Review of Literature
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The kidneys play a critical regulatory role in the balancing of in and out of fluids, excretion of urea, sodium, other salts, and water with fluid reabsorption. Nephrons ensure the renal function and homeostasis as a routine activity (Forster, 1965). It directly and indirectly influences the regulation of several organs (Johns, 2013). The majority of kidney failure cases are associated with hypertension (KDIGO, Clinical practice guideline, 2014), diabetes (Wu et al., 2016), and osteoporosis conditions (Miller, 2014). Indirectly it regulates bone formation and the formation of red blood cells. Another important regulatory role of the kidney is producing erythropoietin and 1, 25-dihydroxy vitamin D₃ (calcitriol), which are involved in the production of red blood cells and in bone formation. Diseases of the kidney are clinically classified into two categories, and majority of them are CKD, influenced by genetic and environmental factors, and another type is acute kidney disease caused by infections. The kidney filtering mechanism is schematically shown in Figure 2.1.

Acute kidney disease

Clinically, acute kidney disease is reported with a reduced kidney action of less than 10% of normal function (Schrier et al., 2004). An overexposure to metals, solvents, and certain antibiotics and medications can lead to acute kidney infection. Infections in the kidney may cause deregulation of the filtration rate and improper function. Obstruction in the urinary tract or renal artery also leads to acute kidney failure tumors (Chawla et al., 2014).
Figure 2.1. The cross sectional diagram of the kidney and the blood flow in the nephron.

**Chronic kidney disease**

The clinical condition of CKD is reported with loss or reduced kidney functions over a period of 3 months to one year. It is a silent process, until the last moment it is difficult to identify the symptoms. The final stage of CKD is called ESRD. At this stage, the kidneys cannot perform their routine activity and remove enough wastes and excess fluids from the body, and thus the patient needs dialysis or a kidney transplant as possible therapy. CKD patients present with severe kidney damage which leads to different complications such as anemia (low blood count), high blood pressure, weak bones, and nerve damage (KDOQI guideline, 2015).
The most common causes of CKD are

- Uncontrolled high blood pressure for many years
- High blood sugar over many years

Other causes that can lead to chronic kidney disease include:

- Kidney disease and infections such as acute kidney injury (AKI), polycystic kidney disease, pyelonephritis and glomerulonephritis.
- A narrowed or blocked renal artery
- Long term use of non-steroidal anti-inflammatory drugs (NSAIDs) such as celecoxib and ibuprofen.

**Public health importance of CKD**

CKD is a public life-threatening problem worldwide. In the US $48 billion dollar is spent annually for the treatment of CKD. Moreover, they spend 6.7% of the total medical care budget for kidney failure treatment. African-Americans and blacks suffer from severe CKD three times higher than Caucasians. In China, it is estimated that $558 billion will be lost in the next decade due to the effects of death and disability to heart disease and CKD. In Australia, $12 billion was allocated for the treatment of kidney failure (World Kidney Day Report – 2016). All these reports substantiate that CKD requires much attention and awareness among the medical society.

**Prevalence of CKD**

Noncommunicable diseases account for 70% of all deaths globally (Hunter and Reddy, 2013). CKD is the second leading noncommunicable disease, and each year millions die because of the unaffordable cost for treatment (Jha et al., 2013; World Kidney Day report – 2016). Currently, over 2 million people receive dialysis or renal replacement therapy just to stay alive (Couser et al., 2011). It is estimated that among
people aged 65 - 74 years, one in five men, and one in four women, have CKD (World Kidney Day report– 2016). Diabetes and hypertension account for two-third of CKD cases in the Western countries (Varma, 2015) and 40 - 60% in India (Rajapurkar et al., 2012). Till date, In India, there is no separate registry for CKD patients. However, as per the Indian council of medical research (ICMR) report, the prevalence of diabetes has increased to 7.1% and 28% in the urban population (Anjana et al., 2011). Likewise, the prevalence of hypertension is 17% (Panesar et al., 2013; Bhadoria et al., 2014). With the rising prevalence of diabetes and hypertension, CKD is also expected to rise in India.

**Estimation of kidney function**

The determination of GFR in correction with the body weight gives a more accurate assessment of the kidney function. Blood samples of creatinine or cystatin C are mainly used to estimate the GFR level. Besides this, other factors such as age, gender and body weight are inserted to the following formula to obtain a relatively good estimate of the renal function.

Cockcroft-Gault formula:

\[
eGFR \text{ (men)} = \frac{(1.23 \times (140 - \text{age}) \times \text{weight})}{\text{serum – creatinine (μmol/L)}}
\]  
\[
eGFR \text{ (women)} = \frac{(1.04 \times (140 - \text{age}) \times \text{weight})}{\text{serum – creatinine (μmol/L)}}
\]

The calculation of GFR (eGFR) allows the physician to observe the extent of kidney disease. Based on these, CKD is classified into five different stages.

Stage I is characterized by a normal or increased GFR with kidney damage. Different markers such as proteinuria are used to detect abnormalities in imaging rests to manifest kidney damage. At this stage, no symptoms can be found, but the lab abnormalities indicate the kidney dysfunction.
The evidence of kidney disease can be observed in stage II as GFR declines to 60 - 89 mL/min. Most of the cases are asymptomatic at this stage, while others may have uremia, anemia, and fluid retention and other lab abnormalities.

