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Association of Vitamin D Receptor Gene Polymorphism with Type 2 Diabetes in the Northern Region of Saudi Arabian Population

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Vitamin D deficiency and vitamin D receptor (VDR) gene polymorphisms are associated with type 2 diabetes mellitus (T2DM), However, its association with type 2 diabetes mellitus) is controversial and has not been established in different ethnic populations. Therefore, we aimed to evaluate the possible association between VDR gene polymorphisms (Fok1, Bsm1, and Taq1and Apa1) and T2DM patients in Saudi Arabia.
Methods: 100 patients with T2DM and 100 healthy age-matched control subjects were enrolled. Fasting blood glucose, lipids profile and HbA1c were measured by autoanalyzer. The circulatory level of 25 hydroxyvitamin D [25(OH)D] was measured by immuno*chem*iluminance. VDR gene polymorphisms detection has been done by polymerase chain reaction through restriction fragment length polymorphism (PCR-RFLP) method.
Results: Our study has shown lower levels of 25(OH) D in patients with T2DM (in comparison with control subjects (44.65 ± 7.19), $p < 0.00$. There were statistically significant differences between patients with type 2 diabetes and controls regarding the distribution of FokI and Taq1 genotypes and alleles ($p<0.004$ and $p<0.04$) and nonsignificant differences regarding Bsm1genotpes and allele.

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Conclusions: Vitamin D deficiency is prevalent in T2DM patients in Saudi Arabia. An association was found between VDR FokI and Taq1 gene polymorphism and susceptibility to T2DM in Saudi Arabian patients.

Keywords: Vitamin D deficiency; type 2 diabetes mellitus; polymorphism; association.

1. INTRODUCTION

Diabetes mellitus (DM) is a multifactorial disorder that occurs due to defects in insulin secretion, insulin action, or, consequentially, hyperglycemia with turbulence in the metabolism of carbohydrates, proteins, and fats [1,2]. Management of diabetes by improving glycemic control is the single most essential therapeutic approach for preventing the complications of diabetes [3]. Diabetes mellitus (DM) is a serious world medical issue, including in Saudi Arabia [4,5]. Modest knowledge concerning the reason for T2DM, but many of its risk factors have been known and investigated. T2DM, as other inflammatory disorders, could be averted if its risk factors are detected through the early onset of the disease and managed [6-9]. The increasing number in T2DM patients is an issue of serious concern. Being a composite disorder, T2DM is recognized to be caused by a vast number of environmental and genetic factors. The role of vitamin D in the pathogenesis and anticipation of DM has sparked pervasive attention. Besides its conventional role in Ca++ homeostasis, vitamin D also regulates insulin secretion from beta cells and its action on mediated a variety of target cells [10-13].

Vitamin D deficiency has been identified as one of the risk factors for the development of type 2 diabetes [14]. Several evidence-based studies have reported the relation between vitamin D deficiency and advancement of T2DM [15], where vitamin D supplementation was found to be associated with the reduced risk in prone patients [16]. Vitamin D has been identified to play a critical role in diabetes, where it regulates the insulin receptor gene [17]. The biological effects of vitamin D mediated after its binding to cytosolic/nuclear vitamin D receptors (VDRs) [18] VDR belong to the steroid hormone receptor family [19]. These receptors spread in various tissues regulating important genes associated with unremitting illness, inflammatory processes and bone disorder [20]. Vitamin D activation through VDRs plays a vital role in regulating insulin secretion from the pancreatic β cells [21]. Many VDR gene polymorphisms have been linked to T2DM, insulin release [22] and metabolic disorder of obesity. Since there is complexity in identifying potential genes associated with T2DM development due to slight changes that possibly occur in the candidate genes and the interaction of these changes with other genetic or non-genetic factors [23]. The VDR gene polymorphisms associated with diabetes is VDR (Fok1, Bsm1, Taq1 and Apa1). The main objective of the present study was to evaluate the possible association of VDR gene polymorphisms in T2DM patients in the Saudi population.

2. MATERIALS AND METHODS

We recruited 200 Subjects.100 patients with type 2 diabetes mellitus, and 100 healthy controls in the present study. Decisive factors such as age, body mass index (BMI), HbA1c, Fasting blood sugar (FBS) and post prandial blood glucose (PPBS) level, urea level, serum creatinine, lipid profiles, Uric Acid, total protein, albumin protein Vitamin D and urine albumin test. All the control subjects were recruited based on the absence of any history of the disease. All biochemical analyses were carried out using the Autoanalyser.

