Emerging Problem of Vitamin D Deficiency in India

T SHALINI, M SIVAPRASAD, AYESHA ISMAIL and G BHANUPRAKASH REDDY*
National Institute of Nutrition, Hyderabad 500 007, India

(Received on 20 May 2016; Accepted on 16 June 2016)

Vitamin D (VD) is unique among the vitamins in the fact that it can either be synthesized in the skin from exposure to sunlight or taken from the diet. Apart from its role in calcium and bone homeostasis, VD and its metabolites are now known to have important roles in various other functions of human health, of which many still await to be discovered. Vitamin D deficiency (VDD) is now known to be rampant globally including the Indian sub-continent. In this review, the classical and non-classical functions of VD are described. Further, we have discussed the prevalence of VDD in general, and in disease conditions, particularly in the Indian population. Studies carried out in pregnant women, cardiovascular disease patients, rheumatoid arthritis patients, both type 1 and type 2 diabetic subjects; all show VD deficient or insufficient status. Genetic and epigenetic variants in the genes involved in the VD metabolism play a fundamental role in individual differences in the VD status. Considering the plethora of VD functions, the recommended dietary allowance (RDA) of VD may need to be revised. Further, randomized control trials across India, and food fortification with VD to combat the VDD epidemic are needed.

Keywords: Vitamin D; Calcium; Osteoporosis; 25-Hydroxyvitamin D3; 1,25-Dihydroxyvitamin D3; 25-Hydroxylase; 1α-Hydroxylase; Vitamin D Binding Protein; Vitamin D Receptor; Vitamin D Deficiency; Vitamin D Insufficiency

Introduction

History of Vitamin D

In 1645, Whistler, Glisson, and Deboot called attention to a bone disease which was later found to be the classic bone disease rickets (Norman, 2012). Two centuries later in 1824, the usefulness of cod liver oil in the treatment of rickets and osteomalacia was observed. In a series of hallmark studies (1919–1924), Mellanby could demonstrate that a nutritional component in the diet was the antirachitic factor that could prevent rickets. In 1919, Hudshinsky showed that UV light was able to ameliorate rickets by increasing calcification in rachitic children. These historical findings were followed by the elucidation of the chemical structure of the fat-soluble secosteroid, vitamin D (VD), in 1936 by Windaus (Bendik et al., 2014).

Sources

(a) Synthesis in skin: The UVB (290–315 nm) component of sunlight converts 7-dehydrocholesterol in the epidermis of the skin to previtamin D3 which further isomerizes to vitamin D3 (Fig. 1) (Norman, 2008; Raghunath et al., 2016). Due to the slow isomerisation rate in the skin, plasma vitamin D3 levels peak two days after sunlight exposure. After its synthesis, vitamin D3 is bound to vitamin D binding protein (VDBP) and carried through the bloodstream to its target organs.

(b) Dietary sources: VD exists in animal products, and the richest sources are fish liver oils. It is present in small and highly variable amounts in mushrooms, egg yolk, and liver. Some plants of Solanaceae family have been found to contain VD like activity in their leaves.

Absorption, Uptake, and Transport

VD being a fat-soluble vitamin, its absorption is similar to lipids. The absorption of VD requires bile salts.
After combining with bile salts, it passes into the lacteal system through the intestinal epithelial cells. Absorption is maximum in the proximal and mid, small intestine. Apart from bile salts, the formation of mixed micelles containing monoglycerides and free fatty acids aid in VD absorption. An impairment of triglyceride breakdown caused by the pancreatic insufficiency through insufficient lipase secretion ultimately leads to decreased VD absorption (Thompson et al., 1966; Vogelsang et al., 1997). Following the uptake into the enterocyte by non-saturable diffusion, VD is packed into chylomicrons and secreted into the lymph (Dueland et al., 1982). The lymph containing VD now enters the circulation through the thoracic duct where the liver scavenges the chylomicron remnants. A fraction of the hepatic VD uptake is mediated through VDBP rather than chylomicrons. The efficiency of absorption process seems to be about 50%.

Metabolism

In the liver, VD is hydroxylated by 25-hydroxylase to form 25-hydroxy vitamin D3 (25(OH)D) or calcidiol. 25(OH)D levels in the blood is a valid indicator of the VD status. Adipose tissue is the major storage organ. Following its synthesis, 25(OH)D binds to VDBP and is transported to the kidney, where it undergoes the second hydroxylation by 25-hydroxy vitamin D 1α-hydroxylase (CYP27B1) to form 1,25-dihydroxy vitamin D3 (1,25(OH)2D) or calcitriol. 1,25(OH)2D is the hormonally active form of VD, which brings about all biological functions in the body. The 24-hydroxylation of 25(OH)D to form 24,25-dihydroxy vitamin D3 (24,25(OH)2D) by the 24-hydroxylase is the primary mechanism and the first step towards the inactivation of VD metabolites.

