Association of Vitamin D Levels and Vitamin D Receptor Gene Polymorphisms with Chronic Kidney Disease

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

Student's Full Name & Signature:

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Approval

The thesis titled "Association of Vitamin D Levels and Vitamin D Receptor Gene Polymorphisms with Chronic Kidney Disease'' submitted by Fatema Akter Liza (20146086), of Spring, 2020 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics Statement

The study does not involve any kind of animal and human trial.

Abstract

Chronic kidney disease (CKD) is a highly prevalent chronic disease representing a serious public health issue worldwide. The decline of renal functions and glomerular filtration rate (GFR) are the hallmarks of CKD. Life expectancy and quality of life are compromised due to CKD. Vitamin D plays a crucial role in the regulation of calcium and bone homeostasis, blood pressure, never transmission, erythropoiesis, etc. Vitamin D Receptor (VDR) gene polymorphism is the genetic alteration of the VDR gene that can cause significant changes in regular biological functions such as calcium metabolism, immune function, and cell proliferation. Vitamin D levels and VDR gene polymorphisms have been reported to be associated with renal problems, cardiovascular diseases, inflammation, osteoporosis, autoimmune diseases, cancer, infectious diseases, and type 1 and type 2 diabetes. This review aims to investigate the association of vitamin D levels and vitamin D receptor gene polymorphisms with CKD.

Keywords: Vitamin D, vitamin D levels, chronic kidney disease, vitamin D receptor (VDR) gene polymorphism, single nucleotide polymorphisms (SNPs).

Dedication

Dedicated to my family, teachers, and friends whose unwavering motivation and support made this academic journey possible.

Acknowledgement

I am thankful to the almighty Allah for infinite blessing and mercy. All praise to Him for allowing me to interact with some truly inspiring individuals throughout my time in School of Pharmacy.

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Secondly, I want to convey my sincere gratitude to Professor A.F.M. Yusuf Haider, PhD (Acting Dean, School of Pharmacy, Brac University) for the invaluable guidance and support that he has provided me with.

Finally, I wish to convey my unending appreciation to my family members for always being there for me. Thanks to their prayers and unwavering support, I was able to push myself patiently toward my goals.

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List of Acronyms

ACE	Angiotensin converting enzyme
ARB	Angiotensin II receptor blocker
BDHS	Bangladesh Health and Demographic Survey
CKD	Chronic kidney disease
CVD	Cardiovascular disease
DBP	Vitamin D binding protein
DHC	Dehydrocholesterol
ECM	Extracellular matrix
eNOS	Endothelial nitric oxide synthase
ESAs	Erythropoiesis-stimulating agents
ESRD	End-stage renal disease
FGF	Fibroblast growth factor
FSGS	Focal segmental glomerulosclerosis
GFR	Glomerular filtration rate
GIT	Gastrointestinal tract
GR	Glucocorticoid receptor
HIV	Human immunedeficiency virus
HMB	Hydroxymethylglutaryl
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A

HPT	Hyperparathyroidism
IBD	Inflammatory bowel disease
IFN	Interferon
IgAN	IgA nephropathy
LVH	Left ventricular hypertrophy
MBD	Mineral bone disease
mRNA	Messenger RNA
PDGF	Platelet-derived growth factor
PTH	Parathyroid hormone
RAAS	Renin angiotensin aldosterone system
RAS	Renin-angiotensin system
RNA	Ribonucleic acid
RXR	Retinoid X receptor
SGLT-2	Sodium-glucose transport protein
SHPT	Secondary hyperparathyroidism
SNGFR	Single nephron GFR
SNP	Single nucleotide polymorphism
TGF	Transforming growth factor
TNF	Tumor necrosis factor
TPMT	Thiopurine methyltransferase

VDBP	Vitamin D binding protein
VDD	Vitamin D deficiency
VDI	Vitamin D insufficiency
VDR	Vitamin D receptor
VDRE	Vitamin D-responsive element
VMDR	Vitamin D metabolite diagnostic ratio

Chapter-1: Introduction

1.1 Background

The prohormone vitamin D is mainly obtained by dermal synthesis that occurs by exposure to sunlight and from the diet (Olajide et al., 2023). This vital component belongs to the fat-soluble secosteroid family. It has two biological forms such as vitamin D2 referred to as ergocalciferol which is found in plants and fish and the other one is vitamin D3 referred to as cholecalciferol which is found in the skin of human body. Vitamin D has three weeks of half-life and it should be supplemented through nutrition or sun exposure (Vahdat, 2020). The primary circulating form of vitamin D is 25-hydroxyvitamin D. The conversion of 25-hydroxyvitamin D into the hormonal form 1,25-dihyroxyvitamin D catalyzed by the enzyme 1-α-hydroxyvitamin D which takes place in the proximal tubules of the kidney (Yokoyama et al., 2012). In adults, based on the parameters, the normal range of vitamin D is 8–30 ng/ml (20–75 nM) and the optimal suggested concentration is 30–32 ng/ml (75–80 nM) (Hewison, 2011).

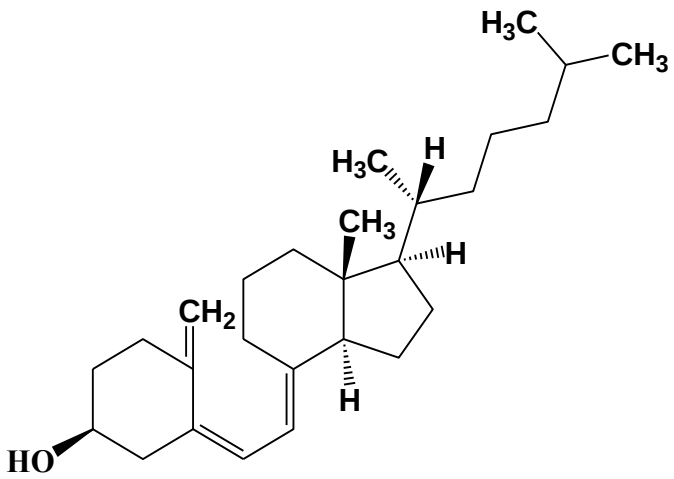


Figure 1.1: Structure of Vitamin D

Vitamin D is crucial for the endocrine system. It acts as a potent regulator of calcium and bone homeostasis. In many target tissues, it also regulates cellular differentiation and replication (Nigel Alexander Morrison et al., 1992). Bone development and mineralization are facilitated by the phosphate and calcium homeostasis and hormonal level (Parathyroid hormone) (Hanafy et al., 2016). It is also associated with many biological functions that include regulation of blood pressure, never transmission, erythropoiesis and so on (Tomei et al., 2020).

Vitamin D receptor (VDR) is a transcriptional factor that is activated through ligands and requires 1,25(OH)2D for the activation (Hussain et al., 2019). The widely distributed Vitamin D receptor (VDR) gene contains single nucleotide polymorphisms (SNPs). The alteration of these influences transcriptional regulation and it further affects VDR protein expression (Elshamaa et al., 2022). The most prevalent kinds of VDR gene SNPs are BsmI (rs1544410), FokI (rs10735810), TaqI (rs731236) and ApaI (rs7975232). VDR gene polymorphisms that are certain mutations or modulation of the consequences have susceptibility to the formation of some major functional disorders including type 1 diabetes mellitus, type 2 diabetes mellitus, rheumatoid arthritis, cardiovascular disease, secondary hyperparathyroidism (sHPT), cancers, etc. (Yang et al., 2015).

Chronic kidney disease (CKD) is a highly prevalent chronic disease representing a serious public health issue with a 10% incidence worldwide. The decline of renal functions and glomerular filtration rate (GFR) are the hallmarks of CKD. Life expectancy and quality of life are compromised at an enormous cost through chronic kidney disease (CKD). The treatment options for patients with CKD are intensive blood pressure management and drugs like angiotensin-converting enzyme inhibitors, angiotensin receptor blockers and statins. Despite the current treatment option, the morbidity and mortality rate remains high for people with CKD (Wang et al., 2016). The findings of the research demonstrated that Bangladesh had a 22.48% overall prevalence of CKD. As reported by Hill et al., the global CKD prevalence was

13.4%. Therefore, Bangladesh exhibited a higher prevalence rate. Based on a systemic review, south Asian countries such as Pakistan, India, and Nepal displayed 21.2, 10.2, and 13.4% of prevalence of CKD respectively. Industrialized countries such as the USA and Japan showed 13% and 10% prevalence of CKD respectively (Banik & Ghosh, 2020).

The three most common causes of CKD are glomerulonephritis, chronic hypertension, and diabetes mellitus (Yoshihara et al., 2018). Regardless the pathophysiology also involves glomerulosclerosis and tubulointerstitial fibrosis. This also includes the structural injury to the renal organ, damage to physiological components, bring about renal impair mechanisms and tissue damage. The renal blood flow rate is 400 ml/100g of tissue per minute which is notably greater than other well-perfused arteries in the body such as brain, heart, and liver (Matovinović, 2009). Consequently, the circulation of potentially hazardous chemicals or compounds may come into contact with renal tissue. Additionally, glomerular filtration hinges on moderately elevated intra-glomerular and trans-glomerular pressure dissimilar to other glomerular capillaries, capillary beds are susceptible to hemodynamic damage. Moreover, depending on immunologic reactions that started with either immune complexes or immune cells, ischaemia and tissue hypoxia, genetic defects, and exogenic agents such as drugs, endogenous substances like glucose or paraproteins, and others, are the dominant reasons for renal damage (Matovinović, 2009).

