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Characteristics of Vitamin D3 Receptor Genotypes in T2DM of Iraqi Obese Women

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Abstract

Objectives: This study aims to examine the association between the (rs1544410) polymorphism of the VDR gene with the pathogenesis of T2DM in Iraqi obese women.

Methods: A case-control study was performed on 50 patients with T2DM and 50 apparently healthy subjects who were admitted to Al-Hussein Teaching Hospital and Al-Hassan Center of Diabetes and Endocrinology unit/Kerbala health directorate—Iraq during (April 2022–March 2023). The T2DM groups were divided into two groups, 25 obese and 25 non-obese; the control group was divided into 25 obese and 25 non-obese as apparently healthy groups. The ELISA Kit was used to measure serum 25(OH)D3, heat shock protein-70, VDBP, insulin and C-peptide. Also HbA1c% and insulin resistance (HOMA-IR) were evaluated. The vitamin D3 receptor gene (VDR) variant and the SNP (rs1544410) polymorphism was determined using allele specific polymerase chain reaction, 1.5% agarose gel electrophoresis and then visualized by gel photo-documentation system.

Results: The result of vitamin D3 variants genotype (rs 1544410) was a clear band with a molecular size of 200 bps. The size of the amplicon was determined by compare with DNA ladder 100–1500 bp. The result of the comparison between observed and anticipated values for SNIP with (rs 1544410) in the tested population was statistically significant, $P = < 0.001$ and the difference between demographic characteristics and (rs 1544410) SNP, age and BMI shows non-significant difference among all groups. The difference between biomarkers and (rs1544410) SNP was performed using one-way ANOVA test to compare the mean levels of HSP-70, VDBP, C-peptide, RBC and HbA1c% which shown a non-significant difference among the variants of VDBP Genotype (rs1544410) in obese women (patients and control) studied groups, P value > 0.05.

Conclusion: The logistic analysis of the (rs1544410) SNP of the patients concluded that HSP-70, VDBP, and C-peptide level was no significantly related to the also C-peptide, was shown to be a related risk factor to both CT and CT alleles (1.003, $P > 0.05$) in comparison with CC alleles. Furthermore, HbA1c% level was demonstrated to be related as a risk factor for the CG allele in comparison with CC and GG alleles (1.009, $P < 0.05$).

Keywords: Diabetes mellitus, type 2, receptors, calcitriol, vitamin D-binding protein, HSP-70, obesity

Introduction

Diabetes mellitus (DM) is a chronic metabolic condition that causes blood glucose levels to rise as a result of either reduced insulin production or body cells that do not react to the effects of insulin (insulin resistance).[1] Although there are various forms of diabetes, type 2 diabetes mellitus (T2DM) is the most frequent type and accounts for 90–95% of cases of diagnosed (DM). T2DM is more common in adults and elderly[2] resulting in anomalies in the metabolism of carbohydrates, lipids, and proteins, as well as disturbance of the regulatory systems that control the storage and mobilization of metabolic fuel.[3] Obesity is mostly measured by means of body mass index (BMI), but anthropometric classification systems do not reflect the presence or severity of comorbidities.[4] Vitamin D3 receptor gene factor nuclear transcription by the mediation of $1,25(OH)_2D3$, VDR influences calcium absorption, bone remodeling, and the rate of mineralization. There are 11 exons in the 3q11 region on the long arm of chromosome 12, and 2 to 9 of them are actively transcribed.[5] Numerous investigations have revealed that the nuclear receptor superfamily's VDR gene, which is found on chromosome 12's long arm (12q13.11), plays a significant role in the etiology of osteoporosis.[6]