Stage III CKD is characterized by a decrease in the GFR by 30 - 59 mL/min. At this stage, patients may have hypertension. Although some older adults are not diagnosed with hypertension, monitoring and screening could help to reduce their risk of developing CKD. High blood pressure has a greater risk for heart attack, heart failure, and kidney diseases.

Stage IV and stage V CKD are marked by severe impaired renal function. In stage IV, the GFR is 15–29 mL/min and individuals may have severe abnormalities including anorexia, edema, apathy, impaired memory, and decreased cognitive function.

Stage V CKD is classified as ESRD with a GFR less than 15 mL/min. In this stage, the patients are no longer able to remove wastes from their body. They will have severe electrolyte imbalances (hyperkalemia and hyperphosphatemia), metabolic acidosis, fluid overload, and uremia (Gomez et al., 2011). Patients with CKD, stage V need either dialysis or kidney transplantation to survive (KDOQI guidelines).

Erythropoietin-deficient anemia (decreased hemoglobin and hematocrit levels), increasing levels of phosphorous, and potassium are other physiological pointers of CKD. In addition to this, calcium levels are below normal limits due to the lack of activated vitamin D. Other physiological changes include elevated parathyroid hormone levels (PTH), dyslipidemia, and altered mental cognition (Melamed and Thadhani, 2012).

CKD is asymptomatic at an early stage, but needs assessment and ongoing monitoring and treatment, as hypertension and diabetes are very common comobidities.
(Gross et al., 2005). The major aim of the treatment is to arrest the progression of CKD by treating hypertension and diabetes. The classification of CKD is summarized in Table 2.1.

Table 2.1. Classification of CKD stages according to the GFR.

<table>
<thead>
<tr>
<th>Stage</th>
<th>GFR* (ml/min/1.73 m²)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>90+</td>
<td>Normal kidney function but urine findings or structural abnormalities or genetic trait point to kidney disease</td>
</tr>
<tr>
<td>2</td>
<td>60-89</td>
<td>Mildly reduced kidney function, and other findings (as for stage 1) point to kidney disease</td>
</tr>
<tr>
<td>3</td>
<td>45-59</td>
<td>Moderately reduced kidney function</td>
</tr>
<tr>
<td>4</td>
<td>15-29</td>
<td>Severely reduced kidney function</td>
</tr>
<tr>
<td>5</td>
<td>&lt;15 or on dialysis</td>
<td>Very severe, or end stage kidney failure</td>
</tr>
</tbody>
</table>

Role of hypertension in the CKD progression

Clinically, hypertension is defined as a systolic blood pressure greater than 140 mm Hg and a diastolic pressure greater than 90 mm Hg based on the average of two or more accurate blood pressure measurements (Frese et al., 2011). Table 2.2 gives the blood pressure classification for adults 18 years and older. Arterial hypertension together with proteinuria is one of the most important factors associated with the progression of both diabetic and nondiabetic CKD. Furthermore, studies from large, observational, prospective trials in the general population showed that hypertension is a strong independent risk factor for the progression of ESRD. The elevated systemic blood pressure causes a hypertropic response that can lead to intimal thickening of the large and small vasculature. Initially, this mechanism is a
compensatory process, but later it leads to glomerular damage and subsequent progression of CKD (Ravera et al., 2006; Tedla et al., 2011).

**Table 2.2.** Classification of blood pressure for adults age 18 or older.

<table>
<thead>
<tr>
<th>BP Classification</th>
<th>Systolic BPP (mm Hg)</th>
<th>Diastolic BP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt;120</td>
<td>&lt;80</td>
</tr>
<tr>
<td>Prehypertension</td>
<td>120–139</td>
<td>80-89</td>
</tr>
<tr>
<td>Stage 1</td>
<td>140-159</td>
<td>90-99</td>
</tr>
<tr>
<td>Stage 2</td>
<td>≥160</td>
<td>≥100</td>
</tr>
</tbody>
</table>

Source: 2014 Evidence-Based Guideline for the Management of High Blood Pressure in Adults (JNC 8). (James et al., 2014).

**Role of diabetes in CKD progression**

Diabetes mellitus (DM) is caused by hyperglycemia. Defects in the insulin secretion lead to this condition (Inzucchi et al., 2015). In addition, damage to vital organs, especially the eyes, kidneys, nerves, heart, and blood vessels are also caused by DM. The basic critical DM classification is given in Table 2.3. In this case, DM damages the kidney blood vessels and this is known as the diabetic kidney disease which further progresses to CKD. Hyperglycemia and high insulin-like growth factor-1 (IGF – 1) concentrations cause a decrease in GFR and chronic hyperglycemia is thought to be the primary cause for diabetic kidney disease (Vallon and Thomson, 2013; Imig and Ryan, 2013). It is also estimated that about 30% of type I diabetes patients and 10%- 40% of type II diabetes patients ultimately suffer from severe kidney damage (World kidney day report – 2016). The major contributors of CKD are listed in Figure 2.2.
Table 2.3. WHO guideline values for the diagnosis of diabetes and intermediate hyperglycaemic conditions.