Based on previous research, elevation of vitamin D deficiency or its insufficiency incidents caused by nutrition is noticed through decreased 25-hydroxyvitamin D levels in CKD. For this, the regulation of 1,25-hydroxyvitamin D levels is getting difficult (Al-Badr & Martin, 2008). As a consequence, the first initiative to treat the abnormalities of bone and mineral metabolism in CKD is to restore vitamin D status by administering native vitamin D. The net amount of 25-hydroxyvitamin D in the circulation is used to determine the vitamin D status. The recommended levels of total 25(OH)D are within 50 ng/mL and 70 ng/mL. The range is below

30 ng/mL in vitamin D deficiency (Sirajudeen et al., 2019). In stage 2 CKD, calcitriol concentration reaches a lower level than normal and it reaches an extremely lower level in the individuals with stages 3 and 4 CKD patients. The increased mortality and cardiovascular morbidity are associated with vitamin D deficiency for patients receiving dialysis. Earlier-stage CKD patients also exhibit a similar association. Low vitamin D status has correlated with diabetic patients and CVD patients remarkably. The observation of treating patients with vitamin D such as ergocalciferol or cholecalciferol for increasing 25-hydroxyvitamin D levels is to enhance calcitriol in CKD patients (Gal-Moscovici & Sprague, 2010).

Vitamin D has been associated with numerous renal, cardiovascular, inflammatory, osteoporosis, cancer, autoimmune diseases, infectious diseases, type 1 and type 2 diabetes, and hypertension in addition to mineral metabolism and bone health. This is mainly due to the vitamin D receptor (VDR) has a wider expression on tissues and cells including heart, muscle, immune cells, kidney, and brain. Based on the restriction sites, several polymorphisms are named including VDR ApaI (rs7975232), Fok1 (rs2228570), and TaqI (rs731236) and so on. Vitamin D signaling pathway is disrupted through ApaI, FokI, and TaqI gene polymorphism which are thought to be reliable markers. Through investigation, it is reported that the susceptibility to CKD increases by this crucial gene polymorphism because of the complex role played by vitamin D in those patients (Zhou et al., 2014).

1.2 Aim and objectives of this study

Aim

This study aims to delineate the potential role of vitamin D levels and VDR gene polymorphism in the susceptibility to CKD.

Objectives

Objectives of this study are:

- to investigate the link between vitamin D levels and CKD
- to identify the association between VDR gene polymorphism and CKD
- to identify the target VDR gene to treat CKD

Chapter-2: Methodology

The present review was primarily started with a thorough screening of scholarly publications from evidence-based sources that were related to the prior mentioned topic. All the essential data was obtained from authentic online search engines and journal databases for this review. Some of them were Google Scholar, PubMed, ScienceDirect, ERIC, ASC Publications, and others. To obtain the title and abstract-specific information, professional websites were ascertained. The outline of this review was designed after the extraction of all the relevant and key details. As a prerequisite, it was essential to find the emerging polymorphisms in the VDR gene in CKD and vitamin D levels in CKD. Literature search was conducted to investigate the association between VDR gene polymorphism in CKD and the interrelation between vitamin D levels in CKD. In this study, it was ensured that only precise and accurate data was used. Finally, all the necessary literature was cited properly for this review.

Chapter 3: Chronic Kidney Disease (CKD)

3.1 Prevalence of CKD

CKD has been acknowledged as a primary public health issue globally. The prevalence of CKD in stages 1-5 patient and stages 3-5 patient is 13% and 6.6% (Duff et al., 2024) respectively that is considered to be a significant burden. The CKD prevalence was 7.5% in women that is comparatively higher compared to men and that is 6.4% in later-stage CKD. Additionally, a noticeably greater prevalence of CKD was reported through investigating on only elderly candidates (≥60 years) and that was 19.3% and 15.0% (Duff et al., 2024) in stages 1-5 and stages 3-5 CKD patients respectively (Duff et al., 2024). Asia had a peak prevalence of CKD in stages 1-5 that was 15.5%, while Australia and Oceania had 8.1% (Duff et al., 2024) prevalence of CKD in 3-5 stages that was the peak. The lowest prevalence of CKD in stages 1-5 was in Europe, which was 10.0%, whereas, the lowest prevalence in stages 3-5 was in Africa, which was 5.7%. Moreover, the lowest prevalence of CKD in stages 3-5 was in the undeveloped countries that was 4.0%, while the lowest prevalence of CKD in stages 1-5 was in developed countries, which was 10.8% (Duff et al., 2024). In Bangladesh, the nationwide prevalence rate of CKD was nearly double than global prevalence, which was 22.48% (Banik & Ghosh, 2020). The higher variation in Bangladesh was because of the design of study eligibility requirements such as regional differences between rural and urban places and the age of participants. In South Asian countries like India, Nepal, and Pakistan, the prevalence of CKD was 10.2%, 13.4% and 21.2% (Banik & Ghosh, 2020) respectively. In 2011, BDHS studies showed a higher prevalence in women compared to men whereas Norway's population-based study showed vice-versa. Two-thirds of the research claimed that women have a higher prevalence of CKD than men (Banik & Ghosh, 2020). Between 1988 to 2004, several national surveys were conducted in the USA and the prevalence of CKD increased from 10% to 13.1%

(Lv & Zhang, 2019) because of the lower estimated GFR and albuminuria. Additionally, the prevalence of CKD in China was 10.8% which was revealed according to a cross-section national survey. Further, the prevalence of CKD in Norway was 10.2% similar to that in developed countries like Japan was 13% (Lv & Zhang, 2019).

3.2 Pathophysiology of CKD

The pathogenesis of CKD is defined by the loss of renal cells and the extracellular matrix (ECM) accumulation. The filtering units of kidneys such as nephrons get damaged and the filtration of blood get hampered. The morphological modifications that characterize progressive renal illness without the underlying insults involve tubular atrophy, tubulointerstitial fibrosis, capillary rarefaction, glomerulosclerosis, and renal inflammation. A devitalizing condition that is consequently spawned by the pathophysiology of renal fibrosis like interstitial fibrosis and glomerulosclerosis requires renal replacement therapy (e.g., dialysis or transplantation) because of the progression of end-stage renal failure (Figure 3.1). Nevertheless, glomerulosclerosis is defined as the eradication of glomerular capillaries and elevation in mesangial matrix accumulation. Firstly, glomerulus-resident cells are disturbed and they are functioning after releasing several chemokines and cytokines that are linked to lymphocytes, monocytes, neutrophils, and the rest of the inflammatory cells. These cells are transferred and gathered in the damaged site based on the dominance of several risk factors including accumulation of immune complexes, hypertension, and dyslipidemia. Secondly, multiple growth factors and cytokines such as tumor necrosis factor (TNF), platelet-derived growth factor (PDGF), transforming growth factor β1 (TGF-β1), angiotensin, fibroblast growth factor (FGF), and interferon-gamma (IFN-γ) are induced by infiltrating cells along with intrinsic cells for producing parietal epithelium cells activation, capillary eradication and

collapse, and capillary loss. Lastly, fibrogenesis produces new ECM compounds for shifting injured cells (J. Yang & He, 2020).

