

Association of Vitamin D deficiency and Vitamin D Receptor Gene Polymorphisms with Type 2 diabetes mellitus Saudi patients

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Abstract:

Background: Type 2 diabetes mellitus (T2DM) is a global problem. Association of multiple genes in T2DM becomes a hot point recently. This study was aimed to evaluate association of vitamin D receptor gene polymorphisms with susceptibility to T2DM.

Subjects and methods: One hundred T2DM Saudi male patients were included in this study and one hundred healthy Saudi men were used as control. For each individual, fasting blood glucose, cholesterol, HDL-C, LDL-C, HbA1c, insulin and 25-(OH) vitamin D were measured. In addition, *Apal*, *BsmI* and *TaqI* genotypes were performed for each subject. Data was analyzed by SPSS version 16, using Spearman's rho and ANOVA tests.

Results: There was significant inverse correlation between 25-(OH) vitamin D level and T2DM ($p < 0.01$). HbA1c was inversely correlated with 25-(OH) vitamin D level ($P < 0.05$). Genotype study showed that tt of *TaqI* genotype was higher in T2DM group compared with control group ($p < 0.05$). Moreover, tt genotype has higher HbA1c than both TT and Tt genotypes ($p < 0.05$).

Conclusion: An association was confirmed between *TaqI* genotypes and T2DM but there is no correlation between *BsmI*, *Apal* and T2DM. In addition, HbA1c is positively correlated with tt genotype of *TaqI*.

Keywords: Vitamin D receptor, diabetes type 2, polymorphism.

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Background

Type 2 diabetes mellitus (T2DM) is still a global problem that affects individuals all over the world. Its prevalence in Saudi Arabia has increased in the last decade and affects the young at 30 years age¹. T2DM prevalence and complications make it the first problem that affects Saudi's life. It is characterized by insulin resistance and alteration in its secretion leading to disturbance in carbohydrate, protein and lipid metabolism². Both genetic and environmental factors influence T2DM development³. Vitamin D is responsible primarily for calcium hemostasis but also involved in diverse types of cells growth and differentiation⁴. Vitamin D deficiency enhances T2DM development and its supplementation induces glucose tolerance⁵. In non-obese diabetic mouse model, vitamin D plays important role in insulin release and normal glucose tolerance⁶. Vitamin D exerts its effect through bind-

ing to specific intracellular receptor called vitamin D receptor (VDR)⁷. The VDR is a member of steroid/thyroid hormone receptor family which is expressed in different cells lines include pancreatic β -cell⁸. Vitamin D and VDR complex induce insulin secretion from pancreatic β -cells and this is may a procedure by which vitamin D induces glucose tolerance⁹. The VDR gene is located at 12q13 chromosome and many polymorphisms were indicated at this region. The most important polymorphisms are *Apal*, *TaqI* and *BsmI* which covered by different studies in different area and ethics. The VDR gene polymorphisms become one of the candidate genes that influence T2DM development¹⁰. Our previous study which was done on Saudi obese males showed an association between these polymorphisms and insulin resistance in these patients¹¹. Accordingly, we aimed in this study to investigate the association of VDR gene polymorphisms and T2DM in Saudi population.

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Subjects and methods:

Subjects: This project consisted of 100 T2DM male patients enrolled from diabetic center of King Abdul-Aziz specialist hospital in Taif and 100 healthy males used as

control. Both groups matched in sex and ethics. All patients diagnosed according to the World Health Organization criteria (fasting blood glucose >126 mg/dL or 2-hour postprandial blood glucose >200 mg/dL)¹². The diagnosis was confirmed by answering the questioner constructed by authors of this project that any patients in this study didn't take any medications or vitamin supplementation and didn't suffering from any chronic that may alter vitamin D metabolism. All subjects in control group did not suffering from any chronic illness such as DM, hypertension, cardiac, hepatic, or urinary illness and did not use any medication affect vitamin D level. After an overnight fast, venous blood samples were collected in EDTA and plain tubes then separated immediately and stored at 4°C until the analysis.

Biochemical parameters: The plasma was used for biochemical marker measurement include glucose, triglyceride, total cholesterol, HDL-C, LDL-C, insulin and 25-(OH) vitamin D. Fasting blood glucose, triglyceride, cholesterol, HDL-C, LDL-C and HbA1c were measured in a Dimension autoanalyzer (Dade Behring Inc). Insulin and 25-(OH) vitamin D were measured by using ELISA technique¹³.