<table>
<thead>
<tr>
<th>Glucose level in mg/dl</th>
<th>Fasting</th>
<th>After eating</th>
<th>2-3 hours of eating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>80-100</td>
<td>170-200</td>
<td>120-140</td>
</tr>
<tr>
<td>Impaired glucose</td>
<td>101-125</td>
<td>190-230</td>
<td>140-160</td>
</tr>
<tr>
<td>Diabetic</td>
<td>126+</td>
<td>220-300</td>
<td>200+</td>
</tr>
</tbody>
</table>

Figure 2.2. The major non-communicable diseases associated with the progression of CKD (National kidney Foundation report - 2015).

CKD Progression and Osteoporosis

FGF23 is the recently discovered regulator of phosphate and mineral metabolism. FGF23 mainly regulates renal phosphate excretion. The increased level of FGF23 is an important clinical diagnosis of CKD patients associated with osteoporotic conditions. Moreover, many cross-sectional studies demonstrate that an inverse relationship is observed in GFR with an improper kidney function (Liu and Quarles, 2007; Damasiewicz et al., 2011; Wan et al., 2012). The elevated serum phosphate and FGF-23 along with 25-hydroxyvitamin D and calcitriol deficiency is associated with adverse clinical outcomes among CKD patients (Nigwekar et al., 2014). These abnormalities particularly lead to osteoporotic conditions among CKD patients.
CKD patients with osteoporotic conditions are clinically known as chronic kidney disease – mineral bone disorder (CKD – MBD) patients (Miller, 2014).

**Pathophysiology of Chronic Kidney Disease – Mineral Bone Disorder**

FGF23, CYP24A1, and VDR genes play an important role in the pathogenesis of CKD - MBD (Cozzolino and Malindretos, 2010; Petkovich and Jones, 2011; Wahl and Wolf, 2012). The increased level of FGF23 leads to the overexpression of CYP24A1 mRNA in the kidney (Bai et al., 2003; Larsson et al., 2004; Shimada et al., 2005; Inoue et al., 2005; Perwad et al., 2007). The CYP24A1 enzyme is responsible for the catabolism of 25 hydroxyvitamin D₃ (25(OH)D₃) and its hormonal form, 1α, 25- di hydroxyvitamin D₃ (1α,25(OH)₂D₃) into 24-hydroxylated products for excretion. 1α,25(OH)₂D₃ is the target hormone to induce the VDR expression (Petkovich & Jones, 2011). Further, the active form of VDR mediates a wide variety of biological actions such as cell proliferation and differentiation, calcium homeostasis, immune modulation, neurological functions, and bone mineralization (Norman, 2008). The overexpression of the CYP24A1 leads to VDR dysfunction as it over metabolizes the 25OHD₃ and 1α,25(OH)₂D₃. Thus, CKD patients experience vitamin D deficiency and subsequent osteoporosis (Loh et al., 2012; Nagamani and Karthikeyan, 2016). The CKD to osteoporosis disease progressive mechanism is schematically shown in **Figure 2.3**.
**Figure 2.3.** The schematic representation of the FGF23 influenced VDR disease progressive mechanism of CKD to osteoporosis (↑ Upregulation; ↓ Down regulation).

**Genetics of CKD - MBD**

CKD – MBD is a complex genetic trait caused by multiple genetic and environmental factors. The polygenic effects on CKD -MBD are modulated by gene-gene and gene-environment interactions. Most investigations for genetic causes of CKD were approached with candidate genes and a few important genes have been identified that belong to four categories: a) phase I enzymes (CYP1A2 and CYP3A5), b) transporters (ABCB1), c) nuclear receptors (VDR and PXR) and d) others (ACE, MTHFR). These genes are highly interconnected as shown in **Figure 2.4**, which synthesizes the crossroads between selected nuclear receptors, drug metabolizing enzymes, and transporters as well as other pharmacogenes for their effects on renal function and disease, in part via renal sodium handling (Bochud et al., 2011).
Figure. 2.4. Top three pharmacogenomics applications (ACE inhibitors treatment, VDR agonists and moderate dietary salt intake); VIP: Very important genes in pharmacogenomics; SS: Putative salt sensitive genes.

Among these genes VDR is an important target for three reasons: 1) The impact of paricalcitol (19-nor-1,25-hydroxy-vitamin D$_2$), a synthetic vitamin D analog, effect on tubulointerstitial fibrosis was investigated and it was identified that paricalcitol significantly attenuated renal interstitial fibrosis compared with vehicle controls in animal models (Tan et al., 2006); 2) Oral consumption of calcitriol and oxalcalcitriol improves glomerulosclerosis by reducing type I and IV collagens in antibody-induced glomerulonephritis (Makibayashi et al., 2001); 3) It has also been observed in three randomized controlled trials the vitamin D analogs reduced albuminuria (de Borst et al., 2013) or proteinuria (Fishbane et al., 2009) in CKD patients.

**Vitamin D History and Significance of Vitamin D**

The use of vitamin D in medical practice was initially identified in the early nineteenth century when sun exposure was the best treatment for people with rickets (Holick, 2006). In 1918, cod liver oil was used to treat rickets in puppies and in 1922, a potential factor, vitamin D led to the amelioration of rickets (Mellanby, 1918;
McCollum et al., 2002). This evoked the exposure to certain foodstuff, especially yeast, to UV light, which was consequently established to have anti-rachitic properties.

