

Short communication

## Vitamin D binding protein gene variants rs4588 and rs7041 and low serum concentration of 25-hydroxy (OH) vitamin D<sub>3</sub> in type-2 diabetes patients: a pilot study

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### Abstract

The current study was designed to evaluate vitamin D<sub>3</sub> levels and its association with common genetic variants of vitamin D binding protein (VDBP) in type-2 diabetic (T2D) patients of local Pakistani population. Serum 25-hydroxy (OH) vitamin D<sub>3</sub> was quantified in 40 patients and 40 healthy individuals through IDS 25-Hydroxy vitamin D enzyme immunoassay (EIA). A common variant of VDBP or group specific component (GC), rs4588 and rs7041 were genotyped by restriction fragment length (RFLP) polymerase chain reaction (PCR). Serum levels of 25-hydroxy (OH) vitamin D<sub>3</sub> were found to be deficient (<10 ng/ml) in 74% of T2D patients, while 20% of T2D patients and 90% healthy individuals revealed insufficient D<sub>3</sub> levels (10-29 ng/ml), indicating the importance of the evaluation of 25-hydroxy (OH) vitamin D<sub>3</sub> levels in T2D patients in Pakistan. The risk alleles of GC (rs4588 and rs7041) were not found to be associated with low serum level of 25-hydroxy (OH) vitamin D<sub>3</sub> ( $p=0.053$ ). This pilot study indicates insufficient levels of vitamin D in our general population and low levels in diabetics and forms the basis to perform a large-scale, population based comprehensive study.

**Keywords:** Vitamin D, hypovitaminosis, type 2 diabetes mellitus

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For the last two decades, there has been an enormous increase in the number of people affected by diabetes mellitus (DM) especially T2D [1]. Pakistan currently ranks the seventh position in the list of countries with major burden of diabetes, but if the present situation persists, it is predicted that it may become fourth [1, 2]. According to a survey more than 10% of the adult Pakistani population suffers from diabetes [3] and its associated secondary complications [2]. This rise in T2DM can be due to various reasons such as unhealthy diet, obesity, sedentary lifestyle etc. [1]. A number of studies suggest a correlation between vitamin D deficiency with the severity and frequency of T2D occurrence. Various studies correlate low serum levels of vitamin D in both T1D and T2D [4]. In line with this, vitamin D supplementation has shown to reduce the risk of developing T2D [5].

Vitamin D is also known as a sun vitamin and a pro-hormone. Vitamin D can be acquired basically by two sources that are: through diet, but mainly through direct ultraviolet B (UVB)-mediated synthesis in the skin. Two hydroxylation steps are needed to activate vitamin D. The first hydroxylation step takes place in the liver with the help of 25-hydroxylase, or CYP2R1 and the other step takes place in the kidneys with the help of 1- $\alpha$  hydroxylase or CYB27B1, leading to 1 $\alpha$ , 25-dihydroxyvitamin D<sub>3</sub> which is an active secosteroid hormone [6]. Vitamin D metabolism, transportation, degradation, or downstream signaling involves different genes. Vitamin D binding protein (VDBP) or group-specific component (GC) is one of the important components of the vitamin D pathway that binds to

vitamin D and its metabolites in circulation. Polymorphisms in VDBP have already been reported to be associated with altered levels of serum 25(OH) in genome wide association studies [7, 8].

In recent years, there is renewed interest, especially in the non-skeletal role of vitamin D and this is mainly due to the realization that the final hydroxylation carried out by the enzyme 1- $\alpha$  hydroxylase or CYB27B1 can also occur in several cells outside the kidney. The expression of 1- $\alpha$  hydroxylase or CYB27B1 enzyme necessary for the final hydroxylation step by cells such as macrophages, neutrophils, pancreatic beta cells allows the paracrine secretion of 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> thus highlighting the importance of vitamin D in processes other than bone health [9]. Therefore a number of studies showed a correlation between vitamin D deficiency and various autoimmune diseases especially insulin resistance. Hypovitaminosis D has been shown to play a significant role in determining the function of pancreatic beta cells [10]. Furthermore, vitamin D has been shown to be an effective immunomodulator which can be used as a supplement therapy and has led to improved outcome in case of DM patients [5].

After the written consent of patients, fresh blood samples were drawn from type 2 diabetes (T2D) patients mainly from the province of Khyber Pukhtunkhwa (KPK) province of Pakistan obtained from Al-Asad Clinic, Rawalpindi. None of the T2D patients were on vitamin D supplements.

For the quantification of serum 25-hydroxy (OH) vitamin D<sub>3</sub>, IDS 25-hydroxy vitamin D EIA kit was used according to manufacturer protocol. Vitamin D

**Table 1** The serum levels of 25-(OH) vitamin D<sub>3</sub> (ng/ml) in patients and healthy control individuals. Numbers in brackets represent percentage of individuals.

