2022 then, decreased to 76 in February 2023.

There are 5 months of sustained elevation and vitamin D levels after 17 weeks of high-dose of Cholecalciferol & Alfacalcidol treatment.

In February 2023, VDBP measured as the after-treatment stage, levels of the same period were reported for vitamin D, Calcium, Phosphorus and albumin, Dec 2022 results for parathyroid hormone.

2.10 VDBP assay procedure

Many methods are currently used to measure VDBP rely on specific anti-VDBP antibodies. One of them, the enzyme-linked immunosorbent assay (ELISA) technique by R&D VDBP DRG kit manufactured by DRG instruments GmbH, Germany used polyclonal rabbitantibodies to measure serum VDBP concentration that is different in accordance with genetic differences in VDBP.

It is an enzyme immunoassay designed to quantify vitamin D binding protein (VDBP) that is free and not actin complex bound in blood, plasma, and urine.

A VDBP sandwich ELISA was used according to the manufacturer's instructions for use[109].

Briefly, serum (diluted 1: 40,000) was added into a micro-titer plate wells which are coated with polyclonal anti-VDBP antibodies and incubated for 1 h at room temperature (15°C - 30°C) on a horizontal shaker.

After washing for 5 times with wash buffer and tapping hardly the plate on absorbent paper, a polyclonal peroxidase-labeled rabbit anti-VDBP antibody for detection was added into each well and incubated for 1 h at room temperature (15°C - 30°C) on a horizontal shaker.

Thereafter washing 5 times again and tapping the plate on absorbent paper firmly, a substrate tetramethylbenzidine was added and incubated for 15 min at room temperature (15°C - 30°C) in the dark.

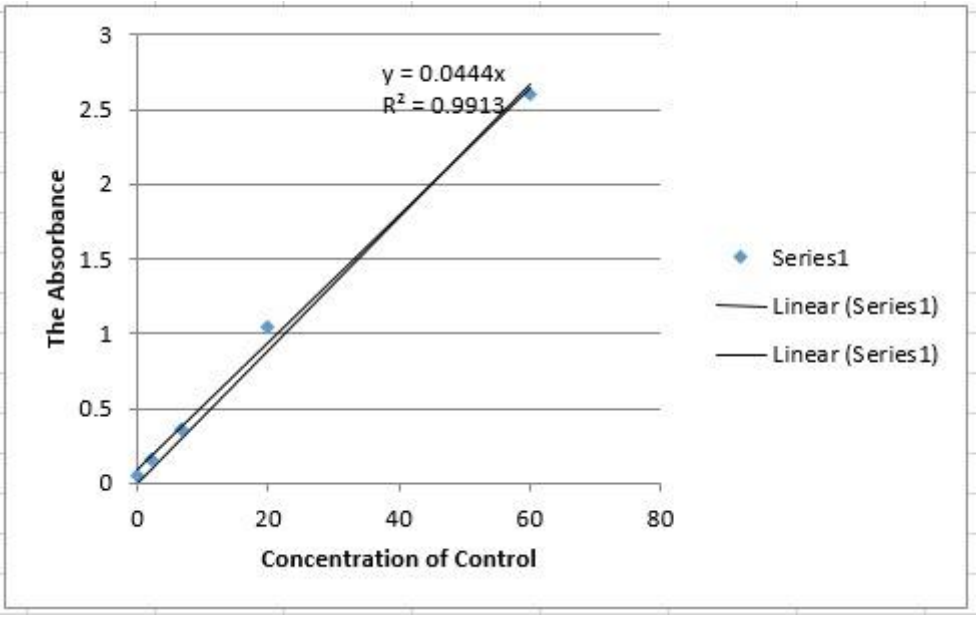
A stop solution was added and absorbance was immediately determined at 450 nm by ELISA reader.

The color converted to yellow as violence of the yellow color is proportional to the VDBP concentration in the sample. The gained VDBP levels of serum samples must be multiplied by a dilution factor which is 40000. Final VDBP levels were calculated by using a standard curve which provided by the supplier based on the value was obtained from the standard[109].

The calibration curve results after using 4 parameter algorithm which is recommended by the supplier is shown in chart 5.

Figure 6

Chart of Calibration curve results



X axis: concentration of control
Y axis: the absorbance

2.11 Other lab test procedures

Vitamin 25(OH)D biochemical analysis by plasma samples were performed with ELISA method while calcium, phosphorus and albumin were quantified by photometric method, (Roche, Cobas C501, North America).

PTH levels were measured by electrochemiluminescence (ECL) method, (Roche, Cobas C601, North America).

2.12 Statistical analysis

Data entry and all of the statistical analyses were processed using the statistical package for social science (SPSS) program.

Descriptive statistics are performed for demographic and clinical characteristics. Continuous variables are presented as mean & standard deviation, whereas frequencies and percentages are used for categorical variables.

