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Original Research

Estimation of vitamin D receptor gene polymorphism in Type 2 Diabetes Mellitus patients in Erbil city

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Abstract: Type 2 diabetes mellitus (T2DM) is a global problem. Recent studies confirmed the association of genes and different single nucleotide polymorphisms with T2DM occurrence and progress. This study was aimed to estimate the vitamin D receptor (VDR) gene polymorphism in Type 2 Diabetes Mellitus kurdish patients in Erbil city. The results showed that the Body mass index (BMI), Systolic blood pressure and Diastolic blood pressure were significantly higher in the diabetic group compared to the control group (P<0.05). In addition, the percent of Glycated hemoglobin (HbA1c), Fasting blood glucose (FBG), and Homeostatic model assessment for insulin resistance (HOMA-IR) were significantly higher in the diabetic group compared to the control group (P<0.05). Among different parameters of lipid profile, only Low-density lipoprotein (LDL) was significantly higher in the diabetic group compared to the control group. It was found that FBG value was significantly higher in patients with GA and AA genotypes of *BsmI* compared with healthy controls. Patients with the GA genotype of *BsmI* had a higher value of triglyceride compared to healthy individuals. Patients with all *ApaI* genotypes had higher FBG values than controls. There were not observed any significant associations among the *BsmI* and *ApaI* polymorphisms and the risk of T2DM. In conclusion, no evidence was found for the association between two VDR polymorphisms and T2DM kurdish patients in Erbil city.

Key words: Type 2 Diabetes Mellitus; Vitamin D receptor; Gene polymorphism; *ApaI*; *BsmI.*

Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disease and a global problem that influences people all around the world (1). Many genetic and environmental factors affect the development of T2DM. However, genetic components play important roles in its occurrence and progress (2). T2DM is known by alteration in the secretion of insulin and also insulin resistance that leads to disturbance in carbohydrate, protein and lipid metabolism. Various researches investigated the association between T2DM and certain genes (3, 4). Long-term diabetes is associated with intense insulin resistance and dysfunction of β cells and can stimulate the release of inflammatory mediators during the diabetes disease (5).

The steroid-derived vitamin D has a wide function including nervous and immune function, bone strength and growth. Different chronic diseases can be caused by low levels of vitamin D. Several studies showed the association of low levels of vitamin D levels and T2DM (6, 7). The relationship between deficiency of vitamin D and insulin resistance, the enhanced risk for T2DM, and glucose intolerance was observed while investigating genes involved in the metabolism of vitamin D (8, 9). Vitamin D regulates the transcriptional function of the insulin receptor gene (10). Vitamin D performs its function through the vitamin D receptor (VDR) which is a candidate gene for T2DM (11). The VDR gene has more than twenty-five different polymorphisms mapped to its locus which is located on chromosome 12q13.1. It has at least 6 untranslated exons, 5 promoter regions, and 8 protein-coding exons that are spliced into TaqI (exon 9), ApaI, and BsmI (intron 8), and FokI (exon 2). Several recent reports are suggesting that these VDR polymorphisms are associated with insulin secretion and type 2 diabetes (12, 13).

In the VDR gene, single nucleotide polymorphisms (SNP) regulate sensitivity and secretion of insulin and glucose intolerance (14). SNPs in the VDR gene may affect the function, transport, and synthesis of vitamin D, cause insulin resistance and have an influence on the secretion of insulin (15). VDR gene located on chromosome 12q12-q14. Through binding to vitamin D response elements (VDRE), the VDR gene regulates the function of vitamin D. VDR is expressed in most tissues of the body including the pancreatic β-cells, which are involved in the regulation of glucose metabolism (16).

The VDR gene polymorphisms become one of the candidate genes that influence T2DM development. The most important polymorphisms of VDR genes include *ApaI* and *BsmI* which are covered by different studies in different areas and populations (1, 11, 17, 18).

Genotype *BsmI* polymorphism of the VDR gene is associated with the beginning of T2DM. The *BsmI* polymorphism is located in the intronic region. It is known to be associated with insulin resistance and obesity in some populations (19). The results of a study found that the SNP of *BsmI* is more frequent in patients with T2DM (20). In addition, some studies investigated the association between polymorphisms of *ApaI* and T2DM

in various populations (21, 22).

