RESEARCH ARTICLE
CURRENT UNDERSTANDING OF ROLE OF VITAMIN D IN TYPE 2 DIABETES MELLITUS

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ABSTRACT
Insulin resistance and its impaired secretion are the core components of type 2 diabetes mellitus. Recently role of vitamin D has been highlighted in the pathogenesis of this disease. Vitamin D has been shown to maintain healthy glucose metabolism by increasing intracellular calcium levels and insulin secretion, by increasing GLUT4 activity and by suppressing the inflammatory response of various cytokines thereby protect the beta cells from oxidative injury. Low vitamin D levels were strongly associated with increased risk of type2 diabetes. Few studies have advocated maintaining vitamin D levels more than 30ng/ml to lower down the risk of this disease. But still more studies are required to establish this cut-off value. The results of vitamin D supplementation trials in preventing type2 diabetes are rather conflicting but it has been shown to be useful in improving impaired glucose tolerance in a pre-diabetic state. Vitamin D mediates its effect by interacting with vitamin D receptors (VDR) which are present in almost every tissue including beta cells in humans which also contains 1αhydroxylase enzyme. Gene polymorphism in VDR gene, Calbindin D-28K(carrier of vitamin D molecule in plasma) and 1α hydroxylase gene were positively linked to type2 diabetes but not in all population studies. In conclusion, deficient vitamin D levels increases the risk of developing type2 diabetes. However, more studies are required to ascertain the role of gene polymorphism and effect of vitamin D supplementation in type2 diabetes mellitus.

INTRODUCTION
Diabetes mellitus type2 is increasing at an alarming pace despite several advancements being made in its diagnosis and treatment. Insulin resistance and impaired insulin secretion are the core components in the pathogenesis of this disease. Various clinical tools are available to predict the risk of type2 diabetes such as fasting and postprandial glucose levels, HBA1c, C-peptide, insulin levels, HOMA-IR etc besides advocating life style modifications and dietary interventions. However, majority of patients are unable to achieve optimal glycemic control and improvement in other metabolic functions. This clearly indicates the importance of other roleplayers affecting beta cell function directly or indirectly. One such important molecule is vitamin D having pleotropic effects in the human body. It has been reported that vitamin D deficiency makes a person 91% prone to insulin resistance and pre-diabetic state even in the presence of normal blood sugar levels (Huang et al,2013) as well as increase the risk of microvascular complications in type 2 diabetes (Bajaj et al., 2014). Vitamin D facilitates insulin secretion from beta cells as well as decreases insulin resistance. Vitamin D also upregulates GLUT4 translocation and glucose utilization as well as decrease inflammatory markers thereby maintaining glucose homeostasis and protect the beta cell from oxidative stress (Manna and Jain, 2012). It has been reported that beneficial effects of vitamin D in type 2 diabetes are attributed to an increase in GSH and decrease in triglyceride levels (Jain et al., 2014). Vitamin D mediates its biological effects by interacting with vitamin D receptors (VDR) which are present in every tissue and cell type including beta cells in the body. The gene polymorphism in candidate genes i.e. VDR, calbindin-D28k and 1α hydroxylase gene have been linked with the increased risk of type 2 diabetes in few (Hitman et al,1998;Ogunkolade et al, 2002) but not in all population studies(Malecki et al., 2003; Angel et al., 2004; Reis et al., 2005). The consequences of this polymorphism are attributed to alteration in calcium metabolism, modulation of insulin secretion and modification of cytokine expression (Palomer et al, 2008). This has radically changed our understanding of the role of vitamin D beyond maintaining good bone health.

Although there is, as yet, no consensus to recommend a target range for serum vitamin D concentrations, vitamin D insufficiency is defined by its levels less than 30ng/ml (75mmol/L) which are proposed by several team of experts (Souberbielle et al, 2010; Adams and Hewison, 2010). It is highly important to generate awareness in this respect so as to decrease the risk of type 2 diabetes mellitus in addition to

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other pathologies. This review will focus on the current understanding of vitamin D in type 2 diabetes mellitus.

Mechanism of Action of Vitamin D In Type 2 Diabetes Mellitus

Vitamin D deficiency was linked to impaired glucose tolerance and type2 diabetes many years ago (Gedik and Akalin, 1986; Chiu et al, 2004). Type 2 diabetes is a state of insulin resistance and insulopenia associated with progressive deterioration in beta cell function and eventual loss of beta cell mass (Prentki and Nolen, 2006). The mechanism of action of vitamin D in type2 diabetes can be better explained by studying its key components i.e.