The variables were assessed for normality, some of this have normal distribution and the others are not-normally distributed.

For variables with normal distribution, Paired sample t-test was used for finding the significance of difference while Pearson correlation coefficient was used to determine the correlations between variables.

Regarding not-normally distributed variables, Wilcoxon test was used for evaluating difference significance while spearman correlation coefficient was utilized to assess the relation between the variables.

After defining the hypothesis for this study, the hypothesis was examined by a significance test (P-value).

Rejection of null hypothesis was advised when P-value was less than 0.05, whereas the null hypothesis was considered not rejected when P-value was more than 0.05, which mean that P value < 0.05 was intended to be statistically significant.

Chapter Three

Results

3.1 Sample distribution according to demographic data.

A prospective study has been enrolled involving 48 patients undergoing hemodialysis as a total of 18 women (37.5%) and 30 men (62.5%).

The mean age was 58.6, ranging from (20-70) years.

BMI (kg/m2) was calculated via the formula, body weight divided by squared height; BMI was in the range of 15.63-48.61 kg/m2, participants with overweight 16 (33.34%) & obesity 10 (20.83%), weight mean of 76.89kg and 1.68m for height.

Table 2

Patients demographics

Variable	Number	Minimum	Maximum	Mean & standard deviation
Age	48	20	77	58.6 ± 13.15
Weight (kg)	48	40	170	76.89 ± 22.23
Height (m)	48	1.5	2	1.6819 ± 0.096
BMI Underweight < 18kg Normal 18.5-24.9kg Overweight 25-39.9kg Obesity > 30kg	48 1 (2.08%) 21 (43.75%) 16 (33.34%) 10 (20.83%)	15.63	48.61	27.1459 ± 7.23
Gender Male Female	48 30 (62.5%) (37.5%) 18			

3.2 Sample distribution according to medical history

Table 3 showed the medical history and co-morbidities among the studies HD patients.

Medical history includes: diabetes mellitus, hypertension and heart disease as the most prevalent chronic diseases.

Cerebrovascular disease, respiratory dysfunction, liver dysfunction, malignancies, Parathyroidectomy and fractures were less prevalent co-morbidities among studied HD patients.

Smoking is also common among sample patients at a rate of 43.7%

Table 3
Existence of co-morbid in the patients

Variable	Frequency	Percentage
Diabetes Mellitus		
No	24	50%
Yes	24	50%
Heart Disease		
No	25	52.1%
Yes	23	47.9%
Cerebrovascular disease		
No	39	81.3%
Yes	9	18.7%
Respiratory Dysfunction		
No	45	93.7%
Yes	3	6.3%
Liver Dysfunction		
No	43	89.6%
Yes	5	10.4%
Hypertension		
No	6	95.8%
Yes	42	4.2%
Parathyroidectomy		
No	46	95.8%
Yes	2	4.2%
Fracture		
No	37	77.1%
Yes	11	22.9%
Smoking		
No	27	56.3%
Yes	21	43.7%
Malignancies		
No	47	97.9%
Yes	1	2.1%

3.3 Biomarkers test used and their results

As shown in table 4., vitamin D pre-treatment levels were significantly low (13.4 ± 8.0), 89.6% of patients were vitamin D deficient, based on this baseline, the patients were treated with different doses of Alfacalcidol and Cholecalciferol, that is result in high levels of vitamin d (66.3 ± 66.3).

VDBP decreased significantly during the treatment of vitamin d, it has results of mean (1733.7835 ± 781.3948) before the treatment and (1146.790 ± 400.0427) after the treatment course, with p value 0.042.

The PTH levels before and after the treatment course, it has means (393.0 ± 259.5) and (530.5 ± 418.8), respectively, which shows higher levels after treatment than before.