Gender	Normal (30-100 ng/ml)		Insufficient (10-29 ng/ml)		Deficient (<10 ng/ml)	
	Healthy	diabetics	Healthy	diabetics	Healthy	diabetics
Male	1	1	21	4	1	15
Female	1	1	15	4	1	14
Total n (%)	2 (5)	2 (5)	36 (90)	8 (20)	2 (5)	30 (75)

Status was classified according to the manufacturer's protocol. Patients and controls (n = 40) were divided into three groups, i.e. normal, insufficient and deficient, on the basis of vitamin D<sub>3</sub> levels. Those whose vitamin D<sub>3</sub> level was equal or greater than 30 ng/ml were included in normal, whereas insufficient had vitamin D value of 10-29 ng/ml and those whose level were below 10 ng/ml were grouped as deficient.

A graph of standards was plotted according to the manufacturer's instructions and with the help of the graph the values of the individual samples were plotted and vitamin D values were calculated. The DNA samples of 24 cases and 26 controls were genotyped for two commonly studied VDBP polymorphisms rs4588 and rs7041. These two SNPs, rs4588 and rs7041, are present at codons 416 (GAT→GAG, Asp→Glu) and 420 (ACG→AAG, Thr→Lys) of exon 11 of the GC gene, respectively [11], recognized by restriction endonucleases (Fermentas): Hae III for T/G at 37°C, Sty I for C/A at 37°C. On the basis of amino acid substitution resulting from rs7041 and rs4588 and glycosylation difference, three electrophoretic variants of VDBP (Gc1f, Gc1 S and Gc2) have been described that can be classified in six common phenotypes of DBP: Gc 1S/1S, Gc 1S/2, Gc 1F/1F, Gc 1S/1F, Gc 1F/2, and Gc 2/2 [12].

In brief, DNA was extracted from whole blood through phenol-chloroform method followed by PCR amplification and restriction fragment length polymorphism (RFLP) analysis as described earlier (11). Bands were analyzed on agarose gel following digestion. Individuals were grouped into 3 genotypes (Gc1/1, Gc1/2 and Gc2/2) based on the type of allele, they carry because Gc1F and Gc1S have similar functional characteristics.

The serum 25-(OH) vitamin D<sub>3</sub> levels of patients and non-diabetic healthy controls are depicted in Table 1. Serum 25-(OH) D<sub>3</sub> levels of T2D patients showed that 20% (n=8) patients were insufficient and 75% (n=30) were deficient while only 5% (n=2) had normal levels of 25-hydroxy (OH) vitamin D<sub>3</sub> (Table 1). While the serum levels of healthy controls, showed that 90% (n=36) patients were insufficient and 5% (n=2) were deficient while only 5% (n=2) had normal levels of 25-hydroxy (OH) vitamin D<sub>3</sub> (Table 1). To determine the impact of Gc polymorphisms on the serum vitamin D level, we analyzed correlation between genotype frequency and 25-hydroxy (OH) vitamin D<sub>3</sub>

concentration in T2D, but there was no significant association (Table 2).

Vitamin D deficiency is associated with increased incidences of diabetes. Reports indicate that diabetic patients and the high risk population have inadequate level of vitamin D in their blood. Low vitamin D levels are also related to secondary disorders related to diabetes. Hypovitaminosis D has been shown to play a significant role in determining the function of pancreatic beta cells [10]. Furthermore, vitamin D has been shown to be an effective immunomodulator which can be used as a supplement therapy and has led to improved outcome in case of DM patients [5].

**Table 2** Genotype frequency of Gc and Serum 25-(OH)D concentration.

25-(OH) levels	Subjects n	Gc Genotypes		$\chi^2$	P value
		Gc1/1	Gc1/2		
< 10 ng/ml	17	14	3	3.744	0.053
≥10 ng/ml	7	3	4		

Diabetes is among the autoimmune diseases shown to involve vitamin D deficiency. Despite ample sunshine in Pakistan, there are reports showing vitamin D deficiency in the population [13]; however, there is a scarcity of data regarding vitamin D levels in our local diabetes patients. Therefore, we focused on evaluating serum levels of vitamin D in Type 2 diabetic patients and non-diabetic healthy controls in order to derive a relationship between hypovitaminosis D and Type 2 diabetes (T2D). Our results indicate low level of vitamin D in patients and relatively better levels in healthy controls, but still lying in the zone of insufficient vitamin D levels (Table 2). It is presumed that sun rich countries like Pakistan will have the normal vitamin D levels; however, there are certain cultural practices such as the indoor sedentary lifestyle, use of veils, and intake of diet poor in vitamin D, body mass index and the amount of melanin in the skin may be the potential factors contributing towards low vitamin D levels [1]. In order to determine the possible molecular basis for low serum concentration of 25-hydroxy (OH) vitamin D<sub>3</sub>, the association of VDBP/Gc polymorphisms; rs7041 and rs4588 was analyzed. But no correlation could be established.

This study indicates insufficient levels of vitamin D in our general population and low levels in diabetics. This would implicate that levels of vitamin D should be monitored regularly in type 2 diabetes patients and its possible use as supplemental therapy. Our study

highlights the potential relationship between vitamin D deficiency and Type 2 diabetes, which could hold enormous public health implications such as the routine evaluation of vitamin D levels in T2D patients and the use of vitamin D supplements. Further study is required in order to explore the possible causes of vitamin D deficiency and consequences of using vitamin D supplements.

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