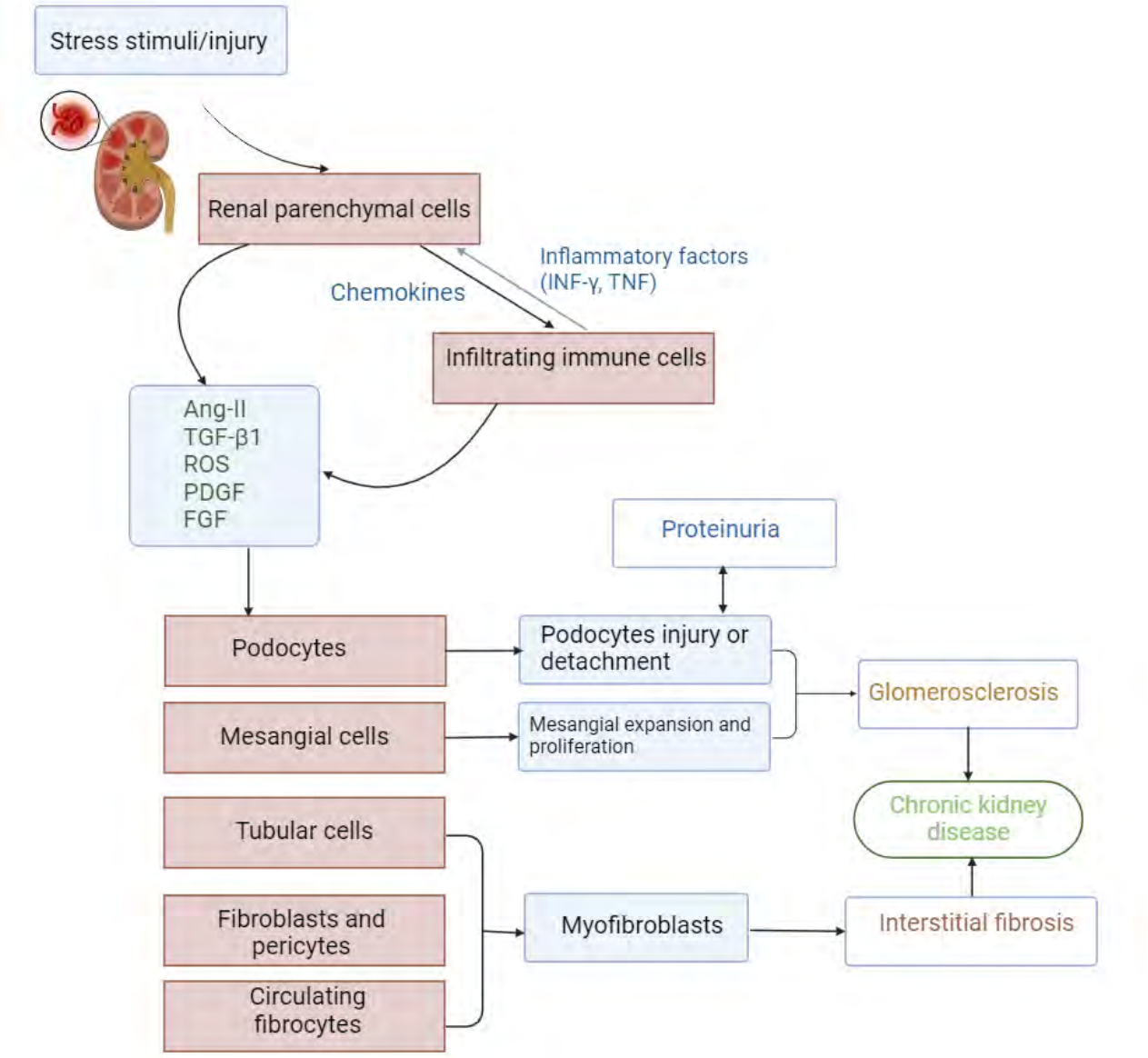


Figure 3.1: A schematic representation of the pathogenesis of CKD.

On the other hand, wound healing failure follows initial damages caused by renal interstitial fibrosis and distinguished by excessive ECM deposition in the interstitial compartment. Some important processes involved in renal interstitial fibrosis are fibroblast activation, generation and deposition of ECM molecules, myofibroblast development, tubular atrophy, and

inflammatory cell infiltration. Inflammatory cells signal to invade site of injury caused by damaged kidney-resident cells, sustained injury such as hypoxemia, high concentration of glucose, proteinuria, and so on. Then the infiltrating inflammatory cells generates components such as ROS and multiple protein factors that includes angiotensin II, IL-1, CTGF, TNF-α, TGF-β1, MCP-1, and prolonged renal cell injury (Figure 3.1). This occurrence leads to fibroblasts as well as different kinds of cells such as epithelial cells, endothelial cells, and pericytes, to generate large quantity of ECM compounds and phenotypic transition or activation. The production of ECM and accumulation of excessive ECM occur through activated myofibroblasts that causes renal tubular apoptosis and atrophy. Excessive buildup of extracellular matrix and its abnormalities in breakdown are responsible for accumulation of excessive matrix in the renal interstitium. Due to proteolysis process of extracellular matrix, fibrosis shows reversibility in earlier stage. Loss of kidney function, microvascular rarefaction, renal parenchyma destruction are the final consequences due to accumulation of excessive ECM. Pericytes separate from endothelium, migrate and proliferate, and ultimately transformed into myofibroblasts. This causes microvasculature to show instability along with myofibroblasts activation that leads to interstitial fibrosis (J. Yang & He, 2020).

3.3 Factors contributing to CKD

CKD is a global public health issue that raises the risk of cardiovascular morbidity and mortality along with ESRD. To enhance personal and public health, recognition of the risk factors of CKD is crucial to prevent or slow down the onset of ESRD. An individual is predisposed to kidney disease as a result of their genetic and phenotypic make-up. Some significant risk factors for CKD include gender, age, African ancestry, dietary habits, socioeconomic status, ethnicity (Noble & Taal, 2019), and family history of kidney disease. Additionally, kidney disease is caused by some other factors such as obesity, diabetes mellitus,

hypertension, smoking, and so on. The progression to ESRD is effortless and rapid for an uncontrolled diabetic or hypertensive patient. Additional hazards involve uncontrolled alcohol consumption, taking analgesic drugs, and being exposed to heavy metals. Over and above that, some further risk factors are a history of cardiovascular disease, previous acute renal damage, glomerulonephritis, multisystem diseases, urinary tract obstruction (Noble & Taal, 2019), metabolic syndrome, malignancy, hepatitis C virus, hyperlipidemia, and HIV infection. For initial assessment, it is satisfactory to determine the serum creatinine levels and urinalysis for the patients at high risk of CKD (Kazancioğlu, 2013).

3.4 Existing treatment options for CKD

The economic burden of CKD will cause a rising burden on the public health care system in order to the increasing prevalence of CKD along with the requirement of therapies for life affirmation. Previously reported studies confirmed that CKD progression in terms of pathogenesis is due to glomerular capillary hypertension and compromised screening function with excessive protein. Besides, a major individual forecaster of progression is proteinuria and it has a renoprotective effect. An updated recent CKD therapy is RAAS. To slow down the progression of ESRD and enhance renal activity descending of the disease is achieved at comparable management of blood pressure, a decline of proteinuria by the renin-angiotensin system (RAS) inhibitors, also angiotensin converting enzyme (ACE) inhibitors, and angiotensin II receptor blockers (ARBs) are highly efficacious than non-renin-angiotensin system (non-RAS) inhibitor therapy. RAS inhibitors postpone the construction of microalbuminuria and its growth into macroalbuminuria in patients with diabetes. Whereas, the overabundance of cardiovascular mortality along with diabetic kidney dysfunctions is decreased by ACE inhibitors. Moreover, along with RAS inhibitors, the optimally controlled several risk factors of CKD, corresponding cardiovascular morbidity, integrative techniques

involving multidrug therapy, and updated lifestyle. Further experiments are required to investigate new efficacious drugs for renoprotective therapies with cost effectiveness (Ruggenenti et al., 2012). Some common drugs for the treatment of CKD are mentioned in Table 3.1 and they are discussed below.

Glucocorticoids:

Glucocorticoids are potent immunosuppressant and anti-inflammatory drugs. Prednisone binds to GRs and forms glucocorticoid receptor/glucocorticoid complex. This complex inhibits several steps in immune response and inflammatory pathways, and changes gene expression. Additionally, this complex engages with activator protein 1 (AP-1) and pro-inflammatory transcription factors nuclear factor κB (NF-κB) and therefore, decreases their functions. Furthermore, prednisone and prednisolone both are used to treat nephrotic syndrome (Schijvens et al., 2018).

Alkylating agent:

Cyclophosphamide (CYC) is an alkylating agent and a nitrogen mustard prodrug. It gets oxidized by the cytochrome P450 (CYP). Phosphoramide mustard is generated by CYP3A5 and CYP2B6 isoforms. This mainly cross-links with the DNA and shows important effects. This includes an alkyl group aggregation in guanine of the imidazole ring on the nitrogen 7. Additionally, the formation of covalent bonds causes disrupts DNA double strands. DNA replication stops and causes cell death (Alamilla-Sanchez et al., 2021).

Purine analog:

Azathioprine is a prodrug as well as an imidazole derivative. It is metabolized into mercaptopurine and 6-thioinosinic acid through a non-enzymatic process and glutathione transferase respectively. These metabolites are excreted by erythrocytes and liver. Inactivation of 6-mercaptopurine follows two routes. Firstly, TPMT enzyme catalyzes methylation and generates methyl-6-mercaptopurine. Secondly, xanthine oxidase catalyzes oxidation reaction

and produces 6-thiopuric acid. Further, methyl monophosphate-thioinosine integrates into DNA, purine synthesis, and inhibits DNA replication. Furthermore, thioguanosine triphosphate stimulates apoptosis by Bcl signaling and inhibits RNA. Therefore, lymphocyte replication is influenced more potently (Alamilla-Sanchez et al., 2021).

Aldosterone receptor antagonist:

Finerenone is an aldosterone receptor antagonist that inhibits aldosterone and cortisol binding and is associated with reduction of inflammation and fibrosis that affect the progression of CKD in patients with diabetes. It shows its action by inhibiting overstimulation of mineralocorticoid receptors. It also causes cardiovascular risk reduction, detains CKD progression, and suppression of endothelial injury. Patients with CKD and type 2 diabetes mellitus (T2DM) cause a reduction in albuminuria due to finerenone (José Luis Górriz et al., 2023).

Inositol monophosphate dehydrogenase (IMPDH) inhibitors:

IMPDH inhibitors such as mycophenolate mofetil (MMF) reduce the cell-mediated immune responses and antibody production by blocking enzyme inosine monophosphate dehydrogenase and inhibiting proliferation of activated lymphocytes (B and T). The nucleic acid synthesis inhibition can be caused by MMF. Moreover, it causes phase S cell arrest, blocks DNA incorporation, and reduces de novo synthesis of guanine (Alamilla-Sanchez et al., 2021).

Loop diuretics:

An example of loop diuretics is furosemide. It blocks sodium-potassium-chloride co-transporter in tubular epithelial cells in thick ascending limb of the loop of Henle. The reabsorption of sodium, chloride, and water from filtered fluid is inhibited by this function in the kidney tubules and urine excretion gets highly increased. In tubular lumen, furosemide binds to albumin and reduce unbound active drug level which can interact with tubular

receptors. Thus, furosemide tubular secretion is decreased in hypoalbuminemia patients (Khan et al., 2023).