Therefore, this research was conducted to estimate the vitamin D receptor gene polymorphism including *ApaI* and *BsmI* variants in Type 2 Diabetes Mellitus in Kurdish patients in Erbil city.

Materials and Methods

Patients and control groups

Totally, 160 individuals participated in this study. The patients attended to Layla Qassim diabetic center, Erbil city, Kurdistan Region, Iraq. The patient group includes 100 T2DM patients (median=50.28 years; min-max = 30-65years; 26 male and 74 female). The control group includes 60 healthy volunteers without T2DM or any metabolic disease (median=45.73 years; min-max = 30-65years; 32 male and 28 female). The patient and control groups were selected from the Kurdish race in Erbil city. Collection of data for patients and control group carried out from October 2020 to March 2021. A questionnaire was designed, and the patients were attended to the center to record various information such as age, gender, smoking, family history, duration of disease. Study participants were asked to sign a consent form provided in the questionnaire. In addition, approval was achieved from the ethics committee of the Department of Biology, College of Education, Salahaddin University-Erbil, Kurdistan Region, Iraq.

Blood collection

Clinical profiling of the patients was carried out by performing different clinical assessments and diagnostic tests. Body Mass Index (BMI) was calculated as weight (in kg) divided by the square of height (in m^2) according to Asian-Pacific guidelines (23). Blood pressure was recorded from the right arm in a sitting position to the nearest 2 mmHg with a mercury sphygmomanometer (Japan). Two readings were taken 5 minutes apart, and the mean of the two was taken as the blood pressure. 7 ml of venous blood was collected from the vein of each person for biochemical tests. 1 ml of blood used for glycated hemoglobin estimated by tina-quant kit (Germany), 3ml of blood used for DNA extraction for gene polymorphism, remained blood was centrifuged 3000r/m for 15 minutes to obtain the serum and used for measurement of fasting blood glucose by Gluc kit, Roche Diagnostics (Germany). Lipid profile levels were estimated by enzymatic kit, Roche (Germany). Also, serum levels of 25 (OH) D (vitamin D) and serum insulin were evaluated by Elycsys kit, Roche (Germany). Liver function test by Cobas Integra kit, Roche (Germany). For all diagnostic tests autoanalyzer Cobas 6000 Roche-Hitachi, Germany was used. For measuring insulin resistance, the formula of homeostasis model assessment of insulin resistance (HOMA-IR) was applied (24).

DNA extraction

Approximately 3 ml of whole venous blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes from each subject and DNA was extracted using a salting-out method. We investigated two VDR gene polymorphisms [*BsmI* (B/b) and *ApaI* (A/a)] in all subjects using the single amplification refractory mutational system method (ARMS)-PCR technique. In the single ARMS-PCR method, two complementary reactions for each polymorphism were performed; one contained a primer specific for the mutant allele and the other contained one for the wild-type allele. Both reactions used a common primer. The mutant and wild-type primers differed by a single nucleotide at the 3' end. Since Taq DNA polymerase lacks the 3' to 5' exonuclease activity, a mismatch at the 3' terminal would reduce the efficiency of the extension. Genotyping was based on whether there was allele-specific amplification in one or both reactions. The validity of amplification reactions was confirmed by size determination and sequencing of PCR products. Sequences of specific and control primers and their mixes and specificities for ARMS-PCR assay are:

BsmI/B 5′ AGCCTGAGTACTGGGAATGT 3′, *BsmI*/b 5′ AGCCTGAGTACTGGGAATGC 3′ and *BsmI*/C 5′ GGGAGGGAGTTAGGCACC 3′; while, sequences for *ApaI* included *ApaI*/A 5′ TGGGATTGAGCAGTGAGGT 3′, *ApaI*/a 5′ TGGGATTGAGCAGTGAGGG 3′ and *ApaI*/C 5′ CCTCATTGAGGCTGCGCAG 3′.