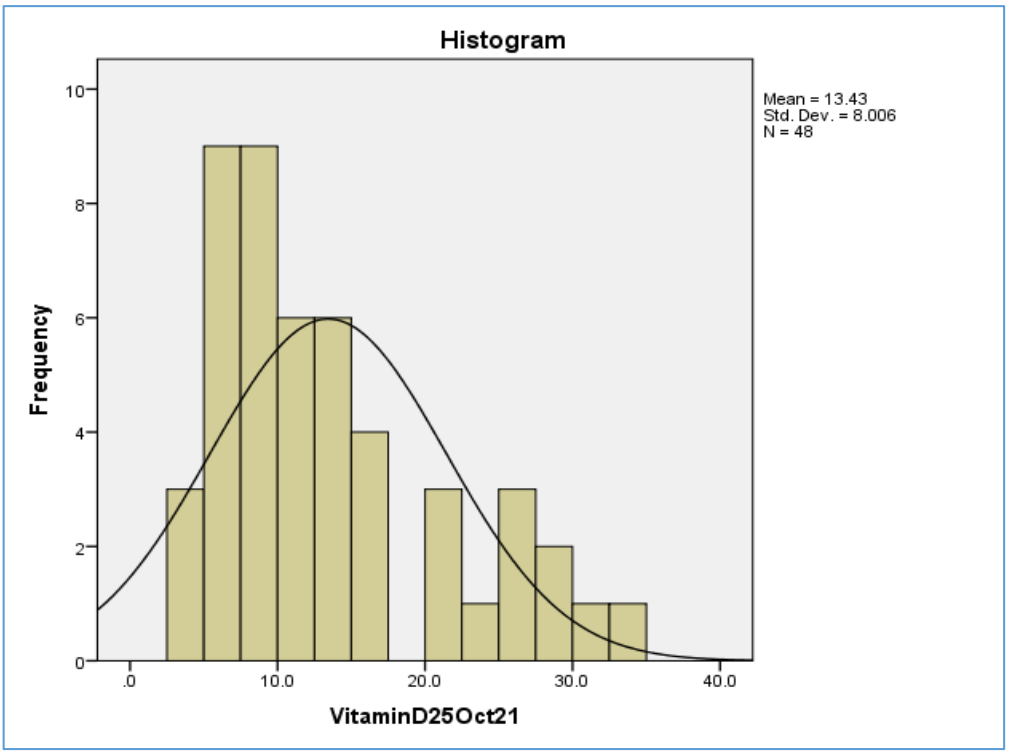
Table 4

Descriptive statistic for the biomarkers results

Variable	Minimum	Maximum	Mean ± SD
Vitamin D pre-treatment (mcg/L)	3.0	33.9	13.4 ± 8.0
Vitamin D post-treatment (mcg/L)	22.7	401.3	66.3 ± 66.3
Vitamin D post – pre	10.8	394.5	52.9 ± 76.9
VDBP pre-treatment (mcg/mL)	532.7314	3153.1530	1733.783 ± 781.394
VDBP post-treatment (mcg/mL)	410.8108	2081.9820	1146.790 ± 400.0427
VDBP pre-post	-648.5810	2164.8647	586.9930 ± 766.0618
PTH pre-treatment (pg/ml)	33.0	1503.0	393.0 ± 259.5
PTH post-treatment (pg/ml)	57.11	2188.0	530.5 ± 418.8
Calcium pre-treatment (mg/dl)	7.3	10.2	9.0 ± 0.57
Calcium post-treatment (mg/dl)	7.12	11.8	9.1 ± 0.78
Albumin pre-treatment (g/dl)	3.4	4.5	4.0 ± 0.25
Albumin post-treatment (g/dl)	3.0	4.5	3.9 ± 0.34
Phosphorus pre-treatment (mg/dl)	2.96	7.7	5.4 ± 1.1
Phosphorus post-treatment (mg/dl)	1.86	9.49	4.5 ± 1.7
Alpha D3 treatment dose (mcg)	0.00	2.0	0.69 ± 0.56
Cumulative doses of Cholecalciferol	200000	1700000	1150000 ± 469154.9
Duration of HD (months)	25	269	76.51 ± 47.3

Figure 7

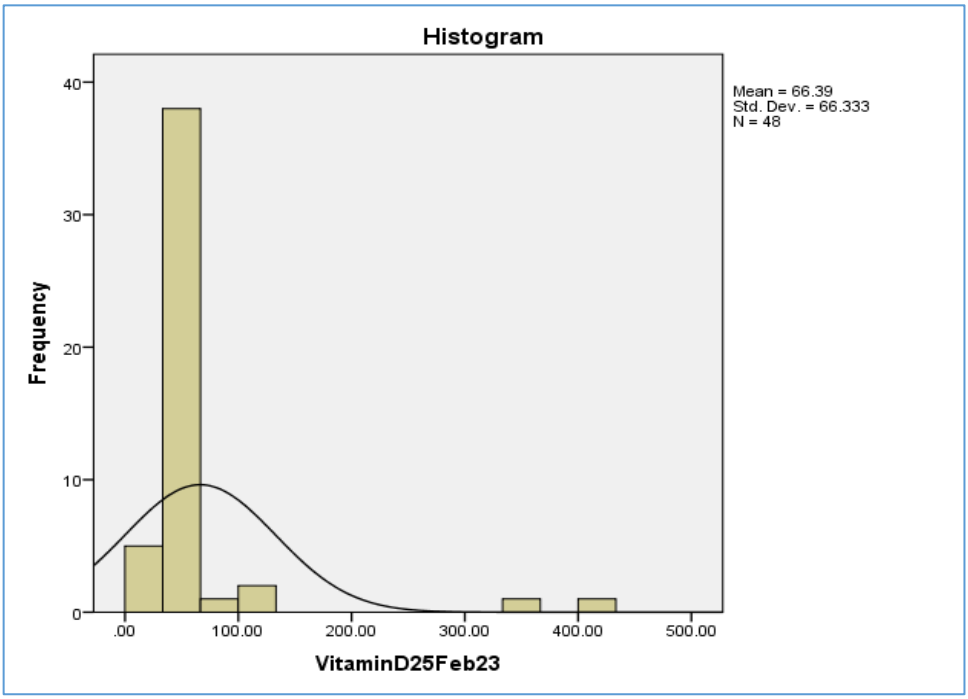
Frequencies of vitamin d levels before treatment



The above curve results from SPSS program shows the frequencies of vitamin d levels before treatment using analyze– frequencies –vitamin d levels) echoosing the variabl t we need the program to show histogram chart withordering tha ,(before the treatment normality curve on histogram.