Thiazide diuretics:

Thiazide diuretics such as chlorothiazide blocks reabsorption of active chloride by Na-Cl cotransporter in early distal tubule. Following this sodium along with chloride, and water excretion increases and causes diuretic effect. Additionally, it binds with thiazide-sensitive sodium-chloride transporter and blocks sodium ion transport across the renal tubular epithelium. Sodium-potassium exchange mechanism elevates excretion of potassium (Minutolo et al., 2022).

Potassium-sparing diuretics:

Spironolactone is a mineralocorticoid receptor antagonist and its working mechanism involves RAAS in the distal tubules of kidney. Spironolactone promotes excretion of sodium and water in urine by the inhibition of aldosterone that prohibits reabsorption. The blood pressure and fluid overload decrease by diuretic effect in kidney patients (Baran et al., 2021).

Angiotensin II receptor blockers (ARB):

Angiotensin II receptor blocker (ARB) such as eprosartan selectively blocks angiotensin II to the AT_1 receptor binding and thus, inhibits aldosterone-secreting effects and vasoconstrictor of angiotensin II (Alamilla-Sanchez et al., 2021).

Statin:

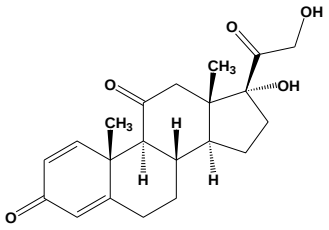
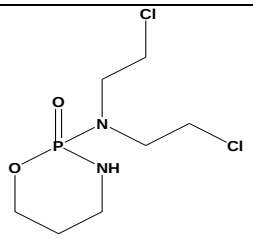
A statin medication is atorvastatin. It is a competitive inhibitor of HMG-CoA reductase enzyme. In cholesterol biosynthesis process, the production of mevalonate from HMG-CoA is an early rate-limiting reaction. It decreases cholesterol production through inhibiting this enzyme. The LDL receptor activity elevates due to this on the lever tissues and thus, LDL

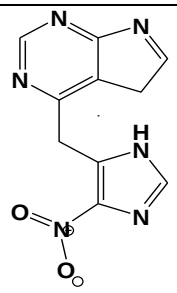
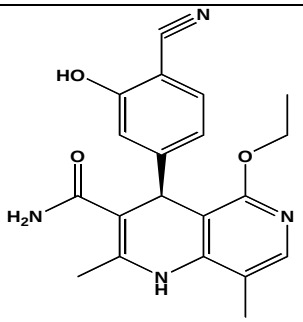
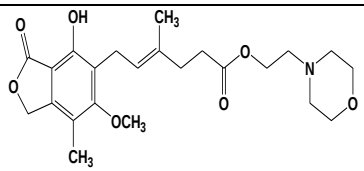
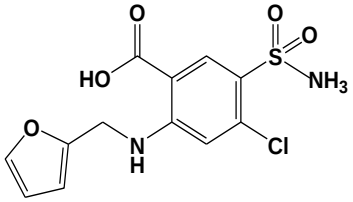
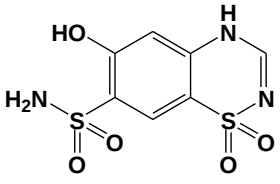
cholesterol is eliminated from the blood. It demonstrates anti-inflammatory activity which diminishes inflammation in CKD (Agarwal, 2007).

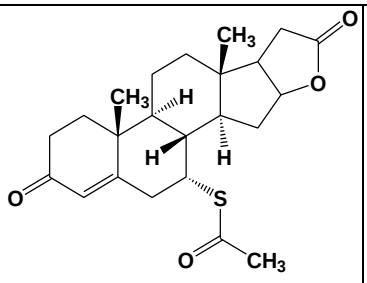
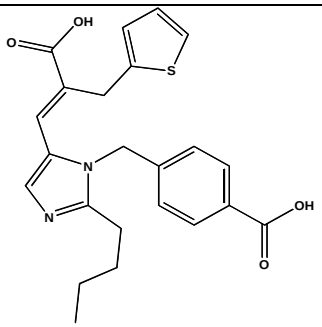
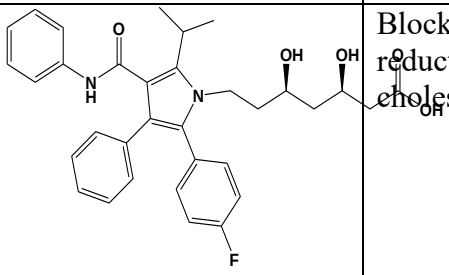
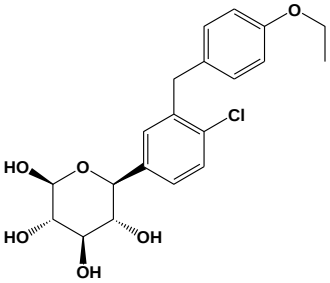
SGLT-2 inhibitors:

Dapagliflozin is a selective SGLT-2 inhibitor that inhibits SGLT-2 protein and causes different protective actions in kidneys. Kidney damage gets decreased by attenuating blood pressure, increasing kidney inflammation and pressure, and prohibiting protein leakage into urine. The severity of declining kidney function, CVD or kidney related death, and end-stage kidney disease is decreased by dapagliflozin in CKD. Some functions of this drug are reducing blood pressure, metabolic effects like minimizing uric acid, glucose, and triglycerides, and hemodynamic effects (Nashar & Khalil, 2022).

Table 3.1: *Common drugs and their mechanism characteristics.*

Classification	Drug name	Structure	Mechanism of action	Reference
Glucocorticoids	Prednisone		Decrease leukocytes migration to the site of inflammation, and decrease vasodilation and permeability of the capillaries.	(Schijvens et al., 2018)
Alkylating agent	Cyclophosphamide		Cell death and cycle arrest via interfering with DNA.	(Alamilla-Sanchez et al., 2021)

Purine analog	Azathioprine		Inhibits purine synthesis causing declining DNA and RNA production, leading to immunosuppression.	(Alamilla-Sanchez et al., 2021)
Aldosterone receptor antagonist	Finerenone		Binds and blocks mineralocorticoid receptors.	(José Luis Górriz et al., 2023)
Inositol monophosphate dehydrogenase (IMPDH) inhibitors	Mycophenolate mofetil (MMF)		Reduces cell-mediated immune responses and antibody production and inhibits proliferation of activated lymphocytes.	(Alamilla-Sanchez et al., 2021)
Loop diuretics	Furosemide		Inhibits the active reabsorption of NaCl in the loop.	(Khan et al., 2023)
Thiazide diuretics	Chlorothiazide		Inhibits cortical distal convoluted tubule Na^+ reabsorption.	(Minutolo et al., 2022)

Potassium-sparing diuretics	Spironolactone (Aldosterone antagonist)		Binds to mineralocorticoid receptors and promotes sodium and water excretion while retaining potassium.	(Baran et al., 2021)
Angiotensin II receptor blockers (ARB)	Eprosartan		Blocks aldosterone secretion and vasoconstriction.	(Alamilla-Sanchez et al., 2021)
Statins	Atorvastatin		Blocks HMB-CoA reductase to inhibit cholesterol synthesis.	(Agarwal, 2007)
SGLT-2 inhibitor	Dapagliflozin		Induces urinary glucose excretion.	(Nashar & Khalil, 2022)

Chapter 4: Vitamin D levels and CKD

4.1 An overview of vitamin D levels in the body

Based on expertise, the concentration of 25-hydroxyvitamin D in the serum is utilized to calculate vitamin D status in the body. It should be equal to or larger than 30 ng/mL (75 nmol/L) (Table 4.1) (Franca Gois et al., 2018). When the level of 25-hydroxyvitamin D in the serum is between 20 to 29 ng/mL (50-74 nmol/L), it is defined as vitamin D insufficiency (VDI). Contrarily, the level of 25-hydroxyvitamin D in the serum is acknowledged as vitamin D deficiency when it falls under 20ng/mL (50 nmol/L). Conversely, peak serum 25-hydroxyvitamin D level has been a subject of investigation. However, overindulgent exposure to sunlight does not induce vitamin intoxication. The maximum serum vitamin D level diagnosed in a farmer in Puerto Rico was 225 nmol/L. Moreover, artificial UVB sources expanded the level of vitamin D that was 273.6. When 25-hydroxyvitamin D gets greater than 150 ng/mL coupled with hyperphosphatemia, hypercalciuria, and hypercalcemia leads to vitamin D intoxication. The intoxication risk might be elevated at above 125-150 nmol/L and it needs to be circumvented. Vascular calcification may also be affected by vitamin D, though, the mechanism of action is not illuminated (Franca Gois et al., 2018). Table 4.1 demonstrates the serum [25(OH)D] concentration. An insufficient level of serum [25(OH)D] concentration can be achieved within 12-20 ng/mL (30-50 nmol/L). Whereas, deficiency of serum [25(OH)D] concentration can be caused between 5-12 ng/mL (12-30 nmol/L). Moreover, severe deficiency can be seen when serum [25(OH)D] concentration is <5 ng/mL (<12 nmol/L) in the human body (Norman, 2008).