Genotyping: Blood samples collected in EDTA tube and the DNA was extracted from peripheral blood leu-

kocytes using the Thermo SCIENTIFIC DNA isolation kit (Thermo SCIENTIFIC). Genomic DNA was amplified and analyzed for VDR genotype by polymerase chain reaction (PCR) and restriction fragment length polymorphisms (RFLP) for, *ApaI*, *TaqI* and *BsmI* genotypes using forward and reverse primers showed in Table 1.1. The PCR mix was contained 5 µL of each primer (10 pmol), 5 µL buffer, 1.5 µL MgCl₂ (50 mM), 5 µL template DNA (50–100 ng), 5 µL dNTPs (2 mmol/L), Taq polymerase (MBI) 2 µL, H₂O 26.5 µL. The DNA template was denatured at 95°C for 2 min. A total of 40 cycles of PCR were performed, consisting of denaturation step for 45 sec at 94°C, an annealing step for 45 sec at optimum temperature (67°C for *ApaI*/*TaqI* and 60°C for *BsmI*), and an extension reaction for 1 min at 72°C. A final extension step at 72°C for 2 min was added after the last PCR cycle. After amplification, the PCR products were digested by incubation with restriction enzymes. For *ApaI* polymorphism, the amplicon incubated with *ApaI* enzyme in 37°C for 5 minutes to get its three genotypes on 1.5% agarose gel designated AA, Aa and aa. Incubation of amplicon with *TaqI* at 65°C for 4 hours produced TT, Tt and tt on 2.5% agarose gel. *BsmI* genotypes were produced after incubation of amplicon with *BsmI* enzyme at 65°C for 15 min, then applied on 2% agarose gel¹⁴. The size of each genotypes *ApaI*, *TaqI* and *BsmI* are shown in Amplified Table 1 and Fig 1,2 and 3 respectively .

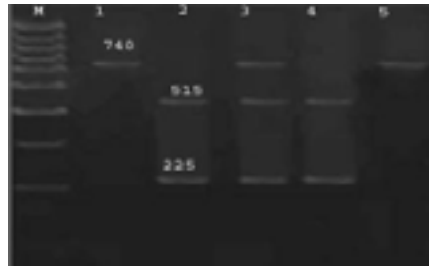


Figure 1. Detection of *ApaI* polymorphism by PCR-RFLP method. Lane 1 and 5: homozygote (AA), Lane 3: heterozygote (Aa), Lane 2 and 4: homozygote (bb). M is 100-bp DNA marker.



Figure 2. Detection of *TaqI* polymorphism by PCR-RFLP method. Lane 5 and 6: homozygote (TT), Lane 1, 2 and 4: heterozygote (Tt), Lane 3 homozygote (tt), M is 100-bp DNA marker.

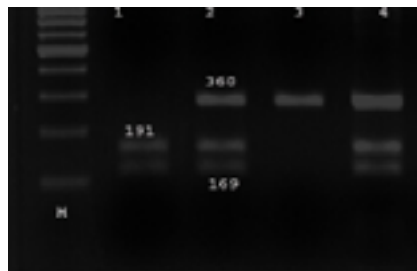


Figure 3. Detection of *BsmI* polymorphism by PCR-RFLP method. Lane 3: homozygote (BB), Lane 2 and 4: heterozygote (Bb), Lane 1: homozygote (bb). M is 25-bp DNA marker.

Statistical analysis

Hardy–Weinberg equation was used to test whether the examined SNPs were in equilibrium. The SPSS software version 16 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The correlations were tested using Spearman's rho. ANOVA was used in comparisons performance. Both comparisons and correlations were considered statistically significant when $p < 0.05$.

Results

The results were represented by Table 1, 2 and 3. Average of population age, BMI, FBG, HbA1c, triglyceride, cholesterol, HDL-C, LDL-C, insulin and 2-(OH) vitamin D were represent in Table 2. Age, weight, BMI, FBG, HbA1c, triglyceride and insulin were significantly higher

in T2DM patients compared with control group ($P < 0.01$). Our focusing parameter 2-(OH) vitamin D showed a significant higher level in T2DM group compared with control group ($P = 0.003$). Based on VDR genotypes, With respect to examined VDR SNPs, all examined genotypes were in Hardy-Weinberg equilibrium (Table 3). The result showed significant differences in *TaqI* in both groups.. The tt genotype was significantly more frequent in T2DM group than control group. The TT genotype of *TaqI* was more frequent in control group than T2DM group. Moreover, the tt allele was more frequent in T2DM group while TT allele was more frequent in control group. The results were not confirmed any significant differences between T2DM group and control group in both *BsmI* and *ApaI* genotypes of VDR (Table 3).

