Original
ISSN 2521-8492

Vitamin D3 receptor gene polymorphism (rs 2228570) as a predictor for coronary artery diseases

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(*Submitted: 14 November 2017 – Revised version received: 10 December 2017 – Accepted: 27 December 2017 – Published online: 26 June 2018*)

Objective Present study aims to explore the association of vitamin D receptor vitamin D receptor (VDR)-FOKI gene polymorphism (rs2228570) with coronary artery disease (CAD) in Iraqi population.

Methods The present study included 300 samples (male and female) with age range between (40 and 70 years), 150 sample of them were randomly selected as normal healthy persons without any known disease as controls, whereas, another 150 cases of CAD subjects who underwent to the angiography block in the cardiology department of Al-Sadder Medical city in Najaf governorate, Iraq and Al-Hussein Medical City Holy Karbala, Iraq. Genotyping of rs2228570 polymorphism is carried out by PCR-RFLP. DNA was extracted from whole blood and genotyping was achieved with specific primers to amplify fragments. The enzyme *FokI* was used for the digestion of VDR gene product followed by electrophoresis on agarose gel.

Results Genotype frequencies of rs2228570 polymorphism were found to be consistent with Hardy–Weinberg equilibrium. Allele frequencies of wild genotype TT (33.3%), heterozygous genotype TC (46.7%), and homozygous genotype CC (20%) in cases of CAD group while 66.7%, 30%, and 3.3% for TT, TC, and CC genotyping, respectively in the control group. The genotype (CC) was significantly (OR = 7.25, 95% CI; 2.74–19.20, $P < 0.001$ (increased the risk of CAD seven and quarter folds with respect to those of (TT), while the TC genotype significantly (OR = 2.04, 95% CI; 1.27–3.28, $P < 0.001$) raised the risk of CAD by two-folds.

Conclusion Our result improved that the gene polymorphism of VDR-FOKI was associated with high risk for development and progression of CAD in Iraqi population.

Keywords CAD, vitamin D receptor, *FokI*

Introduction

Coronary heart disease (CHD) is the leading cause of death in the world and the second most common cause of death in developing countries.[1] Coronary artery disease (CAD) is considered as a multifactorial disorder due to the interaction of genetic and environmental risk factors.[2] Both environmental and genetic factors (such as single nucleotide polymorphism, SNP) play important roles in the pathogenesis and the occurrence of CHD.[3] In some individuals, genetic factors may contribute to coronary atherosclerosis and thrombotic complications.[4] The vitamin D is involved in a wide range of biological processes including bone metabolism, regulation of cell proliferation, and differentiation and modulation of immune responses in the endocrine system.[5] The role of vitamin D and vitamin D receptor (VDR) in the skeletal metabolism is well known.

Vitamin D induces its activity by binding to VDR that is one of the nuclear receptor superfamily, which binds to their specific ligand. VDR is a high-affinity, low-capacity receptor having a molecular weight ofabout 48–55 kD.
VDR is expressed in the majority of humantissues. VDR is prominently present inenterocytes, osteoblast, parathyroid gland cells, and distalrenal tubules cells, which supports the role of vitamin D incalcium absorption, parathyroid hormone suppression, andbone mineral density enhancement. However, the recent investigation has shown a significant role of VDR in all of the major cardiovascular cell types, includingcardiomyocyte, arterial wall cells, and immune cells. Recent studies characterized VDR polymorphisms into four categories: FokI, BsmI, ApaI, and TaqI and these polymorphisms within the VDR gene may potentially influence the vitamin D expression and the stability of VDR mRNA.[5] FokI is located in exon 2 at the 51st coding region,[6] and the polymorphism that results in functional and structural variation in the VDR protein. Studies have demonstrated that VDRs are present in the aortic endothelial, vascular smooth muscle cells, etc. Recent experimental studies suggest that vitamin D and VDR polymorphisms may impact the development of atherosclerotic disease.[7]

This study was performed to investigate the association of SNP of VDR FokI [rs2228570] with risk for CHD in Iraqi populations.

Materials and Methods

Location and duration of the study

This study was conducted at the laboratories of biochemistry department, College of Medicine, University of Kerbala, Iraq from November 2016 to October 2017.