Table 4.1: *25-hydroxyvitamin D [25(OH)D] concentration by nutritional status of Vitamin D in blood plasma.*

Serum 25(OH)D scale	Nutritional status of Vitamin D	Reference
<5 ng/mL (<12 nmol/L)	Severe deficiency	(Norman, 2008)
5-12 ng/mL (12-30 nmol/L)	Deficiency	(Norman, 2008)
12-20 ng/mL (30-50 nmol/L)	Insufficiency	(Norman, 2008)
>30 ng/mL (>75 nmol/L)	Sufficiency	(Franca Gois et al., 2018)

4.2 Synthesis, metabolism and biological functions of vitamin D

Vitamin D is transferred in the liver and metabolized into 25-hydroxyvitamin D after being feasibly ingested through a dietary source or synthesized in the skin resulting from ultraviolet irradiation of 7-dehydrocholesterol (7-DHC) also known as provitamin D3 and the penultimate metabolite in the synthesis of cholesterol (Figure 4.1). UV light absorbs through 7-DHC and generates pre-vitamin through an impact on electrocyclic burst of 9,10 bonds. The former seems a physiologically inactive molecule as it isomerizes to vitamin D_3 impulsively (Zhu & Okamura, 1995). Vitamin D is a substrate for 1-α-hydroxylase enzyme (CYP27B1) and is largely stored as 25-hydroxyvitamin D which is further converted into its principal active metabolite form 1,25-dihydroxyvitamin D. Following this, vitamin D_3 is carried into the liver for metabolic alteration needed for hormonal activity through vitamin D binding protein (DBP), a plasma protein. Plasma parathyroid hormone levels and serum phosphorus and calcium levels strictly control 1,25-dihydroxyvitamin D production through the kidney. In the kidney and in many other tissues, another process is well established that cholesterol side chains cleave by the CYP11A1 enzyme breaks down vitamin D_3 for producing 20-hydroxyvitamin D which has several significant physiological functions in the body. Fibroblast growth factor 23 (FGF23) suppresses the synthesis of 1,25-dihydroxyvitamin D and internalized sodium phosphate cotransporter through small intestine and kidney cells (Bacchetta et al., 2012). 1,25-

dihydroxyvitamin D accompanies the renal calcium absorption effectiveness and the intestinal phosphorus and calcium effectiveness. Plasma delivers hormone 1,25-dihyroxyvitamin D to target cells such as those in the kidney, intestine, bone, and others (Figure 4.1). Furthermore, it promotes the synthesis of 25-hydroxyvitamin D-24-hydroxylase (CYP24) enzyme which converts 1,25-dihydroxyvitamin D and 25-hydroxyvitamin D to fabricate calcitroic acid that is water soluble and biologically inactive (Holick, 2007). Moreover, 1,25-dihydroxyvitamin D binds with hormone receptors in these target cells for example VDR. This receptor or other receptors and ligands association manifest responses either genomic or non-genomic (Zhu & Okamura, 1995).

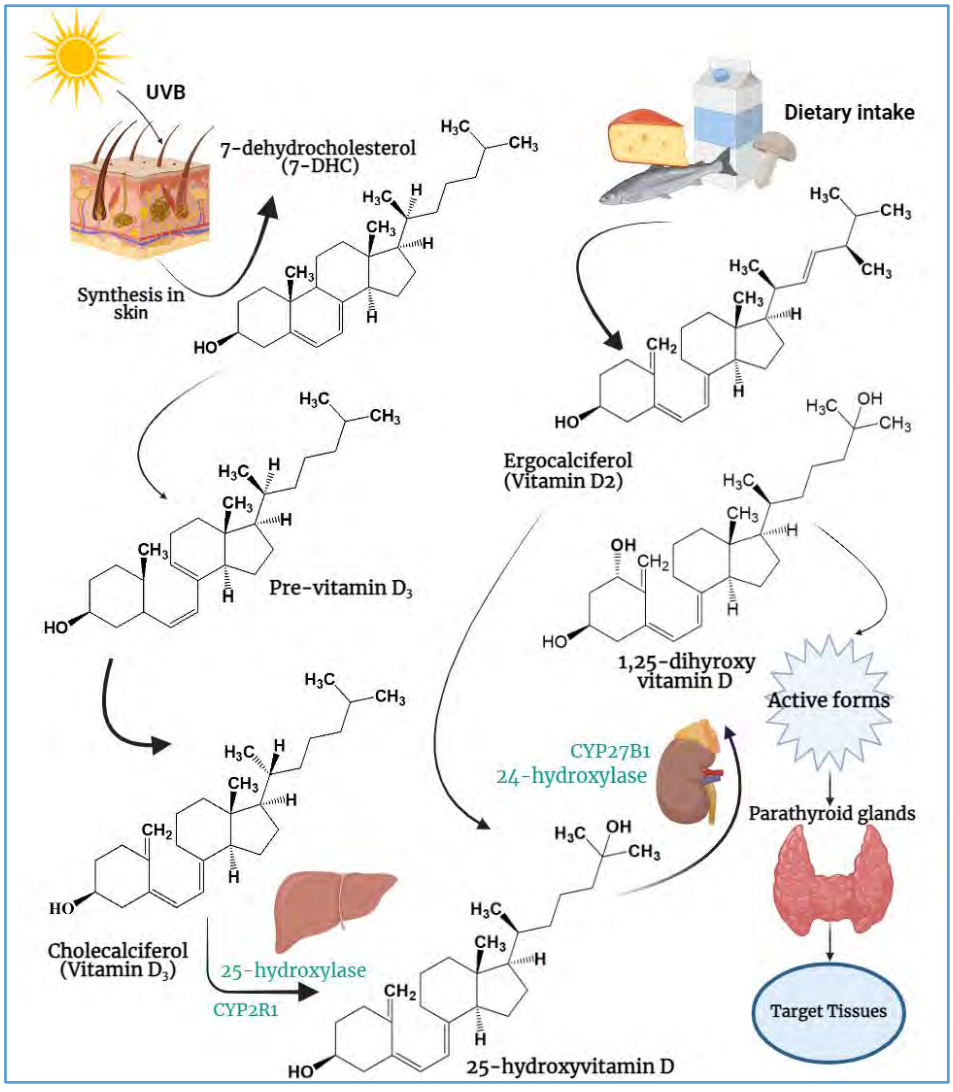


Figure 4.1: Synthesis and metabolism of vitamin D.

4.3 Factors influencing vitamin D levels

Insufficiency or deficiency of vitamin D has affected one billion people globally. Sufficient quantities of Vitamin D are retained by oral consumption and cutaneous photosynthesis process. The bioavailability and photosynthesis of vitamin D are regulated through several factors that increase the risk of inadequate vitamin status (Tsiaras & Weinstock, 2011). The expanding worldwide population, food turbulence, deteriorating agricultural lands, exploitation, pollution, and competition for resources are essential factors for vitamin D deficiency (VDD) that grows in under- and malnutrition. Unemployed or deprived women are mainly affected by food turbulence and have a greater likelihood of having VDD compared to men as a consequence of socio-economic status. One more factor is public obliviousness to VDD, its effects, and preventative measures. Additionally, vitamin D level in the body depends on both endogenous (sun exposure) and exogenous (diet) synthesis. It is produced two to three times more by the skin than by diet. Even so, endogenous synthesis is efficacious for only 15% of the irradiation because of environmental inducement. The level of vitamin D can be enhanced through the portion of skin exposure to solar radiation either weekly three times or 10 to 15 minutes daily at mid-day. Vitamin D_3 is produced at the peak wavelength of 297 nm and the optimum wavelength is between 295 nm and 300 nm. Following that, the factors associated with the duration of exposure are classified into three categories. The first category is based on an individual's environmental circumstances including regions (latitude, altitude, and terrestrial environment), atmospheric conditions such as high levels of nitrogen oxides (NO_x) and nitrogen dioxides (NO_2), particular matter ($PM_{2.5}$ and PM_{10}). This category also includes climate, and season (Table 4.2). Shorter daylight during winter or spring shows 28 ng/mL of vitamin D level in plasma and longer daylight such as summer or autumn shows 30 ng/mL of vitamin D level in plasma. The second category is based on biological aspects including ethnicity, gender, health condition, pigmentation on the skin, and age. Then vitamin

D intake necessity for elderly population is higher than younger population (Wang et al., 2020). The last category is based on social and socioeconomic aspects persuading physical activity, habits, and lifestyle (Edis & Bloukh, 2016). Different ethnic population shows different levels of vitamin D deficiency such as Asians, Chinese, Uighur, Kazak have 15 ng/mL, 20.85 ng/mL, 15.13 ng/mL, and 13.94 ng/mL respectively (Correia et al., 2014) (Table 4.2).