Vitamin D was eventually added with milk at a standard dose of 400 international units (IU) per quarter gallon. This was the first fortification of food with vitamin D (Holick, 2006). Different forms of vitamin D exert different functions. Nevertheless, yeast extracted vitamin D exerted no anti-rachitic properties, whereas cod liver oil was related with an improvement in rickets. Thus, two different types of vitamin D proposed for two different biological effects were named vitamin D\(_2\) (synthesized artificially from yeast) and vitamin D\(_3\) (synthesized naturally from skin). Later, pharmaceutical companies were interested to design different vitamin D derivatives. Table 2.4 gives the major vitamin D compounds, analogs, and their respective function and clinical efficacy.
**Table 2.4.** List of some common vitamin D derivatives and their nomenclature, chemistry, clinical applications and associated diseases.

<table>
<thead>
<tr>
<th>Generic Name</th>
<th>Chemical Name</th>
<th>Molecular Formula</th>
<th>Type of Vitamin D compound</th>
<th>Clinical use</th>
<th>Associated disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previtamin D</td>
<td>7-Dehydro cholesterol</td>
<td>C_{27}H_{44}O</td>
<td>Vitamin D3 precursor</td>
<td>Vitamin D3 precursor</td>
<td>Rickets, Osteomalcia</td>
</tr>
<tr>
<td>VitaminD2</td>
<td>Ergocalciferol</td>
<td>C_{28}H_{44}O_{2}</td>
<td>Nutritional vitamin D</td>
<td>Vitamin D supplement</td>
<td>Rickets, cancer</td>
</tr>
<tr>
<td>Vitamin D3</td>
<td>Cholecalciferol</td>
<td>C_{27}H_{44}O</td>
<td>Nutritional vitamin D</td>
<td>Vitamin D supplement</td>
<td>Osteoporosis</td>
</tr>
<tr>
<td>25-hydroxy vitamin D (25(OH)D)</td>
<td>Calcidiol</td>
<td>C_{27}H_{44}O_{2}</td>
<td>25 hydroxylated vitamin D</td>
<td>Major metabolite of vitamin D</td>
<td>Bone loss</td>
</tr>
<tr>
<td>1,25 hydroxy vitamin D (1α,25(OH)<em>{2}D</em>{3})</td>
<td>Calcitriol</td>
<td>C_{27}H_{44}O_{3}</td>
<td>Active vitamin D metabolite</td>
<td>Major metabolite of vitamin D</td>
<td>Bone loss</td>
</tr>
<tr>
<td>24,25 hydroxy vitamin D</td>
<td>Calcitroic acid</td>
<td>C_{23}H_{36}O_{4}</td>
<td>Inactivated vitamin D metabolite</td>
<td>Inactivated vitamin D metabolite</td>
<td>sHPT and Osteoporosis</td>
</tr>
<tr>
<td>1 – alpha calcidol</td>
<td>1-hydroxy cholecalciferol</td>
<td>C_{27}H_{44}O_{2}</td>
<td>Synthetic vitamin D analogue</td>
<td>PTH suppression, increase calcium in CKD</td>
<td>sHPT and Osteoporosis</td>
</tr>
<tr>
<td>Paricalcitol</td>
<td>19-nor-1,25-(OH)<em>{2}-vitamin D</em>{2}</td>
<td>C_{28}H_{44}O_{2}</td>
<td>Synthetic vitamin D analogue</td>
<td>PTH suppression, increase calcium in CKD</td>
<td>sHPT and Osteoporosis</td>
</tr>
<tr>
<td>Doxercalciferol</td>
<td>1-Hydroxy vitamin D_{2}</td>
<td>C_{28}H_{44}O_{2}</td>
<td>Synthetic vitamin D analogue</td>
<td>PTH suppression, increase calcium in CKD</td>
<td>sHPT and Osteoporosis</td>
</tr>
</tbody>
</table>
**Vitamin D synthesis**

Vitamin D has been synthesized by two ways, viz, 1) synthesized in the skin and 2) ingested in the diet and transported to the liver, where it hydroxylated in the 25th position to yield 25-hydroxyvitamin D, which is the main storage form of vitamin D. Vitamin D nutrition is assessed through the measurement of the vitamin D metabolite. Further, 25-Hydroxyvitamin D is hydroxylated by the enzyme 1α-hydroxylase in the kidney, to yield 1, 25-dihydroxy vitamin D, which is the active form of vitamin D. This metabolite is responsible for the effect of vitamin D on phosphorous and calcium metabolism, bone health, and the regulation of the parathyroid function (Al-Badr and Martin, 2008) (Figure 2.5).

![Diagram of Vitamin D synthesis](image)

**Figure 2.5.** Synthesis mechanism of native vitamin D (1α,25-dihydroxyvitamin D₃).
VDR activation and bone formation

Deficiency of vitamin D and its metabolites affect bone formation, primarily caused by the mineralization dysfunction. It is also well documented that vitamin D directly increases both bone turnover and bone growth (Dardenne et al., 2004; Mathew et al., 2006). Studies of VDR-knockout mice have identified VDR as crucial in maintaining normal bone formation and bone mineralization by directly enhancing osteoblast differentiation (van Driel et al., 2006).

In addition, osteoblasts contain 1α-hydroxylase, apparently to function in an autocrine/paracrine manner to maintain bone formation (Frolik et al., 2003; Jilka, 2007). The self-feedback mechanism of vitamin D, upregulates its own receptor, whereas PTH decreases VDR and blocks homologous upregulation (Reinhardt, 1990; Andress, 2008). VDR activators on bone formation are more important than the PTH inhibition, and thus the effects of active vitamin D compounds and calcimimetics on bone differ under certain experimental conditions in vivo (Nguyen-Yamamoto et al., 2010).

