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Vitamin D receptor gene polymorphism and serum vitamin D level as risk factors for acquiring Type II diabetes mellitus

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ABSTRACT

The studies on the relationship between type 2 diabetes mellitus (T_2DM) risk and vitamin D receptor (VDR) gene polymorphisms are still inconclusive. Therefore, the objective of the study was to assess possible risks of acquiring T_2DM due to polymorphisms in the VDR gene or abnormal serum levels of VD. 362 participants (181 T2DM patients and 181 healthy controls) from the Diabetic Center, Sulaimaniyah/Iraq, from December 2020 to May 2021 were volumtarily enrolled in the study. For each participant, HbA1c, fasting blood sugar (FBS), serum cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride (TG), markers of calcium homeostasis, alkaline phosphatase (ALP), phosphorus, VD and insulin were measured. In addition, *FokI*, *TaqI*, *ApaI*, and *BsmI* genotypes were also performed using Polymerase Chain Reaction (PCR). The results showed that VD level was significantly lower in T2DM compared to the control group. While, HbA1c was significantly higher in T2DM than in the control group. In contrast to AA (*P*=0.034) and CC (*P*=0.011) genotype of *ApaI* (rs7975232) gene polymorphism, which were dominant among the control group, AC-genotype was significantly (P=0.0001) dominat among T2DM group. Meanwhile, TT-genotype of TaqI (rs731236) was significantly (*P*=0.05) dominant among control group. While there were not any significant differences between other genotypes among T2DM and control groups. In conclusion, low VD-level is a possible risk factor for developing T2DM, and an association was found, especially between *ApaI* genotypes and T2DM.

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Introduction

T2DM is still an ongoing chronic health problem that affects people everywhere worldwide. DM affects 1.4 million Iraqis, and Iraq has a reported T2DM prevalence of 8.5% to 13.9% (1). DM is a severe metabolic disorder, which is characterized by insulin resistance and a change its secretion that disturbs carbohydrates, proteins, and lipids metabolism (2). T2DM appears to have a high mortality rate in adult patients (20–79 years old), which was reached 10.7% in 2017. In the MENA region (21 countries and territories, including Iraq), DM-related death toll is estimated to be around 373,557 cases, especially among aged-patients (<60 accounting for 51.8% of the deaths toll) (3). T2DM development is influenced by both genetic and environmental factor. It more likely to be accelerated by VD deficiency (4). VD appears to be crucial for the regulation of the insulin release and seems to have a link with glucose tolerance (5).

The VDR, which is a particular intracellular receptor and a member of the steroid/thyroid hormone receptor family that located on chromosome 12q13, is expressed in various cell types, including pancreatic β-cells. VD produces its effect through binding to VDR. Consequently, VDR-VD complexes induce glucose tolerance through increasing insulin secretion by the pancreatic β-cells (5).

Numerous DNA sequence polymorphisms were found in the VDR gene, including *ApaI*, *TaqI*, *BsmI*, and *FokI*, which have been the subject of numerous studies in various fields and health issues. VD receptor gene seems to be one of the potential genes that might have an influence on the development of T2DM (6).

To the best of our knowledge, there was not any data about VDR gene polymorphism and its association with the T2DM among Kurdish-ethnicity in the Sulaymaniyah city/Iraq. Therefore, the aims of the study was to investigate a relationship between VDR gene polymorphisms and T2DM the region.

Materials and Methods

Study design and setting

In this study 362 participants, including 181 T2DM patients and 181 healthy control individuals, were enrolled at Diabetic Center in Sulaimaniyah City/Iraq, from December 2020 to May 2021.

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Table 1. Forward and reverse primers (Macrogen, Korea) for the vitamin D receptor (VDR) genes.

VDR polymorphism	Location	Primer	The PCR product (bp)	Annealing	Restriction Enzyme	RFLP Products (bp)
rs2228570T>C	Exon 2 (chr12:47879112)	5'- GATGCCAGCTGGCCCTGGCACTG-3 5' GAGGGAGAAGAAGCAAGGTGTTTCCAT-3	272	60°C	FokI	T(F): 272 C(f): 198, 74
rs1544410A>G	Intron 8 (chr12:47846052)	5'-CAACCAAGACTACAAGTACCGCGTCAGTGA-3 5'GCAGCAAATGGGACACAGGC-3	1113	62°C	BsmI	A(B): 1113 G(b): 645, 468
rs7975232A>C	Intron 8 (chr12:47845054)	5'CTGGAGGCTCAAGGAATGGA-3 5'GAGACCTCAGCCATGAGGAGTTGC-3	1215	61°C	ApaI	A(C): 1215 C(a): 527, 688
rs731236C>T	Exon 9 (chr12:47844974)	5'CTGGAGGCTCAAGGAATGGA-3 5'GAGACCTCAGCCATGAGGAGTTGC-3	1215	61°C	TaqI	C(T): 1215 T(t): 763, 252, 200

Inclusion criteria

Patients who had been diagnosed with T2DM according to the World Health Organization's (WHO) standards (FBS level >126 mg/dL or postprandial blood sugar level >200 mg/dL) (7) were involved with the study regardless of their genders.