The optimized PCR reaction condition consisted of 75 ng of genomic DNA in 15 ml of reaction mixture containing specific and control primer mixes, 200 µM of each deoxynucleotide triphosphate (dNTP), 19 ammonium sulfate-based PCR buffer, 1.5–2.5 mM MgCl2, and 0.6 unit Taq DNA polymerase. The reaction was amplified by an Eppendorf gradient Mastercycler PCR system, which started with a heating temperature of 94 0C for 2 min, followed by ten cycles of 10 s at 94˚C, 60 s at 65˚C, and 20 cycles of 10 s at 94 ˚C, the 50s at 61 ˚C, and 30 s at 72 ˚C. The PCR products were analyzed in 2 % agarose gel stained with ethidium bromide. For rapid performance, and to eliminate pipetting errors, primer mixes could be pre-aliquoted and dried in PCR plates or string tubes and then PCR master mix plus sample genomic DNA and Taq polymerase were added.

Statistical Analysis

Statistical analyses were performed using SPSS 20 (SPSS Inc., Chicago, IL, USA). Normally distributed variables were demonstrated as mean ± SE. The P value<0.05 was considered statistically significant. The ANOVA test was applied for between-group comparisons of categorical variables. For gene polymorphism of *BsmI* and *ApaI*, groups were evaluated for significance using the Chi-square test or Fisher's exact test. P-values of 0.05 were considered statistically significant. To assess the consistency of genotype distributions with Hardy–Weinberg equilibrium, the Chi-squared test was used.

Results

Clinical characteristics

Results of biochemical assays are presented in Table l. The average age of the diabetic group was 50.28±1.34 and the control average age was 45.73±1.54. The ratio of males/females in the diabetic group was 26/74, whereas it was 32/28 in the control group. Among 100 diabetic people, 52 individuals had T2DM in their family history. In the control group, only 10 individuals had T2DM in their family history, among 60 people. Duration of

Table 1. Characteristics of the patient and control group (Mean ±SE).

Parameters	Diabetic group (n = 100)	Control group (n = 60)	*P* value
Age (years)	50.28±1.34	45.73±1.54	
Gender (Male/Female)	26/74	32/28	
Family history (Yes/No)	52/48	10/50	
Duration of disease	7.84±0.757	0.00±0.00	<0.0001****
BMI (kg/m^2)	29.4217±0.682	23.014±0.66	<0.0001****
Systolic blood pressure (mmHg)	135.2±1.812	115.7±1.899	<0.0001****
Diastolic blood pressure (mmHg)	93.40±1.171	80.23±0.034	0.0004***
Smoking (Yes/No)	14/86	1/59	
No. of children	2.58±0.3587	4.4±0.3699	<0.0001****

Table 2. Mean ±SE of HbA1c, fasting blood glucose, fasting insulin and HOMA- IR in patients and control group.

Parameters	Diabetic group (n = 100)	Control group (n = 60)	P value
HbA1c (%)	7.634±0.2652	5.724±0.086	0.0026**
FBG (mg/dl)	193.0±1.202	87.75±2.612	0.0003***
Fasting insulin(U/l)	11.56±1.644	6.882±0.8818	0.3161ns
HOMA- IR	5.659±1.072	1.527±0.2239	0.0002***

Table 3. Mean ±SE of lipid profile in patients and control group.

Parameters	Diabetic group (n = 100)	Control group (n = 60)	*P* value
TC(mg/dl)	164.3±7.292	156.3±6.669	>0.9999ns
TG(mg/dl)	166.3±1.473	125.4±1.529	0.9924ns
HDL(mg/dl)	36.58±1.146	37.57±1.831	>0.9999ns
LDL(mg/dl)	102.8±0.695	95.27±0.551	0.0003***

Table 4. Mean ±SE of liver function test in patients and control group.

Parameters	Diabetic group (n = 100)	Control group (n = 60)	*P* value
ALT(U/I)	20.10±1.566	17.38±1.667	>0.9999ns
AST(U/I)	19.17±0.9204	17.37±1.138	>0.9999ns
ALP(U/I)	75.72±3.668	72.27±4.299	>0.9999ns

disease in T2DM was 7.84±0.757 Years. The BMI of the diabetic group (29.4217±0.682) was significantly higher than the control group (23.014±0.66) (P<0.05). Systolic blood pressure was significantly higher in the diabetic group (135.2±1.812) compared to the control group (115.7±1.899) (P<0.05). In addition, diastolic blood pressure was significantly higher in the diabetic group (93.40±1.171) compared to the control group (80.23±0.034) (P<0.05). The number of children in the diabetic group (2.58±0.3587) was significantly lower than the healthy control group (4.4±0.3699) (P<0.05).