The majority of research on VDR polymorphisms has been done in Caucasian populations and has concentrated on five SNPs: (1) rs10735810 or *FokI* in exon 2; (2) rs1544410 or *BsmI* in intron 8; (3) rs731236 or *TaqI* in exon 9; (4) rs7975232 or *ApaI* in intron 8 and (5) rs757343.[7] Vitamin D receptor gene sigle nucleotide polymorphism of BsmI variant modulate glucose intolerance, insulin secretion and sensitivity.[8] VDR gene's variants can alter insulin secretion, leading to insulin resistance, as well as vitamin D3 biosynthesis, transportation, and action.[9] Reported about T2DM patients have a considerably higher prevalence of the *BsmI* SNP. Several studies showed a similar connection between *BsmI* polymorphism and T2DM in other groups.[10] *BsmI* SNP and risk of T2DM in various ethnic groups are thus not conclusively linked. SNP is connected to therapeutic responsiveness and illness susceptibility.[11] T2DM) and VDR polymorphisms are still not clearly linked. Many genetic VDR polymorphisms have been identified in studies carried out in different places with varying populations of people. Just one research has examined the relationship between the VDR *BsmI* (rs1544410) polymorphism and vitamin D3 insufficiency, obesity, and insulin resistance among non-diabetic subjects across various age groups to date, and it was conducted in the central area of Malaysia.[12] Accordingly, the aforementioned study had found that the BsmI (rs1544410) polymorphism was associated with increased risk for vitamin D deficiency and insulin resistance among the Malaysian population.[12] The risk of metabolic

syndrome elements including abdominal obesity has also been linked to vitamin D insufficiency.[13] The control of hormone sensitive genes and the modulation of vitamin D pathways are both important functions of the VDR gene. It's interesting to note that the VDR gene is expressed in both pancreatic beta cells and adipocytes. As a result, it may affect body composition either directly by controlling adipocyte development and metabolism or indirectly by modulating insulin levels.[14] The objective of the presented work was to examine the association between the (rs1544410) polymorphism of the VDR gene with the pathogenesis of T2DM in Iraqi obese women.

Materials and Methods

A case-control study was performed on 50 patients with T2DM and 50 apparently healthy subjects who were admitted to Al-Hussein Teaching Hospital and Al-Hassan Center of Diabetes and Endocrinology unit/Kerbala health directorate—Iraq during (April 2022–March 2023). The T2DM groups were divided into two groups, 25 obese and 25 non-obese; the control group was divided into 25 obese and 25 non-obese as apparently healthy groups. Seven ml of blood was drawn from the vein of all subjects by using a disposable syringe and then divided into two parts: The first part (3 ml) was placed in a gel tube and left at room temperature for about (30 min) for clotting, then put in the centrifuge at 4000 x g to obtain serum which was used for the determination of biomarker levels, including blood glucose by using the enzymatic colorimetric method and Heat shock protein-70 (HSP-70), vitamin D3 binding protein (VDBP), 25(OH)D3, insulin, and C-peptide levels by using ELISA specialized kits. The remaining blood (4 ml) was put in tow (EDTA) containing. The first EDTA tube containing (2 ml) of blood used to determine the HbA1c% level and the second EDTA tube was stored by freezing at –20˚C until using for genomic DNA extraction, and then performing various molecular analyses.

The (rs1544410) polymorphism of the VDR gene was determined by using genomic DNA extracted by (Promega kit), and then by allele specific polymerase chain reaction. Maximum absorbance for proteins and nucleic acids occurs at 260 and 280 nm, respectively. The ratio of absorbance at these wavelengths has been used as a measure of purity in both nucleic acid and protein extractions. When the ratio is between 1.8 and 2.0, DNA is commonly considered to be pure.

The target gene (VDR gene) was amplified using allele-specific PCR with a specific primer indicated below which were [purchased from Bioneer, Korea].

FC 5-AGAACCATCTCTCAGGCTCC-3
FT 5-AGAACCATCTCTCAGGCTCT-3
R 5-CCTCACTGCCCTTAGCTCTG-3

The reactions were conducted in PCR tubes under sterile conditions; the total volume of the reaction mixture was brought to 25 liter using deionized distilled H_2O, and the master mix containing optimal concentrations of reaction requirements ($MgCl_2$, 1.5 mM, each dNTPs 200 M) was used. Different volumes of primer (0.5 µL,1 µL, 1.5 µL) with different volumes of template DNA (1 µL, 2 µL, 3 µL, 4 µL, 5 µL, 6 µL) and different temperatures of the reaction conditions were trailed to optimize the conditions of the reaction. PCR tube was centrifuged for 30 seconds at 2000 x g in a micro-centrifuge to mix solutions well at room temperature then tubes were placed in the thermocycler to start the reaction. Programs of the PCR protocol reaction for VDR gene polymorphism for *BsmI* (rs1544410) was employed by gel electrophoresis by using 1.5% agarose in 1X TBE buffer (tris borate EDTA) was prepared by diluting 10X TBE buffer with deionized water.