Different VDR activators also influence the bone function. Nakane et al., (2006) found that paricalcitol caused less Ca ion release than calcitriol and doxercalciferol in an in vitro study. Furthermore, paricalcitol is helpful in the effective preparation in stimulating collagen synthesis. The authors concluded that paracalcitol contributes a lower calcemic profile on the bone effect, which may be effective in stimulating new bone formation. Notably, the opposite effect is observed on calcitriol and paricalcitol in the PTH ratio (whole PTH (1-84)/carboxy-terminal PTH) is described among CKD 5D stage patients (Monier-Faugere et al., 2001).
Vitamin D and kidney disease

CKD patients often experience bone fracture due to vitamin D deficiency (Obi et al., 2015). The association of kidney disease with vitamin D deficiency is supported by several reports (Holick, 2005; Dusso et al., 2005; Andress, 2006; Honda et al., 2006; Jones, 2007; Cheng and Coyne, 2007; Gal-Moscovici and Sprague, 2007; Ali et al., 2009; Mirkovic et al., 2011; Nigwekar et al., 2014; Drueke and Massy, 2016). As the kidney function declines, there is a progressive degeneration in mineral homeostasis, with a disruption of the normal serum and tissue concentrations of phosphorus and calcium, and changes in circulating levels of hormone which includes the parathyroid hormone (PTH), 1,25-dihydroxyvitamin D (1α,25(OH)2D3), and other vitamin D metabolites, FGF-23 and growth hormones (Phelps et al., 2016). The decreasing level of 1,25-dihydroxyvitamin D in the pathogenesis of CKD is supported by the demonstration of the negative effects of 1,25-dihydroxyvitamin D on the parathyroid hormone (PTH) gene transcription (Dusso et al., 2005).

Vitamin D Deficiency in CKD

The available lower limit of normal 25-hydroxyvitamin D values is < 30 ng/ml in the majority of CKD patients (Al-Aly et al., 2007). It is also observed among patients with severe proteinuria. It is also noted that in the CKD patients group, there is a positive relationship between the 25-hydroxyvitamin D and 1α, 25-dihydroxyvitamin D levels, in contrast to a normal individual’s levels. Thus, patients administered with 25-hydroxyvitamin D levels would anticipate increased levels of 1α, 25-dihydroxyvitamin D (Al-Badr and Martin, 2008).

Numerous mechanisms are involved in CKD patients while the 1α, 25-dihydroxyvitamin D level declines. Thus, renal mass decrement will obviously limit the 1α-hydroxylase quantities which are essential for the active vitamin D production.
On the other hand, decreased GFR levels limit the capability of the kidney to produce 1α, 25-dihydroxyvitamin D (Nykjaer et al., 1999; Nykjaer et al., 2001; Hilpert et al., 2002; Willnow et al., 2002).

**Vitamin D therapy for CKD patients**

Treatment of CKD includes administration of active vitamin D therapies to suppress the PTH levels (Kramer et al., 2014; Morrone et al., 2016). The structural and functional differences among vitamin D therapies are mainly related to their ability to directly activate the VDR. Calcitriol and paracalcitol are the two important active vitamin D therapies. Both the compounds differ structurally, and as a result paracalcitol has a reduced calcemic and phosphatemic effect compared with calcitriol (Slatopolsky et al., 1995). Ergocalciferol, cholecalciferol, calcifidiol, and doxercalciferol are inactive vitamin D therapies that require activation by the kidney or liver before they can bind with high affinity to VDR. The liver should activate the doxercacifeol for use among CKD patients (Coburn et al., 2004).

**Ergocalciferol**

Ergocalciferol is used for CKD patients (stages 3 – 4) with vitamin D deficiency and elevated PTH levels. It is approved by the Food and Drug Administration (FDA) for the vitamin D deficiency treatment. It is a prohormone and should be converted to the biologically active forms of vitamin D by the liver and kidney (Hudson, 2006). Although the NKF-KDOQI guidelines recommend ergocalciferol for the vitamin D deficiency treatment, the effectiveness of this drug in reducing the PTH levels has not been established (Andress, 2006). However, the dose recommended by the NKF-KDOQI guidelines is not sufficient and subsequently higher doses are essential (Zisman et al., 2007).
Calcitriol

The 1, 25 dihydroxyvitamin D₃ (calcitriol) is an approved drug for CKD patients in stages 3–5 for the treatment of shPT and in patients on dialysis for the treatment of metabolic bone disease. Among regular dialysis patients, the administration of calcitriol greatly influences the correcting of vitamin D deficiency. Calcitriol inhibits the PTH secretion and prevents the parathyroid gland from hyperplasia among CKD patients (Brown et al., 1999). The major disadvantage of this drug is hypercalcemia and increased hyperphosphatemia in CKD patients (Slatopolsky, 2003). Calcium-based phosphate binders also increase the risk of hypercalcemia from calcitriol therapy (Friedman, 2005; Mangoo-Karim et al., 2015).

Doxercalciferol

Doxercalciferol (Hectorol, 1-hydroxyvitamin D₂) is a second-generation analog, which requires biological activation in the liver (Coburn et al., 2004). It is an approved drug for CKD patients in stages 3–5, including dialysis patients. This drug also effectively suppresses the PTH levels in hemodialysis patients. However, statistically significant elevations in serum calcium and phosphorous level were observed in patients with doxercalciferol treatment (Coburn et al., 2004).