Exclusion criteria

Participants who took any medications, vitamin supplements and had conditions that might have had an effect on VD metabolism, were excluded from the study.

Study protocol

Venous blood (10 mL) was collected from each participant, and divided between an EDTA tube (5 mL) for genotyping/HbA1c% and a gel tube (5 mL) for biochemical tests. Cobas c311 (Roche, Hitachi Modular, Japan) was used to measure HbA1c%, FBS, cholesterol, HDL, LDL, TG, markers of calcium homeostasis, ALP, and phosphorus**.** while Diasorin XL Inc. (Germany) was used to measure VD and serum insulin.

DNA Extraction and PCR

Total genomic DNA (gDNA) was extracted from fresh whole blood using an AddPrep Genomic DNA extraction kit (Korea). Then, gDNA was amplified (8).Briefly, 20µl PCR reaction was set by mixing 10 µL of Add Start Taq master mix (Addbio, Inc), 2.0 µL of gDNA, 1 µL each of VDR forward and reverse primers (Table-1), then the volume was completed with ultrapure nuclease-free water. Then, the PCR reaction was subjected to an amplification program that started with 95°C for 10 min initial denaturation, 32 cycles of denaturation (95 °C/35 sec), annealing (60-62°C/20 sec(and extension for 70 sec at 72 °C, then the PCR reaction was completed by a final extension for 5 min at 72 °C and hold at 4°C. The integrity of the DNA bands were checked by visualizing them using 1.5% agarose gel and UV Transilluminator (Ingenius, USA).

Genotyping

Restriction fragment length polymorphism (RFLP) was used to detect VDR single nucleotide polymorphisms (SNP) in *FokI* (rs2228570), *TaqI* (rs731236), *ApaI* (rs7975232), and *BsmI* (rs1544410) sites in VDR gene. Each site was digested separately with a site specific restriction enzyme to reveal the genotypes for each SNP. For *BsmI*, 6.5 µL of PCR product was digested with 0.4 µL of *BsmI* enzyme (Biolabs, New England), 1.5 µL of 10X restriction buffer (150 mM Tris-HCl, pH 7.5, 250 mM NaCl, and 35 mM MgCl2) and 11.6 µL H_2O, incubated for 90 min at 65 ºC, and deactivated at 80 ºC for 20 min. For *FokI*, the PCR product (6.0 µL) was digested with 0.4 µL of *FokI* enzyme (Biolabs, New England) for 110 min at 37 ºC in a mixture of 1.5 µL of 10X restriction buffer and 12.1 µL free nuclear H_2O, incubated for 90 min at 37 ºC, and deactivated at 50 ºC for 10 min. *TaqI*, and *ApaI* SNPs were digested by mixing 6.0 µL of each PCR product in 2 separate tubes; then 0.1 µl of each the digestive enzyme (*TaqI* or *ApaI)* (Promega, USA) was added into their corresponding tubes. Then, a mixture of 1.5 µL of 10X restriction enzyme buffer, 0.1 µL of BSA and 12.3 µL of H_2O was added to each. The *ApaI* reaction mix was incubated for 45 min at 37 ºC. While *TaqI* mix was incubated for 50 min at 65 ºC. Finally, the digestion products were visualized using 1.5% agarose gel and UV Transilluminator (Ingenius, USA) (Figures 1-4).

Ethical considerations

The proposal of the current study was approved (No: 237 on October 25, 2020) by the Ethics Committee at the College of Medicine, University of Sulaimani, Sulaimaniyah city/Iraq. The participants were handled according to the Declaration of Helsinki, and a written consent was taken from each participant before starting the study to ensure their willingness participation in the study.