2.1 Sample Collection

Isolated DNA was collected from King Khalid Hospital, Hail, Saudi Arabia.

2.2 VDR Genotyping

For TaqI polymorphisms of the VDR gene, 740 bp fragment was amplified using forward primer 5'CAGAGCATGGACAGGGAGCAAG-3' and reverse primer 5'-CAACCAAGACTACAAGTACCGCGTCAGTGA-3'. To generate PCR products in the final volume of 25μl containing 1.2μl genomic DNA, 0.4 μl of forward and reverse primers (10 pmoles conc.), 0.7μl of 10mM of each dNTP's and 4 unit of *Taq* polymerase were added. The PCR protocol was: 94^0C for 5 min followed by 30 cycles of 94^0C for 1 min, 69^0C for 35secs, 72^0C for 35secs and final extension at 72^0C for 5 min. VDR gene fragment was digested with FokI (New England Biolabs

Inc, Ipswich, MA, USA) restriction endonuclease and electrophoresed in 2% agarose gel. TaqI site yielded bands of 245 and 495 bp, whereas the presence of homozygous site yielded fragments of 205, 245 and 290 bp. Heterozygous conditions for the TaqI site exhibited fragments of 490, 290, 245 and 205 bp. A 745 base pair (bp) fragment containing the ApaI polymorphism in the intron 8 of VDR gene was amplified by Polymerase chain reactor (PCR) (Applied Biosystems, Foster City, USA) using the forward primer 5'AGAGCATGGACAGGGAGCAAG-3' and reverse primer 5'-GCAACTCCTCATGGCTGAGGTCTCA -3'. To generate PCR products in the final volume of 25μl containing 1.2μl genomic DNA, 0.3 μl of forward and reverse primers (10 pmole conc.), 0.5μl of 10mM of each dNTP's and 5 unit of Taq polymerase were added. The PCR condition was: 94^0C for 5 min followed by 35 cycles of 94^0C for 45seconds, 68^0C for 45seconds, 72^0C for 45 seconds and final extension at 72^0C for 5 min. After amplification, VDR gene fragment was digested with ApaI restriction endonuclease for 1 hour at 65^oC (New England Biolabs Inc, Ipswich, MA, USA) followed by electrophoresis in 2% agarose gel. The Apa I genotype was named as follows: AA (absence of the restriction), aa (presence of the restriction site), Aa (heterozygous for the restriction site.

DNA amplification for PCR (Applied Biosystems, Foster City, USA) analysis of VDR (FokI) gene polymorphism in exon 2 was determined using the primers: F-5'ATGGAAACACCTTGCTTCTTCTCCCT3'andR 5'AGCTGGCCCTGGCACTGACTCTGGCTCT3' producing and 265 bp fragment. The final volume of 25ml containing 1.2μl genomic DNA, 1.2 μl of forward and reverse primers (10 pmoles conc.), 0.4ml of 10mM of each dNTP's and 3 unit of Taq(Applied Biosystems , Foster City, USA) polymerase were used to obtain PCR product. The PCR protocol was: 940 C for 5 min followed by 30 cycles of 940 C for 45secs 620 C for 45secs 720 C for 45secs and final extension at 720 C for 7 min. After, amplification of PCR products was digested with FokI restriction endonuclease (New England Biolabs Inc, Ipswich, MA, USA) at overnight at 37oC. Then, 10 ml of the products were loaded onto 3% agarose gel containing ethidium bromide for electrophoresis. The presence of the restriction site that generates two fragments of 196 bp and 69 bp was indicated with f. In contrast, its absence, resulting in a single uncut 265 bp fragment, was indicated with F. Subjects were scored as ff homozygous, Ff heterozygous and FF homozygous according to the digestion pattern. Detection of the BsmI (VDR gene) site in intron 8 was performed by PCR amplification of a region carrying the BsmI site with primers in exon7(Forward primer)5'CAACCAAGACTACAAGTACCGCGTC AGTGA-3' and intron 8 (Reverse primer)- 5'-AACCAGCGGAAGAGGTCAAGGG-3' producing an 825-basepair (bp) fragment. PCR products were generated in the final volume of 25ml containing 1.2μl genomic DNA, .4 μl of forward and reverse primers (10 pmoles conc.), 0.5ml of 10mM of each dNTP's and 5 unit of Taq polymerase was used to obtain PCR product. The PCR protocol was: 940 C for 5 min followed by 30 cycles of 940 C for 45secs 620 C for 45secs 720 C for 45secs, and final extension at 720 C for 7 min. After, amplification PCR products were digested with BsmI (New England Biolabs Inc, Ipswich, MA, USA) restriction endonuclease at 650C for 1 hour. Digested products were electrophoresed in a 2% agarose gel containing ethidium bromide. The presence of the BsmI restriction site produced 175 and 650 bp fragments, whereas the absence of this site yields one band of 825 bp fragments.

