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Investigation of the VDR Gene Polymorphism in Unrelated Gujarati Group with and without Diabetic Mellitus Type-2.

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ABSTRACT

Vitamin D receptor (VDR) polymorphism influences susceptibility to type- 1diabetes, but the association with type 2 diabetes is not clear. A small group comprising 33 males and 24 females out of which 34 diabetic patients and 23 normal individual from unrelated Gujarati individuals above 45 years of age for the study. DNA was extracted from their blood samples for PCR and RFLP using specific primers BmsI endonuclease. VDR gene polymorphism with single band of 822bp (AA) two bands of 650bp and 172bp (BB) and three bands of 822bp, 650bp and 172bp (AB) genotypes were detected in normal as well as patients with T2DM. The genotype frequency and gene frequency of the VDR polymorphism did not differ between patients and controls in our present studies. It is therefore concluded that single polymorphism of BsmI is not associated with T2MD, however, further studies considering all types of polymorphisms in VDR gene are required for their association with T2MD and or T1MD/ gestational diabetes.

Keywords: VDR gene, Polymorphism, T2DM, DNA, PCR-RFLP

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INTRODUCTION

Majority of diabetes could be defined as type-1 and type -2 insulin and non- insulin dependent respectively. Occasionally, diabetes occurs during pregnancy and it is called gestational diabetes. Type-1 diabetes is an autoimmune condition in which body immune cells due to genetic defects could not recognize and attack insulin-producing β-cells of the islets of Langerhans in the pancreas, leading to insulin deficiency. Type-1 diabetes mostly develops in adolescence. The first signs and symptoms of the disorder are caused by high blood sugar and may include frequent urination (polyuria), excessive thirst (polydipsia), fatigue, blurred vision, tingling or loss of feeling in the hands and feet, and weight loss. These symptoms may recur during the course of the disorder if blood sugar is not well controlled by insulin replacement therapy. Type-2 diabetes make insulin but either their pancreas do not make enough insulin or the body cannot use the insulin well enough. This is called insulin resistance or metabolic syndrome. Diseases or conditions associated with insulin resistance include: obesity, high blood pressure, abnormal cholesterol levels, heart disease, polycystic ovary syndrome and above 45 years of age. People with type-2 diabetes mellitus (T2DM) have high levels of insulin in the blood as a marker of the disease rather than a cause. The development of type 2 diabetes is caused by a combination of lifestyle [1,2] and genetic factors[3]. Most cases of diabetes type-2 involve many genes in β-cell functions. There are a number of rare cases of diabetes that arise due to an abnormality in a single gene, monogenic condition of diabetes.

Vitamin D (1,25- dihydroxyvitamin D3) receptor (VDR) gene synthesize vitamin D receptor. Vitamin D has role in many biological actions; calcium homeostasis, cell proliferation and cell differentiation to many target tissues. Major role of vitamin D is to control the absorption of calcium and phosphate from the intestines into the bloodstream. Vitamin D plays an important role in insulin secretion. Most of these biological actions of vitamin D are now considered to be exerted through the nuclear vitamin D receptor[4]. VDR binds to the active form of vitamin D, known as calcitriol. The VDR gene locates on human chromosome 12q13.11[5]. VDR is a member of the steroid/thyroid hormone-receptor superfamily[6].

Allelic variations in the VDR gene were reported to be associated with a variety of phenotypes[7,8,9,10,11,12,13]. Earlier documented studies indicate that VDR gene polymorphisms have been found associated with type 1 diabetes in a Taiwanese [14], German [12], Japanese[15] etc. There is also evidence that this steroid may influence the insulin sensitivity. Thus genes involved in its metabolic pathway have been regarded as good candidates for T2DM[16]. Some of studies indicated possible association of VDR polymorphism with diabetes type-2 in north Indian [1], Iranian [2], Saudi [3] population. These findings prompted us to investigate the role of VDR as a candidate gene for susceptibility to type 2 diabetes mellitus (T2DM) in Gujarati population.

Recent studies have indicated many polymorphisms exist in the vitamin D receptor (VDR) gene, but the influence of VDR gene polymorphisms on VDR protein function and signaling is largely unknown. So far, three adjacent restriction fragment length polymorphisms for BsmI, ApaI, and TaqI, respectively, at the 3'end of the VDR gene have been the most frequently studied[17]. Present study has aimed to find out BsmI polymorphism in VDR gene, estimation of gene and genotype frequencies of VDR gene and their association with diabetes type 2.

MATERIALS AND METHODS

A set of 57 Blood samples comprising 33 males and 24 females out of which 34 diabetic patients and 23 normal individual was collected from unrelated Gujarati individuals above 45 years of age with their consent. Approximately, 2ml blood was taken which were transported to the laboratory in cool condition. DNA was extracted from blood samples and the quality and quantity of DNA were determined using agarose gel electrophoresis and Nanodrop ND-1000 Spectrophotometer. Nanodrop and the samples were diluted to 50 ng/ µl by autoclaved MilliQ water for final concentration and 3 µl of DNA was used as template for PCR reaction. For detection BsmI polymorphism in an VDR gene coding for vitamin D receptor, 822bp DNA fragment was amplified by PCR, which was set by adding forward and reverse primers (CAACCAAGACTACAAGTACCGCGTCAGTG)-(AACCAGCGGGAAGAGGTCAAGGG)[18].