Table 4.2: *Risk factors that influence vitamin D status.*

Factors	Influence on Vitamin D Level	Reference
Regions (latitude, altitude, and terrestrial environment)	25(OH)D decreases as follows: Germany (3.03 ng/mL) Finland (3.28 ng/mL) Scotland (5.41 ng/mL) Spain (9.28 ng/mL)	(Oskarsson et al., 2021)
Atmospheric conditions such as high levels of nitrogen oxides (NO_x) and nitrogen dioxides (NO_2), particular matter ($PM_{2.5}$ and PM_{10})	25(OH)D decreases in 9.11, 2.47, and 0.56 nmol/L due to 10 $\mu g/m^3$ increase in $PM_{2.5}$, PM_{10}, NO_x, and NO_2 respectively.	(Yang et al., 2021)
Climate	Below 30 ng/mL	(García-Dorta et al., 2021)
Season	Shorter daylight (Winter/Spring) (28 ng/mL) Longer daylight (Summer/Autumn) (30 ng/mL)	(Wang et al., 2020)
Ethnicity	Asians (15 ng/mL) Chinese (20.85 ng/mL) Uighur (15.13 ng/mL) Kazak (13.94 ng/mL) 25(OH)D decreases as follows: Hispanic (3.41 ng/mL) Non-Hispanic (7.47 ng/mL)	(Correia et al., 2014)
Gender (Female)	Female (38.40±12.37 nmol/L) Male (43.49±14.78 nmol/L)	(Yan et al., 2019)
Health condition (Impaired 25(OH)D tubular reabsorption, Adiposity, Calcineurin inhibitor Prescriptions, Proteinuria, Peritoneal dialysis, Diabetes mellitus)	Greater than 28 to 32 ng/mL.	(Hossein-nezhad & Holick, 2013)

Skin pigmentation (Reduced skin synthesis of vitamin D)	Dark skin color (8.6 ng/mL) Light skin color (11.8 ng/mL)	(Richard et al., 2017)
Age	26–35 years (26 ng/mL) 36–45 years (27 ng/mL) 46–55 years (30 ng/mL) 56–65 years (33 ng/mL)	(Wang et al., 2020)
Diet, physical activity (Low)	18.1±6.3 ng/mL	(Wang et al., 2020)
Lifestyle and habits	Indoor workers (28.61 ± 8.92 ng/mL)	(Wang et al., 2020)

4.4 Association of vitamin D levels and CKD progression

CKD progression is ascribed to the cellular function activation and contribution that is generated in different compartments in the kidney, along with that, this mechanism gets expanded through biochemical pathways of cellular impairments and extended from their origin and the reason behind the increasing number of dysfunctional nephrons and the progression of CKD. Then, the extracellular matrix prolongation can be caused by angiotensin II induced TGF- β1. Moreover, it leads to oxidative stress that stimulate TGF- β1 in surrounding cells and induces fibrosis response (Agarwal & Nath, 2020). Further, VMDR is the assessment of CYP24A1-mediated vitamin D clearance that is significantly associated with the reasons linked with mortality in CKD patients regardless of the progression in ESRD. Furthermore, renal ischemic injury slowly progresses due to elevated vitamin D status according to the previous reports. Angiotensin II decreases renal damage and inflammation demonstrated by the suppression of vitamin D of hypertension in CKD patients (Bansal et al., 2019). Additionally, renal incident is associated with vitamin D insufficiency by increasing risk of severe CKD stage. CKD progression may be delayed by regulating the serum calcitriol level above 23 ng/ml. The primacy site of vitamin D bio-activation to 25(OH)D is liver. Also, vitamin D deficiency cannot be treated through either calcitriol or its analogs (Lee et al., 2023). Vitamin D therapy is compromised in CKD patients due to abnormalities in vitamin D levels for example in patient with renal damage, CV damage, systemic inflammation, hypertension,

and so on. Therefore, it is necessary to maintain vitamin D status appropriately to decrease the progression of CKD and to increase the patient outcomes (Dusso et al., 2021). Vitamin D deficiency is common in CKD patients and 50–100% of CKD patients under dialysis have below 75 nmol/L (30 ng/mL) of 25(OH)D levels (Shroff et al., 2015). On the other hand, low vitamin D levels cause significant improvement and death risk in CKD. The mortality risk decreases by 14% with the elevation of every 4 ng/mL (10 nmol/L) vitamin D level (Pilz et al., 2011). Nonetheless, there is a complicated association between CKD and vitamin D levels. The rapid cure was seen in CKD patients due to inflated doses of calcitriol because of the elevated level of phosphate in serum (Schön et al., 2021). According to Table 4.3 the controlled healthy individuals has higher levels of 25(OH)D than the levels of 25(OH)D in CKD patients in different countries including Finland, Austria, Netherlands, UK (England), North America, USA, Indonesia, South Korea**,** Thailand**,** South Africa, Gambia, Nigeria. In Thailand, the controlled group population has 67·6 nmol/L while the CKD patients have 55.9, 46.3 nmol/L of 25(OH)D level. Then, the controlled group population has 43·6 nmol/L while the CKD patients have 11.2 ± 6.5 nmol/L (Hilger et al., 2013) of 25(OH)D level in Finland. Further, the controlled group population has 36.9 nmol/L while the CKD patients have 47.5 ± 35.0 nmol/L of 25(OH)D level in South Africa. Moreover, in UK (England), the controlled group population has 40·0†k; 37·4‡k 58·3†; 49·4‡ nmol/L (Kim et al., 2011) while the CKD patients has 37.2 nmol/L of 25(OH)D level. In South Korea, the controlled group population has 46·1nmol/L while the CKD patients has 52, 47 nmol/L of 25(OH)D level (Hilger et al., 2013) (Table 4.3).

Table 4.3: *Comparison of Vitamin D levels in controlled healthy individuals and CKD patients.*

Country	**25(OH)D (nmol/L) in CKD patients**	**25(OH)D (nmol/L) in controlled healthy individuals**	**Reference**
Austria	25	52·2	(Hilger et al., 2013)
Finland	11.2 ± 6.5	43·6	
Netherlands	35	53·2	

UK (England)	37.2	40·0†k; 37·4‡k 58·3†; 49·4‡	(Kim et al., 2011)
North America	61.5	67·7	
USA	65	67·4†; 57·7‡	
Indonesia	50.25, 49.75	68·2	
South Korea	52, 47	46·1	
Thailand	55.9, 46.3	67·6	
South Africa	47.5 ± 35.0	36.9	
Gambia	25.6‡	97.7	
Nigeria	43.2	66.8	

† 25(OH)D is the value for male.

‡ 25(OH)D is the value for female.

4.5 Treatment of CKD with vitamin D supplements

The necessity of vitamin D supplementation in CKD patients is due to bone and mineral metabolism through the effectiveness of vitamin D along with their impact on other physiological actions because of the broad expression of vitamin D. These includes blood pressure regulation through the direct renin-angiotensin aldosterone system (RAAS) inhibition, insulin secretion regulation, anti-inflammatory action, lipid metabolism, immune system modulation, and decline in CVD, which prevents the advancement of CKD. The oversight of CKD such as hyperparathyroidism regulation has been accomplished with vitamin D and derivatives. Based on the mechanisms, the effectiveness the physiological activity of vitamin D analogs is differentiated. Supplementation can be done either by adequate dietary sources enriched with vitamin D, that is the nonbiological active forms including cholecalciferol (vitamin D_3), ergocalciferol (vitamin D_2), or by oral intake of vitamin D. Currently, the establishment of four different vitamin D analogs such as falecalcitriol, paricalcitol, oxacalcitriol, and doxercalciferol enhance CKD therapy (Table 4.4). Oral paricalcitol reduces PTH level, TGF- β1, and C-reactive protein concentration in plasma and also minimizes urinary protein-creatinine ratio. Besides, paricalcitol demonstrates an antiproteinuric effect and

residual proteinuria is decreased by other active vitamin D analogs including calcitriol, and paricalcitol in patients with CKD. This ensures a protective barrier to enhance the advancement of renal dysfunction (Zhu et al., 2015).

Cholecalciferol:

Cholecalciferol has multiple mechanism of action in CKD including antiproteinuric effect, anti-inflammatory, antifibrotic effect, and anti-anemic effect. cholecalciferol can be used as an adjuvant to renin-angiotensin system (RAS) blockers to lower residual proteinuria in individuals with CKD. It also acts as a substitute in patients who are intolerant to these medications. It stimulates phosphate and calcium absorption from small intestine, enhance phosphate resorption of renal tubule, and aid calcium secretion from bone to blood. Moreover, it has potential to alleviate anemia along with lowering ESA dosage due to PTH suppression and anti-inflammatory effect. Cholecalciferol administration reduces C-reactive protein levels along with decrease in erythropoietin dosing and inflammation reduced by enhancing ESA responsiveness. The release of profibrotic cytokines such as TGF-β is suppressed by exhibiting nephroprotective effect. The level of urinary TGF-β1 and albuminuria decrease by cholecalciferol administration (Giannini et al., 2017).

Ergocalciferol:

Ergocalciferol has mechanism of action in CKD involves in improving nitric oxide generation, decreasing oxidative stress, and increasing eNOS expression for enhancing microcirculatory endothelial functions and eliminate adverse effects in endothelium of uremia in CKD. eNOS Expression is enhanced by ergocalciferol to increase nitric oxide generation for maintaining endothelial functions. Moreover, microcirculatory function and vasodilation regulated by increases nitric oxide production. Further, tissue oxidative stress gets decreased through

ergocalciferol administration to reduce risk of cardiovascular events and improve endothelial function (Mangoo-Karim et al., 2015).

Calcifediol:

Calcifediol regulates calcium homeostasis in CKD patients. Some important mechanism of action involves suppressing FGF23 levels, PTH levels regulation, and calcium homeostasis regulation to improve secondary hyperparathyroidism. The increase in intestinal calcium absorption through calcifediol regulates calcium homeostasis in blood. The regulation of PTH level get suppressed due to elevated calcium level by calcifediol. Calcitriol generation also persuaded by calcifediol. The serum 25(OH)D levels get elevated by calcifediol to regulate calcium levels and bone health. Calcifediol enhances 25(OH)D status and decreases PTH status in CKD by modification in metabolism pathways and peak onset (Nigwekar et al., 2012).