Mechanism of Action and Regulation of 1,25(OH)2D

The majority of VD and its plasma metabolites are transported bound to the VDBP, with a small percentage bound to albumin (Bikle et al., 1986). 1,25(OH)2D dissociates from the VDBP on reaching the target cells and binds to the nuclear VD receptor (VDR). The liganded VDR complexes with the retinoic X receptor (RXR) to form heterodimers (Guessous, 2015). The VD-VDR-RXR complex then interacts with VD response elements (VDRE) in the 52 promoter region of the regulated gene resulting in transactivation or transrepression. About 913 human genes containing the VDRE in their promoter regions are reported to alter their expression in response to calcitriol (Wagatsuma and Sakuma, 2014).

Renal production of 1,25(OH)2D is primarily through the actions of the parathyroid hormone (PTH) and the fibroblast growth factor 23 (FGF-23) secreted by the bone and are thus tightly regulated by a network of feedback loops. Low circulating Ca triggers the PTH secretion resulting in the activation of the CYP27B1 (Hofman-Bang et al., 2010; Quraishi and Camargo, 2012). 24-hydroxylase is activated to initiate degradation of metabolites (Jones et al., 2012). FGF-23 negatively regulates the CYP27B1 activity together with the transmembrane protein, Klotho, which acts as a co-receptor essential for the activation of the FGF signaling by FGF-23 (Martin et al., 2012; Olmos-Ortiz et al., 2015). FGF-23 synergises with the PTH to increase the renal phosphate excretion in the proximal tubule (Bergwitz and Juppner, 2010).

Rapid non-genomic responses to the calcitriol follow ligand-binding to the non-nuclear VDRs, as in the cytoplasmic membrane caveolar pits. This results in the enhancement of the gut Ca absorption, opening the Ca channels in osteoblasts, increased endothelial cell migration, and the Ca enhancement of insulin release from the islet beta cells (Boucher, 2012).

Extra-renal synthesis of 1,25(OH)2D is reported in many tissues, such as the parathyroid glands, keratinocytes and the cells involved in immunity (T cells (Gombart, 2009; Prietl et al., 2013), activated macrophages and dendritic cells) (Barragan et al.,...
Unlike in the kidney, regulation of the extra-renal CYP27B1 is generally under the control of the immune stimuli and not by the classical feedback loop involving the PTH and FGF-23 (White, 2008). CYP27B1 is induced by the Toll-like receptor (TLR) ligation or stimulation by the interferon-γ in macrophages (Stoffels et al., 2006). The VD metabolism, mode of action and regulation of 1,25(OH)₂D is depicted in Fig. 2 (Suaini et al., 2015).

**Excretion**

In the liver, the metabolites of VD combine with a glucuronic or sulphuric acid, get excreted in the bile, then into the intestine, and pass into the enterohepatic recirculation. Only 3% of the VD metabolites circulating in the blood are excreted in urine and faeces. In humans, about 1-7 µg of VD is excreted daily, mainly in the faeces, with the aid of bile salts; and small amounts appear in the urine.

**Classical Functions**

VD is a key regulator of the Ca and P homeostasis. First, through gene expression, calcitriol enhances the active transport of Ca in the small intestine, stimulates synthesis of Ca binding proteins (including calbindin) in the mucosal brush border, and these proteins then increase the Ca absorption (Fleet and Schoch, 2010). Another mechanism unrelated to gene expression is by the opening of voltage-gated Ca channels. Phosphorus absorption increases by enhancing acid phosphatase activity, which cleaves the phosphate esters and allows its absorption. Secondly, the PTH alone or with calcitriol or oestrogen or both mobilizes the Ca and P from the bone to maintain normal blood levels. This process involves either increased osteoclast activity, or increased number of new osteoclasts through cell differentiation, or both (Penido and Alon, 2012). Finally, in the kidney, calcitriol increases the renal tubular reabsorption of the Ca and P. The thyroid hormone stalls the activity of calcitriol and PTH by suppression of the bone mobilization and increases the renal excretion of Ca and P.

**Non-classical Functions**

The non-classical functions of VD include the regulation of hormone secretion, immune function and cellular proliferation and differentiation (Bikle, 2009; Zehnder et al., 2001). VD has been shown to play a vital role in the non-classical target organs such as adipose tissue (Bhat et al., 2014), skin, muscle,
pancreas, thymus, breast and colon (Al-Shoumer and Al-Essa, 2015; Ayesha et al., 2001; Harinarayan, 2014; Ismail and Namala, 2000; Leung, 2016).