The PCR mix containing 3 µl (30ng/µl) genomic DNA, PCR Master mix (Cat no. K0171, MBI Fermentas) containing 0.05U/µl Taq DNA polymerase (recombinant) in reaction buffer, MgCl2 (4mM) and dNTPS (0.4 mM

of each), and finally added with sterilized distilled water to make a final volume of 25 µl. The PCR reaction included the following steps: Predenaturation for 5 minutes at 94°C followed by 35 cycles of 30 seconds at 94 °C, 45 seconds at 62 °C, and one minute at 72°C and final extension for 10 minutes at 72°C for utilization of extra dNTPs in mixture. The amplified product 822bp was visualized on 1.5% agarose gel. The amplified or Polymerase Chain Reaction (PCR) products were digested by using BsmI restriction enzyme (@10 U/ µl), 10X reaction buffer at 37°C for overnight. The digested product was visualized on 2% agarose gel. Genotypic and allelic frequencies of VDR gene polymorphism in normal as well as diabetic patients in Gujarati population were determined manually.

RESULTS AND DISCUSSION

Absence of restriction site on the DNA strand resulted in appearance of single band of 822bp (AA genotype). The presence of one restriction site on both the strands resulted in the appearance of two bands of 650bp and 172bp (BB genotype). While one double stranded DNA with having one restriction sites and another strand with no restriction site, resulted in three bands of 822bp, 650bp and 172bp (AB genotype) as indicated in figure 1. In the present study, all three genotypes i.e., AA, BB and AB were observed in the samples (table 1). Among all genotypes, genotypic frequency of BB (48%) was maximum in Non Diabetic group than in Diabetic, while genotypic frequency of AA (29.5%) was maximum in Diabetic than in Non Diabetic individuals and frequency of genotypes AB (43.3%) was maximum in Non Diabetic individuals. Frequency of allele B was found on higher side in both groups; 0.57 in Diabetic and 0.70 in Non Diabetic individuals. The genotype frequency and gene frequency of the VDR polymorphism did not differ between patients and controls. It is in agreement with Ye et al [19] observations where they found that VDR is not a major gene for T2DM in French Caucasians. However, polymorphisms in the VDR gene are associated with the susceptibility to obesity in subjects with early-onset T2DM. Findings of Al-Daghri et al.[3] observed that polymorphisms in exon 9 (TaqI) and intron 8 (BsmI) of the VDR gene were significantly associated with T2DM, whereas the genotype distribution and allele frequency of the polymorphisms in exon 2 (Fok1) and intron 8 (ApaI) of VDR did not differ significantly between patients and control group. The prevalence of VDR polymorphisms in 4 restriction fragment length polymorphism sites including BsmI, FokI, ApaI and TaqI were analysed in patients and controls. The frequencies of 3 genotypes (Aa, FF and Bb) were significantly higher in the type I diabetes patient group in Iran[20]. However, the relationship between VDR gene polymorphisms and onset pattern of diabetes was not significant. Observations of Tawfeek et al.[21] are similar to our studies as they revealed that the gene frequency, allele frequency and carriage rate of the VDR polymorphism BsmI did not differ between patients and controls with no significant association with any clinical parameters. It is therefore concluded that single polymorphism of BsmI is not associated with T2MD, however, further studies considering all types of polymorphisms in VDR gene are required for their association with T2MD and or T1MD/ gestational diabetes.

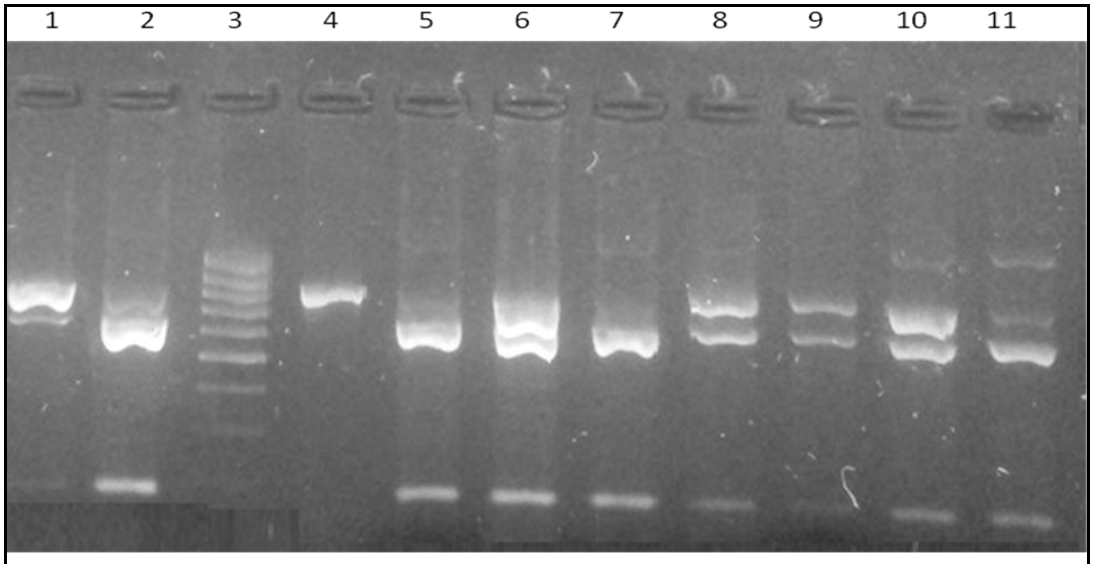

Fig 1: lane 1: PCR product of 822bp, lane 2, 5, 6 & 7: BB-genotype of 650bp & 172bp, lane 3: Marker (MassRuler) 100-1000bp and lane 8-11: AB genotype of 822bp, 650bp and 172bp

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Table 1: Genotypic and allelic frequency of VDR gene

Group	Genotypic Frequency			Allelic Frequency	
	AA	AB	BB	A	B
Diabetic	29.5% (n=10)	26.5% (n= 9)	44% (n=15)	0.43	0.57
Non Diabetic	8.7% (n=2)	43.3% (n=10)	48% (n=11)	0.30	0.70

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