The comb was removed and the glass plates were placed in a vertical electrophoresis chamber, 1 X TBE buffer was added inside the chamber until reaching above the level of wells, and then the samples were loaded into the gel wells by using 10 µl micropipettes. The electrophoretic conditions include negatively charged nucleic acids move across the gel toward the positive (+) electrode as a result of an electric field being given to the system (60 V, 45 mA for 35 min.) after sample loading (anode). Ethidium bromide was used to dye the agarose gel. Agarose gel was placed above the UV transilluminator device and exposed to UV light. The photos were captured using a digital camera and visualized by a PC connected to the transilluminator. The UV transilluminator device was covered with a protective shield to avoid exposure to UV light when the light was on. The study protocol has been approved by the Research Ethics Committee of the College of Medicine at the University of Kerbala, as well as a committee from Al-Hussein Teaching Hospital and Al-Hassan Centre of Diabetes and Endocrinology Unit, under the Kerbala Health Directorates. This ensures that the research is conducted with the highest ethical standards and guidelines.

Results

The results of variants genotype (rs1544410) indicate a clear band with a molecular size 200 bps (Figure 1).

The size of amplicon was determined by compare with DNA ladder 100–1500 bp. gene polymorphisms. The distribution of genotyping groups of patients shows in Table 1.

Result of comparison between observed and anticipated values for SNIP with (rs 1544410) in the tested population were shown in Figure 2, and Table 2. The distribution and percentage of individuals having (rs 1544410) differ from those expected under Hardy–Weinberg equilibrium [number of

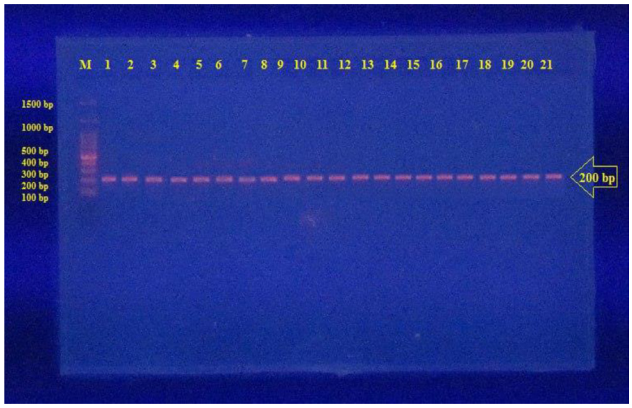

Fig. 1 **Genotyping of gene variants of VDR (rs 1544410). Genotype variants of VDR of gene (rs 1544410) SNP which were classified into three genotypes.**

1. The major genotype group (CC) homozygous for the allele C.
2. The minor genotype group (TT) homozygous for the allele T.
3. Heterozygous (CT).

observed vs expected], which were: CC (36%, 25.6); TT (30%,19.6); CT (24%, 44.8) (goodness-of-fit $\chi 2$ for (rs 1544410), 19.397, $P = < 0.001$ and therefore it was statistically significant.

The difference between demographic characteristics and (rs 1544410) SNP (Table 3), was performed using one-way ANOVA test to compare the age, BMI and groups of study. No significant difference was found between all groups.

The difference between biomarkers and rs (1544410) SNP (Table 4) was performed using one-way ANOVA test to compare the mean levels of HSP-70, VDR, C- peptide, RBC and while blood test, HbA1c was shown all no significant difference among the variants of VDR Genotype (rs 1544410) in obese (Patients & Control) studied groups, P value > 0.05.

The odds ratios of the detected genotypes of the (rs 1544410) of the patients with levels of biomarkers. The logistic analysis of the (rs 1544410) SNP of the patients concluded that HSP70, VDR, C. Peptide level was no significantly related to the Also C. Peptide, was shown to be related risk factor to the both CT and CT alleles (1.003, $P = 0.338$) in comparison with CC alleles. Furthermore, HbA1c level was demonstrated to be related risk factor for the CG allele in comparison with CC & GG alleles (1.009, $P = 0.917$).

Table 1. **Distribution of gene variants of VDR Genotype (rs 1544410) different genotypes in studied groups**

Variable	Group	Frequency	Percentage
Genotype	CC (wild)	36	40
	CT (hetero)	24	26.7
	TT (homo)	30	33.3

Data presented by numbers and percentage.

