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Genotyping of vitamin D receptor (foki and BSML) gene polymorphism in male patients with coronary artery disease in diabetic verses non diabetic

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Abstract

The present study aims to correlate the Vit.D levels and frequency of the VDR gene polymorphisms FokI (rs2228570), BsmI (rs1544410) in male patients with coronary artery disease with and without Type 2 diabetes. 40 healthy controls, 40 individual experiencing T2DM, 55 patients with CAD and 55 patients with both T2DM and CAD made comprised the 4 groups of participants in this case control study which include totally 190 male individuals. This investigation was conducted during the period from February 2022 to November 2022 at the catheterization unit in cardiologic clinics of Iraqi Center for Heart Disease and specialized center for endocrinology and diabetes, Baghdad Teaching Hospital/ Medical City. Diagnosis depend on physical examination and complete medical history. Two-gene polymorphism were assessed in the current study, 1st one was FOKI in which 3 genotypes were detected in participations. 45.8% of all participant had CC genotype, 33.2% had CT and 21.1% had TT. Regarding each group, in DM group 65% had CC, 30% had CT and 5% had TT, the odd ratio and p-value were 0.89 (0.35-2.26), 0.81; 1.28 (0.48-3.44), 0.61; and 0.64 (0.10-4.11), 0.64, respectively. Regarding the group of patients with CAD only, 32.7% had CC genotype, 40% had CT and 27.3% had TT, patients with CAD had 4.62 (1.23-17.27) times higher than control to have TT genotype, p-value 0.02, respectively. The 2nd polymorphism was BSMI, 3 genotype were detected 28.4% had AA, 36.3% had AG and 35.3% had GG genotype, with statistical significant association between the studied group (CAD group and CAD with DM) with development of GG genotype, p-value <0.001. Regarding DM group 40% had AA, 55% had AG and 5% had GG, DM patients had 3.22 (1.26-8.18) times of getting AG in compare to control, p-value 0.01. Regarding CAD group, 10.9% had AA, 38.2% had AG and 50.9% had GG genotype, patients had 40.44 (5.18-315.4) times higher chance of getting GG in compare to control, p-value 0.0004.

Keywords: Coronary heart disease, Diabetes mellitus, Vitamin D receptor genes

Introduction

The vitamin D receptor (VDR) gene provides instructions for making a protein called vitamin D receptor, which allows the body to respond to vitamin D [1-2]. VDR is an intracellular receptor that belongs to the Steroid/thyroid nuclear receptor family and is found in various tissues, including brain, immune cells, and peripheral blood monocytes [3-4]. The vitamin D receptor protein is consisting of three distinct regions, an N-terminal DNA binding domain containing two zinc fingers that binds to specific sequences of DNA called hormone response elements (HRE), a C-terminal ligand binding domain as well as an extended and unstructured region that connects the two functional domains of this protein together [45-6]. In addition, the genetic variation FokI (rs2228570) VDR SNP, also referred as the start codon polymorphism (SCP), was defined using the FokI (Flavobacterium okeanokoites) restriction enzyme in a restriction fragment length polymorphism test (RFLP) , Also FokI (rs2228570) is located in exon 2 at the 5' portion, and is considered a non-synonymous polymorphism, because the change of C > T (ancestral allele T) [6]. More specifically, a variation of T to C at the start codon of the VDR gene the change from ATG (FokI T) to ACG (FokI C) results in a VDR protein that is three amino acids shorter compared with the full-length VDR, this shorter form may have altered biological activity [7]. Genetic mutations in the VDR associated with increased risk of developing CAD [8]. VDR is consider is a nuclear hormone receptor the function of this nuclear receptor is influenced by the presence of several genetic polymorphisms [1,20]. The VDR gene, it is present on the long arm of chromosome (12q 12-14) it consists of nine exons and eight introns, which contain four polymorphic areas. Several pathways and cell types that are relevant to cardiovascular Physiology and pathology

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are influenced by vitamin D metabolites [9]. VDR gene polymorphisms may potentially affect CVD due to the presence of VDRs in all main cardiovascular cell types Firstly, the VDR gene is found in vascular smooth muscle cells (VSMC), endothelial cells (ECs), cardiomyocytes, platelets, and most immune cells, potentially affecting their growth and proliferation [10]. Otherwise, the most study reported VDR gene polymorphisms that influence T2DM development is VDR (Fok1, Bsm1, Taq1 and Apa1) [11-12]. However, most Genotype have been related with T2DM include ApaI and BsmI which are covered by different studies in different areas and populations [13].