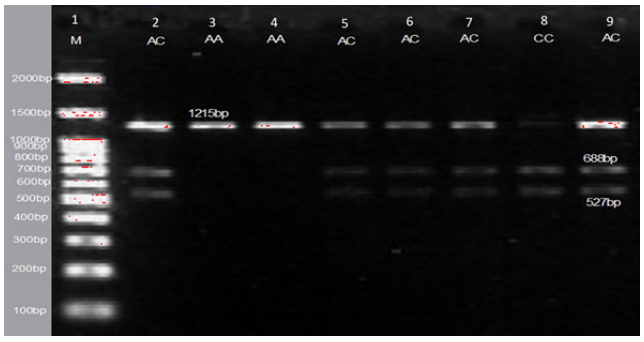

Figure 1. *ApaI* gene polymorphism gel electrophoresis. Lane-1; Ladder-100. Lane-3 and 4; homozygous AA genotype exhibited only a band of 1215 bp. Lane-2,5,6,7,and 9; The heterozygous AC genotypes yielded three bands of 1215, 688, and 527 bp. Lane-8; the homozygous CC genotype exhibited two bands of 688 and 527 bp.

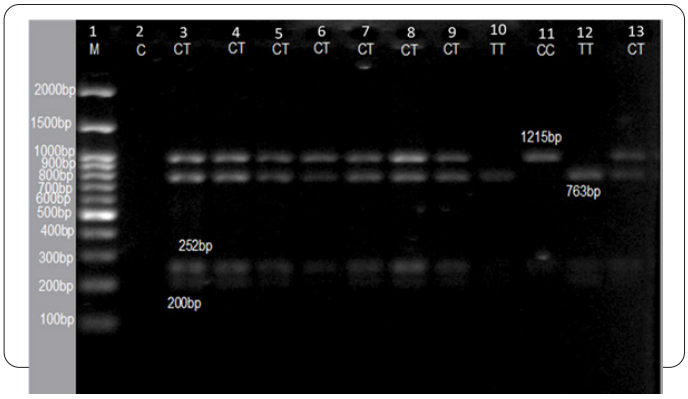

Figure 2. *TaqI* gene polymorphism gel electrophoresis. Lane-1; Ladder-100. Lane-11; homozygous CC genotype exhibited only a band of 1215 bp. Lane-3,4,5,6,7,8,9,and 13; The heterozygous CT genotype yielded four bands of 1215, 763, 252. Lane-10 and 12; homozygous TT exhibited three bands of 763, 252, and 200 bp. Lane-2; Control gegative showed no band.

Figure 3. *BsmI* gene polymorphism gel electrophoresis. Lane-1; Ladder 100. Lane-3,4,6,7,and 12; homozygous AA genotype exhibited only a band of 113 bp. Lane-2,8,9 and 10; heterozygous AG yielded three bands of 113, 645, and 468 bp. Lane-11; homozygous GG exhibited two bands of 645 and 468 bp. Lane-5; Control gegative showed no band.

Statistical analysis

Collected data were analyzed using the statistical package for social sciences (SPSS, version 26). Descriptive data were expressed as mean±standard division. Comparisons between groups were analyzed using independent T-tests and One-Way ANOVA. Cross-tabulation was used to examine the variations of genotype and genotype frequencies in groups. Medical calculator was used to analyze the Hardy-Weinberg equation to find allele frequency in the population, logistic regression, the Odds ratio and their 95% confidence interval (CI) using diabetes as a dependent variable. A $p \leq 0.05$ was considered statistically significant, and a $p \leq 0.001$ was highly effective.

Results

The mean age of diabetic patients was 54.7±8.5 years, aged 35-70 years. There were no significant differences between the control group and the diabetic group regarding BMI, BFP, cholesterol, LDL, creatinine, calcium, inorganic phosphate, and PCV ($p \geq 0.05$). On the other hand, diabetic patients had significantly ($p \leq 0.05$) higher FBG, HbA1c, TG, fasting serum insulin, urea, ALP, and HOMA with lower HDL, HOMA-B, QUICKI, VD, and Hb than the control group (Table 2).

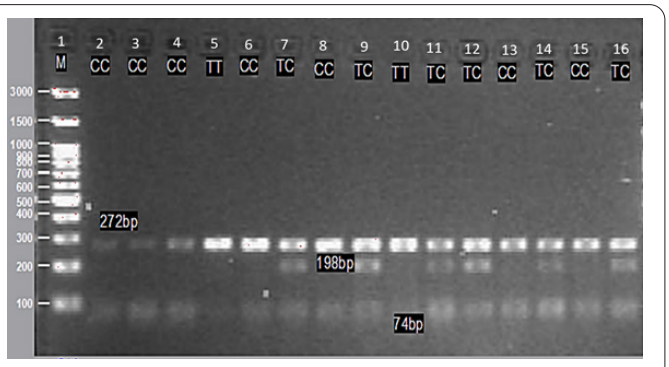

Figure 4. *FokI* gene polymorphism gel electrophoresis. Lane-1; Ladder-100. Lane-5 and 10; homozygous TT genotype exhibited only a band of 272 bp. Lane-7,9,11,12,14,and 16; heterozygous TC genotype yielded three bands of 272, 198, and 74 bp. Lane-13 and 15; homozygous CC exhibited two bands of 272 and 74 bp.