Samples, age, and experimental design and grouping

The current study group included 300 samples (male and female) with the age range between (40 and 70 years), 150 sample of them were randomly selected as normal healthy without any known disease as controls, whereas another 150 cases of CAD subjects who underwent to the angiography block in the cardiology department of Al-Sadder Medical city in Najaf governorate, Iraq and Al-Hussein Medical City, Holy Karbala, Iraq. Subjects with pregnancy, polycystic

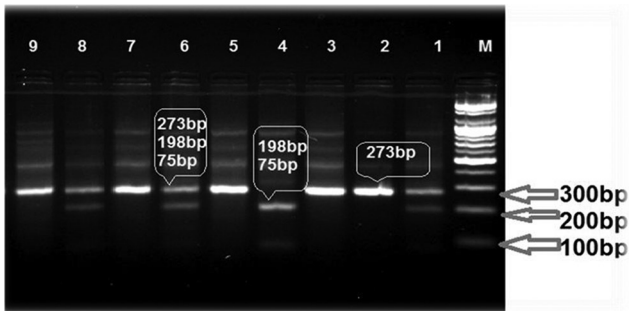

Fig. 1 **TT, TC, and CC genotype bands on gel electrophoresis.**

ovarian disease any known endocrine disorders, and those on vitamin D supplementation, malignancies were excluded. The study was approved by the institutional ethics committee. After taking an informed written consent, whole blood was collected in EDTA tubes for genotyping. The EDTA samples were analyzed by PCR-RFLP method.

Genotyping: Genomic DNA was extracted from peripheral blood using the Relia Prep™ Blood gDNA Miniprep System kit (Promega) as per the manufacturer's protocol. Amplification of the gene of VDR was performed using primers described previously by Mishra et al., 2013.

Forward primer 5'- GAT GCC AGC TGG CCC TGG CAC TG-3'

Reverse primer '5-ATG GAA ACA CCT TGC TTC TTC TCC 3'.

The Reaction was set up with standard PCR reagents in a 25 µL reaction volume containing 5 µL DNA sample, 12.5 µL master mix, 1.5 µL forward primer, 1.5 µL reverse primer, and 4.5 µL nuclease-free water at 25°C then, centrifuged for 30 seconds at 2000 × g in amicrocentrifuge for mixing the sample tubes and then placedin the thermocycler.

The PCR reactions were carried out as follows: 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min; 30 cycles of rotation allowed and final extension at 72°C rotated for 4 min. PCR amplification was confirmed by 2% agarose gel electrophoresis. 100 bp DNA Leader (BioNeer, South Korea) was used to confirm the amplicon size and the gel was visualized in the gel documentation system. FokI genotypes was analyzed by using with 5 unit of Fok-I restriction enzyme and the reaction buffer are incubated at 37°C for 1 h; 10 µL micropipette of the digested reaction mixture was then loaded into 2% agarose gel containing ethidium bromide. The wild genotype (TT) lacked a Fok-I site and showed only one band of 273 bp. The homozygote genotype (CC) generated two fragments of 75 and 198 bp. The heterozygote displayed three fragments of 273, 75 and 198 bp, designated as (TC). Genotype quality and validation were done by performing repeated assay on 20% of the samples selected at random. Samples with known genotypes were included in the reaction to confirm the genotypes.

Statistical analysis

The expected genotype and allele frequencies for the observed variations were calculated for the cases and controls. These frequencies were used to test if the population followed Hardy–Weinberg equilibrium. The interaction between the genotypes was evaluated by calculating the odds ratio and CI. A difference was considered to be statistically significant when *P*-values were <0.05. All the analysis was done using the statistical analysis program IBM SPSS version 24 (SPSS Inc., Chicago, IL).

Results

Table 1 shows the distribution of VDR FokI genotype. Among 150 cases, CC homozygous genotype was observed in 30 cases, TC heterozygous genotyping in 70, and TT wild genotyping in 50 subjects. In controls, among the 150 subjects, 5 were CC, 30 were TC, and 100 were TT genotypes. 95% CI and odds ratio were calculated, which show significant association with CAD. Table 2 shows the allele frequency in cases and controls. The odds ratio is significant about twice for the development of CAD in TC. The genotypes and seven and quarter in CC the genotype compared to TT genotype.