Biochemical assays

Table 2 demonstrates the Mean ±SE of glycated hemoglobin (HbA1c) test, fasting blood glucose (FBG), fasting insulin and HOMA- IR in patients and control group. The percent of HbA1c was significantly higher in the diabetic group (7.634±0.2652) compared to the control group (5.724±0.086) (P<0.05). Also, FBG increased significantly in the diabetic group (193.0±1.202) when compared with the control group (87.75±2.612). In addition, HOMA-IR was significantly higher in the diabetic group compared to the control group (P<0.05). Fasting insulin was not significantly different between the two groups (P>0.05).

The lipid profile of patients and control groups are presented in Table 3. Different parameters including total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were assessed. The results showed that only the LDL levels of the diabetic group were significantly higher than the control group (P<0.05). However, the difference between two groups in TC, TG and HDL was not significant (P>0.05).

The liver function was assessed through different tests including Alanine Aminotransferase (ALT) test, aspartate transaminase (AST) test and Alkaline Phosphatase (ALP) test (Table 4). There are not any significant differences between the two groups considering these liver function tests (P>0.05).

A significant association was found between vita-

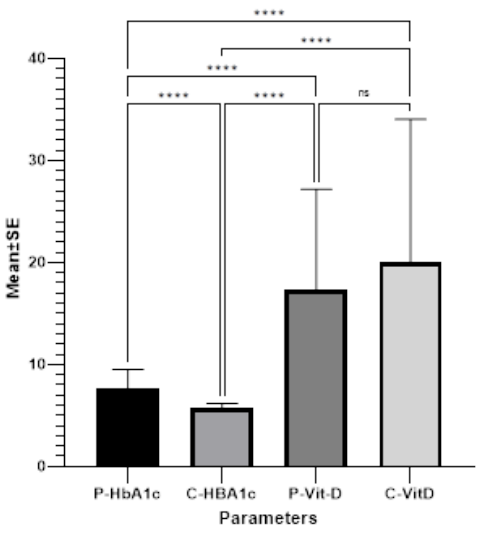

Figure 1. Significant relation between vit. D and HbA1c.

Table 5. Probability values for the association of biochemical parameters and *Bsm*I genotypes.

BSMI and parameters	PATIENTS	CONTROL	P VALUE
FBG			
GG	191.4±17.87	87.50±3.83	0.136
GA	168.3±14.68	89.83±4.47	0.0005
AA	228.1±27.34	85.85±4.37	0.0001
INSULIN			
GG	13.39±3.85	6.490±2.12	0.95
GA	13.94±2.93	6.650±1.25	0.99
AA	6.901±0.82	7.329±1.66	0.99
P-HbA1c			
GG	7.492±0.41	5.423±0.23	0.81
GA	7.254±0.44	5.685±0.16	0.99
AA	8.265±0.45	5.782±0.11	0.90
VIT D			
GG	18.17±3.07	25.78±12.99	0.90
GA	16.65±1.96	20.73±4.97	0.99
AA	16.75±2.72	19.47±2.62	0.95
TC			
GG	139.9±9.05	158.2±7.3	0.71
GA	173.7±10.94	167.7±10.06	0.99
AA	168.7±15.16	149.1±11.26	0.80
TG			
GG	139.6±28.29	152.3±48.9	0.97
GA	178.9±24.89	100.9±19.72	0.0005
AA	167.4±23.37	148.8±27.22	0.76
HDL			
GG	38.07±1.89	43.70±11.34	0.99
GA	36.16±1.94	37.88±2.86	0.99
AA	37.34±2.09	36.67±2.17	0.95
LDL			
GG	79.17±7.2	84.00±6.42	0.96
GA	116.1±9.39	109.5±7.65	0.99
AA	102.1±15.16	86.62±9.42	0.90
AST			
GG	19.50±1.35	14.83±0.48	0.90
GA	19.66±1.52	17.68±1.72	0.99
AA	18.24±1.75	18.51±1.97	0.99
ALT			
GG	20.84±2.69	11.57±1.18	0.98
GA	22.34±2.97	15.14±2.1	0.99
AA	16.48±1.63	21.35±3.00	0.99
ALP			
GG	77.02±7.62	79.67±20.85	0.90
GA	71.66±4.3	68.50±3.84	0.99
AA	80.33±8.16	76.23±7.59	0.95