The VDR genotypic and allelic distribution was performed for T2DM patients and controls by looking at the *ApaI*, *TaqI*, *BsmI*, and *FokI* gene polymorphism. The ApaI genotype frequency was 32.6%(AA), 15.0%(AC) and 36.3%(CC) among control group. While they were 48.8%(AA), 48.8%(AC) and 18.6%(CC) among T2DM group. There was a significant association between each genotype in the control group and the same genotype in the T2DM group. The frequency of the A and C alleles was non-significantly (p=0.89) higher among patients compared to the controls (Table 3).

The results of *TaqI* polymorphism revealed that the frequency of the CC, TC and TT genotypes were vary among healthy and control groups. They were 35.0%(CC), 38.8% (TC) and 26.5%(TT) among control healthy, and 32.6%(CC), 48.8%(TC) and 18.6%(TT) among T2DM patients. There was a substantial associations (*P*=0.058 and 0.05, respectively) between CT and TT genotypes among control and T2DM patients. The frequency of the C allele (59.3%) was higher among T2DM group than the control (54.4%) group. While the T allele frequency was higher in controls (45.6%) than in patients (40.7%).

The frequency of *BsmI* genotypes was 30.0% (AA), 47.5% (AG) and 22.5% (GG) among the control group. Meanwhile, it was 23.3%(AA), 55.8%(AG) and 20.9%(GG) among T2DM group. There was no significant associations between the genotypes of both studied groups. The allele distribution was 31.4% (A) and 68.6%(G), and 42.5%(A) and 58.8%(G) among T2DM and control groups.

Regarding *FokI* polymorphism, the frequency of the TT, TC and CC genotypes was 52.5%, 11.3% and 36.3% among control group and it was 43.2%, 17.4% and 39.5% among T2DM group, respectively. There was no significant association between TT, TC, and CC genotypes between both groups (p=0.22, 0.8, and 0.66, respectively), with no significant differences in frequency of the T and C alleles between both groups (p=0.24) (Table 3).

Heterozygous mutant polymorphism of *ApaI* (AC genotype), *TaqI* (TC genotype), and *BsmI* (AG genotype) was the most frequent polymorphism among these genes in T2DM, while the wild TT was the most frequent *FokI* genotype among T2DM; and only *ApaI* (AC genotype) was significantly higher than the corresponding genotype in controls. The allelic frequency of all the genotypes was

Table 2. Demographic and biochemical data for studied subjects.

Variable	Diabetic group (n = 181)	Non-Diabetic group (n = 181)	p-value
	Mean±SD		
BMI (kg/m²)	26.0±2.3	26.2±3.8	0.4
BFP%	33.5±6.5	32.4±7.5	0.12
Fasting plasma glucose (mg/dl)	193.5±82.3	93.2±6.0	0.001**
HBA1C%	8.2±1.9	5.2±0.37	0.001**
Serum cholesterol (mg/dl)	172.4±46.1	165.4±38.6	0.12
Serum triglycerides (mg/dl)	187.3±105.9	163.8±83.7	0.02*
HDL cholesterol (mg/dl)	38.7±10.1	41.8±11.6	0.009
LDL cholesterol (mg/dl)	107.1±35.1	102.6±34.9	0.22
Insulin (µmol/ml)	9.7±5.7	8.2±3.6	0.02*
HOMA IR	4.6±3.5	1.88±0.82	0.001**
HOMA-B	38.1±36.0	96.1±78.7	0.001**
QUICKI	0.31±0.03	0.35±0.02	0.001*8
Urea (mg/dl)	32.5±8.4	30.3±7.6	0.011*
Serum creatinine (mg/dl)	0.89±0.87	0.80±0.18	0.11
Calcium (mg/dl)	9.3±0.59	9.4±0.89	0.6
Inorganic phosphorus (mg/dl)	4.5±0.34	4.4±0.42	0.069
Vitamin D (ng/ml)	13.4±6.9	17.5±8.3	0.001**
Alkaline phosphatase (IU/L)	94.9±28.5	83.4±20.9	0.001**
PCV%	40.6±4.6	41.2±3.3	0.15
Hb (g)	13.5±1.48	13.6±1.0	0.039*