**Recommended Dietary Allowance (RDA)**

India being a tropical country, the VD requirement can be met by regular exposure to sunlight. In the absence of exposure to sunlight, a daily intake of 400 IU of VD is suggested by Indian Council Medical Research (ICMR, 2010). The intake of VD is low when compared to the recommendations, and this gap needs to be addressed. Keeping in view the current wealth of information on the non-classical functions of VD in a variety of tissues, revision of the RDA for VD has been recommended. This step helps to maintain optimum circulating levels of 25(OH)D not only for bone health but also for the maintenance of general health.

**Toxicity**

VD intoxication is rare and can occur at dosages of more than 20,000 IU per day over a long-term or when serum 25(OH)D level crosses 200 ng/mL (Gallagher, 2013). Hypervitaminosis D is a progressive intoxication, where there is an excessive calcification of bone and soft tissues (kidney, heart, lung), leading kidney stones and hypercalcaemia. Patients often complain of weakness, headache, nausea, and vomiting. Excess of VD may also increase the risk of abnormal immune responses and an increased risk of food allergy. VD hormone has potential therapeutic applications in some human diseases. However, therapeutic doses of 1, 25(OH)\( \_{2} \)D lead to substantial hypercalcaemia and systemic toxicity. This resulted in the development of synthetic VD analogues which have specific therapeutic properties and reduced calcaemic activity. Few examples of VD analogues are calcipotriol, ED-71, and Gemini. These analogues are now being used for the treatment of osteoporosis, psoriasis, secondary hyperthyroidism and cancer of colon and prostate.

**Vitamin D Deficiency (VDD)**

VDD occurs in individuals who are not adequately exposed to sunlight or due to inadequate intake of VD through the diet and is characterised by inadequate mineralization of the bone. Foods high in fibre content, phosphates and phytates can deplete the VD stores and increase the Ca requirement. VDD leads to mild oxidative stress in the muscle which may act as a trigger for increased proteolysis. The ubiquitin-proteasome pathway is the major pathway involved in VDD-induced muscle protein degradation (Bhat and Ismail, 2015; Bhat et al., 2013). In children suffering from rickets, the bones cannot withstand normal mechanical stress and tend to undergo rachitic deformities such as bowlegs and knock-knees. In adults, VDD leads to under-mineralization of the bone matrix osteoid, and in extreme conditions, bone fractures (osteomalacia) and diminished bone mass (osteoporosis). The prevalence of osteomalacia is high in the women of child bearing age with Ca depletion due to multiple pregnancies, inadequate intake or insufficient exposure to the sun. It can also occur due to gastrointestinal diseases as well as chronic renal diseases, wherein the absorption of Ca and synthesis of the vitamin are impaired. A good number of epidemiological studies have reported osteoporosis as a major public health problem in the Indian women (Khadilkar and Mandlik, 2015; Raska et al., 2016; Vaidya et al., 2012). Loss of 24-hydroxylase function usually leads to idiopathic infantile hypercalcaemia, a rare disease characterized by failure to thrive, vomiting, dehydration, and nephrocalcinosis (Schlingmann et al., 2011).

**Classification of VDD**

25(OH)D is considered as a valid indicator of the VD status since its half-life is 15 days and it lacks the hormonal control of the hepatic 25-hydroxylase (Jones, 2008). VD levels are distributed into deficient (<20 ng/mL), insufficient (20–30 ng/mL) and sufficient (>30 ng/mL) groups (Reddy et al., 2015). HPLC and competitive protein binding assay are used for measuring 25(OH)D in serum.

**Prevalence of VDD**

Ecological and observational studies have demonstrated that the VDD, defined as a low serum total 25(OH)D concentration, is associated with increased risks of various diseases such as cardiovascular diseases, malignancies, infectious diseases, diabetes, autoimmune diseases, and kidney diseases (Bendik et al., 2014; Khadilkar and Khadilkar, 2013). However, more than a billion people worldwide are thought to have VDD or insufficiency (Ruwanchathirana et al., 2014). The probable reasons for the high prevalence of hypovitaminosis D are
either; reduced synthesis of VD is owing to low exposure of the skin to sunlight or decreased consumption of VD (Grober et al., 2013). Indian epidemiologic studies reported higher than 70% prevalence of VDD in all age groups, including toddlers, school children, and pregnant women (Babu and Calvo, 2010). A study reported that depending on the type of season, and latitude, the prevalence of VD insufficiency (VDI) in the female athletes ranged between 33 and 42% (Deldicque and Francaux, 2015). A study in Delhi school girls (48% lower socioeconomic strata), reported 91% VDD (Puri et al., 2008). Seventy percent of healthy volunteers in Mumbai had VDD with a slightly higher prevalence (76%) in females (Shivane et al., 2011). A rural study from Lucknow reported a high prevalence of VDD among the pregnant women (74%) and the adolescent girls (88.6%) (Sahu et al., 2009). VDD is reported among the adult population and the postmenopausal women, where the levels of 25(OH)D correlated with adverse effects on bone mineral homeostasis (Harinarayan, 2005; Harinarayan et al., 2008; Harinarayan et al., 2004; van der Meer et al., 2011). Longer sunshine exposure of subjects residing in a rural area had better mean 25(OH)D values when compared to the subjects in an urban area (Goswami et al., 2000; Goswami et al., 2008; Goswami et al., 2009).