Figure 8

Frequencies of vitamin d levels after vitamin d treatment



The above curve results from SPSS program shows the frequencies of vitamin d levels before treatment using analyze– frequencies –choosing the variable (vitamin d levels after the treatment), ordering that we need the program to show histogram chart with .normality curve on histogram

3.4 Biomarkers results difference between vitamin d pre-treatment phase and vitamin d post-treatment phase

Table 5 illustrated the significance of difference between the biomarkers results before and after 17 weeks of vitamin d treatment course. Wilcoxon test (P value < 0.05) was used to evaluate the effect of vitamin d treatment on the levels of vitamin d, VDBP and parathyroid hormone levels in the blood.

Moreover, Paired sample T-test (P value < 0.05) was used to find the correlation between calcium, phosphate and albumin levels in the blood before and after vitamin d treatment, as shown in the below table, P-value is significant in all of the mentioned biomarkers except in the calcium level.

The differences are represented as marked increase in vitamin D and parathyroid hormone levels, slight increase in calcium levels, noteworthy decrease in VDBP levels and minor decrease in albumin and phosphorus levels after vitamin d treatment course.

Table 5

Difference significance in biomarkers between the two stages

Variable 1	Variable 2	Difference significance (Wilcoxon test p-value)
Vitamin D pre treatment	Vitamin D post treatment	0.000
VDBP pre treatment	VDBP post treatment	0.000
Albumin pre treatment	Albumin post treatment	0.014
PTH pre treatment	PTH post treatment	0.000
Calcium pre treatment	Calcium post treatment	0.152
Phosphorus pre treatment	Phosphorus post treatment	0.000

3.5 Relationships between tests results

Pearson and Spearman tests were used to find and understand some relationships between the biomarkers (P value 0.05). It is shown in table 6 that there is an association between VDBP before and after treatment, but there is no significant relationship between vitamin d and VDBP levels.

There is no clear correlation between VDBP with calcium, phosphorus, parathyroid hormone, and albumin levels before and after vitamin d treatment.