*Independent sample T-test was used to anlyze the data. HOMA-IR: Homeostatic model assessment for insulin resistance, BFP: Body fat percentage, HOMA-B: Homeostatic model assessment β-cell function, BMI: Body mass index, QUICKI: Quantitative Insulin Sensitivity Check Index, HbA1c: Glycated hemoglobin, Hb: Hemoglobin, PCV: Packed cell volume, HDL: High-density lipoprotein, LDL: Low-density lipoprotein. *p≤0.05, **p≤0.001.

Table 3. *ApaI*, *TaqI*, *BsmI*, and *FokI* genotypes with allelic frequencies among studied groups.

Genotype	T_2DM (n=86)	Control (n=80)	P-value
	Number, %		
	*Apa*I (rs7975232)		
AA	28 (32.6)	39 (48.8)	0.034*
AC	42 (48.8)	12 (15)	0.0001**
CC	16 (18.6)	29 (36.3)	0.011*
Allele A	98 (57)	90 (56.3)	0.89
Allele C	74 (43.0)	70 (43.8)	
	TaqI (rs731236)		
CC	28 (32.6)	28 (35)	0.7
TC	46 (53.5)	31 (38.8)	0.058
TT	12 (14)	21(26.5)	0.05*
Allele C	102 (59.3)	87 (54.4)	0.3
Allele T	70 (40.7)	73 (46.2)	
	BsmI (rs1544410)		
AA	20 (23.3)	24 (30)	0.32
AG	48 (55.8)	38 (47.5)	0.28
GG	18 (20.9)	18 (22.5)	0.21
Allele A	88 (51.1)	86 (53.7)	0.6
Allele G	84 (48.8)	74 (46.2)	
	FokI (rs2228570)		
TT	37 (43)	42(52.5)	0.22
TC	15 (17.4)	9 (11.3)	0.8
CC	34 (39.5)	29 (36.3)	0.66
Allele T	89(51.74)	93(58.13)	0.24
Allele C	83(48.26)	67(41.88)	

*Medical calculator software was used to calculate allele and genotypes distribution among T2DM and control groups. P-value. *p≤0.05, **p≤0.001.

not significant among T2DM patients.

In T2DM paticipants, the results showed that the level of HOMA-B was significantly higher among AA genotype of *ApaI* and TT genotype of *FokI* in comparison to AC and CC genotypes of *ApaI*, and TC and CC genotypes of *FokI*, respectively. The VD level was although dramatically low among AC genotypes of *ApaI* gene polymorphism if compared to AA and CC genotypes.

The insulin hormone level was marked low among CC genotypes of *FokI* in comparison to TT and TC genotypes. All the variables were non-significantly different among CC, CT, and TT genotypes of *TaqI*, and AA, AG, and GG genotypes of *BsmI* (Table 4).

The *ApaI* AA, AC, and CC genotypes were present in controls (39%, 12%, and 29%, respectively). In normal control group, HbA1c% was significantly lower among TC genotypes of TaqI and TC genotype of FokI in comparison to other genotypes of TaqI and FokI. There was

Table 4. Comparison of the demographic and clinical characteristics among different genotypes of *ApaI*, *TaqI, BsmI,* and *FokI* genes in diabetic patients.