Prevalence of VDD in Disease Conditions

Reproduction

VD is involved in many functions of the human reproductive system in both genders as evident from animal and human studies. VD plays a role in the human spermatogenesis (Vanni et al., 2014). VDD is linked to the development of insulin resistance and impaired glucose clearance in the polycystic ovary syndrome (PCOS) (Dasgupta et al., 2015; He et al., 2015). Lower 25(OH)D levels are implicated in uterine fibroids. Both in vitro and animal studies have shown inhibition of development and/or growth of uterine fibroids and endometriotic lesions by VD supplementation or with a VDR agonist (Vanni et al., 2014).

Pregnancy

Studies have shown an association between serum 25(OH)D with various pregnancy outcomes such as gestational diabetes mellitus (GDM), hyperglycaemia and caesarean section. A study reported, that 26.3% of serum 25(OH)D inadequacy increases the risk of emergency caesarean section in Indian women (Loy et al., 2015). Mysore Parthenon Study, on 568 pregnant women (28-32 weeks of gestation), reported 67% of VDD and association of intra-uterine exposure to low 25(OH)D concentrations with lower muscle mass and higher insulin resistance in children (Krishnaveni et al., 2011). 42% of VDD and 14% of VDI in pregnant females (n=50) aged 20-40 yrs was reported in a study from the north eastern part of India (Dasgupta et al., 2012). A Mumbai study reported that mothers whose serum 25(OH)D levels were <20.0 ng/mL had significantly lower mean sun exposure index than women with VDI (serum 25(OH)D (Jani et al., 2014). VD deficiency is highly prevalent among the pregnant women in Northern India and thus raises concern about the health consequences of mother and the offspring (Sharma et al., 2016). Another study found that the maternal serum 25(OH)D (14±9.3 ng/mL) correlated positively with the cord blood (8.4±5.7 ng/mL) and negatively with the PTH (Sachan et al., 2005).

Body Mass Index

Mounting evidence shows an inverse association between VDD and body mass index (BMI). A 10% increase in BMI led to 4% decrease in 25(OH)D concentrations in a recent meta-analysis of 42,000 general adult patients (Vimaleswaran et al., 2013). Andhra Pradesh children and parents study (APCAPS) done in rural South India reported 41% VDD, which is inversely related to fat percentage but not BMI (Baker et al., 2015). Reduced sun exposure, inadequate dietary intake, urban lifestyle and higher BMI led to 92% of VDD in pregnant women (12-16 weeks gestation). The deficiency was associated with gallbladder stasis (Singla et al., 2015). 53% VDD and 58% of osteopenia were reported in healthy South Indian males (Shetty et al., 2014).

Asthma and Respiratory Infections

A study from Lucknow reported the association of low 25(OH)D levels with bronchial asthma (Awasthi and Vikram, 2014). VD supplementation may increase the anti-inflammatory property of corticosteroid by enhancing the glucocorticoid-induced mitogen-activated protein kinase phosphatase-1 expression in
asthmatic patients (Barnes, 2011). Sub-clinical VDD and non-exclusive breastfeeding in the first four months of life were significant risk factors for severe lower respiratory infection in Indian children under five years (Wayse et al., 2004).

**Cardiovascular Effects**

VD has a cardioprotective effect by influencing endothelial and vascular smooth muscle cell function as well as controlling inflammation and affecting the regulation of blood pressure through the renin-angiotensin-aldosterone system. Abnormalities in lipid profiles are associated with reduced VD status, but ApoA1 and HDL-C are directly, and independently, associated with VD status (Kienreich et al., 2013a; Kienreich et al., 2013b). APCAPS study in rural south India found an association between serum VD levels and cardiovascular disease risk factors (Baker et al., 2015). A study reported a higher prevalence of VDD (98.3%) among myocardial infarction patients after adjusting for conventional risk factors (Roy et al., 2015). Patients in the first VD quartile showed 2.54 times greater risk for coronary artery disease than those in the fourth quartile and the levels were lower in vegetarians. There was an inverse association of the VD levels with body weight, triglyceride, and BMI (Shanker et al., 2011). An association was seen between the high levels of VD with arteriotoxicity and increased the incidence of ischemic heart disease (Rajasree et al., 2001). A study found a correlation between VDD and chronic stable angina. Low levels may be an independent, potentially modifiable cardiovascular risk factor (Raina et al., 2016).