Materials & Methods

Ethical considerations

Ethical approval was received from the scientific committee of the Biochemistry department and College of Medicine, University of Baghdad-Iraq. The Institutional Board of the Specialized Center for Endocrinology and Diabetes & Cardiologic Clinics of Ibn- Al-Bitar Hospital (Bagdad- Iraq) approved the study protocol. All participants volunteered for research and provided written informed consent before enrolment.

Study design

The study statistically considered as [case- control study], the participants were divided as following:

- 40 apparently healthy male were recruited through advertisement among laboratories and hospital staff in the three medical centers previously mentioned and from friends in Baghdad.
- 40 male with T2DM were recruited from the specialized center for endocrinology and diabetes.
- 55 male who had CAD,
- 55 male who have had T2DM with CAD.

Sample collection

2 ml of blood was collected from patients and healthy controls individuals from peripheral vein after 15 minutes of rest and participants were sitting inside a relatively quiet room during blood drawing. Disposable samples were used for blood aspiration under sterile conditions. Patients and healthy individuals had their blood drawn into disposable plastic tubes (EDTA).

Genotype measurements

DNA was extracted from blood using DNA isolation kit (promega). Genotyping was carried out by PCR-restriction fragment length polymorphism (RFLP) for VDR Gene SNPs (rs2228570,1544410), primers and a Hot Green master mix kit (Promega) were used, PCR products were digested with restriction enzymes (Promega) or (Biolabs), the digested products were separated on a 2% agarose gel.

Statistical analysis

Various statistical analyses were applied to analyze the research data, student t-test was used for calculating the probability using (PAST version 3.09, 2004) the statistical analysis software. Two-way ANOVA and Least significant differences (LSD) post hoc test were performed as well as paired t-test. $P<0.05$ was considered statistically significant.

Results and Discussion

Gene polymorphism distribution and odd ratio in the four groups:

Two gene polymorphism were assessed in the current study, 1st one was FOKI in which 3 genotypes were detected in participations. 45.8% of all participant had CC genotype, 33.2% had CT and 21.1% had TT. Regarding each group, in DM group 65% had CC, 30% had CT and 5% had TT, the odd ratio and p-value were 0.89 (0.35-2.26), 0.81; 1.28 (0.48-3.44), 0.61; and 0.64 (0.10-4.11), 0.64, respectively. Regarding the group of patients with CAD only, 32.7% had CC genotype, 40% had CT and 27.3% had TT, patients with CAD had 4.62 (1.23-17.27) times higher than control to have TT genotype, p-value 0.02, respectively. Regarding patients with CAD with DM, 29.1% had CC genotype, 34.5% had CT and 36.4% had TT, patients with CAD with DM had 7.04 (1.92-25.82) Times higher than control to have TT genotype, p-value 0.003, respectively. Regarding control group 67.5% had CC genotype, 25% had CT and 7.5% had TT, a statistical significant association between groups of the study and presence of CC and

CT genotype, p-value <0.001. Regarding allele frequency, C allele was presented in 80%, 52.73%, 46.36% and 80% in DM group, CAD group, CAD with DM group and control group respectively. The hardy weinberg p-value was 0.69, 0.14, 0.02, 0.16 for each group, patients in CAD with DM group didn't follow hardy Weinberg equilibrium. Patients in CAD group had 3.58 (1.84-6.92) times than control of getting T allele, p-value 0.0002. While patients in CAD with DM had 4.62 (2.38-8.98) times of getting T allele, p-value < 0.001, as presented in tables 1, 2 and figure 1.

Table 1. FOKI polymorphism distribution between the groups

FOKI		**Group**				**Total**
		DM	**CAD group**	**CAD with DM**	**control**	
CC	N.	26	18	16	27	87
	%	65.0%	32.7%	29.1%	67.5%	45.8%
CT	N.	12	22	19	10	63
	%	30.0%	40.0%	34.5%	25.0%	33.2%
TT	N.	2	15	20	3	40
	%	5.0%	27.3%	36.4%	7.5%	21.1%
	N.	40	55	55	40	190
	%	100.0%	100.0%	100.0%	100.0%	100.0%
p-value		<0.001*				
C	N.	64	58	51	64	/
	%	80%	52.73%	46.36%	80%	/
T	N.	16	52	59	16	/
	%	20%	47.26%	53.64%	20%	/
p-vale		0.69	0.14	0.02*	0.16	/