min D and HbA1c (Figure 1). The HbA1c of patients (P-HbAc1) was significantly higher than the HbA1c of healthy controls (C-HbAc1) ($P<0.05$).

Parameters based on *BsmI* Genotypes

After amplification of VDR gene polymorphisms through ARMS-PCR, probability values for the association of biochemical parameters and genotypes of *BsmI* and *ApaI* were calculated (Table 5 and Table 6).

A significantly higher FBG value was observed in patients with GA and AA genotypes of *BsmI* compared with healthy controls. Patients with the GA genotype of *BsmI* had a higher value of triglyceride comparing to healthy individuals. However, no significant differences were observed between the insulin, HbA1c, vitamin D, TC, HDL, LDL and liver enzyme parameters of the two groups.

Table 6. Probability values for the association of biochemical parameters and *ApaI* genotypes.

*Apa*I and parameters	PATIENTS	CONTROL	P VALUE
FBG			
AA	192.6±24.68	84.82±1.43	0.0001
Aa	180.2±16.19	88.71±4.67	0.0001
aa	208.2±29.11	86.92±5.11	0.0001
INSULIN			
AA	16.38±6.06	10.71±3.18	0.97
Aa	11.74±2.23	7.101±1.43	0.99
aa	8.219±1.24	5.359±0.86	0.99
P-HbA1c			
AA	7.661±0.54	5.328±0.19	0.96
Aa	7.792±0.39	5.765±0.15	0.99
aa	7.284±0.58	5.838±0.13	0.90
VIT D			
AA	16.56±3.77	22.98±7.95	0.96
Aa	17.84±2.14	19.35±2.83	0.99
aa	16.61±2.69	20.50±5.3	0.89
TC			
AA	173.7±13.62	174.8±14.63	0.99
Aa	171.8±9.34	167.2±7.09	0.99
aa	155.7±18.21	137.8±13.57	0.96
TG			
AA	181.7±37.32	123.8±36.00	0.93
Aa	168.8±25.65	131.6±23.04	0.90
aa	156.7±19.72	128.1±30.23	0.96
HDL			
AA	36.45±2.36	43.18±5.67	0.93
Aa	37.92±1.56	38.23±2.32	0.99
aa	35.69±2.63	33.26±3.17	0.99
LDL			
AA	113.8±12.11	106.8±15.62	0.97
Aa	110.1±9.13	102.6±5.39	0.98
aa	95.57±16.8	83.45±11.48	0.99
AST			
AA	19.81±2.09	17.08±2.29	0.99
Aa	19.91±1.66	19.66±1.72	0.99
aa	17.46±1.28	15.28±2.16	0.99
ALT			
AA	20.21±3.57	20.26±5.89	0.99
Aa	22.83±2.53	19.53±2.77	0.96
aa	15.70±2.76	14.27±2.06	0.99
ALP			
AA	89.11±11.29	68.80±8.68	0.90
Aa	71.90±5.24	83.00±7.00	0.96
aa	73.48±5.8	62.55±6.8	0.99

Parameters based on *ApaI* Genotypes

All *ApaI* genotypes of patients had higher FBG values than controls. However, no significant differences were observed between other parameters of the two groups (Table 6).

Statistical evaluation of *BsmI* and *ApaI* variants

Statistical evaluations of *BsmI* genotypes or alleles, Relative risk, and Etiological or Preventive Fraction of T2DM patients were calculated and shown in Table 7.

Relative risks were between 0.58 (95% CI=0.23 –1.47) and 1.97 (95% CI=0.58 –6.69) respectively for AA and GG genotypes of BsmI. However, none of these associations were significant.

Statistical evaluations of *ApaI* genotypes or alleles, relative risk, and Etiological or Preventive Fraction of T2DM patients were calculated (Table 8).

