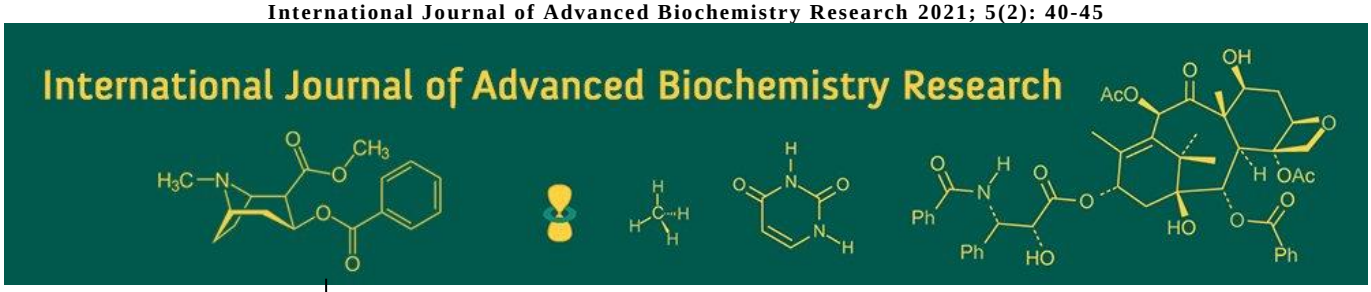

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Role of vitamin D and its receptors genes in the pathophysiology of nephrotic syndrome: Review article

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Abstract
Nephrotic syndrome (NS) is a common paediatric kidney disease characterised by leakage of protein from the blood into the urine through damaged glomeruli. It is classically defined by nephrotic-range proteinuria (≥40 mg/m^2/hour or urine protein/creatinine ratio ≥ 200 mg/mL or 3 + protein on urine dipstick), hypoalbuminaemia (<25 g/L) and edema. During NS, vitamin D-binding globulin (DBG), which binds up to 98% of the 25(OH)D and has a molecular weight less than that of albumin may be lost in the urine causing a low 25(OH)D.
Low levels of 25-hydroxycholecalciferol [25(OH)D] have been documented in NS patients during relapse due to the loss of both 25(OH)D and its binding protein in urine at this time. However, since most NS relapses are short lasting, these low levels do not reflect the steady state of body stores. Intracellular, vitamin D acts through the vitamin D receptor (VDR), a nuclear transcription factor to which vitamin D binds through the carboxyl-terminal ligand binding domain. Genetic sequence encoding VDR may differ producing polymorphic forms similar to the variants formed on digestion with restriction enzymes like ApaI and TaqI. A subset of VDR polymorphic variants are linked with decreased vitamin D activity and may result in increasing risk and tendency to several bone and endocrinal disorders.

Keywords: Nephrotic syndrome, Vitamin D receptors, gene polymorphisms, children

Introduction
Severe proteinuria (more than 40 mg/m^2 per hour) and hypoalbuminemia (less than 30 g/L) are hallmarks of nephrotic syndrome (NS), and various other conditions. For example, viral or thrombo-embolic damage to the basement membrane of the renal glomerulus increases permeability. Congenital infections, type 2 diabetes, lupus, and neoplasia can all lead to renal glomerular permeability abnormalities, which can be caused by a variety of diseases affecting the kidneys particularly [1, 2, 3]. Protein loss from the kidneys is the primary cause of nephrotic syndrome, a constellation of clinical symptoms. When you look at it in this way, it's not a sickness at all, but an expression of many other illnesses that affect the glomeruli. Post-infectious glomerulonephritis, for example, can be acute and temporary, but disorders like focal segmental glomerulosclerosis are chronic and progressive (FSGS). Additionally, certain disorders, such as minimum change renal syndrome (MCNS) [4], may recur and then go away. During the first four to six weeks of corticosteroid therapy, At 60 mg/m^2/day, up to a maximum daily dosage of 80 mg, oral prednisone was indicated by KDIGO. First, 40 mg/m^2 should be taken twice a day for the next two to five months, with additional decreases in dosage. Therapy should last at least a year if possible [5]. Vitamin D (1, 25(OH)$_2$D$_3$ insufficiency and hypocalcemia can aggravate secondary hyperparathyroidism and reduced bone mineral density in children with nephrotic syndrome. Hyperphosphatemia and renal phosphate loss can be caused by osteoblastic cells overproducing FGF-23 [6]. Hydroxylase in the liver transforms vitamin D3 to 25(OH) in the kidneys, which is subsequently metabolised by the1 alpha-hydroxylase. These active calcium forms are synthesised in part by the combination of calcium, phosphorus, and parathyroid hormone [7]. As a fat-soluble vitamin, Vitamin D The only naturally occurring source of vitamin D is fatty fish livers, so this is the most common method of obtaining it.