**Ageing**

VD levels in the body gradually decrease as the synthesis of VD in the skin declines with age due to reduced outdoor activity, low food intake and also decreased gut absorption of VD, leading to VDD in the elderly. Leukocyte telomere length decreases with age and enhanced VD status is associated with longer telomeres even after adjustment for age, menopausal status, obesity, the use of hormone replacement therapy and physical activity. Experimental evidence suggests that VD reduces the oxidative nuclear damage and also reduces loss with age thereby protecting against the DNA damage (Gombart, 2009).

**Auto-immune Diseases**

VD is associated with various autoimmune diseases such as multiple sclerosis, rheumatoid arthritis (RA), type 1 diabetes mellitus, inflammatory bowel disease and systemic lupus erythematosus (SLE). Plasma 25(OH)D correlated inversely with SLE disease activity index scores, plasma interferon-α and its gene expression (Mandal et al., 2014). A study reported 68% prevalence of VDD in RA and supplementation of 1,25(OH)2D could relieve the RA pain (Gopinath and Danda, 2011).

**Immune System and Infection**

VD has protective effects against bacterial and viral infections by stimulating monocyte maturation into macrophages. Cathelicidin, an antimicrobial peptide promotes wound healing by enhancing the bactericidal killing of pathogens and clearance of tissue breakdown products (Liu et al., 2006; Suaini et al., 2015). Cathelicidin was demonstrated to be a VD target protein, induced by 1, 25(OH)2D3 in macrophages (Shanker et al., 2011). VD protects against acute lung infections by reducing alveolar surface tension. VD repletion may reduce chronic inflammatory problems such as periodontitis (Boucher, 2012). A study from Pune reported 55% of VDD in patients suffering from TB. The disease appeared to be treated with the sunshine and/or VD before anti-tuberculosis drugs emerged (Jubulis et al., 2014).

**Type 1 Diabetes (T1DM)**

Recent studies suggest that the VDD may increase the risk of developing T1DM. Urinary loss of the VDBP was higher in the T1DM compared to healthy individuals suggesting that the loss might contribute to the lower levels of serum 25(OH)D (Yousefzadeh et al., 2014). A study from North India showed 58% of VDD and 86% of VDI in children, newly diagnosed with T1DM (Borkar et al., 2010). A study indicated that VDD was seen in 91% of the subjects with T1DM. Whereas the mean 25(OH)D was significantly lower in subjects than controls (Daga et al., 2012). Factors and mechanisms affecting bone health in Indian patients with T1DM have been reported (Dhaon and Shah, 2014).

**Type 2 Diabetes (T2DM)**

VD plays a major role in the pathogenesis of T2DM.
It decreases the systemic inflammation by directly modulating the expression of calcibindin-D28K, which in turn protects beta cells from cytokine-mediated cell death (Valdes-Ramos et al., 2015). Apart from Ca homeostasis, VD directly induces β-cells to secrete insulin through an increase in intracellular Ca concentration through Ca channels or by mediating β-cell Ca-dependent activation to facilitate the conversion of proinsulin to insulin (Pittas et al., 2007). Our earlier work demonstrated that VDD leads to glucose intolerance, decreased insulin secretion and turnover in rats (Ayesha et al., 2001; Ismail and Namala, 2000). Pancreatic islet renin-angiotensin-system activity increases hyperglycaemia but can be blocked by VD in islets. We reported 25(OH)D levels ranging between 18-45 ng/mL in long-standing T2DM subjects (Ayesha et al., 1998). A study conducted in Varanasi demonstrated 45% VDD in patients with both diabetes and TB, which implies that T2DM and severe VDD predispose to pulmonary TB (Chaudhary et al., 2013). Mean VD was lower in T2DM than healthy subjects aged 18-70 yrs. VDD was significantly associated with neuropathy, retinopathy, and nephropathy due to T2DM (Bajaj et al., 2014). Recently we reported 40% prevalence of VDD in adults >50 yrs, the prevalence being much higher in patients with diabetes and diabetic retinopathy (Reddy et al., 2015).

Chronic Kidney Disease (CKD)

Severe hypertension reduces the synthesis of calcitriol by the kidneys. Unless there is a secondary renal damage which is irreversible; VD supplementation blocks renal renin secretion which reduces the blood pressure. Decreased endogenous production of 1,25(OH)₂D, decreased reabsorption of 1,25(OH)₂D due to tubular damage results in a reduction in the megalin expression in the epithelial cells and decreased enzyme activity to form 1,25(OH)₂D in the kidneys is observed in patients with CKD. Also, increased urinary loss of 25(OH)D and VDBP is exhibited by CKD patients (Nakashima et al., 2016). Urinary VDBP is a potential biomarker for early detection of diabetic nephropathy. It is raised in microalbuminuria or macroalbuminuria (Tian et al., 2014). A study in North Indian adults showed that 77% and 22% of the CKD population had VDD and VDI respectively, and an inverse correlation was found between VD and the PTH (Jabbar et al., 2009). A hospital-based cross-sectional study from Vellore reported hyperparathyroidism and VDD in 75% of CKD patients. Further, patients with vascular calcification had low 25(OH)D levels (Valson et al., 2014).