Paricalcitol:

VDR agonist and reduces PTH in CKD. VDRs get selectively activated to reduce bone mobilization and intestinal calcium absorption. In order to decrease PTH level and elevate serum calcium and phosphorus levels, paricalcitol is used. Paricalcitol shows pleiotropic effects in CKD patients (Cheng & Coyne, 2006).

Falecalcitriol:

Falecalcitriol suppresses PTH and decrease PTH status and phosphate in secondary hyperparathyroidism. The decreased inactivation and altered side chain metabolism cause strong and long-lasting effects of falecalcitriol. It gets metabolized by Cyp24 enzyme to a metabolite named C-23S hydroxylated (Nigwekar et al., 2012).

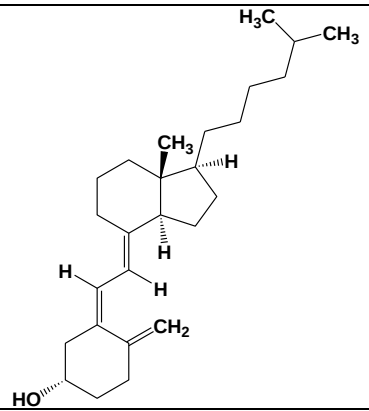
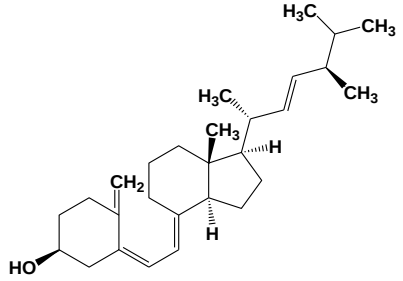
Oxacalcitriol:

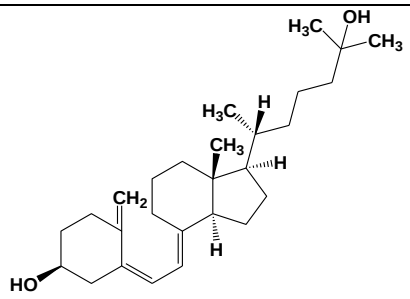
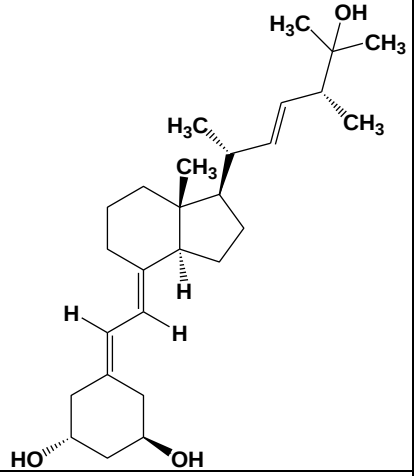
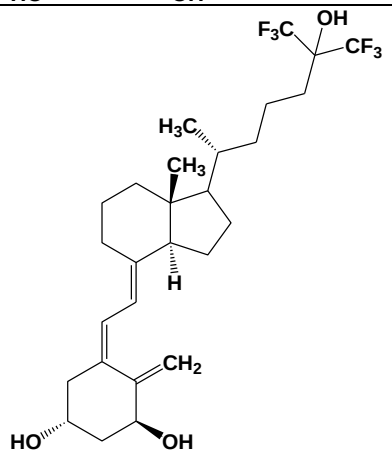
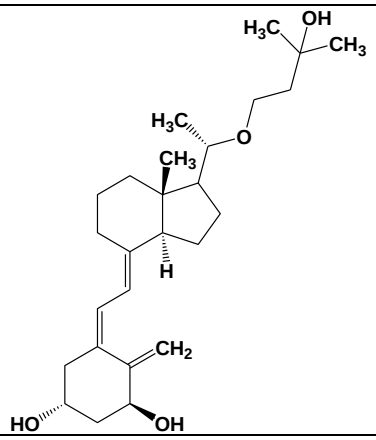
Oxacalcitriol detects secondary hyperparathyroidism in CKD by converting calcitriol structure to accumulate PTH suppression and increase VDR affinity. Oxacalcitriol enhances calcium absorption from GIT to regulate blood calcium levels. Also, calcium reabsorption in the renal tubules caused by oxacalcitriol to decrease loss of calcium in urine. Moreover, due to the activation of osteoclasts, calcium release from bone is maintained by oxacalcitriol (Pavlovic et al., 2015).

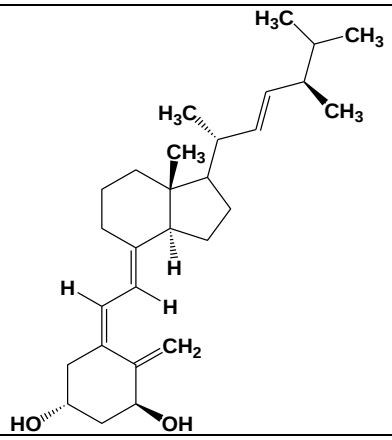
Doxercalciferol:

Doxercalciferol inhibits the production and secretion of PTH by producing active 1,25-$(OH)_2D_2$ by hepatic metabolism that binds to VDR to stimulate specific response (Pavlovic et al., 2015).

Table 4.4: *Available drugs with their working mechanism.*

Drugs	Structure	Mechanism of action	References
Cholecalciferol [25(OH)D_3]		Stimulates phosphate and calcium absorption from small intestine, enhance phosphate resorption of renal tubule, and aid calcium secretion from bone to blood.	(Giannini et al., 2017)
Ergocalciferol [25(OH)D_2]		Improve endothelial functions and eliminate adverse effects in endothelium of uremia in CKD	(Mangoo-Karim et al., 2015)

Calcifediol		Enhances 25(OH)D status and decreases PTH status in CKD by modification in metabolism pathways and peak onset.	(Nigwekar et al., 2012)
Paricalcitol		VDR agonist and reduces PTH in CKD.	(Cheng & Coyne, 2006)
Falecalcitriol		Suppress PTH and decrease PTH status and phosphate in secondary hyperparathyroidism.	(Nigwekar et al., 2012)
Oxacalcitriol		Detect secondary hyperparathyroidism in CKD by converting calcitriol structure to accumulate PTH suppression and increase VDR affinity.	(Pavlovic et al., 2015)

Doxercalciferol		Inhibits the production and secretion of PTH by producing active 1,25-$(OH)_2D_2$ by hepatic metabolism that binds to VDR to stimulate specific response.	(Nigwekar et al., 2012)

Chapter 5: VDR gene polymorphism and CKD

5.1 Vitamin D receptor and its significance in functioning of vitamin D

Vitamin D receptor (VDR) is a nuclear receptor that mediates the most common features of the active metabolite of vitamin D which is 1,25-dihydroxyvitamin D [1,25$(OH)_2$ D_3]. The VDR gene is composed of 8 protein-coding exons (exons 2–9) and 6 untranslated exons (1a-1f) those are distinctively spliced and is located on chromosome 12q12.14 (Santoro, Lucisano, et al., 2015). Vitamin D shows multiple biological operations in cell proliferation and cell differentiation, immune response, calcium homeostasis, electrolyte, and blood pressure regulation. These important biological functions of vitamin D are executed via VDR-mediated control of target genes. VDR works as a ligand-inducible transcription factor and pertains to the nuclear hormone receptor superfamily (Haussler et al., 2012). VDR activation of any compound can be done by VDRA (Vitamin D receptor activator) (Zhu et al., 2015). VDR is responsible for the biological reaction to 1,25$(OH)_2$ D_3 hormone and is detected as a chromatin-associated protein which interacts with 1,25$(OH)_2$ D_3. VDR ligands stimulate the relationship between VDR and RXR (retinoid X receptor) isoform which is its heterodimeric partner. The formation of this liganded VDR-RXR heterodimer permits the identification of VDREs (vitamin D-responsive elements) regulated by vitamin D in the gene sequences of DNA. 1,25$(OH)_2$ D_3-VDR shows its activity by genomic processes while the hormone receptor complex exhibits its functions quickly by non-genomic processes. Additionally, 1,25$(OH)_2$ D_3 in intestinal calcium transport stimulation is regulated through VDR-independent mechanisms (Haussler et al., 2012).

5.2 VDR gene polymorphisms and vitamin D metabolism

The binding ability of VDRs to its ligands can be altered through different gene polymorphism mechanisms. The metabolism of vitamin D to its active form, calcitriol can be affected by VDR gene polymorphisms. The VDR genes encode a member of the steroid or thyroid hormone receptor superfamily and can be found on chromosome 12. 427 amino acid residues contain VDR protein that is soluble and found in the nucleus, cytoplasm, and cell membrane. The metabolism of calcium, neurological system, immunological cells are suppressed by this VDR protein. Moreover, VDR isoform is the most significant one among the three distinct isoforms with 427 amino acids and 48kDa. The four most commonly investigated SNPs are BsmI (rs1544410), FokI (rs2228570), TaqI (rs731236), and ApaI (rs7975232) which may be associated with alterations in VDR protein structure and function (Ruiz-Ballesteros et al., 2020). VDR gene FokI and BsmI polymorphism are a great marker of altered vitamin D signaling pathways in CKD. A stubby VDR protein is induced in case of FokI polymorphism accompanied by transactivation capacity, whereas, mRNA sustainability and gene expression of VDR are altered in ApaI and BsmI polymorphisms. Therefore, the binding affinity of vitamin D metabolite and cellular consequences have been affected by this alteration of genes. These mainly impact genes such as CYP24A1 and CYP27B1 that influence the maintenance of vitamin D metabolism (Santoro, Lucisano, et al., 2015).