2.3 Statistical Analysis

Demographic and clinical data were reported as mean ± standard deviation (SD). The unpaired student t-test made statistical comparisons between group means. Genotype and allele frequencies of the T2DM were compared to the respective frequencies of the control groups using the chi-square test. The odds ratios (ORs) and the 95% confidence interval (CI), comparing the allelic distribution in the study groups were also calculated. Two-tailed P-value <0.05 were considered significant.

3. RESULTS

The baseline characteristics of Type-2 diabetes and healthy controls are presented in Table 1. In this study, we observed significantly higher BMI in diabetic patients compared with controls. Fasting sugar, RBS, HbA1C, LDL, VLDL TG and cholesterol were significantly higher levels found in T2DM patients when compared with controls. Moreover, a decreased circulatory level of vitamin D was observed in T2DM patients when compared with controls.

The distribution of genotypes and alleles of *VDR* (*BsmI* & *FokI*) gene polymorphism in type 2

diabetes patients and controls is shown in Table 2. In *BsmI* polymorphism, the frequency of BB, Bb and bb genotypes in patients was 21%, 52% and 27%, respectively. While in controls, their frequencies were 20 %, 60% and 20%, respectively. FokI polymorphism, the frequencies of FF, Ff and ff genotypes in patients were 38%, 51% and 11%, respectively, while their frequency in control was 53%, 45%, 2%, respectively. The ff genotype was significantly higher in patients than control (p<0.004, OR (95% CI): 7.671 (1.606-36.636).f allele of *FokI* polymorphism was also significantly higher in patients than control (p<0.006, OR (95% CI): 1.771 (1.150-2.729).

The frequency distribution of genotypes and alleles of TaqI and ApaI polymorphism among diabetic patients and control is shown in Table 3. A significant difference was seen in the genotype distribution in Taq1 among diabetic patients and controls (p<0.04). The frequencies of TT, Tt and tt genotypes in patients were 11%, 57% and 32%, respectively, while in controls, their frequency was 5%, 50% and 45%, respectively. In ApaI polymorphism, the frequencies of AA, Aa and aa among patients were 33%, 56% and 11%, respectively, while their frequency in controls were 52%, 51% and 7%, respectively. The TT genotype was found higher in patients than in controls. The frequency distribution of genotypes for VDR Apa I between diabetic patients and controls did not show any significant difference (p<0.14). A statistically significant difference was found in T and t allele distribution in Taq1 polymorphism among diabetic patients and controls (p<0.03). The frequency of T and t allele in patients were 39.5% and 60.5%, respectively, and their frequency in controls were 30% and 70%. There was no significant difference found in alleles A and ApaI polymorphism (p<0.10).

Pearson Correlation between Vitamin D and other variable are presented in Table 4.

The correlations between the circulatory level of vitamin D and biochemical markers by Pearson Correlation analysis are presented in Table 4 and Fig 1. Vitamin D levels exhibited a significant negative correlation with HbA1c (R = -0.1975, p<0.0489), However, there were no significant correlation observed between vitamin D and another biochemical marker.

4. DISCUSSION

Vitamin D has known to play a vital role in insulin synthesis, secretion and function also, it has an important function on elements of inflammation which may affect the progression of T2DM [24]. Vitamin D stimulates insulin secretion from the pancreatic β cells by enhancing the intracellular Ca ion concentration converting the pro-insulin to insulin. Moreover, it increases the sensitivity of cells to insulin by raising the expression of insulin receptors and by keeping a sufficient supply of calcium pool [21]. We have investigated VDR gene polymorphisms with T2DM in Saudi people.