Table 1. Forward and reverse primers.

SNP	Sequences	Restriction products (bp)
ApaI	Forward 5'-CAGAGCATGGACAGGGAGCAAG-3'	Allele AA: 740 Allele
	Reverse 5'-GCAACTCCTCATGGCTGAGGTCTCA-3'	Aa: 740+515+225 Allele aa: 515+225
TaqI	Forward 5'-CAGAGCATGGACAGGGAGCAAG-3'	Allele TT: 495+245 Allele
	Reverse 5'-GCAACTCCTCATGGCTGAGGTCTCA-3'	Tt: 495+290+ 245+205 Allele tt: 290+245+205
BsmI	Forward 5'-AGTGTGCAGGCGATTCGTAG-3'	Allele BB: 360
	Reverse 5'-ATAGGCAGAACCATCTCTCAG-3'	Allele Bb: 360+191+169 Allele bb: 191+169

Table 2. Comparison of biochemical parameters between type 2 diabetes mellitus patients and control using t-test (Mean±SD)

	Control N= 100	T2DM patients N= 100	P value
Age	25.25±6.30	38.25±10.36	0.004**
Weight (kg)	62.9±5.4	89.8±7.2	0.001**
BMI	26.49±2.04	30.7±1.81	0.003**
FBS (mg/dl)	90.33±7.31	152.68±30.66	0.002**
TG (mg/dl)	158.44±21.22	183.59±33.49	0.008**
Cholesterol (mg/dL)	198.21±16.49	223.75±17.32	0.241
HDL (mg/dL)	51.96±7.73	47.65±5.01	0.566
LDL (mg/dL)	102.32±9.44	123.91±22.56	0.311
Insulin (pmol/L)	17.83±2.32	8.22±6.03	0.004**
25-H-Vit-D (ng/mL)	37.41±8.12	18.09±4.17	0.003**

Table 3. Comparison of *ApaI*, *TaqI* and *BsmI* genotypes and allelic frequencies between type 2 diabetes mellitus patients and control

Genotypes <i>ApaI</i>	Control (100) %	T2DM (100) %	P value	Hardy-Weinberg P value
AA	30 (30%)	32 (32%)	0.689	0.735 ^s
Aa	48 (48%)	47 (47%)	0.815	
aa	22 (22%)	21 (21%)	0.809	
Allele A	108 (54%)	111 (55.5%)	0.665	0.571
Allele a	92 (46%)	89 (44.5%)	0.571	
Genotype <i>TaqI</i>				
TT	53 (53%)	24 (24%)	0.041*	0.208 ^s
Tt	36 (36%)	34 (34%)	0.602	
tt	11 (11%)	42 (42%)	0.013*	
Allele T	142 (71%)	82 (41%)	0.036*	0.011*
Allele t	58 (29%)	118 (59%)	0.011*	
Genotypes <i>BsmI</i>				
BB	50 (50%)	45 (45%)	0.417	0.263 ^s
Bb	38 (38%)	41 (41%)	0.632	
bb	12 (12%)	14 (14%)	0.801	
Allele B	138 (69%)	131 (65.5%)	0.406	0.558
Allele b	62 (31%)	69 (34.5%)	0.558	

S: In Hardy-Weinberg equilibrium

Table 4 represents a comparison of all biochemical parameters between each genotype of *ApaI*, *TaqI* and *BsmI* in all individual included in this study. The results showed

a significant higher HbA1c value in tt genotype of *TaqI* (P=0.029). According to *BsmI* genotypes, BB genotype showed a significant higher 25-(OH) vitamin D level (P=0.036)

Table 4. Comparison between fasting blood glucose, HbA1c, insulin and 25-(OH) vitamin D in each genotypes of *ApaI*, *TaqI* and *BsmI* in both groups

<i>ApaI</i> genotypes	AA	Aa	aa	P value
FBG	136.21±20.64	129.88±30.46	142.11±36.83	0.309
HbA1c	7.99±2.08	8.12±3.22	8.33±2.06	0.752
Insulin	18.03±4.36	20.45.03±6.36	19.79±5.36	0.601
25-(OH) vit D	31.14±7.22	37.11±4.22	35.66±5.63	0.447
<i>TaqI</i> genotype	TT	Tt	tt	P value
FBG	140.24±28.01	126.44±35.19	125.34±21.79	0.335
HbA1c	5.21±2.11	7.89±1.57	10.55±1.85	0.029*
Insulin	17.26±4.04	21.69±7.28	24.81±2.36	0.466
25-(OH) vit D	29.49±8.22	32.65±4.28	27.56±7.23	0.486
<i>BsmI</i> genotype	BB	Bb	bb	P value
FBG	111.20±20.21	120.66±18.93	133.67±30.51	0.298
HbA1c	6.34±2.22	6.45±3.06	6.93±2.45	0.814
Insulin	19.84±5.55	20.59±4.77	22.35±6.71	0.619
25-(OH) vit D	40.21±3.22	38.58±5.92	37.73±4.44	0.671