Ingestion or skin absorption are the only two ways that vitamin D may be processed by enzymes for its active form [8]. Vitamin D's role in bone mineralization is well-known. A wide range of chronic illnesses, including type 1 diabetes, SLE and MS, cardiovascular disease and cancer, have been related to vitamin D deficiency [9]. As a result of these latest studies, vitamin D insufficiency is getting more attention. There are a variety of genetic variants, including TaqI and Apal restriction fragment length polymorphism (RFLP) [10] that affect vitamin D receptor (VDR) activity, resulting in NS.

Vitamin D in nephrotic syndrome

A greater than two-thirds chance of having a relapse occurs. Non-responsive patients are treated with immunosuppressive medicine in addition to prednisolone for relapses. Prednisolone treatment for children with NS has been linked to a decrease in bone mineral density and an increase in fracture risk in a meta-analysis of adults [11].

Vitamin D deficiency

The deficiency of vitamin D has been shown to induce rickets, osteomalacia, and hypocalcemia [12] in children and adults. As a result, it has been linked to a number of health issues, including diabetes, hypertension, ulcerative colitis, cancers, and asthma. In addition to ensuring healthy bone health, appropriate vitamin D levels are necessary for vitamin D's non-osseous effects [13].

Table 1: Effect of Vit. D deficiency.

	References
Rickets	Rathi *et al*. [12]
Osteomalacia	Boullon *et al*. [25]
Hypocalcemia	Rathi *et al*. [12]
Hypertension	Rathi *et al*. [12]
Inflammatory bowel -disease	El-Mohamdy *et al*. [57]
Malignancies	Kulie *et al*. [13]

Vitamin D and pathogenesis of nephrotic syndrome

Due to its prevalence in paediatric renal illness, non-specific bone loss (NSBDL) is closely associated with the disease. As a result of low 25(OH)D levels, DBG, which may bind up to 98% of 25(OH)D and has a molecular weight lower than albumin, may be lost in urine throughout the course of the NS [11, 14]. 25-hydroxycholecalciferol [25-OHD] levels have been seen in NS patients during relapse due to the loss in urine of 25(OHD) and its binding protein. Because NS relapses are so brief, these low levels may not correctly reflect the condition of physical reserves [15, 16].

Calcium homeostasis in nephrotic syndrome

Numerous studies have linked vitamin D deficiency, hypocalcemia, and inadequate intestinal calcium absorption to bone abnormalities in NS children (PTH). These readings are generally within safe ranges for phosphorus. These symptoms are considered to be caused by the use of steroids and the loss of plasma proteins and minerals in urine. As renal failure is common in certain nephrotic syndrome individuals, their calcium and vitamin D deficiency is a result of this in these people, even when they are otherwise healthy (GFR). When a kid has low bone mineral density or a poor bone histology, it's likely that they have osteomalacia, which is a condition that affects the metabolism of the bones. Early detection and treatment of renal osteodystrophy and growth retardation in children with nephrotic syndrome can be beneficial [17]. Although some investigations have found normal blood calcium levels in NS patients, hypocalcemia appears to be a prevalent characteristic of NS.

Table 2: Clinical features of INS

	References
Reduced serum vitamin D metabolites	Greenbaum *et al*. [15]
Hypocalcemia	Banerjee *et al*. [11]
impaired intestinal absorption of calcium	Peacock*et al*. [42]
elevated levels of immunoreactive parathyroid hormone	Koşan *et al*. [14]
High risk of metabolic bone disease	Lisa *et al*. [17]

Complication of hypocalcemia

Neuromuscular conditions as Tetany, heart disease, and mental issues can all be caused by low levels of calcium in the blood. Patients may experience anything from a moderate case of carpopedal spasm and muscular cramps to severe cases of laryngospasm and seizures [18].