*p-value ≤ 0.05

Table 2. Odd ratio of FOKI polymorphism distribution between the groups

FOKI		**Group**			
		DM	**CAD group**	**CAD with DM**	**Control**
CC	Odd, 95%CI	0.89 (0.35-2.26)	0.23 (0.09-0.55)	0.19 (0.08-0.47)	Reference
	p-value	0.81	0.001*	0.003*	
CT	Odd, 95%CI	1.28 (0.48-3.44)	2.00 (0.81-4.90)	1.58 (0.63-3.91)	Reference
	p-value	0.61	0.12	0.32	
TT	Odd, 95%CI	0.64 (0.10-4.11)	4.62 (1.23-17.27)	7.04 (1.92-25.82)	Reference
	p-value	0.64	0.02*	0.003*	
C	Odd, 95%CI	1.0 (0.46-2.17)	0.27 (0.14-0.54)	0.21 (0.11-0.41)	Reference
	p-value	1.0	0.0002*	< 0.001*	
T	Odd, 95%CI	1.0 (0.46-0.2.17)	3.58 (1,84-6.92)	4.62 (2.38-8.98)	Reference
	p-value	1.00	0.0002*	< 0.001*	

*p-value ≤ 0.05

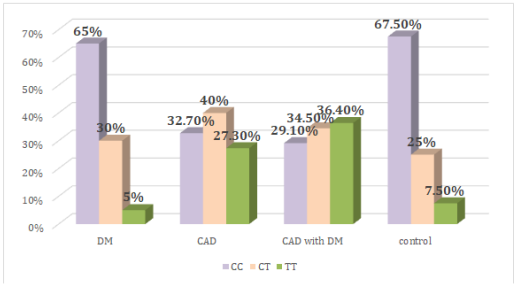

Figure 1. Vitamin D gene (FOKI) polymorphism

The 2nd polymorphism was BSMI, 3 genotype were detected 28.4% had AA, 36.3% had AG and 35.3% had GG genotype, with statistical significant association between the studied group (CAD group and CAD with DM) with development of GG genotype, p-value <0.001. Regarding DM group 40% had AA, 55% had AG and 5% had GG, DM patients had 3.22 (1.26-8.18) times of getting AG in compare to control, p-value 0.01. Regarding CAD group, 10.9% had AA, 38.2% had AG and 50.9% had GG genotype, patients had 40.44 (5.18-315.4) times higher chance of getting GG in compare to control, p-value 0.0004. Patients in CAD with DM group, 7.3% had AA, 27.3% had AG and 65.5% had GG, patients in this group had 37.89 (9.40-580.5) higher chance of getting GG genotype in compare to control, p-value < 0.001. In control group 70% had AA, 27.5% had AG and 2.5% had GG genotype. Regarding allele frequency, A was found in 67.5%, 30%, 20.91% and 84.75% in DM group, CAD group, CAD with DM group and control group respectively. The hardy weinberg p-value was 0.10, 0.50, 0.19 and 0.95 , indicate that all group follow hardy Weinberg equilibrium, patient in CAD group and CAD with DM had 12.05 (5.85-24.71) and 19.49 (9.19-41.31) higher chance of having G allele in compare to control, p-value was < 0.001 and < 0.001, respectively, as presented in table 3, 4 and 2.

Table 3. BSMI polymorphism distribution between the groups

BSMI		Group				Total
		DM	CAD group	CAD with DM	control	
AA	N.	16	6	4	28	54
	%	40.0%	10.9%	7.3%	70.0%	28.4%
AG	N.	22	21	15	11	69
	%	55.0%	38.2%	27.3%	27.5%	36.3%
GG	N.	2	28	36	1	67
	%	5.0%	50.9%	65.5%	2.5%	35.3%
	N.	40	55	55	40	190
	%	100.0%	100.0%	100.0%	100.0%	100.0%
p-value		< 0.01*				
A	N.	54	33	23	67	/
	%	67.5%	30%	20.91%	84.75%	/
G	N.	26	77	87	13	/
	%	32.5%	70%	79.09%	16.25%	/
p-value		0.10	0.50	0.19	0.95	

*p-value ≤ 0.05

Table 4. Odd ratio of BSMI polymorphism distribution between the groups

BSMI		Group			
		DM	CAD group	CAD with DM	Control
AA	Odd, 95%CI	0.28 (0.11-0.72)	0.052 (0.01-0.15)	0.03 (0.009-0.11)	Reference
	p-value	0.008*	< 0.001*	< 0.001*	
AG	Odd, 95%CI	3.22 (1.26-8.18)	1.62 (0.67-3.93)	0.98 (0.39-2.46)	Reference
	p-value	0.01*	0.27	0.98	
GG	Odd, 95%CI	2.05 (0.17-23.5)	40.44 (5.18-315.4)	37.89 (9.40-580.5)	Reference
	p-value	0.56	0.0004*	< 0.001*	
A	Odd, 95%CI	0.38 (0.18-0.82)	0.08 (0.04-0.17)	0.05 (0.02-0.18)	Reference
	p-value	0.01*	< 0.001*	< 0.001*	
G	Odd, 95%CI	2.84 (1.16-5.28)	12.05 (5.85-24.71)	19.49 (9.19-41.31)	Reference
	p-value	0.01*	< 0.001*	< 0.001*	