CAD is a common heart disease with global health problems. Different studies conducted in the past decade, vitamin D3 has a strength associated with a low level of vitamin D3 in CAD patients,[8] and vitamin D3 has a positive impact on cardiovascular health.[9] (Li, 2011). The efficiency of vitamin D3 was linked with the nuclear receptor VDR for the whole genome action. VDR gene has various polymorphisms in coding region. They are termed as *FokI,* which are located in starting codon of exon 2 in the restriction site. While BsmI, ApaI, and TaqI are located in exons 8, 9, 10, respectively.[10] The VDR-*FokI* was revealed in the current study due to impact of *FokI* polymorphism on vitamin D3 protein. The *FokI* polymorphism is important because it was located in exon 2, which is the starting codon translation site at 5 end of VDR gene and the change of sequence from T to C in the initiation codon that clues to alteration the codon sequence from ATG to ACG. If this happened ACG converts the first initiation site quite than ATG codon to start the encoding of VDR protein then search the sequence to find next ATG as a result of this defect, VDR that created from polymorphic variant T (427 amino acids) was longer than C variant (424 amino acids).Variant T was longer than variant C by three amino acids which are methionine, glutamic acid, and alanine that had been revealed functionally less effective as described by various studies.[5] .

It is clear from the present study that reducednumber of patients group with wild TT genotype polymor-phism by 33.3% when compared with 66.7% found in healthycontrol group. Also, the present study demonstrated that VDR-*FokI* gene polymorphism was linked with the danger of expanding coronary artery disease. There was elevated risk of CAD in patients who carried out heterozygous alleles

Table 1. **Distribution of VDR Fok-I genotype**

Genotype	Patients no.	%	Control no.	%	OR	CI 95%	*P*-value
(Met 1 Thr) TC	150		150				
TT	50	33.3	100	66.7	0.25	0.15–0.40	<0.001
TC	70	46.7	45	30	2.04	1.27–3.28	<0.001
CC	30	20	5	3.3	7.25	2.74–19.20	<0.001

Table 2. **Fok-I Allele frequency in cases and controls**

Genotype	No.	Allele frequency	HW—observed frequency	percentage %	HWE—expected frequency	percentage %	X2	*P*-value HWE
Cases		T: 0.57						
TT	50	C: 0.43	50	33.3	48.2	32.1	0.37	>0.05
TC	70		70	46.7	73.7	49.1		
CC	30		30	20	28.1	18.8		
Control		T: 0.82						
TT	100	C: 0.18	100	66.7	100.1	66.8	0.0005	>0.05
TC	45		45	30	44.9	29.9		
CC	5		5	3.3	5.0	3.3		

HWE: Hardy–Weinberg equilibrium; $P > 0.05$ is Non-significant.

TC by approximately two times and in homozygous CC alleles genotype by about seven and quarter times when compared with patients who carried out common homozygous allele TT genotype after adjustment for age and BMI. Such observations strongly suggested a role of VDR gene polymorphism (rs2228570) in the pathogenesis of CAD in Iraqi patient. The results of this study are in covenant with the results of one Arab populations Egyptians.[11] They are also an agreement with data of studied populations of Han Chinese (HE, 2015),[9] reported the role of vitamin D3 through VDR-*FokI* rs 2228570 on CAD in Iranian population,[9] but Pan et al. (2009)[12] reported the FokI polymorphism was not found to be associated with CAD incidence in Chinese. Sowjanya et al.[3] reported that there is no significant association of VDR FokI polymorphism with CAD in south Indian population.[13,14]

Conclusion

According to the observed data, we conclude that there is a significant association of *FokI* polymorphism with CAD in Iraqi population. We proved that the carriers of the homozygous genotype (CC) have seven and quarter folds more risk for the development of CAD as compared with (TT) genotyping, while the risk in heterozygous genotype (TC) carriers was two-folds more risk when compared with wild genotype (TT).

Therefore, we conclude that the carriers of the VDR gene polymorphism (rs2228570) of VDR-*FokI* are associated with high risk of the progression of CAD in Iraqi populations.

Conflict of Interest

None.

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