***ApaI* genotype**	**AA (n=28)**	**AC (n=42)**	**CC (n=16)**	***P*-value**
		Mean ± SD		
Age (Years)	52.1±7.4	55.3±8.8	55.7±7.6	0.2
FBG (mg/dL)	195.2±89.2	202.4±94.6	234.5±87.9	0.33
HbA1c%	8.2±1.88	8.0±1.82	9.6±3.2	0.1
Insulin (µmol/L)	8.8±6.4	8.7±7.1	9.4±5.2	0.9
VD (ng/mL)	17.7±9.8	13.6±5.4	17.3±8.5	0.00**
HOMA-IR	3.7±2.6	5.5±4.4	4.7±2.9	0.36
HOMA-B	39.1±34.7	32.4±25.4	25.8±18.7	0.038**
BMI (Kg/m^2)	26.4±2.3	25.8±2.2	26.8±2.5	0.79
T_2DM Duration (Years)	1.2±0.75	1.1±0.65	1.1±0.34	0.7
***TaqI* genotype**	**CC (n=28)**	**CT (n=46)**	**TT (n=12)**	***P*-value**
Age (Years)	54.7±9.0	53.5±7.6	56.0±8.6	0.25
FBG (mg/dL)	187.6±80.0	232.7±97.3	146.8±53.6	0.00**
HbA1c%	7.9±1.6	8.9±2.5	7.4±1.6	0.11
Insulin (µmol/L)	8.5±6.6	9.2±6.7	8.3±5.6	0.15
VD (ng/mL)	15.0±7.19	16.6±8.1	13.2±8.2	0.7
HOMA-IR	3.9±3.6	5.3±4.9	3.2±2.9	0.3
HOMA-B	34.28±24.05	28.32±24.14	50.6±42.1	0.06
BMI (kg/m^2)	26.2±2.3	25.9±2.3	27.3±2.1	0.066
T2DM Duration(Years)	1.0±0.69	1.2±0.60	1.3±0.65	0.34
***BsmI* genotype**	**AA (n=20)**	**AG (n=48)**	**GG (n=18)**	***P*-value**
Age (Years)	53.8±9.2	55.6±7.59	51.1±8.1	0.4
FBG (mg/dL)	205.1±87.3	202.0±90.7	207.7±95.7	0.9
HbA1c%	8.4±1.9	8.5±2.4	8.2±1.9	0.6
Insulin(µmol/L)	11.7±9.4	86.9±4.3	8.8±6.5	0.13
VD (ng/mL)	16.5±7.1	15.6±8.3	14.7±7.6	0.7
HOMA-IR	6.1±6.0	3.5±3.2	4.3±3.7	0.3
HOMA-B	38.4±29.9	32.0±26.2	31.24±30.8	0.64
BMI (Kg/m^2)	25.3±2.5	26.4±2.3	26.6±1.89	0.6
T2DM Duration(Years)	1.2±0.69	1.1±0.59	1.2±0.73	0.9
***FokI* genotype**	**TT (n=37)**	**TC (n=15)**	**CC (n=34)**	***P*-value**
Age (Years)	53.7±7.8	54.0±9.5	54.9±8.2	0.64
FBG (mg/dL)	196.±97.7	245.2±100.8	199.6±77.9	0.2
HbA1c%	8.3±1.9	8.5±2.39	8.5±2.5	0.7
Insulin(µmol/L)	10.3±7.7	10.6±7.0	6.5±3.7	0.03*
VD (ng/mL)	15.5±8.7	18.0±8.0	14.7±6.7	0.1
HOMA-IR	5.1±5.3	5.9±3.9	3.4±2.9	0.1
HOMA-B	42.1±32.5	29.2±22.6	25.6±21.7	0.007**
BMI (Kg/m^2)	26.2±7.8	25.2±1.47	26.7±2.26	0.08
T2DM Duration(Years)	**1.2±0.64**	1.0±0.65	1.2±0.64	0.4

*One-way ANOVA was used to analyze the data. HOMA-IR: Homeostatic model assessment for insulin resistance, FBG: Fasting blood glucose, HOMA-B: Homeostatic model assessment β-cell function, BMI: Body mass index, HbA1c: Glycated hemoglobin, T2DM: Type 2 Diabetes Miletus; VD: Vitamin D. *p≤0.05, **p≤0.001.

not any observed significant differences between other parameters (FBG, insulin, VD, HOMA-IR, HOMA-B, and BMI) and reported genotypes (Table 5).

Discussion

It was reported that VDR gene polymorphism appears to have an influence on the development of T2DM in some populations, including United Arab Emarate (9), Italian (10) Jordian (11) and Asian populations (12). A meta-analysis of 39 case-control studies showed that there might be an association between ethnicity, VDR polymorphism, and T1DM (13). And the VDR genotypes seems to have an impact on insulin sensitivity, fasting insulin, and HOMA-IR (14). Therefore, the rational behind the study was to investigate an association between VDR gene polymorphism and T2DM among kurdish-ethnicity in Sulaymaniyah city/Iraq. In T2DM participants, the results showed high level of FBG, HBA1c, TG, fasting serum insulin, urea, ALP, and HOMA-IR with lower HDL, QUICKI, VD, and Hb than the control group. The results also revealed an alarming increase in the levels of dyslipidemia in T2DM, inclusing TG and low HDL These outcomes align with another study (15). It seems that the early development of insulin resistance and the eventual onset of DM brought on by cell death and is induced by VD insufficiency. Insu-

Table 5. Comparison of the demographic and clinical characteristics among different *ApaI*, *TaqI, BsmI*, and *FokI genotypes* in controls.