Relative risks were 0.56 (95% CI=0.22 –1.42) and 1.41 (95% CI=0.45 –4.47) respectively for aa and AA

Table 7. Statistical evaluations of *BsmI* Genotype or Allele.

BsmI Genotype or Allele	Statistical Evaluations			
	Relative Risk	Etiological or Preventive Fraction	Fisher's Exact Probability	95% Confidence Intervals
GG	1.97	0.12	Not Significant	0.58 to 6.69
AG	1.11	0.05	Not Significant	0.45 to 2.77
AA	0.58	0.19	Not Significant	0.23 to 1.47
G	1.62	0.18	Not Significant	0.83 to 3.14
A	0.62	0.25	Not Significant	0.32 to 1.20

Table 8. Statistical evaluations of *ApaI* Genotype or Allele.

ApaI Genotype or Allele	Statistical Evaluations			
	Relative Risk	Etiological or Preventive Fraction	Fisher's Exact Probability	95% Confidence Intervals
AA	1.41	0.06	Not Significant	0.45 to 4.47
Aa	1.38	0.13	Not Significant	0.56 to 3.42
aa	0.56	0.19	Not Significant	0.22 to 1.42
A	1.47	0.15	Not Significant	0.77 to 2.82
a	1.47	0.15	Not Significant	0.77 to 2.82

Table 9. Genotype and allele frequencies of the studied VDR gene polymorphism.

	Genotypes			Alleles		
BsmI						
	GG	**AG**	**AA**	**G**	**A**	**H-W (P ≤?)**
T2DM	12 (24)[a]	22 (44)	16(32)	46 (46)	54 (54)	Not Significant
Controls	4 (13.8)	12 (41.4)	13 (44.8)	20 (34.5)	38 (65.5)	Not Significant
ApaI						
	AA	**Aa**	**aa**	**A**	**a**	**H-W (P ≤?)**
T2DM	11 (22)	24 (48)	15 (30)	46 (46)	54 (54)	Not Significant
Controls	5 (16.7)	12 (40)	13 (43.3)	22 (36.7)	38 (63.3)	Not Significant

[a] Values are presented as No. (%).

genotypes of *ApaI*. The relative risk of both alleles (A and a) was equal (1.47). However, none of them were significantly associated with T2DM.

Genotype and allele frequencies of the studied VDR gene polymorphic sites including *BsmI* and *ApaI* were in agreement with the Hardy-Weinberg equilibrium. There was no statistically significant deviation from the expected Hardy–Weinberg equilibrium for both polymorphisms in this study (Table 9).

As a result, there were not observed no significant associations among the *BsmI* and *ApaI* polymorphisms and the risk of T2DM.

Discussion

The fatality caused by diabetes is high and the treatment of diabetic patients is a complex condition. Complications of diabetes can lead to enhancing the mortality of patients with diabetes. In diabetic complications, the mechanism of hyperglycemia role was not completely explained, therefore, genetic factors may involve in complications of diabetes (25).

Various studies were investigated factors involved in the diabetic disorder. A study was performed in Tokyo and its results represented that smoking and family history of diabetes is the risk factor for T2DM prevalence (26).

There is a negative correlation between BMI and serum 25 (OH) D concentrations. The vitamin D fat solubility causes sequestration of this vitamin in the adipose tissue, therefore its bioavailability will be decreased. This can be the cause of a negative correlation between metabolic syndrome and low levels of vitamin D (27).

The result of a study on postmenopausal women represented that there is no association between the BsmI polymorphism and susceptibility to insulin resistance and obesity. However, there is a creation between it was related to a higher level of LDL-C and BsmI polymorphism (28).

In this study, two vitamin D receptor gene polymorphisms including *BsmI* and *ApaI* were investigated in T2DM patients in Erbil city. It was found that the BMI, the number of children, systolic blood pressure and diastolic blood pressure of patients with T2DM were significantly higher than the control group ($P<0.05$). The higher BMI in patients with T2DM was observed in the various study (1, 11). In addition, different studies were reported significantly higher blood pressure in patients with T2DM comparing with the control group (2, 11).