Table 1. Demographic data and biochemical parameter of study subjects

Variables	Control (N=100)	Type 2 Diabetes (N=100)	p = Value
Age (Years)	54.48±6.98	54.92±6.29	0.32
Duration (Years)		10.04±3.02	-
Weight (Kg)	56.90±5.94	57.77±10.40	0.23
Height (cm)	155.22±7.04	152.22±6.39	0.001**
BMI (Kg/cm^2)	23.59±2.59	24.92±4.25	0.004**
HbA1c (%)	5.41±0.59	9.85±2.97	0.001***
Fasting Blood Sugar (mg/dl)	92.70±7.88	183.56±86.61	0.001***
Postprandial Blood Sugar (mg/dl)	127.43±14.49	267.90±107.98	0.001***
HDL (mg/dl)	40.80±15.15	38.41±12.79	0.11
LDL (mg/dl)	71.39±25.77	86.10±41.49	0.001**
VLDL (mg/dl)	25.24±8.11	35.62±24.20	0.001***
TG (mg/dl)	126.49±40.69	179.27±119.92	0.001***
Cholesterol (mg/dl)	132.90±25.86	161.37±51.80	0.001***
Vitamin D	27.41±9.20	12.21±6.32	0.001***

All data are shown as mean ± SD

** signifies p < 0.05, " ** " signifies p < 0.01, " *** " signifies p < 0.001*

Table 2. Genotype and allele frequency of VDR BsmI and FokI among controls and T2DM patients

BsmI Genotype	**Control N=100 (%)**	**T2DM N=100 (%)**	**OR (95% CI)**	**P- Value**
BB	20 (20%)	21 (21%)	-	-
Bb	60 (60%)	52 (52%)	0.82 (0.403-1.690)	0.36
bb	20 (20%)	27 (21%)	1.26 (0.553-2.985)	0.35
Allele				
B	100 (50%)	94 (47%)	-	-
b	100 (50%)	106 (53%)	0.25 (0.761-1.670)	0.30
FokI Genotype	**Control N=100 (%)**	**T2DM N=100 (%)**	**OR (95% CI)**	**P- Value**
FF	53 (53%)	38 (38%)	-	-
Ff	45 (45%)	51 (51%)	1.581 (0.886-2.818)	0.08
ff	02 (02%)	11 (11%)	7.671 (1.606-36.636)	**0.004****
Allele				
F	151 (75.5%)	127 (63.5%)	-	-
f	49 (24.5%)	73 (36.5%)	1.771 (1.150-2.729)	**0.006****

p< 0.05 is considered statistically significant

Table 3. Genotype and allele frequency of VDR TaqI and Apa1 among controls and patients

TaqI Genotype	**Control N=100 (%)**	**T2DM N=100 (%)**	**OR (95% CI)**	**P- Value**
tt	45 (45%)	32 (32%)	-	-
Tt	50 (50%)	57 (57%)	1.603 (0.887-2.896)	0.07
TT	05 (05%)	11 (11%)	3.09 (0.979-9.775)	0.04*
Allele				
t	140 (70%)	121 (60.5%)	-	-
T	60 (30%)	79 (39.5%)	1.52 (1.006-2.306)	0.03*
ApaI Genotype	**Control N=100 (%)**	**T2DM N=100 (%)**	**OR (95% CI)**	**P- Value**
AA	42 (42%)	33 (33%)	-	-
Aa	51 (51%)	56 (56%)	1.398 (0.772-2.529)	0.17
aa	07 (07%)	11 (11%)	2.00 (0.698-5.726)	0.14
Allele				
A	135 (67.5%)	122 (61%)	-	-
a	65 (32.5%)	78 (39%)	1.328 (0.880-2.002)	0.10

p< 0.05 is considered statistically significant

Table 4. Pearson correlation between vitamin D and other parameter

Variable	**R = Value**	**p = value**
Vitamin D vs HbA1c	-0.1975	**0.0489***
Vitamin D vs FBS	-0.1254	0.2137
Vitamin D vs PPBS	-0.1138	0.2594
Vitamin D vs HDL	0.1154	0.2529
Vitamin D vs LDL	0.07500	0.4583
Vitamin D vs VLDL	-0.09652	0.3394
Vitamin D vs TG	-0.04582	0.6508