- Status of Pancreatic beta cell function and insulin resistance
- Role of inflammation

Status of pancreatic beta cell function and insulin resistance

The biological effects of vitamin D on beta cells is mediated by its binding to vitamin D receptor (Holick,2003). Vitamin D directly induce beta cell insulin secretion by increasing intracellular calcium concentration via non-selective voltage dependent calcium channels or by activation of beta cell calcium dependent endopeptidase which facilitates the conversion of proinsulin to insulin. Furthermore, 1α hydroxylase enzyme is expressed in beta cells suggesting that active vitamin D molecule is important in governing the insulin status. Vitamin D increases the responsiveness of cells to insulin by stimulating the expression of insulin receptors and by assuring normal calcium influx through cell membranes, particularly maintaining the adequate intracellular cytosolic calcium pool (Ojuka,2004; Mathieu and Gysemans, 2006). Hypocalcemia is associated with impairment of insulin release even in non-diabetics (Yasuda et al, 1975). Variations in ionic calcium in primary insulin target tissues contribute to peripheral insulin resistance via impaired insulin signal transduction leading to reduced GLUT-4 activity (Mathieu and Gysemans, 2006). GLUT-4 is a key player in glucose metabolism and maintenance of glucose homeostasis in the body (Huang and Czech, 2007). It has been reported that GLUT-4 heterozygous knockout mice had increased levels of serum glucose and insulin associated with reduced muscle glucose uptake, hypertension and diabetic complications (Stenbit et al, 1997). Manna and Jain (2012) for the first time showed that vitamin D upregulates GLUT-4 translocation to the cell surface leading to glucose uptake and utilization in adipocytes having treated with high glucose. They further reported that vitamin D also suppresses the action of inflammatory markers.

Vitamin D binding protein, also known as Calbindin–D28k, encoded by the Gc (group specific component) gene functions as a specific transporter of circulating vitamin D metabolite (Daiger et al,1975). Calbindin D is a glycoprotein synthesized by the liver and forms a complex with vitamin D so that the circulating vitamin D is delivered to the target tissues (Chun,2012). This complex comprising vitamin D interacts with vitamin D receptors and mediates its biological pleotropic effects. The expression of Calbindin D has been shown to protect beta cells from the destructive action of cytokines (Mathieu et al, 2005). At the molecular level, vitamin D activates the transcription of the human insulin gene and vitamin D response element is also present in human insulin gene promoter region (Norman, 2006). This clearly indicates that the interaction of vitamin D with insulin production is a highly conserved phenomenon.

Role of Inflammation

Systemic inflammation is associated with type 2 diabetes (Duncan et al, 2003; Hu et al, 2004). Vitamin D has been proposed as an anti-inflammatory molecule. Elevated cytokine levels led to insulin resistance and beta cell apoptosis. 1,25 (OH)2D3 behaves like an immunomodulator as it stimulates phagocytosis and suppresses the antigen presenting capacity and activation of IL-12 (Penna and Adorini, 2000; Adorini, 2003). Vitamin D also down regulates the activation of nuclear factor k-beta which is an important regulator of genes encoding pro-inflammatory cytokines implicated in insulin resistance (Van Etten and Mathieu, 2005). Macrophages and dendritic cells express 25-hydroxylase and 1α hydroxylase enzymes and can synthesize active molecule of vitamin D which can suppress the expression of TLR2 (Toll like receptors) and TLR4 molecules (Fritsche et al, 2003; Sadeghi et al, 2006). As mentioned earlier, calbindin-D also protects the beta cells from the destructive action of cytokines. Role of vitamin D as an immunomodulator is well defined especially in type1 diabetes which comprises the component of autoimmunity. Vitamin D suppresses the expression of MHCII and adhesion molecules necessary for full T cell activation. It has been reported that 1, 25(OH)2D3 preserve the insulin content of human islets and prevent MHCII expression, IL-6 production and NO release (Riahy et al, 2001).

These data clearly support the role of vitamin D in lowering the inflammatory response in the body thereby protect the vital tissues such as beta cells from free radical mediated injury.

Role of Gene Polymorphism In Vitamin D Related Molecules In Type 2 Diabetes Mellitus

Gene polymorphism in three candidate genes i.e. VDR, Calbindin –d 28K and CYP1 alpha gene have been shown to play an important role in type 2 diabetes mellitus.