An independent risk factor for high levels of HbA1c in T2DM patients especially in females is 25(OH) D. It was reported that 25(OH) D is not a risk factor for

high levels of HbA1c in males. The differences between the male and female can be because of the influence of exercise, hormones, or other factors (29). The results of a study in a non-diabetic population demonstrated that people with a 25(OH) D level equal to or higher than 20ng/mL were less likely to show enhanced HbA1c levels compared with people with a 25(OH) D level less than 20ng/mL (30).

There are receptors for active vitamin D in beta cells of pancreatic islets which enable vitamin D to operate the response of insulin to enhanced levels of blood glucose. Also, 1α- hydroxylase is expressed by these cells. 1α- hydroxylase can convert the biologically inert 25 (OH) D to active vitamin D which by suppressing inflammation can also contribute to insulin-mediated responses (31).

Elevated FBG levels may be due to defects in insulin action, insulin secretion, or both of them. Two processes control Blood glucose that includes the secretion of insulin through pancreatic B cells and the action of insulin on target organs such as adipose tissue and liver (32). The results of a study demonstrated a negative correlation between HOMA-IR and 25(OH) D levels (33).

In the present study the HbA1c percent, FBG and HOMA-IR were significantly higher in the diabetic group compared to the control group ($P<0.05$). It was reported in different studies that the percent of HbA1c and the level of FBG were significantly higher in patients with T2DM compared to healthy controls (2, 11).

In T2DM patients, abnormal lipid metabolism enhanced the formation of ketone bodies and catabolism, also it decreased TG and FA synthesis. Half of an ingested glucose load is usually transformed to H2O and Co2, about 30 to 40 percent is converted to fat storage, and five percent is converted to glycogen. In T2DM patients, less than 5% is converted to fat even if the amount is burned to H2O and Co2. Therefore, glucose accumulates in the blood and is excreted in the urine (34).

InT2DM patients, the most important impairment in the metabolism of lipoprotein is represented through increasing plasma triglyceride, decreasing levels of HDL-C with near-normal levels of LDL-C. In diabetes, this part of LDL contains a higher proportion of small, dense LDL particles that are thought to be more atherogenic (35).

The overall enhance in serum lipids in diabetic patients may be mainly due to enhanced mobilization of free fatty acids from adipose tissue. Extra fatty acids in the serum are then converted to cholesterol, phospholipids, and triglycerides in the liver (36).

Among lipid profile parameters, only LDL was significantly higher in the diabetic group compared with healthy controls. This result was in contrast with another study that compared biochemical parameters of T2DM patients and controls and reported a significant difference in only TG among lipid profile parameters (1). A significant association was found between vitamin D and HbA1c. In addition, the HbA1c of patients was significantly higher than the HbA1c of healthy controls.

The deficiency in vitamin D can have several causes, including excretion vitamin D from the blood through fat cells due to obesity, Gastrointestinal problems because of certain diseases that impair the absorption of vitamin D, having dark skin that decreases the skin's ability to synthesize vitamin D, restrictions on exposure to the sunlight for a variety of reasons, limit consumption of fish and fish oil, that are rich in vitamin D, or eating fiber-rich foods that influence vitamin D absorption (37).

One of the reasons for the limited exposure to sunlight in diabetes could be that most people had indoor work environments, mild physical activity, and lack of sunlight (38).

In this study, most cases were women. For religious and cultural reasons, most Kurdish women in Erbil city wear clothing that completely covers their bodies, thus blocking sunlight and preventing the synthesis of vitamin D. This reason may be responsible for deficiency of vitamin D and diabetes.

In investigating of *BsmI* genotypes, it was found that the FBS value was significantly higher in patients with GA and AA genotypes of *BsmI* compared with healthy controls. Patients with the GA genotype of *BsmI* had a higher value of triglyceride comparing to healthy individuals.

BsmI Polymorphism has a functional influence because its location is within intron 8 that is removed after transcription of mRNA. However, VDR alone may not be a locus that influences disease, but it can be considered as a marker locus in linkage disequilibrium with the residual locus. Another feasible mechanism is that BsmI alternation in intronic sequence can affect protein expression (39, 40).