Paricalcitol

Paricalcitol (Zemplar, 19-nor-1, 25-dihydroxyvitamin D₂) is a third-generation vitamin D analog. It mimics the calcitriol action when it binds with VDR (Hudson, 2006). It has been recommended for CKD stages 3 and 4, and it is recommended to patients that are not in dialysis as an oral form. It suppresses the PTH levels significantly with less impact on serum calcium and phosphorous levels in hemodialysis patients (Lindberg et al., 2003). From the clinical studies, 91% of patients receiving paricalcitol achieved two consecutive reductions of 30% or greater.
in PTH levels (Coyne et al., 2006). Comparing paricalcitol with calcitriol in head-to-head multicenter studies of patients on dialysis those treated with paricalcitol reached a 50% or greater reduction in the baseline PTH significantly faster than patients treated with calcitriol (Sprague et al., 2003). The 2D representation of the drugs is shown in **Figure 2.6.**

![Figure 2.6. 2D representation of selective VDR analogs.](image)
**Structure of VDR gene**

The VDR gene structure contains eight protein codon exons (exon 2 – 9) and six untranslated exons (1a-1f), which are alternatively spliced and are located on chromosome 12q12.14. Labuda et al., (1992) first proposed the location of the VDR gene on the physical map of chromosome 12 by linkage mapping. The first genomic structure of human VDR was proposed by Miyamoto et al., (1997). Many SNPs and point mutations repeatedly occur in the introns or the 3’ untranslated (UTR) of the VDR gene. Changes in this region may affect the mRNA stability or protein translation efficacy, transcriptional regulation, which ultimately affect the VDR protein levels (*Figure 2.7*). A high-resolution analysis of linkage disequilibrium (LD) and tag SNPs in the VDR gene divulged that three VDR SNPs (*ApaI G>T [rs7975232], BsmI [rs1544410] and TaqI C>T [rs731236]*) fall within an area between exon 8 and exon 9, whereas *FokI T>C* (rs10735810) exists in a different block that includes exon 2 and all of the noncoding exons (Santoro et al., 2013). A summary of the associated studies of the VDR gene is given in Table 2.5.

*Figure 2.7.* Genomic structure of VDR gene and positions of known polymorphisms.
Table 2.5. Summary of association studies on VDR gene.

<table>
<thead>
<tr>
<th>S. No</th>
<th>SNPs</th>
<th>Significant observations</th>
<th>Population Size (n)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>BsmI and TaqI</td>
<td>“tt” or “BB” genotypes may develop more severe secondary hyperparathyroidism</td>
<td>90</td>
<td>Pourfarzam et al., 2014</td>
</tr>
<tr>
<td>2.</td>
<td>FokI and ApaI</td>
<td>FokI polymorphism may play a role in iPTH levels</td>
<td>46</td>
<td>Shahbazloo et al., 2014</td>
</tr>
<tr>
<td>3.</td>
<td>ApaI, BsmI, FokI and TaqI</td>
<td>“BB” and “TT” genotype had higher serum calcium concentrations</td>
<td>31</td>
<td>Hernandez et al., 2012</td>
</tr>
<tr>
<td>4.</td>
<td>FokI</td>
<td>FokI polymorphism associated with higher 1,25 OHD levels</td>
<td>410</td>
<td>Yokoyama et al., 2012</td>
</tr>
<tr>
<td>5.</td>
<td>ApaI, BsmI, FokI and TaqI</td>
<td>“TT” variat of TaqI influence the development of hyperparathyroidism in hemodialysis patients</td>
<td>186</td>
<td>Ozdemir et al., 2005</td>
</tr>
<tr>
<td>6.</td>
<td>BsmI BB genotype</td>
<td>Hyperparathyroidism in every stage of CRF and HD</td>
<td>248</td>
<td>Marco et al., 1999</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>Marco et al., 2001a</td>
</tr>
<tr>
<td>7.</td>
<td>BsmI BB genotype</td>
<td>Increased risk for mortality and shorter life expectancy in HD patients with relation to age, diabetes and calcitriol treatment</td>
<td>143</td>
<td>Marco et al., 2001b</td>
</tr>
<tr>
<td>8.</td>
<td>BsmI BB genotype</td>
<td>Strong predictor for inadequate response to EPO administration in HD patients</td>
<td>91 patients and 85 controls</td>
<td>Erturk et al., 2002</td>
</tr>
<tr>
<td>9.</td>
<td>BsmI bb genotype</td>
<td>Higher rhUPEO doses needed for management of ESRD-related anemia</td>
<td>128</td>
<td>Sezer et al., 2007</td>
</tr>
<tr>
<td>10.</td>
<td>ApaI aa genotype</td>
<td>Increased parathyroid response after dialysis</td>
<td>58</td>
<td>Yokoyama et al, 2001</td>
</tr>
<tr>
<td>12.</td>
<td>FokI FF genotype</td>
<td>Higher PTH levels in CKD</td>
<td>64</td>
<td>Vigo Gago et al, 2005</td>
</tr>
<tr>
<td>14.</td>
<td>FokI F allele</td>
<td>Nearly two-fold increased risk for stone disease</td>
<td>50 patients</td>
<td>Bid et al, 2005</td>
</tr>
</tbody>
</table>
| 15. | *TaqI* TT genotype | Five to 8-fold increased risk for stone disease | 83 patients and 83 controls | Nishijima *et al.*, 2002
| | | | 64 patients and 90 controls | Ozkaya *et al.*, 2003
| 16. | *ApaI* AA genotype | Nearly four-fold increased risk for hypercalciuria | 64 patients and 90 controls | Ozkaya *et al.*, 2003
| | | | 80 patients and 86 controls | Soylemezoglu *et al.*, 2004
| 17. | a/t/F/b haplotype | A 11.0-fold increased risk for ESRD | 258 patients and 569 controls | Tripathi *et al.*, 2010
| 18. | bb/ff/TT haplotype | Risk factor for urolithiasis | 31 patients and 50 controls | Zhu *et al.*, 2010
| 19. | *BsmI* bb genotype | Higher incidence and faster progression of sHPT and earlier time of parathyroidectomy in HD patients | 170 patients and 120 controls | Fernandez *et al.*, 1997
| | | | 90 | Schmidt *et al.*, 1997
| | | | 877 | Nagaba *et al.*, 1998
| | | | 56 hypo and 43 hyper parathyrodism patients | Tagliabue *et al.*, 1999
| | | | 121 | Borras *et al.*, 2003