Discussion

The number of people with diabetes is expected to skyrocket from 382 million (8.3% of the population) in 2013 to 592 million (10.3%) in 2035. Previous research has established a causal

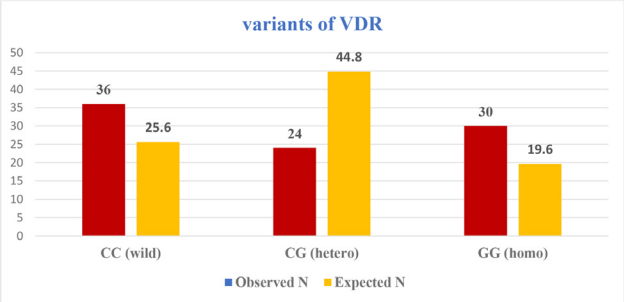

Fig. 2. **Variants of VDR Observed (Obs.) vs expected (Exp.) genotype frequencies % of rs 1544410 among individuals' sample.**

Table 2. **Hardy–Weinberg equilibrium for (rs 1544410) genotype in studied groups**

Genotypes			Alleles		Hardy–Weinberg equilibrium X^2 test
			C	T	
Genotype $N = 100$	Frequency	%			
CC (Wild Type)	36	40			
CG (heterozygous mutant type)	24	26.7	0.533	0.467	19.397 $P < 0.001$ [S]
GG (homozygous mutant type)	30	33.3			

Table 3. **Difference between demographic characteristic in (rs 1544410) SNP in studied groups**

Demographic parameters		rs 1544410 (N = 90)			*P*-Value
		CC ($N = 36$)	CT ($N = 24$)	TT ($N = 30$)	
Age		42.33 ± 8.89	46.75 ± 12.32	47.07 ± 12.08	0.156 [NS]
BMI		29.35 ± 6.39	28.45 ± 6.19	27.18 ± 5.74	0.363 [NS]
Group	Patient	18	12	15	0.903 [NS]
	Control	18	12	15	
Study Group	Patient with obese	10	6	6	0.953 [NS]
	Patient without obese	8	6	9	
	Control with obese	10	6	6	
	Control without obese	8	6	9	
Bp	Yes	12	11	13	0.563 [NS]
	No	24	13	17	
Smoking	Yes	1	1	0	0.564 [NS]
	No	35	23	30	

Results are presented as mean ± SD, or n = number of subjects and percentage, $P < 0.05$ considered significantly different, [S] = Significant, [NS] = non-significant

Table 4. **Difference between alleles of variants of VDR Genotype (rs 1544410) with mean levels of biomarkers in obese studied groups**

Biomarkers	rs 1544410 (*N* = 50)			*P*-value
	CC (*N* = 10)	CG (*N* = 12)	GG (*N* = 28)	
HSP-70	30.22 ± 6.28	28.88 ± 7.06	29.63 ± 7.88	0.769 [NS]
VDR	73.16 ± 21.42	72.34 ± 16.58	68.94 ± 17.75	0.669 [NS]
C. Peptide	139.72 ± 68.61	163.72 ± 126.68	130.73 ± 46.30	0.334 [NS]
RBS	184.19 ± 96.81	197.46 ± 113.47	173.23 ± 92.75	0.678 [NS]
HbA1c	7.19 ± 3.06	7.28 ± 3.69	6.45 ± 2.40	0.521 [NS]
W.H.R	0.97 ± 0.11	0.94 ± 0.11	0.93 ± 0.12	0.337 [NS]

Results are presented as mean ± SD, $P < 0.05$ considered significantly different, [S] = Significant, [NS] = non-significant.

Table 5. **The odds ratios of VDR Genotype (rs 1544410) with biomarkers level**

Variables	SNIP	OR (95% CI)	*P*-value
	CC	1[a]	–
HSP-70	CT	0.972(0.901 – 1.048)	0.464 [NS]
	TT	0.988(0.921 – 1.059)	0.732 [NS]
	CC	1[a]	–
VDR	CT	0.998(0.969 – 1.027)	0.875 [NS]
	TT	0.988(0.961 – 1.015)	0.384 [NS]
	CC	1[a]	–
C-Peptide	CT	1.003(0.997 – 1.009)	0.338 [NS]
	TT	0.998(0.990 – 1.006)	0.593 [NS]
	CC	1[a]	–
HbA1c%	CT	1.009 (0.853 – 1.193)	0.917 [NS]
	TT	0.918 (0.778 – 1.084)	0.315 [NS]
	CC	1[a]	–
RBS	CT	1.001 (0.996 – 1.006)	0.618 [NS]
	TT	0.999 (0.994 – 1.004)	0.648 [NS]