In a study on Egyptian T2DM patients, there were not observed any significant statistical differences between patients with T2DM and controls in genotypes and alleles distribution of TaqI and BsmI polymorphisms. In addition, there was not find any significant association between VDR polymorphisms and BMI in these patients (41).

In another study, the prevalence of vitamin D deficiency was higher in T2DM patients compared to healthy controls. In addition, a significant relationship between T2DM and BsmI was observed (2). The results of a study on French patients with T2DM represented that TaqI and BsmI polymorphisms were associated with obesity. However, there was not any significant correlation between ApaI polymorphisms and obesity (42).

The investigation results of *ApaI* genotypes showed that all *ApaI* genotypes of patients had higher FBG values than controls. There were not observed any significant associations among the *BsmI* and *ApaI* polymorphisms and the risk of T2DM. There was no statistically significant deviation from the expected Hardy–Weinberg equilibrium for both polymorphisms in this study.

Association between *BsmI* genotype and T2DM is reported by the various study. However, findings of these studies were inconsistent with those studies that reported no association between *BsmI* genotype and T2DM.

The association of T2DM susceptibility and four polymorphisms of the VDR gene including *TaqI*, *FokI*, *ApaI*, and *BsmI* were investigated through a meta-analysis study. The results showed that only FokI polymorphism was associated with enhanced T2DM risk. It was

not observed no associations among T2DM risk and *TaqI*, *ApaI* and *BsmI* polymorphisms (43).

A study investigated three VDR gene polymorphisms among Emirati patients with T2DM (11). These polymorphisms were included *BsmI*, *TaqI* and *FokI*. The *TaqI* variant was associated with high cholesterol and LDL levels in patients with T2DM. However, *BsmI* was associated with lower levels of LDL and BMI. Another study investigated VDR Gene Polymorphisms and their association with T2DM in a Polish population (18).

A study on the Moroccan population showed that ApaI and BsmI polymorphisms were not correlated with T2DM. The genotypic distribution of these VDR polymorphisms did not represent any statistical difference between healthy controls and T2DM patients (44).

Another study on the ApaI site showed that there is a high correlation between T2DM and VDR gene, whereas it has not been reported in many other studies from different populations. Gene pool exchange, ethnic background, and allele distributions are the most important causes which influence these results (45). Also, positive correlations were found between ApaI polymorphism and obesity in a Chinese population (46).

Four VDR variants including *TaqI*, *FokI, BsmI* and *ApaI* were evaluated. The outcomes did not show any associations between these VDR polymorphisms and T2DM in a Polish population. A study was performed to determine the association of *BsmI* polymorphism of the VDR gene with T2DM in the Pakistani population (2). The results showed that the *BsmI* genotype association with T2DM was not significant. In conclusion, it was not no associations between *BsmI* polymorphism of the VDR gene with T2DM in the Pakistani population. Another study investigated the association of VDR Polymorphisms with T2DM in Saudi patients (1). Among three polymorphisms of the VDR gene assessed, only the association between TaqI genotypes and T2DM was significant. There was no correlation between T2DM and polymorphisms of *ApaI* and BsmI. The results of a study showed that the *ApaI* genotype is associated with T2DM in American population (47). However, another study did not find any associations between *ApaI* genotypes and T2DM (22).

Different studies reported conflicting results about the effect of VDR gene polymorphisms on T2DM in various populations. Some of them support and the others contrast this study findings (2, 22, 48). Limited information of underlying mechanisms and heterogeneity in different populations can be the cause of these discrepancies. In relation to diabetes, more studies are needed in terms of genetics, polymorphism, the nature of the disease and other influencing factors (49-52).

In conclusion, the present study represented there was not any association between Bsm1 and Apa1 polymorphisms and T2DM in Kurdish patients. GA and AA genotypes of *Bsm1* show significantly higher FBGvalue compared with healthy controls and patients with GA genotype of *Bsm1* had higher value of triglyceride comparing to healthy individuals. In addituion, all *Apa1* genotypes of patients had higher FBGvalue than controls. However, there was not observed any significant relationship between T2DM patients and *Bsm1* and *Apa1* polymorphisms of VDR gene in this population. However, further studies are needed to investigate the association of the other VDR polymorphisms and T2DM.

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