Discussion

Recently, T2DM affects young people and its complications are increased in Saudi Arabia. Vitamin D is steroid derived vitamin and has a large variety of functions. It is involved in growth, bone strength, immune and nervous function. Low levels of vitamin D can involve in development of various chronic diseases such as cancers, osteoarthritis and cardiovascular disease¹⁵. Vitamin D deficiency is becoming a global problem in all countries including sunny areas. A study done by Alharbi et al., showed that 98% of all Saudi men included in their study have vitamin D deficiency and this may occur as a result of less exposure of people to sun light due to high temperatures of this area during spring, summer and autumn¹⁶. Several studies found that low vitamin D levels are associated with T2DM. Mercedes and his colleagues found that low vitamin D levels are associated with T2DM in Spanish people¹⁷. Also another study done by Romero and Lozano found that vitamin D deficiency is common in T2DM in patients in Southern Spain¹⁸. The results are in agreement with the previous studies, which showed that 97% of T2DM patients have vitamin D deficiency and the group has a lower level of this vitamin compared with normal people. In 2017, Al-Hazmi and his colleagues also found

that vitamin D deficiency is dominant in obese people¹¹. also found that vitamin D deficiency is dominant in obese people¹¹. In the same year, Mahmodenia et al., also found that vitamin D deficiency is common in obese and T2DM Iranian patients¹⁹. Both obesity and vitamin D insufficiency induce insulin resistance²⁰. Vitamin D supplementation induces insulin production by pancreatic β -cells and It also improves insulin resistance and impaired glucose tolerance in T2DM patients²¹. Vitamin D produces its signals either directly by binding to VDR in β -cells or indirectly by induction of release of calcium from these cells²².

Several studies found inverse correlation between insulin level and 25-(OH) vitamin D level in T2DM patients²³. This observation was not confirm in this study, but an inverse correlation between HbA1c and 25-(OH) vitamin D was confirmed in this study as previous studies concluded^{24,25}. Previously, several studies were done to evaluate association of VDR with different chronic diseases. Results of previous studies to indicate association of VDR genotypes and T2DM are conflict. In 2011, Reza et al found a significant difference in *TaqI* genotype between T2DM patients and control group in Iranian popu-

lation. Their result showed that TT genotype is decreased in T2DM patients group, but did not confirm any difference in *TaqI* allele²⁶. Obesity is a predisposing factor for T2DM and previously, our group found that tt genotype of *TaqI* is more frequent in obese people¹¹. Similarly, this study showed that tt genotype of *TaqI* is more frequent in T2DM patients group.

In addition, t allele is more frequent in T2DM patients group than control group. Other studied done on Turkish population found no association between *TaqI* genotype of VDR and T2DM²⁷. About *ApaI* genotype, Oh et. al., found that *ApaI* genotype is associated with T2DM in American population²⁸. On the other hand, Dilmec et al found no association between this *APaI* genotypes and T2DM²⁷. The finding also did not confirm association of *AqaI* genotype and T2DM. Association between *BsmI* genotype and T2DM is reported by Speer et al., and Ortlepp et. al.,^{29,30}. The study findings are inconsistent with those of Mackawy et al who found no association between *BsmI* genotype and T2DM³¹. The reason for differences in results of studies that focus on association of VDR gene polymorphisms with T2DM may be due to genetic, ethical and environmental differences in population. The strength of this study was studying of more than one genotype in vitamin D receptor and the subjects were matched in both race and sex. Moreover, The study demonstrated the correlation of vitamin D genotypes with some diabetic parameters such as FBS, HbA1c, and insulin. The limitation of this study was that it used different ages for T2DM patients.

Recommendation and conclusion

An association was confirmed between *TaqI* genotypes and T2DM but there is no correlation between *BsmI*, *ApaI* and T2DM among Saudi population. In addition, HbA1c is positively correlated with tt genotype of *TaqI*. This is the first study done on Saudi population to evaluate association of VDR gene polymorphisms with T2DM. Vitamin D supplementation is recommended to preventing insulin resistance and T2DM.

Conflicts of interest

Authors have no competing interests to declare.

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