Cancer

VD and its analogues are reported to regulate cell growth, differentiation and induce apoptosis in tumour cells in vitro and act as antiproliferative and anticancer agents. Association of the VDR gene polymorphism is reported in cancers of the breast, colorectal, prostate, and skin. VDR poly-A polymorphism at 32 UTR region of this gene is associated with the risk and progression of breast carcinoma (Chakraborty et al., 2009). VDR (Fok-I) polymorphism is involved in the increased susceptibility to the development and progression of multiple myelomas (Shafia et al., 2013). An inverse association was shown between the incidence rates of leukaemia and UVB irradiation (Cuomo et al., 2015) and also between the risk of pancreatic cancer and serum VD levels (Iqbal and Naseem, 2016). Recent research has shown the role of zinc in carcinogenesis, and it was found to be affected by VD supplementation (Iqbal and Naseem, 2016).

Genetics and Epigenetics of VD Metabolism

Several genes have been identified which control pathways that synthesize, transport, and degrade VD metabolites. Genetic variants in these genes play a role in individual differences in VD status. It is now known that VD status is to some extent under genetic control (McGrath et al., 2010) and that the heritability of VD levels may be greater than previously assumed (Arguelles et al., 2009; Hunter et al., 2001; Shea et al., 2009; Wjst et al., 2007). The group-specific complement gene (GC) on chromosome 4 encodes the VDBP that is the main transporter of VD and its metabolites in the circulation (Speeckaert et al., 2006). Single nucleotide polymorphism in the GC gene is associated with circulating 25(OH)D levels (McGrath et al., 2010). Two genome-wide association studies of 25(OH)D levels in populations of European ancestry found strong evidence for an association between a single nucleotide polymorphism (SNP) in GC (rs2282679 in intron 12 and rs7041 and rs4588 in exon 11) (Ahn et al., 2010; Wang et al., 2010). Other VD pathway genes (e.g., CYP27B1, CYP24A1, CYP2R1, VDR) also contribute to the genetic basis
of VD status (Engelman et al., 2008; Sinotte et al., 2009).

In India, though there are few studies on the protein variants of GC (Kamboh and Ferrell, 1986), no studies have reported SNPs in GC. Some studies reported the association of VDR gene polymorphisms with different disease conditions in correlation with VD status. Fok-I, Bsm-I and Taq-I gene polymorphisms of VDR were analysed in North Indian individuals with T2DM and found that the genotype distribution, allele and haplotype frequencies of VDR polymorphism did not differ significantly between patients and controls. However, combination studies showed that individuals carrying the combination of the three genotypes such as FF Bb tt increased the risk of T2DM (Bid et al., 2009). The association pattern of VDR polymorphisms (Cdx2, Fok-I, Apa-I, and Taq-I) with polycystic ovary syndrome (PCOS) among Indian women was investigated. Only Cdx2 genotype and allele frequency distributions were significantly different between the PCOS cases and control women. The study revealed the protective role of this SNP in PCOS phenotype and also two haplotypes, ACCA, and ACTA which were found to be significantly associated with PCOS indicating haplotype-specific risk (Dasgupta et al., 2015). 25(OH)D levels were significantly associated with the Taq-I SNP but not with the Fok-I (Bhanushali et al., 2009). In a study, of the four polymorphic sites (Fok-I, Apa-I, Taq-I, and Bsm-I) in VDR gene in idiopathic nephrotic syndrome, a significant difference was found at three polymorphic sites except at Taq-I (Jafar et al., 2009). VDR gene Fok-I polymorphism is associated with the risk of developing essential hypertension (Swapna et al., 2011). The FF genotype (or F allele) of the VDR gene plays a crucial role in determining the risk of prostate cancer and could be postulated as a good candidate genetic marker (Mishra et al., 2005). The prevalence of VD deficiency and VDR gene polymorphisms are significantly higher in people with pulmonary and spinal tuberculosis (Panwar et al., 2016).

Epigenetic alterations are molecular modifications to DNA and chromatin without alteration to the underlying DNA sequence that are mitotically heritable yet potentially reversible. VD interacts with the epigenome on multiple levels. Genes in the VD signalling system, such as those coding for VDR and the enzymes 25-hydroxylase, 1α-hydroxylase, and 24-hydroxylase have large CpG islands in their promoter regions and therefore can be silenced by DNA methylation. The VDR alone or in interaction with other transcription factors can recruit histone modifying enzymes like histone acetyltransferases or histone deacetylases and epigenetically direct transcriptional expression of downstream genes. Though maternal VDD has been related to adverse pregnancy outcomes or potential susceptibility for diseases, the trans-generational epigenetic inheritance of VD triggered epigenome modification is not entirely explored (Burrell et al., 2011; Hossein-nezhad et al., 2013).