Effect of Nephrotic syndrome on calcium regulating hormones

Although some individuals have signs of secondary hyperparathyroidism, PTH levels are not usually increased in patients with NS [19] despite low ionised blood calcium and histological evidence. In a small number of investigations, iPTH (immune reactive parathyroid hormone) levels were shown to be elevated. Differences in patient demographics or the settings in which the tests were taken may explain the discrepancies in iPTH and serum ionised calcium readings. In the majority of the investigations on these individuals, the phosphorus levels in the blood were within the normal range [14].

Effect of Glucocorticoids

Although these alterations may be caused by a prolonged proteinuria condition or low vitamin D level, they are not always the primary cause of these changes. The regular use of corticosteroid medication in children with NS has been linked to osteoporosis [18].

The renal tubule's ability to reabsorb calcium is reduced by glucocorticoids (GCs), resulting in a negative calcium balance and an increase in urine calcium excretion [20]. Supplementation with calcium and vitamin D does not appear to have a direct effect on blood calcium levels, but rather, illness remission following steroid medication has been linked to better serum calcium levels, regardless of supplementation. Instead of decreasing the risk of low BMD, supplementation may raise blood calcium or vitamin D levels [21].

Risedronate, a bisphosphonate, can be used to prevent steroid-induced osteoporosis. As a result, the administration of a bisphosphonate may be beneficial in NS patients receiving steroids [11].

Table 3: Effect of glucocorticoids

	References
Osteoporosis	Panczyk-Tomaszewska [18]
decreased gastrointestinal calcium absorption	Niaudet [20]
increased urinary calcium excretion	Dasitania *et al*. [19]

Role of Vitamin D in Ca homeostasis

The parathyroid gland's calcium-sensing receptor becomes less sensitive in hypocalcemia, increasing PTH secretion. Vitamin D (PTH) stimulates the kidney enzyme 1-alpha-hydroxylase, which produces 1,25$(OH)_2$D in the presence of low calcium and high phosphate levels in the blood [22]. Vitamin D is linked to the carboxyl-terminal ligand binding domain of a transcription factor in the nucleus (VDR). During digestion, polymorphic forms identical to those made by restriction enzymes, such as ApaI and TaqI, are produced. There is some evidence that some polymorphic V1DR variants may increase the risk of bone and hormone disorders such as osteoporosis, rickets, urolithiasis, and type 1 diabetes [23].

Role of vitamin D in the treatment of nephrotic syndrome

Vitamin D's significance in calcium and phosphate metabolism and bone health is well-known [24]. Studies on the influence of inflammation, infection, allergies, and cancer has been rising in recent years [25, 26]. People who are at risk of vitamin D insufficiency need to make sure their bodies have adequate vitamin D to carry out their regular activities, including skeletal and extraosseous processes [12, 27-28]. However, there is a lot of debate over what constitutes "normal" levels in healthy populations and in non-skeletal illness situations [13, 29-30].

Vitamin D dynamics inside the body

Vitamin D levels in the body are frequently tested, which includes both 25hydroxy D3 (cholecalciferol) and 25$(OH)_2$ (cholecalciferol) (ergocalciferol). Because of its short half-life (a few hours) and fast reactivity to calcium and parathyroid hormone (PTH) fluctuations, 1,25-dihydroxyvitamin D levels stay stable for 3-4 weeks in healthy persons [30, 31, 32]. Vitamin D-binding protein (VDBP), albumin, and other proteins bind most of the total serum 25(OH)D, with just 0.03% to 0.04% remaining in the free state [32]. It is not known whether or not the VDBP-linked part of 25(OH)D has any physiological effect. Albumin-bound portion's bioavailability is unclear. Both of these substances are lost in the urine of NS patients [33].