*p-value ≤ 0.05

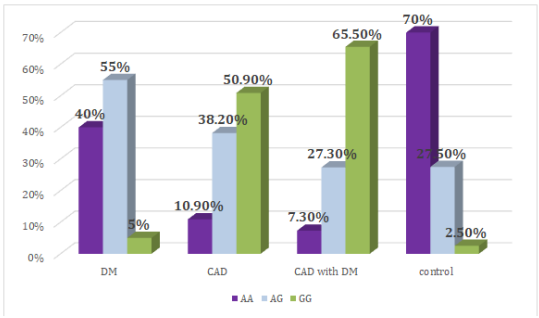

Figure 2. Vitamin D gene (BSMI) polymorphism

In the current study, 2 of vitamin D receptors polymorphism were assessed in the studied groups. The significance of Fok I polymorphism is that It was found in exon 2, which is the start codon translation location at the 5 end of the VDR gene. The shift from T to C in the initiation codon provides a hint that the codon sequence was changed from ATG to ACG. As a result, when the C variation is present, a different start site is utilized, resulting in a protein of a different size. Methionine, glutamic acid, and alanine, three amino acids that have been shown in studies to be less therapeutically beneficial, made up three amino acids longer in variation T than in variant C [14]. The most prevalent genotype for the FOKI polymorphism was CC in 67.5% of the control group and 65% of the DM group, CT in 40.0% of the CAD group, and TT in 36.4% of the CAD with DM group. There was a statistically significant correlation between the CT and TT genotypes and the development of CAD, with a p-value of 0.001. In terms of allele frequency distribution, patients in the CAD with DM group did not adhere to the hardy Weinberg equilibrium. Patients with the TT genotype had increased odds of developing CAD (4.62, CI 95% 1.23-17.27) and CAD with DM (7.04, CI 95% 1.92-25.82) compared to controls. In the CAD group, those who carried the VDR FokI-T allele had a higher chance of developing CAD (3.58, CI 95%, 1.84-6.92) and (4.62, CI 95%, 2.38-8.98). In Rahi et al [15] study, TT genotype was most frequent in control while in CVD group CT was the most frequent consisting with current finding. CAD patients with CT and CC genotype had 2.04 and 7.25 higher risk of development CAD. The findings of this study are consistent with those of the populations of Chinese, Egyptians, and Iranians [16,21]. In another study [17] that analyze the relationship between CAD in T2DM and VDR polymorphism and vitamin D insufficiency, FokI CT and TT genotypes had higher risks of CAD than controls in both T2DM groups, however these risks were not statistically significant (OR=1.56, 2.04, and p=0.18, 0.08, respectively). In contrast to the controls in the Egyptian population, Ewida et al. [12] found that the CT genotype and T allele of the VDR FokI genotype (rs2228570) were more common in CAD patients with and without T2DM. The VDR protein does not alter structurally as a result of BsmI polymorphisms, however, have the potential to alter the VDR gene's expression and raise the risk of getting CVD. According to current research, the VDR BsmI polymorphism is linked to an increased risk of cardiovascular disease (CVD). the study demonstrate that owing G allele carry high risk of developing CAD which was (2.84, CI 95%, 1.16-5.28) in DM, (12.05, CI 95%, 5.85-24.71) in CAD group and (19.49, CI 95%, 9.19-41.31) in CAD with DM in compare control. Numerous investigations in Caucasian populations contradict our findings, including those by Ewida et al. [12] and Nakhl et al. [18] studies, which found no connection between those polymorphisms and CVD. However, the findings of the study conducted by Abouzid et al. [19] suggest that the VDR BsmI-AA genotype may be protective against CVD (p = 0.04). Declaration of Ethics Standard: This research adhered to the Declaration of Helsinki's ethical standards and received ethical approval from the scientific committee of the Biochemistry Department and College of Medicine, University of Baghdad, Iraq (Ref. no: 134). Funding: This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Clinical trial number: This research does not involve clinical trials; therefore, a clinical trial number is not applicable.

Data Availability Declaration: All research data have been included in the submitted manuscript and there is no additional research data.

Competing Interest Declaration: The authors declare that they have no competing interests.

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