Conclusions and Future Directions

Apart from its classical role in the calcium and bone homeostasis, VD and its metabolites are known to have important roles in various other functions of human health, of which many still await to be discovered. Despite abundant sunshine (8.4 and 37.6°N latitude), VDD is a pandemic in India. VDD is prevalent among individuals irrespective of their age, gender, race, and geography. VD status and its role in a variety of disease states are becoming apparent slowly. The causal relationship of many of the associations observed has to be investigated. In-depth studies to provide insights into VD role in these conditions in the Indian context are very essential. Improvement in the VD status of Indians is necessary and urgent. The Indian government must take substantive measures in this direction. VD fortification appears to be the need of the hour, keeping in view the very high prevalence of VDD across the country. Population-based strategies must be employed, to increase the awareness of the problem, and to provide affordable VD either alone or with Ca supplementation or via VD fortified foods. Milk was the preferred food vehicle, and the amount of micronutrient used varied significantly among the studies. India being the largest producer of dairy milk in the world, does not fortify milk. To increase the mean daily dietary intakes in India, VD could be added to staple foods such as wheat flour, refined wheat flour, rice and rice flour. Random Clinical Trials showed that fortification of foods with VD significantly increased the serum concentration of 25(OH)D and reduced serum parathyroid hormone concentration (Ritu and Gupta, 2014). Establishing a genetic basis of VD levels among
Indians, particularly those who are at high risk for VDD would be a significant advancement in our understanding of the VD status and may offer better understanding into the causes of certain racial health differences. Future nutritional research on identification and validation of epigenetic biomarkers that could serve as risk assessment tools for VD related susceptibility to develop a disease later in life is needed.

References

Awasthi S and Vikram K (2014) Serum 25 hydroxy vitamin D insufficiency associated with bronchial asthma in Lucknow, India Indian journal of pediatrics 81 644-649
Ayesha I, Bala T S, Reddy C V and Raghuramulu N (2001) Vitamin D deficiency reduces insulin secretion and turnover in rats Diabetes, nutrition & metabolism 14 78-84
Babu U S and Calvo M S (2010) Modern India and the vitamin D dilemma: evidence for the need of a national food fortification program Molecular nutrition & food research 54 1134-1147
Bergwitz C and Juppnner H (2010) Regulation of phosphate homeostasis by PTH, vitamin D, and FGF23 Annual review of medicine 61 91-104
Bhat M and Ismail A (2015) Vitamin D treatment protects against and reverses oxidative stress induced muscle proteolysis The Journal of steroid biochemistry and molecular biology 152 171-179
Bhat M, Kalam R, Quadri S S, Madabushi S and Ismail A (2013) Vitamin D deficiency-induced muscle wasting occurs through the ubiquitin proteasome pathway and is partially corrected by calcium in male rats Endocrinology 154 4018-4029
Bhat M, Noolu B, Quadri S S and Ismail A (2014) Vitamin D deficiency decreases adiposity in rats and causes altered expression of uncoupling proteins and steroid receptor coactivator3 The Journal of steroid biochemistry and molecular biology 144 Pt B 304-312

Acknowledgements

The authors acknowledge the help of Dr. Swapna Nagalingam and Ms. Shayari Das in the manuscript preparation. We are grateful to Dr. M. S. Bamji for the critical comments in writing the manuscript.

Study (APCAPS) PloS one 10 e0129468
Bergwitz C and Juppnner H (2010) Regulation of phosphate homeostasis by PTH, vitamin D, and FGF23 Annual review of medicine 61 91-104
Bhat M and Ismail A (2015) Vitamin D treatment protects against and reverses oxidative stress induced muscle proteolysis The Journal of steroid biochemistry and molecular biology 152 171-179
Bhat M, Kalam R, Quadri S S, Madabushi S and Ismail A (2013) Vitamin D deficiency-induced muscle wasting occurs through the ubiquitin proteasome pathway and is partially corrected by calcium in male rats Endocrinology 154 4018-4029
Bhat M, Noolu B, Quadri S S and Ismail A (2014) Vitamin D deficiency decreases adiposity in rats and causes altered expression of uncoupling proteins and steroid receptor coactivator3 The Journal of steroid biochemistry and molecular biology 144 Pt B 304-312

Borkar V V, Devidayal Verma S and Bhalla A K (2010) Low levels of vitamin D in North Indian children with newly diagnosed type 1 diabetes Pediatric diabetes 11 345-350