**Significant considered as P<0.05. (2-tailed)*

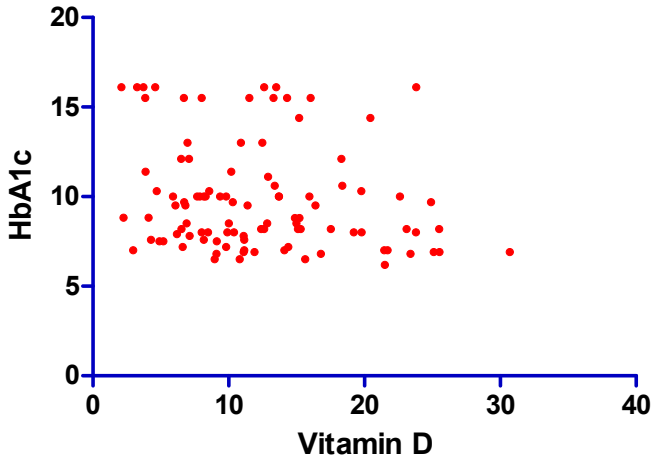

Fig. 1. Pearson correlation between vitamin D level and HbA1c

We evaluated VDR (Fok1, Taq1, Bsm1 and Apa1) gene polymorphisms in our patients. We found significant statistical differences in comparing Taq1 and FokI genotypes and allele frequency distribution between T2DM patients and controls with increased frequency of and TT and ff genotype in patients with T2DM. Our results are in good agreement with Gendy et al. [25] finding that ff genotype and the f allele of the VDR Fok1 polymorphism are significantly more frequent in type 2 diabetic Egyptian patients. Mackay and Badawi [26] found the ff genotype and the f allele of the VDR Fok1 polymorphism significantly more frequent in diabetic patients with metabolic syndrome. Also, the study done by Errouagui et al. [27] on the Moroccan population reported a significant association regarding FokI distribution in T2DM. In the Saudi population of the Riyadh region, Aldaghri et al. [28] examined the polymorphism of four SNPs in VDR gene (ApaI, FokI, TaqI and BsmI) and an association of BsmI T allele and C/T genotype and TaqI A/G genotypes and T2DM was observed. This study is in accordance with our results. We also found a significant association of Taq TT genotypes and the tallele.

Several studies have been studied to identify the relationship of VDR polymorphism in a mixture of populations. In Polish subjects, Malecki et al. have evaluated the polymorphism of four single nucleotide polymorphisms (SNPs) of VDR gene (BsmI, TaqI, FokI and ApaI), and they found that the genotype and allele distribution does not differ in both controls and T2DM. [29] Furthermore, in the French Caucasian population, Ye et al. studied the same SNPs of the VDR gene (BsmI, TaqI, FokI and ApaI), and they did not observe that both genotype and allele distribution in both controls and T2DM. [30] Furthermore, in the Turkish population, Dilmec et al. found no significant difference in genotype and allele frequencies of the same four SNPs (BsmI, TaqI, FokI and ApaI) of the VDR gene between both controls and T2DM [31]. Moreover, European Caucasians, Bertoccini et al. studied the association between VDR FokI polymorphism and T2DM. They did not found any difference of the genotype distributions and allele frequencies between T2DM subjects and controls in Italians [32].

There are many possible reasons for these discrepancies, including allelic heterogeneity between different ethnicities, population admixture in the studies, small or insufficient sample size, and linkage disequilibrium or environmental factors that might mask the genetic effects. There is a limitation in our study that the sample size is relatively small. Therefore, the relationship between VDR polymorphisms and T2DM is unclear and will require additional studies.

5. CONCLUSION

In conclusion, our study that vitamin D deficiency was highly prevalent in T2DM patients in Saudi Arabia. There were statistically significant differences between patients with T2DM and controls regarding FokI and Taq1 genotypes and allele distribution, which could be a risk factor for

patients with T2 DM in Saudi Arabia. The possible role of vitamin D in the pathogenesis of T2DM is still not completely understood. Further knowledge and studies are needed to identify new gene polymorphisms, which can play a significant role in the treatment and prevention of T2DM. Thus, the relevance of several studies from different populations, like ours, may provide support to narrowing down the reported variation in results associated with these gene polymorphisms and disease risk.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study has been reviewed and approved by the Research Ethics Committee (REC) at the University of Hail, and approved by University president letter-number 28252/5/42.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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