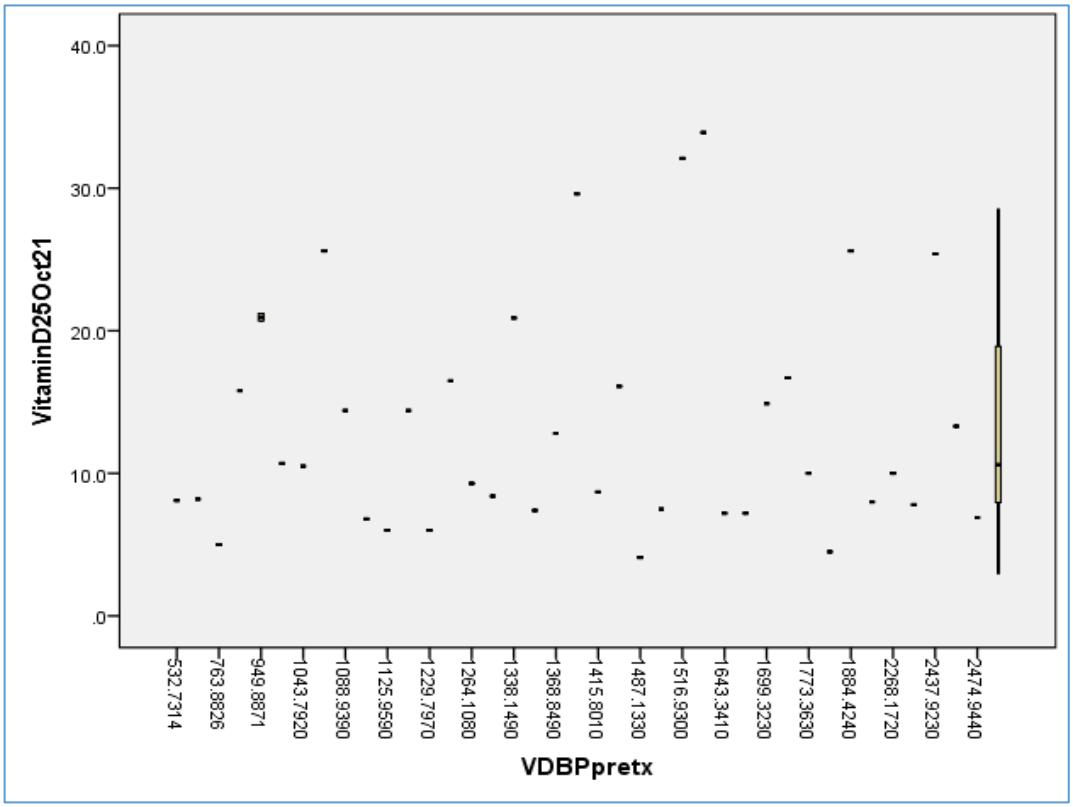
Table 6

Relations between the results

Variable 1	Variable 2	P=value
Vitamin D pre treatment	VDBP pre-treatment	0.971
Vitamin D post treatment	VDBP post-treatment	0.960
Vitamin D difference post-pre	VDBP difference pre-post	0.937
VDBP pre treatment	VDBP post-treatment	0.043
VDBP pre treatment	Albumin pre-treatment	0.465
VDBP post treatment	Albumin post-treatment	0.956
VDBP pre treatment	PTH pre-treatment	0.832
VDBP post treatment	PTH post-treatment	0.600
VDBP pre treatment	Calcium pre-treatment	0.457
VDBP post treatment	Calcium post-treatment	0.167
VDBP pre treatment	Phosphorus pre-treatment	0.857
VDBP post treatment	Phosphorus post-treatment	0.319

Figure 9

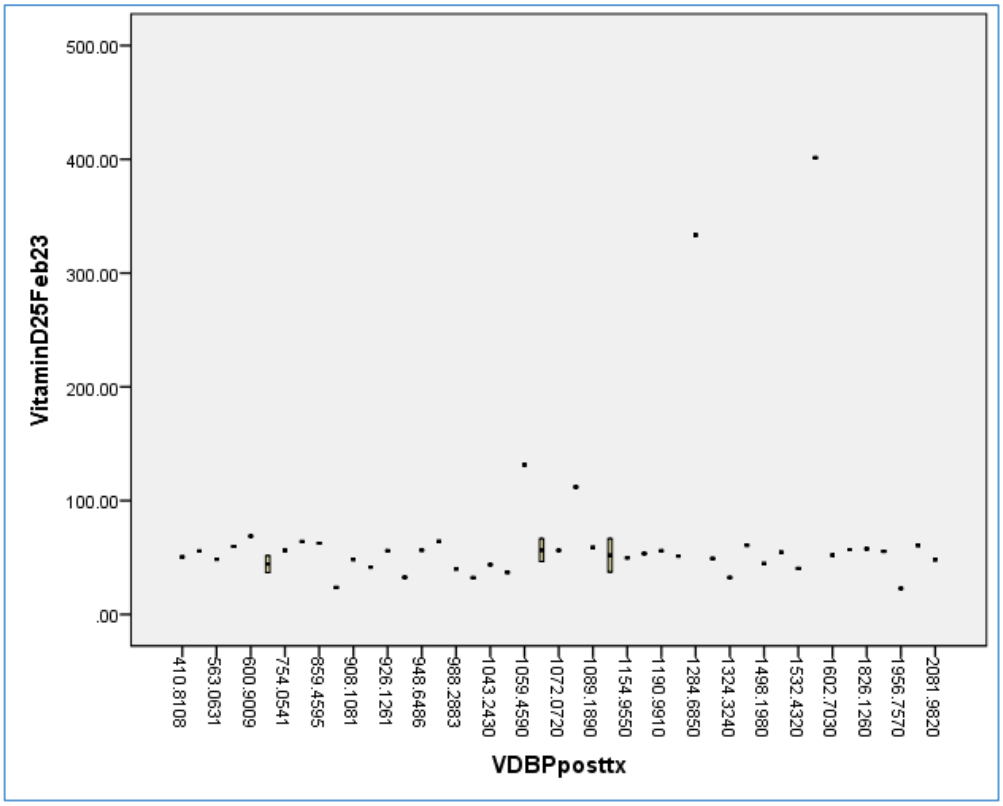
Distribution of vitamin d and VDBP results before vitamin d treatment course



The above curve results from SPSS analysis showing the distribution of vitamin d and VDBP results before vitamin d treatment course by using analyze – descriptive statistics – explore (dependent list: vitamin d levels before the treatment, Factor list: VDBP levels before the treatment).

Figure 10

Distribution of vitamin d and VDBP results after vitamin d treatment course



The above curve results from SPSS analysis showing the distribution of vitamin d and VDBP results after vitamin d treatment course by using analyze – descriptive statistics – explore (dependent list: vitamin d levels after the treatment, Factor list: VDBP levels after the treatment).

3.6 Effect of some medication on VDBP levels

Phosphate binder is a common medication used in hemodialysis patients, and the dose is determined based on their food quality, quantity, and phosphate content.