Chaudhary S, Thukral A, Tiwari S, Pratyush D D and Singh S K (2013) Vitamin D status of patients with type 2 diabetes and sputum positive pulmonary tuberculosis Indian journal of endocrinology and metabolism 17 S670-673


Dhaon P and Shah V N (2014) Type 1 diabetes and osteoporosis: A review of literature Indian journal of endocrinology and metabolism 18 159-165


Gallagher J C (2013) Vitamin D and aging Endocrinology and metabolism clinics of North America 42 319-332

Gombart A F (2009) The vitamin D-antimicrobial peptide pathway and its role in protection against infection Future microbiology 4 1151-1165


Harinarayan C V (2005) Prevalence of vitamin D insufficiency in postmenopausal south Indian women Osteoporosis international: A journal established as result of cooperation between the European Foundation for Osteoporosis and
the National Osteoporosis Foundation of the USA 16 397-402
Harinarayan C V (2014) Vitamin D and diabetes mellitus Hormones 13 163-181
Harinarayan C V, Ramalakshmi T, Prasad U V and Sudhakar D (2008) Vitamin D status in Andhra Pradesh: a population based study The Indian journal of medical research 127 211-218
Hossein-nezhad A, Spira A and Holick M F (2013) Influence of Vitamin D status and vitamin D3 supplementation on genome wide expression of white blood cells: A randomized double-blind clinical trial Plos one 8 e58725
ICMR (2010) Nutrient requirements and recommended dietary allowances for Indians
Ismail A and Namala R (2000) Impaired glucose tolerance in vitamin D deficiency can be corrected by calcium The Journal of nutritional biochemistry 11 170-175
Khadilkar V V and Khadilkar A V (2013) Use of vitamin D in various disorders Indian journal of pediatrics 80 215-218
Leung P S (2016) The Potential Protective Action of Vitamin D in Hepatic Insulin Resistance and Pancreatic Islet Dysfunction in Type 2 Diabetes Mellitus Nutrients 8
Association of Maternal Vitamin D Status with Glucose Tolerance and Caesarean Section in a Multi-Ethnic Asian Cohort: The Growing Up in Singapore Towards Healthy Outcomes Study PloS one 10 e0142239


Martin A, David V and Quarles L D (2012) Regulation and function of the FGF23/klotho endocrine pathways Physiological reviews 92 131-155


Nakashima A, Yokoyama K, Yokoo T and Urashima M (2016) Role of vitamin D in diabetes mellitus and chronic kidney disease World journal of diabetes 7 89-100

Norman A W (2008) From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health The American journal of clinical nutrition 88 491S-499S


Olmos-Ortiz A, Avila E, Durand-Carbajal M and Diaz L (2015) Regulation of calcitriol biosynthesis and activity: focus on gestational vitamin D deficiency and adverse pregnancy outcomes Nutrients 7 443-480


Prietl B, Treiber G, Pieber T R and Amrein K (2013) Vitamin D and immune function Nutrients 5 2502-2521


Quraishi S A and Camargo C A Jr (2012) Vitamin D in acute stress and critical illness Current opinion in clinical nutrition and metabolic care 15 625-634


Raska I Jr, Raskova M, Zikan V and Skrha J (2016) High Prevalence of Hypovitaminosis D in Postmenopausal Women with Type 2 Diabetes Mellitus Prague medical report 117 5-17


Ritu G and Gupta A (2014) Fortification of foods with vitamin D in India Nutrients 6 3601-3623


Vitamin D Deficiency in India


Shafia S, Qasim I, Aziz S A, Bhat I A, Nisar S and Shah Z A (2013) Role of vitamin D receptor (VDR) polymorphisms in susceptibility to multiple myeloma in ethnic Kashmiri population Blood cells, molecules & diseases 51 56-60


Shivane V K, Sarathi V, Bandgar T, Menon P and Shah N S (2011) High prevalence of hypovitaminosis D in young healthy adults from the western part of India Postgraduate medical journal 87 514-518


Swapna N, Vamsi U M, Usha G and Padma T (2011) Risk conferred by FokI polymorphism of vitamin D receptor (VDR) gene for essential hypertension Indian journal of human genetics 17 201-206


Vaidya S V, Ekbote V H, Khadilkar A V, Chiplonkar S A, Pillay D and Divate U (2012) Bone status of women over 40 years of age from two socioeconomic strata Endocrine research 37 25-34


van der Meer I M, Middelkoop B J, Boeke A J and Lips P (2011) Prevalence of vitamin D deficiency among Turkish, Moroccan, Indian and sub-Sahara African populations in Europe and their countries of origin: an overview Osteoporosis international : A journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 22 1009-1021

reproduction technologies: current concepts Reproductive biology and endocrinology: RB&E 12 47

Vimala


White J H (2008) Vitamin D signaling, infectious diseases, and regulation of innate immunity Infection and immunity 76 3837-3843