***ApaI* genotype**	**AA (n=39)**	**AC (n=12)**	**CC (n=29)**	**p-value**
		Mean ± SD		
Age (Years)	45.01±9.8	43.6±9.4	43.8±9.6	0.8
FBG (mg/dL)	93.1.2±5.6	92.7±7.1	92.9±6.1	0.97
HbA1c%	5.1±0.42	5.1±0.32	5.1±0.46	0.3
Insulin (µmol/L)	8.8±3.2	8.0±2.8	8.9±4.6	0.7
VD (ng/mL)	18.5±7.4	23.2±11.9	20.9±10.0	0.17
HOMA-IR	2.0±0.75	1.8±0.66	2.0±1.0	0.76
HOMA-B	99.3±42.3	91.2±36.0	103.0±72.6	0.37
BMI (Kg/m²)	25.3±3.1	23.3±2.0	27.0±3.8	0.39
***TaqI* genotype**	**CC (n=21)**	**CT (n=38)**	**TT (n=28)**	**p-value**
Age (Years)	43.8±9.6	45.0±8.5	49.4±10.9	0.22
FBG (mg/dL)	92.9±5.7	92.8.3±5.5	93.2±7.3	0.9
HbA1c%	5.2±0.38	5.0±0.5	5.2±0.27	0.044*
Insulin (µmol/L)	8.6±0.37	9.3±4.2	8.2±3.1	0.7
VD (ng/mL)	18.9±9.0	20.7±8.3	21.7±10.6	0.6
HOMA-IR	2.0±0.84	2.1±0.92	1.9±0.79	0.8
HOMA-B	104±56.6	100.9±64.5	91.2±21.7	0.5
BMI (Kg/m²)	25.4±3.7	25.5±2.9	26.0±4.1	0.64
***BsmI* genotype**	**AA (n=24)**	**AG (n=38)**	**GG (n=18)**	**p-value**
Age (Years)	46.0±9.2	44.3±9.4	48.5±10.8	0.7
FBG (mg/dL)	92.71±6.2	92.8±6.3	93.6±5.1	0.8
HbA1c%	5.1±0.46	5.1±0.3	5.2±o.16	0.12
Insulin (µmol/L)	8.5±4.0	8.7±3.8	9.0±3.2	0.9
VD (ng/mL)	20.4±9.4	19.3±9.0	21.2±9.7	0.7
HOMA-IR	1.95±0.86	2.0±0.88	2.0±0.77	0.12
HOMA-B	100.7±71.4	99.6±50.2	97.3±34.3	0.9
BMI (Kg/m²)	26.4±3.9	24.9±2.8	26.0±4.0	0.34
***FokI* genotype**	**TT (n=42)**	**TC (n=9)**	**CC (n=29)**	**p-value**
Age (Years)	47.8±9.8	44.5±8.2	43.2±9.5	0.6
FBG (mg/dL)	94.1±4.9	90.2±8.4	92.1±6.3	0.1
HbA1c%	5.2±0.35	5.0±0.7	5.2±0.35	0.001**
Insulin (µmol/L)	8.8±3.5	8.86±4.7	8.5±3.8	0.9
VD (ng/mL)	21.5±9.3	18.4±6.9	18.5±9.5	0.6
HOMA-IR	2.0±0.76	1.97±1.0	1.95±1.0	0.8
HOMA-B	97.6±57.7	113.5±69.5	97.6±43.8	0.8
BMI (Kg/m²)	25.3±3.1	23.6±3.0	26.4±4.0	0.35

*Oneway ANOVA was used to analyze the data. HOMA-IR: Homeostatic model assessment for insulin resistance, FBG: Fasting blood glucose, HOMA-B: Homeostatic model assessment β-cell function, BMI: Body mass index, HbA1c: Glycated hemoglobin, VD: Vitamin D. *p≤0.05, **p≤0.001

lin resistance is mainly caused by inflammation; however, VD works to diminish it (16). DM-related dyslipidemia is related to several reasons, including insulin's effects on the formation of apo-proteins in the liver, the control of lipoprotein lipase, the activities of cholesteryl ester transfer protein, and the peripheral effects of insulin on adipose and muscle tissue (17).

The current study showed no significant differences in BMI values between both groups. This result does not agree with that of Gray et al., who reported an association between a mild raise in BMI and an increase in the chance of getting DM (18). Previous studies verified that inactivity and being overweight are linked to a higher risk of acquiring several illnesses, especially T2DM (19, 20). The word was defined as "diabesity" in 2000 since being overweight significantly predicts T2DM (21). Moreover, we found non-significant high cholesterol and LDL in patients than controls, which is inconsistent with another study that mentioned that LDL is significantly lower in T2DM patients than controls (22).