**3D - Structure of VDR protein and its regulatory mechanism**

Rochel *et al.*, (2000) first reported the crystal structure of the ligand binding domain (LBD) of VDR. Till date, 57 different VDR-LBD crystal structures are available in the protein data bank (PDB) (Berman *et al.*, 2000). The VDR crystal structure consists of three domains namely DNA-binding domain (DBD: 24-89 aa; domain C), ligand binding domain (LBD: 126-427 aa; domain E), and a connectivity hinge (domain D) between these domains and a short A/B domain located at the N-terminus. The LBD comprises of three layer anti-parallel α-helical sandwich. Overall, the VDR structure (254 amino acids) contains 15 helices, 3 strands, and 13
The LBDs of the respective nuclear receptors (NR) exhibit high similarity based on the functional properties. The 3D structure of VDR is shown in Figure 2.8.

**Figure 2.8.** The 3D structure of the VDR protein.

The LBD of the VDR plays a major role in active ligand recognition and in partnering proteins interactions such as coregulators, and RXR in order to form the functionally active VDR-RXR hetero dimer. Especially helices 3, 4, and 10–12 play a major role in the protein partner’s interaction. All published VDR analog crystal structures to date display one clear active conformation with the tightly packed helix12 toward the VDR-LBD. This is expected for VDR agonists but rather surprising for VDR antagonists, since they are assumed to destabilize helix12 and to shift the co-factor equilibrium toward co-repressors. However, the VDR analogs that show antagonistic activity have been crystallized in the presence or absence of co-activator peptides. This suggests that the associated co-factor peptide influences the VDR conformation, and thus can change the property of an antagonist to a partial agonist. Therefore, in future the design and synthesis of the new analogs may take the route to
influence particularly the strength and specificity of the co-activator/co-repressor interaction, in order to create selective VDR modulators (Nakabayashi et al., 2008; Carlberg et al., 2012).

The correcting folding helix 12 in the VDR protein is an important mechanism for the VDR agonistic activity. The helix 12 moves close to the drug molecules that might turn into “closed” conformers. This particular change is very important for the VDR agonistic activity. However, the opposite effect was found in VDR antagonists. Here, the helix 12 moved away from the active site and thereby turned into “opened” conformer (Nakabayashi et al., 2008; Nagamani and Karthikeyan, 2016). Thus the VDR antagonist was not able to induce the expression of VDR. The diagrammatic agonistic and antagonistic mechanism of VDR is shown in Figure 2.9.

Figure 2.9. Illustration of molecular mechanisms of the VDR regulation: agonistic Vs antagonistic regulation.
Potency and efficacy are the two important functions essential to activate nuclear receptors, especially in VDR regulation. Potency is described as the required amount of the ligand to achieve effective function. It was simply measured by the inverse of the EC$_{50}$ for that ligand. Meanwhile, the maximum strength of the effect was calculated by the efficacy at saturated ligand concentrations (Asano et al., 2013). The binding pattern at the VDR active site plays a vital role in the efficacy of VDR analogs which may depend on direct interactions with helices H6-H7 loop and helix H5. The potency is regulated by the ligand-specific formation and rearrangement of the interaction network between helices H3 and H5 as shown in Figure 2.10.

![Figure 2.10](image)

**Figure. 2.10.** Illustrate mechanism of VDR agonist – Ligand binding domain interactions on VDR mediated transcription.

**Role of Computer Aided Drug Discovery (CADD) in drug discovery process**

Discovery of new drugs and bringing up therapeutic solutions is a time-consuming and expensive process. It is estimated that it can take up to 14 years for a typical drug discovery cycle from lead identification to clinical trials (Myers & Baker, 2001) with an estimated cost of 800 million US dollars (DiMasi et al., 2003).
In the early 1990s, rapid development in the combinatorial chemistry and high-throughput screening technologies brought out an environment to synthesize and screen compounds in a short period of time. However, these attempts not only failed to increase the number of successfully launched new molecular entities, but seemingly aggravated the situation (Lahana, 1999). Thus it leads to failure in the hit rates without entering into preclinical studies (Hann et al., 2001; Oprea et al., 2001; Klebe, 2006). Among the late stage failures are adsorption, distribution, metabolism, excretion and toxicity (ADME/T) deficiencies (Venkatesh & Lipper, 2000; Hou & Xu, 2004). Collectively, these concerns underscore the need to develop alternative strategies that can be helpful to remove unsuitable compounds before proceeding with cost-effective methods (Klebe, 2006).