VDR Gene Polymorphism (Vitamin D Receptor)

VDR is a member of nuclear receptor superfamily of ligand activated transcription factors which also include thyroid hormone receptor, retinoic acid receptor, peroxisome proliferator activated receptor etc. In humans, gene encoding VDR is located on chromosome 12cen-q12 and shows extensive polymorphism (Haussler et al, 1998). Till date, more than 25 different polymorphisms have been described in the VDR locus (Tuorkey and Abdul Aziz, 2010). VDR receptors have traditionally been associated with calcium and phosphorus homeostasis but its identification in many cell types including the beta cell has led to the recognition of non-calcemic action of VDR ligands. It has been proposed that genetic alterations in the VDR contribute to the pathogenesis of type2 diabetes mellitus by altering calcium metabolism, modification of adipocyte function and insulin secretion as well as by modification of cytokine expression (Palomer et al, 2008). VDR functions as a transcription factor when bound to 1,25(OH)2D3. Polymorphism in the VDR gene such as Taq1, Bsm1, Apa1 and Fok1 has been linked with insulin secretion
and sensitivity in few but not in all population studies. Apa1 polymorphism was associated with insulin secretion in healthy Bangladeshi Asian population (residing in London) with vitamin D deficiency (Hitman et al, 1998). On the other hand, Taq1 polymorphism has been reported to be an independent predictor of insulin secretion while Bsm1 and Apa1 were associated with post-prandial C-peptide levels, fasting glucose, HOMA-IR and FokI VDR polymorphism was linked with insulin resistance (Angel et al, 2004). On the contrary, in some populations i.e. in Polish, Chile and Finnish populations, no positive association of VDR polymorphism with type 2 diabetes mellitus has been reported (Malecki et al, 2003; Angel et al, 2004 and Reis et al, 2005).

Therefore, the evidence supporting the association of VDR polymorphism with diabetes mellitus is still conflicting and require more studies in this respect.

**Gene polymorphism in CalbindinD-28k gene**

Vitamin D signaling occurs by binding of circulating 1,25(OH)2D3 to VDR. Calbindin is essential for vitamin D endocytosis and its metabolism. Two frequent missense polymorphisms at codons 416GAT→GAG (Asp→Glu) and 420 ACG→AAG(Thr→Lys) in exon 11 exists in Calbindin gene (Blanton et al, 2011). Variants of this protein are the carriers of vitamin D metabolites in serum. Polymorphism in this gene has been associated with increased risk of type2 diabetes and pre-diabetic phenotype in many (Sathmary, 1987; Iyengar et al, 1989 ; Hirai et al, 2000) but not in all studies (Ye et al, 2001; Klupa et al, 1999).

**Gene polymorphism in CYP1 alpha gene**

CYP1alpha gene encodes 1 alpha hydroxylase enzyme which is responsible for the synthesis of active vitamin D molecule. Polymorphism in this gene may influence the risk of type2 diabetes mellitus due to deficient vitamin D production. A study in polish population reported the dominance of T-C/T-T heterozygous haplotype combination in subgroup of obese type 2 diabetics as compared to controls (Malecki et al, 2003). More studies are required to establish a firm association of CYP1alpha gene polymorphism with type 2 diabetes mellitus. The accumulated evidence indicates that although gene polymorphism in VDR and in other candidate genes influences the risk of developing type2 diabetes, the conclusions are relatively variable in different cohorts. The reasons may be variable because of ethnic variations, study design, gene-environment interactions, dietary and life style factors. This strongly suggests the need to conduct more studies in different populations in order to draw any definite conclusions.