The average of doses used in our study in an attempt to find its effect on VDBP levels in the blood, but no relationship was discovered between phosphate binder ingestion and VDBP levels as seen in table 7.

Table 7

Effect of common used medications in hemodialysis patients on VDBP levels

Variable 1: Phosphate binder medication ingestion	Variable 2: VDBP levels (pre-post)	Pearson P-value:
Sevelamer Yes: 19 No: 29	VDBP level	0.603
Calcium carbonate Yes: 27 No: 21	VDBP level	0.743
Calcium acetate Yes: 11 No: 37	VDBP level	0.825

3.7 Effect of medical history, demographic variables and vitamin d doses on VDBP levels

In this study, several details were collected and analyzed to determine the effect of pathological conditions on VDBP and changes in VDBP levels as shown in table 8 below.

Spearmen test (P-value 0.05) used to evaluate the relations, in a result, there are no significant relations between the presence of chronic diseases as diabetes mellitus, heart diseases or hypertension, cerebrovascular disease, respiratory or liver dysfunction, history of fractures or Parathyroidectomy and malignancies with VDBP.

The smoking also has no effect on this too. Neither doses of alfacalcidol and cholecalciferol nor duration of hemodialysis have been investigated. Furthermore, none of the demographic parameters influence VDBP levels.

Table 8

Effect of medical history, demographic variables and vitamin d doses on VDBP levels clarified values-by P

Variable	VDBP pre	VDBP post	VDBP pre - post
Diabetes Mellitus	0.353	0.263	0.124
Heart Disease	0.346	0.985	0.341
Cerebrovascular Disease	0.811	0.641	0.625
Respiratory Dysfunction	0.166	0.123	0.545
Liver Dysfunction	0.865	0.407	0.545
Hypertension	0.721	0.706	0.574
Parathyroidectomy	0.604	0.920	0.634
Fracture	0.343	0.581	0.207
Smoking	0.115	0.292	0.297
Malignancy	0.373	0.964	0.377
Age	0.780	0.442	0.908
Gender	0.945	0.363	0.687
Weigh	0.590	0.384	0.314
BMI	0.482	0.549	0.301
Duration of hemodialysis	0.789	0.799	0.684
Cumulative Doses of Cholecalciferol	0.298	0.070	0.898
Alpha D3 treatment dose	0.665	0.863	0.595

Chapter Four
Discussion

4.1 Introduction

Vitamin D deficiency is one of the most common complications for ESRD that requires huge researches and interventions due to its consequences throughout the body.

By understanding the physiology of vitamin d and going through the factors that may have a role in its deficiency, in addition to extended research on literature focusing on the causes and how to control or prevent that deficiency, we thought that VDBP may be one of the stones that affect vitamin d level and can support to find therapeutic and preventable intervention.

Up to date, there is no studies about relation between VDBP and other biomarkers, also, about the role of VDBP through the body in normal and pathological condition in Palestine.

Furthermore, although there are several studies regarding VDBP around the world, a little of available knowledge describes the relation between VDBP with vitamin d in hemodialysis patients.

We recruited the demographic data and lab tests results of 48 hemodialysis patients from dialysis unit of al-Najah hospital in Nablus city to participate in the current study, their information and lab results were collected and analyzed in order to be discussed.

4.2 Vitamin D treatment in HD patients

Supporting to our results, randomized trial in maintenance HD patients studying therapeutic options to treat vitamin D deficiency were conducted by Annich and his colleagues.

It's found that administering of 25,000 IU of cholecalciferol on weekly basis for 13 weeks lead to moving the 25(OH)D levels (30 ng/mL) in 62% of patients to normal level and also normalization of 1,25(OH)2D levels (20 pg/mL) in 54%[110].

Our results also show that vitamin d treatment course resulted in normalization and markedly increase of vitamin d levels in hemodialysis patients.

In view of the high prevalence of vitamin d deficiency in patients with kidney diseases, maintenance of vitamin d normal levels is one of the important challenges in hemodialysis patients.

It is important also, due to the essential roles of vitamin d in human body as vitamin d deficiency complication may affect the immunity cascade in this patients who are vulnerable to several types of infections[111].

In addition to the bone homeostasis and vascular health status which is negatively affected with vitamin d deficiency[111].

4.3 VDBP and Vitamin D levels

In this study, after vitamin d treatment course of 17 weeks, VDBP levels were significantly reduced while vitamin d levels were markedly increased without correlation between them, which is mean that no relationship between vitamin d levels and VDBP levels in hemodialysis patients.

These findings are comparable with Linda and others conclusion that high-dose of vitamin D supplementation has no effect on VDBP levels in plasma after 6 months.

Furthermore, the results they obtained imply that VDBP levels don't correlate with free 25-OHD levels in the circulation[50].