Results are presented as numbers and percentage, $P < 0.05$ considered significantly different, [S]; Significant, [NS]; Non significant, OR: Odds Ratio, CI; Confidence Interval, a; reference category

link between VDR polymorphisms and metabolic parameters related to type 2 diabetes.[15] The human VDR gene is found on chromosome 12q13.1 to give a rapid rundown. there are both coding and non-coding exons in the VDR gene, which undergo alternative splicing.[16] Increased secretion from cells, which may be caused by a genetic variation in the VDR gene, is linked to type 1 and type 2 diabetes.[17] Homozygous dominant model analysis was used to verify the role of the critical allele in the VDR gene's connection to type 2 diabetes. There were 40% of the groups were homozygous for CC, 26% for CT, and 33.3% for TT. Analysis using a recessive model also helped shed light on how the minor allele contributed to the link between the VDR gene and type 2 diabetes.

Specifically, the current study found that the A-allele of the VDR gene's rs1544410 polymorphism was linked to higher insulin secretion, as evaluated by disposition index, in women with a history of DM.

VDR belongs to the nuclear receptor family of transcriptional regulators. Including beta cells in the pancreas, it is found ubiquitously and forms a heterodimer with a retinoid X receptor (RXR).[18] Consistent with previous research, this study found that vitamin D may play a role in the prevention and treatment of various health problems.[19] Potentially beneficial effects on insulin secretion and sensitivity have been postulated. Possible processes include modulation of calcium homeostasis and activation of vitamin D receptor (VDR) on pancreatic beta cells and insulin-sensitive organs.[20] The risk of diabetes, metabolic syndrome, insulin secretion, and insulin resistance has been shown to be inversely related to circulating concentrations of vitamin D.[21] Polymorphisms in the VDR gene have been linked to diabetes and insulin resistance in multiple studies, including a meta-analysis and those conducted by Malik et al.[22] There are currently about 25 distinct polymorphisms associated with the VDR locus. These VDR polymorphisms have been linked to type 2 diabetes and altered insulin secretion in a number of studies.[23] Metabolic alterations associated with obesity are known as metabolic syndrome, and they are linked to VDR polymorphisms.[24] According to the results of the current study, those with the rs1544410 genotype of the VDR gene are more likely to develop type 2 diabetes and, by extension, obesity.

In people with vitamin D3 deficiency, the (rs10735810) polymorphism of the VDR gene was discovered to be an additional independent driver of insulin secretion. In addition, the level of VDR mRNA expression has been linked to insulin secretion.[25] The likelihood of cluster disease has been linked to polymorphisms in the vitamin D receptor gene, according to a number of studies.[26] But there's a lack of consistency in findings, which may be because study populations are of different races. It is not yet understood how VDR polymorphisms contribute to the pathogenic landscape of MetS at the molecular level.

Furthermore, linkage disequilibrium (LD) and haplotypes for the four previously indicated VDR SNPs and their relationships with metabolic syndrome in the Thai population have not been documented.[27] Several other researchers agreed that the underlying pathophysiological processes of these correlations are still poorly understood. Because VDR is found in pre-adipocytes, it is plausible that vitamin D has a direct effect on adipocyte development and metabolism.[28]

Conclusion

VDR polymorphisms could have a pivotal role in the presence of T2DM. Vitamin D3 binding protein (VDBP) has gained attention as a new target to generate a drug for the treatment of endocrine nutritional and metabolic disorders like obesity and type 2 diabetes mellitus**.** The logistic analysis of the (rs1544410) SNP of the patients concluded that HSP-70, VDBP, and C-peptide level was no significantly related to the also C-peptide, was shown to be a related risk factor to both CT and CT alleles (1.003, $P > 0.05$) in comparison with CC alleles. Furthermore, HbA1c% level was demonstrated to be related as a risk factor for the CG allele in comparison with CC and GG alleles (1.009, $P < 0.05$).

Conflict of Interest

None.

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