**Evidence Based Data Regarding Role of Vitamin D Deficiency And Its Supplementation In Type 2 Diabetes**

There are multiple reasons of vitamin D deficiency in humans. The major source of vitamin D is the sunshine; hence role of vitamin D in type 2 diabetes has been linked with seasonal variations in glycemic control which is worst in winters due to hypovitaminosis D (Mathieu et al, 2005). Therefore, seasonal variations, food habits, lack of mandatory vitamin D fortification programs and genetic factors may be responsible for vitamin D deficiency in humans. In the past few years, several studies have been conducted to study the association between vitamin D and type 2 diabetes. Gedik and Akalin (1986) first reported impairment in insulin secretion in four relatively healthy subjects with vitamin D deficiency. In these subjects, insulin secretion was normalized after 6 months of vitamin D supplementation. Others also reported significant improvement in insulin secretion with vitamin D intervention (Borisssov et al, 2003). Insufficient vitamin D and calcium hinders the glycemic control and supplementation of both the nutrients is essential to optimize glucose metabolism (Pittas et al, 2007). Substantial data has revealed that native Indian population is deficient in optimal vitamin D levels (Goswami et al, 2000; Marwaha et al, 2005; Harinarayan et al, 2007;Bachhal et al, 2015). India is also becoming the diabetic capital of the world. It has also been predicted that by 2030, Asian Indians would bear the maximum burden of this disease globally (Ramachandran et al, 2001; Chow et al, 2006). A study on Indian Punjabi population showed insufficient as well as deficient vitamin D levels in type 2 diabetics as compared to healthy controls (Khanna et al, 2014). A meta-analysis of 21 prospective studies revealed that higher 1,25(OH)2D3 levels were associated with lower risk of type2 diabetes and this association was not affected by age, sex, duration of follow-up, sample size, diabetic diagnostic criteria and assay procedure. They further stated that each 10nmol/l increase in 1,25(OH)2D3levels were associated with a 4% lower risk of type 2 diabetes (Song et al, 2013). Nurses Health Study reported an increased risk of type 2 diabetes in 8,3779 females in the age group of more than 20 years who had deficient vitamin D levels. The study advocated the combined daily intake of more than 800IU of vitamin D and 1000mg of calcium to lower the risk of diabetes by 33% (Pittas et al, 2006).

 Destruction of beta cells usually begin in infancy or early childhood until active diabetes is diagnosed. Hence it is important to start vitamin D supplementation soon after birth if one is detected with its deficiency (Rifkin, 2009). In vitro study suggested that 1,25(OH)2D3 induces the biosynthesis of insulin in rat pancreatic islet cells and also inhibit free fatty acid induced insulin resistance (Bourlon et al, 1999). Intravenous administration of vitamin D also improves insulin sensitivity (Gunal et al, 1997). A cross sectional survey of 5677 individuals in Newzealand concluded that serum concentration of vitamin D were altered in patients with newly diagnosed type2 diabetes and impaired glucose tolerance(Scragg et al, 1995). In addition to these reports, few studies reported conflicting results regarding vitamin D supplementation. A meta-analysis reported that vitamin D supplementation did not reduce the risk of developing diabetes over 7 years of follow-up. They further concluded that probably higher levels of vitamin D are required to affect the risk of type 2 diabetes (Boer et al, 2008). Some studies also reported no improvement in insulin sensitivity after vitamin D supplementation (Fliser et al, 1997).

It is important to mention here that relatively higher vitamin D levels are required to maintain the non-calcemic activities in the human body. Effect of 1,25(OH)2D3 on tissues other than bone can only be manifested at concentration of 10-100mol/L which exceeds the physiological levels required to maintain calcium and bone homeostasis by a factor of 100-1000. It has been suggested that in type2 diabetic patients, there is a need to maintain vitamin D levels around
30ng/ml (Cavalier et al, 2011). A study in Indian population also recommended to maintain vitamin D levels more than 30ng/ml (Harinarayan et al, 2007).

SUMMARY AND CONCLUSIONS
It can be firmly concluded that low vitamin D levels and its prolonged deficiency is a major risk factor for the development of type 2 diabetes. Relatively increased levels of vitamin D are required to maintain the healthy state of beta cells and other non-calcemic activities in the body. The important actions of vitamin D pertaining to type2 diabetes have been highlighted in Fig1. Few studies recommended the levels to be more than 30ng/ml. However more studies are required to firmly establish these cut-off values. The efficacy of Vitamin D supplementation once the person becomes active diabetic or how far it is beneficial in preventing the development of type 2 diabetes in near future is still an active area for research and need more population studies but certainly this intervention would never be negative if not highly beneficial. Maintenance of optimal levels of vitamin D is required right from the childhood to lower the risk of this disease. Gene polymorphism in VDR and in other candidate genes showed a positive association with increased risk of diabetes in few but not in all the population studies. Hence, the results cannot be generalized. This may also suggest that while designing a study for vitamin D status, role of gene polymorphism should also be taken into account as it may or may not affect the vitamin levels and its outcome after supplementation in a particular subset of population. There should be mandatory fortification of food stuffs with vitamin D in order to meet the daily needs and moreover vitamin D estimation should be included in the routine panel in order to monitor its status regularly.

Vitamin D directly/indirectly modifies the insulin response and glucose homeostasis in a positive manner.

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Legend: Fig 1
Previtamin D is synthesized by cutaneous synthesis by non-enzymatic photoysis. The action of 25 hydroxylase and 1α hydroxylase enzyme produces the active vitamin D molecule which binds with calbindin-D28k and interacts with VDR receptors present in all cell types including the beta cells.
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