In line with the results of previously conducted study in the Netherland to assess the effect of urinary loss of VDBP on vitamin D and VDBP status in patients with chronic kidney disease, there was no relationship discovered between plasma VDBP and vitamin D3 levels[112].

In a cross-sectional analysis for pediatric participants including patients with CKD stages 2-3, others undergoing hemodialysis, and renal transplant patients, Evgenia and colleagues found no association between VDBP and total-25(OH)D concentrations in any of the studied groups [52].

In accordance with our results, the levels of VDBP and vitamin D showed no relation either before and after the treatment course.

4.4 VDBP and demographic variables

In comparison with our results and the previous research about relation of age and gender with VDBP level, we found that no definite link between age or gender and VDBP level in HD patients.

In agreement with published results by Marta and his colleagues that correlation between age and VDBP was not proven in HD patients[113].

Despite healthy individuals have not been subjected to similar studies in Palestine, a previous study operated by USA concluded that VDBP was found to be inversely linked with age in healthy female, implying that age may be an independent factor influencing VDBP and 25(OH)D levels.

Also VDBP levels were shown to be lower in males than in females, possibly due to estrogen effects on VDBP by several studies[22, 93, 114].

In contrast of our result, Marta and others found that VDBP has slight negative correlation with BMI among HD patients in Prague[113] while our result showed that there is no relationship between them.

4.5 VDBP and other Lab results

Vitamin D is required for calcium absorption and parathyroid hormone (PTH) increase prevention, which can expand bone resorption.

One of our objectives was to examine serum levels of 25-hydroxyvitamin D [25(OH)D] and PTH, and understand the relation with VDBP.

Zari and others investigated the influence of VDBP concentration on vitamin D action by studying the association between 25(OH)D concentrations and serum PTH a biologic biomarker of 25(OH)D activity while controlling VDBP levels.

By controlling VDBP levels, their findings demonstrate that the extent of the association between total vitamin D and PTH is hardly affected.

This implies that VDBP levels have no effect on the relationship between 25(OH)D and PTH in normal status and pathophysiological conditions that do not affect VDBP metabolism directly[115].

In this study, a minor decrease in albumin levels was resulted after vitamin d treatment course, with no significant relationship found between serum albumin and VDBP, a previous study resulted in a slight positively link between them[113].

The little decrease in phosphorus levels after vitamin d course ingestion shown in our study may be due to phosphate level control in the patients by phosphate binder treatment based on routine lab test for them.

In contrast of several previous studies result which indicate that cholecalciferol supplementation elevates serum phosphorus levels. The greater phosphate levels found in their studies could be attributed to higher levels of serum phosphate baseline, as well as dietary factors[71].

4.6 VDBP concentration

Here in Palestine, there is no previously known value as baseline or reference range values for VDBP in normal population or in any of physiological and pathological conditions since our study is the first one addressed VDBP.

Also, the kit supplier didn’t establish reference concentration of VDBP in renal diseases and recommended each laboratory to define its own concentration range.

Due to genetic variants of VDBP among worldwide races, it is hard to debate certain concentrations or reference ranges to compare with our results as a result of the lack of similar studies in neighboring countries.

However, we can review some of VDBP concentrations worldwide in normal population and in patients with renal diseases [113, 116].

In table 9, VDBP levels in multi-ethnic healthy older people with vitamin d deficiency[116].

Table 9

Vitamin d and VDBP levels in healthy older people from 3 different populations

Country	Chinese	Malay	Indian
25-OH D levels (mcg/l)	29.1 ± 10.1	19.2 ± 8.3	19.5 ± 10.1
VDBP levels (mcg/ml)	169.6 ±106.7	188.8 ± 127.9	220.1 ± 84.6

In Prague, the vitamin d and VDBP results of the study conducted to evaluate vitamin D status and its relation with VDBP in healthy control and patients with chronic kidney disease and long-term hemodialysis patients are shown in table 10[113].

Table 10

Vitamin d and VDBP results of a study in Prague

	Healthy control	Chronic kidney disease	Hemodialysis patients
25-OH D (mcg/L)	48.72 ± 18.35	30.16 ± 16.74	18.85 ± 15.85
VDBP (mcg/mL)	222.0 ± 87.7	273.2 ± 93.8	213.8 ± 70.9

4.7 Conclusion

We discovered no correlation between VDBP and vitamin D levels in patients with end-stage renal disease undergoing hemodialysis.

We also discovered no link between VDBP levels and calcium, phosphorus, parathyroid, and albumin levels.

A vitamin D treatment course maintains vitamin D levels while significantly reducing VDBP levels, with no apparent correlation between the changes in both variables during treatment.

There is a significant need for vitamin D treatment due to the decreased baseline levels in these patients without treatment as several complications such as secondary hyperparathyroidism, CKD-MBD and the risk of fractures which is probable and requires attention.