Table 4: Difference between vit. D and 25(OH)D

	References
For 3-4 weeks, the circulation amount of vitamin D remains consistent, however, the 1,25-dihydroxy vitamin D only lasts for a few hours	Herrmann [30]
Serum 25(OH)D is strongly linked associated to vitamin D-binding protein (VDBP) in the circulation (85% to 90%).	Schwartz *et al.* [31]
5% to 10% of the serum (OH) In the free state, 0.03 to 0.04% of D is released from albumin and enters the bloodstream.	Bikle *et al.* [32]

25(OH)D and Nephrotic syndrome

Several studies indicated that the overall 25(OH)D level in the NS was low [34-37]. It appears that NS has no effect on bone indicators such as blood calcium, phosphate, Alkaline Phosphatase (ALP), and intact Parathyroid Hormone (iPTH) [11, 38]. A large increase in 25(OH)D levels in the blood did not translate into an increase in BMD or BMC with vitamin D treatment [34]. Kidney disease that is steroid sensitive Patients with Steroid Sensitive Nephrotic Syndrome (SSNS) and healthy controls will be compared for total and free 25(OH)D blood levels. We'd like to investigate if blood levels of 25(OH)D free or total, calcium, phosphate, ALP, and iPTH are more closely connected with (1) the severity of the nephrotic syndrome as defined by proteinuria, or (2) bone biochemistry. Vitamin D deficiency and the requirement for supplementation in SSNS patients may be better predicted by "free" rather than "total" 25(OH)D concentrations, according to this study. Urine samples were obtained and analysed in order to determine the 25(OH)D protein–creatinine ratios total and free (PCR). A formula can be used to make up for low levels of albumin in the blood, such as those found in hypoalbuminemia [39]. Written parental agreement was obtained from the Institute for Child Health, Kolkata after ethics approval was obtained.

Vitamin D receptor genes: relation to kidney

The vitamin D receptor (VDR) is the only one that interacts with vitamin D in its active state. 'By interacting with the nucleus of the target cell, it is possible to induce growth, bone mineralization, and cell differentiation. A polymorphism in the BsmI restriction enzyme of the vitamin D receptor (VDR) gene has been linked to diabetes in several studies [40, 41].

VDR polymorphism as a cause of Nephrotic syndrome

There is still a lot of debate over how the immune system plays a role in the pathophysiology of nephrotic syndrome. Many skeletal and non-skeletal illnesses have been linked to frequent variants in the VDR gene [42]. NS pathogenesis has been linked to metabolic disorder because VDR functions as an immunomodulator and NS pathogenesis has been linked to biochemical abnormalities.

Vitamin D receptor gene polymorphism

Fok1's coding amino acid sequence is not affected by the silent single nucleotide polymorphisms (SNPs) as BsmI and ApaI, but their capacity to regulate mRNA stability may have an impact on gene expression [43]. A mutation from guanine (G) to adenine (A) in the VDR gene's promoter region is known as the Cdx2 polymorphism., where a particular transcription factor known as Cdx2 binds. In terms of transcriptional activity, the A allele has an enhanced affinity for the Cdx2 transcription factor. Due to its effect on intestinal VDR expression, the A allele may improve bone mineral density [43].

Table 5: Pleomorphism of Vit. Receptor genes.

	References
BsmI	Bodoki L *et al.* [43]
ApaI	Amal *et al.* [44]
Fok1	Bodoki L *et al.* [43]
TaqI	Liu *et al.* [45]

Vitamin D receptor polymorphism

Vitamin D's carboxyl-terminal ligand binding domain activates this nuclear transcription factor, the vitamin D receptor (VDR). There are three types of nuclear receptors that make up the VDR: retinoic acid (VDR), thyroid, and steroid nuclear receptors. 2, 3 Genes that encode VDR can be polymorphic, resulting in forms like ApaI and TaqI-digested variations of the gene sequence [44]. ApaI (rs7975232 c.1025-49G > T) and TaqI (rs731236, C > T, p. Ill352Ile) are located at the 3′ end of the gene [45]. The codon 352 (p.Ala352Ala) polymorphism in TaqI (rs731236,

c.1056T > C, p.Ala352Ala) has no effect on amino acid sequence [46]. Near the ApaI and TaqI polymorphisms, the 3'UTR of mRNA is placed near to the gene's regulatory three-UTR. Certain haplotypes' VDR mRNA transcript stability and rate of transcription are affected by ApaI and TaqI polymorphisms [47].