5.3 Role of VDR gene polymorphism in CKD

VDR gene polymorphism has a significant role in CKD that includes frequency of the disease, progression of the disease, renal dysfunction, and so on. These may affect the binding affinity of active vitamin D to VDR due to the receptor protein sequence variation. Hence the level of

vitamin D goes down and causes a deficiency of vitamin D. VDR gene polymorphism leads to several CKD incidences that include the following:

Influence on activity and expression of VDR: VDR gene polymorphism can affect the binding ability to the active vitamin D, that further leads to CKD. The function and expression of VDR might be changed. Particularly, CKD is associated with TT allele of BsmI genotype, and the potential to bind with VDR decreases due to shorter proteins by this genotype, and thus, CKD development can progress (Elshamaa et al., 2022).

Correlation with emergence and progression of CKD: The development and occurrence of CKD have been linked to the VDR gene polymorphisms for example ApaI, BsmI, FokI, and TaqI. This cause circumferential ailments that includes diabetic nephropathy, lupus nephritis, and IgA nephropathy. CKD diagnosis may be affected through certain polymorphisms that may cause renal dysfunction, renal insufficiency to the patients with immunoglobulin A nephropathy (IgAN) (Mo et al., 2019).

Genetic deviation and pathogenesis: VDR polymorphisms highly affect the pathogenesis of Chronic kidney disease–mineral bone disorder (CKD-MBD). The calcium/PTH/calcitriol axis may be modified by the variation in VDR gene sequences that distort the bone health and mineral metabolism of CKD patients. Additionally, ethnic diversity in VDR polymorphisms impacts racial differences in CKD-MBD markers (Elshamaa et al., 2022).

Impact on CVD and mortality in CKD: VDR polymorphisms impact on protein status of VDR or affinity towards target organs or tissues. As a consequence, this influences the incidence of mortality in CKD. Moreover, CVD is correlated with VDR gene polymorphism in CKD patients which shows a significant influence of this in CKD (Santoro et al., 2015).

5.4 Mechanisms of VDR gene polymorphisms in CKD

VDR mRNA transcription and splicing can be damaged by specific polymorphisms including TaqI and BsmI which cause variations in expression in the VDR gene and its functions (Waziri et al., 2018). Additionally, transcriptional functions of VDR protein may be impacted as its length is altered by FokI polymorphism (Machado et al., 2007). Therefore, the pathways associated with CKD including fibrosis, calcium metabolism, vitamin D metabolism, and PTH level may be affected (Santoro et al., 2014). The susceptibility and complication to CKD can be changed by VDR polymorphisms (Mo et al., 2019). The risk of CKD is increased by TaqI and BsmI polymorphisms in certain populations. IgA nephropathy patients may suffer from renal dysfunction by FokI polymorphism (Santoro et al., 2014). The variation in PTH and vitamin D levels may emerge from CKD-MBD through VDR polymorphisms (Waziri et al., 2018).

5.5 Specific polymorphisms identified of CKD patients

Based on some studies, there is a correlation between VDR BsmI and TaqI gene polymorphism and CKD. Additionally, the susceptibility to vitamin D deficiency is a crucial finding due to BsmI TT and TaqI AG genotypes (Elshamaa et al., 2022). According to another study, CKD susceptibility is not correlated to VDR TaqI, ApaI, and FokI gene polymorphism in Caucasians and overall populations. Nevertheless, Asian population are susceptible to the risk of CKD because of FokI f allele, ff genotype, and FF genotype (Zhou et al., 2014). There are four different groups including Asian, African, Egyptian, and Caucasian according to ethnicity (Table 5.2). This finding might explain the mechanism for the different and contradictory results from generated association analysis between the VDR gene polymorphism and CKD in different ethnic populations. According to subsequent studies, the main factor influencing the

persistence of pre-existing secondary hyperparathyroidism after renal transplantation is its extent. The VDR BB genotype is linked to the lowest PTH levels both exhibiting lower set point values than the Bb and bb genotypes during transplantation and after stabilization. Despite considering calcium and phosphorus levels, patients with the BB genotype has more genetic alteration than those with the BB genotype. Additionally, the impact of the BsmI polymorphism in pre-dialysis patients show that BB individuals had lower levels of PTH and higher levels of calcitriol than bb individuals. These happen even after controlling for other factors that affect parathyroid function, such as diabetes, hyperalbuminemia, parathyroid hypofunction, and hypercalcemia. Then, FokI polymorphisms influence serum PTH levels in CKD patients because a higher PTH levels is found in FF group than those in both the Ff and ff groups (Santoro et al., 2012). Moreover, the bb genotype defends against immunosuppressive therapies after kidney transplantation and sHPT induced bone loss. Besides, VDR TaqI t allele is increased the risk of developing post-transplant diabetes mellitus. Whereas, the risk of adult severe stone disease increases due to the allele was linked to a 5.2-fold. Also, Tt and tt genotypes patients has higher urinary calcium levels than TT genotype patients. In contrary, children with a Tt/tt genotype compared to juvenile patients with the VDR TaqI TT genotype had an eight-fold increased risk for repeated stone episodes. Children who develop hypercalciuric stones have a considerably greater frequency of the ApaI AA genotype than healthy controls. Compared to the Aa/aa genotype, ApaI AA genotype is also linked to a 3.5-fold higher risk of idiopathic absorptive hypercalciuria (Santoro et al., 2012). In Caucasians or other general groups, the polymorphisms of the VDR Fok1, TaqI, and ApaI genes were not linked to the risk of CKD. But the FokI gene polymorphism was linked to an increased risk of CKD among Asians (Table 5.2).

Table 5.2: *The effects of VDR gene polymorphism on CKD patients and controlled healthy individuals.*

Ethnicity	SNP	Genotype	Analytical method/SA method	CKD patients (% Genotype)	Controlled healthy individuals (% Genotype)	Reference
African	ApaI	AA Aa aa	Fisher's exact test	41.6% 56.5% 1.86%	38.6% 61.4% 0.0%	(Waziri et al., 2018)
Egyptian	Apa I A/C (rs7975232)	AA AC CC	Kruskal-Wallis and Mann-Whitney tests	44.6% 44.3% 11.1%	50.0% 37.0% 13.0%	(Elshamaa et al., 2022)
Caucasian	ApaI	AA Aa aa	Cochrane Review Manager Version 5	34.09% 51.14% 14.77%	26.17% 53.74% 20.09%	(Zhou et al., 2014)
African	TaqI	TT Tt tt	Fisher's exact test	54.5% 35.5% 10.1%	44.1% 50.0% 6.0%	(Waziri et al., 2018)
Egyptian	TaqI A/G (rs 731236)	AA AG GG	Kruskal-Wallis and Mann-Whitney tests	18.0% 45.2% 36.7%	31.0% 34.0% 35.0%	(Elshamaa et al., 2022)
Caucasian	TaqI	tt Tt TT	Cochrane Review Manager Version 5	17.04% 47.72% 35.22%	34.09% 45.79% 40.18%	(Zhou et al., 2014)
African	FokI	FF Ff ff	Fisher's exact test	57.1% 41.7% 1.1%	52.3% 45.3% 2.3%	(Waziri et al., 2018)
Egyptian	FokI A/G (rs 2228570)	AA AG GG	Kruskal-Wallis and Mann-Whitney tests	25.9% 40.7% 33.4%	19.0% 39.0% 42.0%	(Elshamaa et al., 2022)
Caucasian	FokI	ff Ff FF	Cochrane Review Manager Version 5	9.65% 35.86% 54.48%	8.46% 34.61% 56.92%	(Zhou et al., 2014)

Asian	FokI	ff Ff FF	Cochrane Review Manager Version 5	37.5% 50% 12.5%	15.73% 43.82% 40.45%	(Zhou et al., 2014)
African	BsmI	BB Bb bb	Fisher's exact test	32.5% 65.7% 1.87%	27.4% 65.5% 7.14%	(Waziri et al., 2018)
Egyptian	BsmI C/T (rs1544410)	CC CT TT	Kruskal-Wallis and Mann-Whitney tests	34.1% 48.9% 17.0%	33.0% 57.0% 6.0%	(Elshamaa et al., 2022)

Chapter 6: Conclusion and future perspectives

Vitamin D deficiency is known to play a role in CKD progression. vitamin D level falls as a result of the insufficient binding to the VDR, therefore nucleotide variations in the VDR gene may influence transcript sustainability, transcript levels, or functional integrity of the VDR protein to some extent. Thus, vitamin D downstream pathways get troublesomely influenced. Nucleotide variations mainly occur as SNPs in the VDR gene. After reviewing several studies, the polymorphisms in TaqI and BsmI genes are mostly found in CKD patients and the polymorphism of FokI and ApaI genes in CKD patients are also found in some studies but the evidences are not sufficient enough. There is a need to investigate a broader range of ethnic groups or countries to explore the relationship between VDR gene polymorphism and vitamin D levels in CKD.

CKD patients with insufficient vitamin D levels need to seek advice from physicians and take vitamin D supplements to treat CKD. Moreover, CKD patients with reduced vitamin D levels require to get exposure to sufficient UVB to facilitate vitamin D synthesis. Additionally, vitamin D rich foods can also be recommended for CKD patient to help them meet the required vitamin D levels. In the future, if any other VDR gene polymorphisms are found that are linked to CKD, then the appropriate drugs need to be discovered/developed for the treatment of CKD by resolving VDR gene polymorphism.

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