Later, a new prototype came into sight in the drug discovery process for early measurement of potency (activity) and selectivity of lead candidates, as well as their potential ADME/Tox responsibilities. This helps to reduce the last stage failures and increases successful development of new molecular entities. Computer-aided drug design (CADD) is a universal term that signifies computational tools and resources for the management, storage, analysis, and design of compounds. It includes digital repository development for the chemical interaction relationship analyses, compounds designing with interesting physiochemical features using computer programs, and generation of tools for systematic assessment of potential lead candidates before they are synthesized and tested. In the past decade, X-ray crystallography was an expensive and time-consuming process, distributing its inconceivability for large-screening in industrial laboratories (Congreve et al., 2005). Over the past few years, new technologies such as comparative modeling have emerged and initiated to be exploited in lead design (Blundell, 1996). Altogether, with advances in combinatorial chemistry
and high-throughput technologies, computational infrastructures have rapidly bridged the gap between theoretical studies and medicinal chemistry. Numerous success stories of computer aided drug design are reported in books and reviews (Bohacek et al., 1996; Babine and Bender, 1997; Kubinyi, 1998; Davis et al., 2003; Congreve et al., 2005). Designing of the antihypertensive drug captopril (Cushman et al., 1977), saquinavir, indinavir, and ritonavir for the treatment of HIV infection (Wlodawer and Vondrasek, 1998), Zanamivir for therapeutic and prophylactic treatment of influenza (von Izstein et al., 1993), Dorzolamide for the treatment of glaucoma, Boceprevir for the development of anti-HCV therapy are a few examples (Baldwin et al., 1989).

CADD plays a critical role in searching new entities (Congreve et al., 2005; Klebe, 2006). The pipeline inspection of a typical virtual screening crusade from data preparation to post screening analysis is shown in Figure 2.11.

Figure 2.11. Illustrate the applications of CADD to the various stages of drug development.

Computational analysis of deleterious SNPs

The effects of disease causing nsSNPs on the functional property of a gene are valuable to expose the most important function toward pathogenesis. Analyzing the effect of nsSNPs in biological functions by experimental methods is a challenging task. Moreover, it is also time-consuming and laborious process to investigate the molecular basis of diseases like cancer, since the number of mutations is very high. In contrast, different in silico approaches are readily available to study the effect of nsSNPs on the protein structure. The tested prediction methods are more useful for
further experimental analysis by computing the most interesting and likely pathogenic cases (George Priya Doss *et al.*, 2008; Lahti *et al.*, 2012). Sequence and/or structural information as well as physiochemical property of amino acid for phenotype prediction are taken into consideration while applying computational methods. Most importantly, integrating the nsSNPs information with the protein 3D structure forms the basis to study the individual variability in drug response (McCammon *et al.*, 1977). As a consequence, great effort has been made to study the structural details governing drug target interactions for recently approved therapeutics agents.

The important role of nsSNPs in protein flexibility and their efficacy for ligand binding can be clearly demonstrated by crystallographic studies. Further, extensive labor and expenses are required to predict the protein motions upon mutation experimentally. Later in the 1970s, molecular dynamics simulation methods were developed to overcome this limitation using simple approximations based on Newtonian physics to simulate atomic motions; thus it can reduce the computational complexity (Zhang *et al.*, 2010). Many studies have given fruitful results in studying the structural information along with molecular dynamics simulation which predicts the impact of mutation on proteins (Miteva *et al.*, 2004; Zhang *et al.*, 2010; Witham *et al.*, 2011; John *et al.*, 2013) and protein-drug complexes (Dasgupta *et al.*, 2003; Yellapu *et al.*, 2013; Nagasundaram and George Priya Doss, 2013; George Priya Doss *et al.*, 2013; George Priya Doss *et al.*, 2014). Besides, good concordance between computational and experimental measurements of macromolecular dynamics has reported in many studies (Miteva *et al.*, 2004; Zhang *et al.*, 2010; Zhang *et al.*, 2011). Overall, with constant improvements in both computer power and algorithm design, it is promising to identify the genetic determinant of different drug responses across a population to allow for the development of “individualized therapy”.
Moreover, molecular dynamics simulation studies play an increasingly important role in the development of novel pharmacological therapeutics.

Based on these supportive literatures, the study was designed and explained in four different chapters. In Chapter 1, the role of Apal and TaqI polymorphism of VDR gene has been studied in South Indian population. Further in Chapter 2, other than the studied polymorphisms (Apal and TaqI), deleterious polymorphisms were identified in dbSNP database using different computation tools and subsequently the role of the identified polymorphisms on drug effect has been studied in detail. The agonist and antagonist mechanism of VDR analogs were studied in chapter 3. Moreover, the role of amino acids in VDR active site and their contribution for ligand binding were also discussed in detail. Finally, the best charge calculation methods for VDR analoges have been identified and tested using QSAR analysis and further four potential VDR agonists have been identified in Chapter 4.