Pathogenesis of idiopathic nephrotic syndrome

An imbalance between T-helper cell subtype 1 (Th1) and type 2 cytokines, which is controlled by 1,25-dihydroxy D3, may explain this phenomenon, according to controversial research [48]. Vitamin D inhibits the transcription of cytokine genes, which in turn impacts the release of cytokines. Previous studies have established the prevalence of VDR polymorphism genotypes in diverse populations [49].

Vitamin D activates the carboxyl-terminal ligand binding domain of vitamin D (VDR), a nuclear transcription factor. During digestion, restriction enzymes like ApaI and TaqI produce polymorphic forms that are similar to those produced by restriction enzymes. Osteoporosis, rickettsial infections, urolithiasis, and type 1 diabetes have all been related to vitamin D insufficiency [49, 50].

Only the vitamin D active formula interacts with VDR, an intracellular hormone receptor. Intermingling the nuclei of target cells can have a variety of biological effects, including increased immunity, growth, bone mineralization, and cell differentiation [41, 51]. A BsmI restriction enzyme polymorphism has been found in the vitamin D receptor gene (VDR). Immune system involvement in the pathogenesis of nephrotic syndrome remains controversial. Recently, the VDR gene has been linked to a wide variety of skeletal and non-skeletal conditions [42].

Effect of different allele on VDR

Biochemical abnormalities that contribute to metabolic disorders have been implicated in the pathogenesis of NS. VDR functions as a strong immunomodulator. Single nucleotide polymorphisms, or SNPs, such as those found in Fok1 have no effect on its coding sequence, but their potential to control the stability of mRNA might affect gene expression. In the promoter region of the VDR gene, a transcription factor called Cdx2 interacts to a change in the guanine (G) to adenine (A) polymorphism known as Cdx2. The Cdx2 transcription factor exhibits a greater affinity for the A allele in terms of transcriptional activity. Due to greater calcium absorption in the intestines, the A allele may boost VDR expression, which may contribute to an increased bone mineral density [43]. The Vitamin D receptor VDR gene polymorphisms in nephrotic syndrome children were the subject of this study.

Mechanism of action of the alleles

The polymorphisms (BsmI, ApaI, and TaqI) in exon 8 and intron 8 can be utilised to construct a haplotype. The RfLPs' linkage disequilibrium is caused by the 3.2-kilobase untranslated region (UTR), which is located close to exon 9. vitamin D3 receptor (VDR) gene is located at 12q12-q14 [52, 53]. The entire length of this gene is 75 Kilobases (kb). The noncoding 5-prime end of the VDR gene is comprised of exons 1A, 1B, and 1C. The product is encoded by exons 2-9 [54, 55]. Two exons, 2 and 3, are involved in DNA binding, whereas three exons, 7, 8, and 9, are involved in vitamin D binding. Interacts with an intracellular hormone receptor that is specific to vitamin D3 (VDR). Target cell nuclei may have biological implications [56, 57]. VDR is a member of the nuclear receptor transcription factor family that is ligand dependent. Vitamin D–response elements (VDREs) on target genes are activated when $1,25(OH)_2D_3$ or another ligand for the VDR binds to the RXR and vitamin D–response elements (VDREs). Increased or decreased gene expression is possible as a result of this [56].

Th1 and Th2 disturbance as a pathogen of Nephrotic Syndrome

The pathophysiology of INS remains a mystery. INS pathology has been connected to a malfunction in Th1 and Th2 cytokine production in recent studies [58]. Cell-mediated immunity is enhanced by IFN- and interleukin-2, whereas IL-4 and IL-10 increase antibody production in Th2 type cytokines, as seen in this figure. A possible role for VDR and $1,25(OH)_2D_3$ in regulating the Th1/Th2 ratio may be to block the transcription of cytokine genes. With $1,25(OH)_2D_3$, a dose-dependent reduction in Th1 cell production of IFN was found. Retinoic acid (RX) and the vitamin D3 receptor (VDR) create a heterodimer as RX enters cells [59].

In the promoter region of target genes, this heterodimer complex binds to VDRE (vitamin D responsive elements) and thereby controls its own transcription [56]. According to the results of one research, $1,25(OH)_2D_3$ suppresses the formation of NFATp/AP-1 complexes, which reduces the transcription of the IL-2 gene. Skeletal and non-skeletal problems can be caused by variations in the VDR gene's sequence [42].

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