In DM patients, the mean levels of creatinine, calcium, inorganic phosphate, PCV, and Hb were non-significantly different from controls; and they were within normal range but may be changed to undesirable levels on longer DM duration; thus, continuous monitoring of patients with T2DM is essential to correct any metabolic aberration and prevent complications. Fang et al. recorded significantly lower serum phosphorus in patients with T2DM, which may indicate a problem with phosphorus metabolism (23). Arkew et. Al. noted that anaemia among T2DM patients was found to be a mild public health problem, which is against our results as we recorded a mean Hb of 13.5±1.48 mg/dl (24)

VDR gene polymorphisms (*BsmI, FokI, ApaI,* and *TaqI*) have been implicated as possible genetic contributors to Nevertheless, some published investigations' outcomes are still erratic (25). In the current study, we compared the polymorphisms in VDR gene, including *ApaI, TaqI, BsmI,* and *FokI*, with the controls to explore the possible significant differences between the polymorphism prevalence in the T2DM group and controls. The results revealed the presence of these genes in all participants. Moreover, the alleles for each gene polymorphism were differently distributed among patients and controls.

Three allelic polymorphisms for each of the VDR genes polymorphism were tested in the study. The prevalence of different allelic polymorphisms for *TaqI, BsmI,* and *FokI* genes in T2DM patients was non-significantly different from the controls. Still, the *ApaI* gene's heterozygous allele, AC genotype, was significantly higher in T2DM patients than in controls. In contrast, the homozygous alleles of the same *ApaI* gene, AA or CC, were significantly higher in controls than patients.

In a systematic review, it was found that single-nucleotide polymorphisms or direct alterations in the amino acid sequence of proteins are 2 types of genetic polymorphisms that have been linked to T2DM. These polymorphisms can potentially indicate how to treat T2DM (26). Similar to these results, a meta-analysis study on a mixed population observed a considerably higher risk of T2DM on the VDR *ApaI* polymorphism. Except for the *FokI* AA genotype and G allele, no significant changes in the frequency of the genotypes and alleles of the *BsmI*, *TaqI, ApaI*, and *FokI* VDR genes between individuals with DM and hypertension and those without these medical disorders were seen (27). Malecki et al. found that VDR allele frequencies for T2DM patients and controls for *FokI, ApaI, BsmI,* and *TaqI* gene polymorphism were non-significant, with no association between the gene polymorphisms and the risk of T2DM (28).

We did not observe significant differences in *ApaI* genotype variants relationship and BMI, HbA1c, insulin, HOMA-B, cholesterol, TG, HDL, LDL, urea, creatinine, calcium, inorganic phosphate, ALP, PCV, and Hb. Still, we found a significant increase in FBS and HOMA-IR in the AC variant compared to AA and CC variants, while the inverse was found for the VD level. Moreover, the participants having heterozygous CT variant of *the TaqI* gene also had significant increase in FBG, HbA1c, and HOMA-IR when compared to homozygous variants CC and TT of the same *TaqI* gene; for *the FokI* gene, the TC people with heterozygous TC were having significant increase in FBG and insulin when compared to the homozygous variants of the same *FokI* genotype. In contrast to the previous genotypes, people carrying different variants of the *BsmI* genotype did not have any significant differences in any of the parameters mentioned previously.

In the current study, we compared the presence of VDR gene *FokI* polymorphism in T2DM patients and controls to assess the SNP as a risk factor for T2DM. Genotype and allele frequencies in the T2DM group (TT=37, TC=15, CC=34) were non significantly different from those of the control group (TT=42, TC=9, CC=29), which is comparable to another study (29). Inverse to these results, researchers in the United Arab Emirates (UAE) found that *FokI* is a possible candidate for T2DM susceptibility (30). Regarding the association of VDR gene *ApaI* polymorphism in studied participants, the results demonstrated a significant value of this SNP in controls than in patients.

In conclusion, our study revealed that there was not any association between VDR gene polymorphism and T2DM. while there was an association between VD level and *ApaI* genotype in T2DM among Kurdish-ethnithity in Sulayamaniyah city.

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Interest conflict
The authors declare that they have no conflict of interests.

Author contribution
The study was planned, designed and supervised by SAA and BSAJ. The data acquisition was done by MSA. The data analysis, interpretation and revision were by SAA and MSA.

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