Immune deficiency and the risk for many types of infections including respiratory infections and catheter related infection, in addition to immunity defect diseases predicted to occur as rheumatoid arthritis, vascular and cardiovascular complications.

4.8 Recommendations

It is highly recommended to maintain normal levels of vitamin d among patients with end stage renal disease undergoing hemodialysis, to adopt routine vitamin d examination every three to six months.

Also, to assure patient awareness of the consequences of vitamin d deficiency and the importance of the treatment and working to raise patient compliance for the treatment.

Studying the status of VDBP among normal human and patients diagnosed with several diseases as liver disease, some types of tumors in Palestine and giving that topic more attention.

4.9 Limitations

Using higher sample size may be more preferable, measuring all lab tests from the same sample can result in more accurate outcomes than using some results of lab tests from patient files but that needs higher budget than offered for the study.

Since VDBP is polymorphic protein and has many variants which differ by race, it was difficult to compare our results with others similar due to the lack of previous studies on that subject in Palestine and in the neighboring countries whose populations may be somewhat similar genetically to the population of Palestine.

Moreover, there is no previous research conducted for understanding the status of VDBP in normal human in Palestine.

List of Abbreviation

Abbreviation	Meaning
KDIGO	Kidney Disease Improving Global Outcomes
GFR	Glomerular filtration rate
CKD	Chronic Kidney Disease
HD	Hemodialysis
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
MBD	Mineral Bone Disorders
ESRD	End Stage Renal Disease
PTH	Parathyroid Hormone
Vitamin D2	Ergocalciferol
Vitamin D3	Cholecalciferol
VDBP	Vitamin D Binding Protein
GC	Group-specific Component
25(OH)D	Calcidiol
1,25(OH)2D	Calcitriol
UVB	Ultraviolet B
FGF23	Fibroblast Growth Factor 23
VDR	Vitamin D Receptor
ECF	Extra-Cellular Fluid
LC-MS-MS	Liquid Chromatography with tandem mass spectrometry
RIA	Radioimmunoassay

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جـامعـــة النجاح الوطنية

كليـــة الدراســـات العليـــا

العلاقة بين البروتين الناقل لفيتامين د ومستوى فيتامين د عند مرضى غسيل الكلى

إعداد

آيات تيسير توفيق حمادنة

إشراف

د. لبني خراز

قدمت هذه الرسالة استكمالا لمتطلبات الحصول علي درجة الماجستير في الكيمياء الحيوية السريرية، من كلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس– فلسطين.

2024

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الملخص

نقص فيتامين د هو مشكلة شائعة بين مرضى الفشل الكلوي في المرحلة النهائية الذين يخضعون للغسيل الكلوي، مما يستدعي البحث الواسع والتدخلات بسبب تداعياتها المنتشرة في الجسم. فهم فيزيولوجيا فيتامين د، فضلاً عن تحديد العوامل المساهمة في نقصه، كان محوراً للبحث. بالإضافة إلى ذلك، تم استكشاف بروتين رابط فيتامين د (VDBP) ودوره المحتمل في التأثير على مستويات فيتامين د، على أمل العثور على تدخلات علاجية.

حاليًا، هناك بحث محدود حول العلاقة بين VDBP وغيره من العلامات الحيوية، خاصة في سياق مرضى الغسيل الكلوي في فلسطين. هدفت هذه الدراسة إلى معالجة هذا الفجوة من خلال التحقيق في العلاقة بين VDBP ومستويات فيتامين د في مرضى الغسيل الكلوي.

أشارت النتائج إلى أنه بعد دورة علاج بفيتامين د، انخفضت مستويات VDBP بشكل كبير بينما ارتفعت مستويات فيتامين د بشكل ملحوظ، دون وجود ترابط بين الاثنين. كانت هذه النتائج متسقة مع الأبحاث السابقة التي أظهرت عدم وجود علاقة معنوية بين VDBP ومستويات فيتامين د في مجموعات سكانية مختلفة.

لم تظهر المتغيرات الديموغرافية مثل العمر والجنس رابطًا قاطعًا مع مستويات VDBP بين مرضى الغسيل الكلوي، وهو ما يتناقض مع النتائج من الدراسات الأخرى. بالإضافة إلى ذلك، لم يكن هناك علاقة

معنوية بين مستويات VDBP ونتائج الاختبارات المعملية الأخرى مثل الألبومين والكالسيوم والفوسفور وهرمون الغدة الدرقية.(PTH)

بشكل عام، تؤكد هذه الدراسة أهمية علاج فيتامين د في مرضى الغسيل الكلوي وتسلط الضوء على الحاجة إلى مزيد من البحث لفهم تمامًا دور VDBP في استقلاب فيتامين د وآثاره على الإدارة السريرية.

الكلمات المفتاحية: فيتامين د، بروتين رابط فيتامين د، المرحلة النهائية من مرضى الكلى، مرضى الغسيل الكلوي.