Vitamin D3 receptor gene polymorphism (rs 2228570) as a predictor for coronary artery diseases
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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) raised 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 of about 48–55 kDa. VDR is expressed in the majority of human tissues. VDR is prominently present in enterocytes, osteoblast, parathyroid gland cells, and distalrenal tubules cells, which supports the role of vitamin D in calcium absorption, parathyroid hormone suppression, and bone mineral density enhancement. However, the recent investigation has shown a significant role of VDR in all of the major cardiovascular cell types, including cardiomyocyte, arterial wall cells, and immune cells. Recent studies characterized VDR polymorphisms into four categories: FokI, BsmI, Apal, and TaqI and these polymorphisms within the VDR gene may potentially influence the vitamin D expression and the stability of VDR mRNA.6 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
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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, and vitamin D3 has a positive impact on cardiovascular health. (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, Apal, and TaqI are located in exons 8, 9, 10, respectively. 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.

It is clear from the present study that reduced number of patients group with wild TT genotype polymorphism by 33.3% when compared with 66.7% found in healthy control 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

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients no.</th>
<th>Control no.</th>
<th>%</th>
<th>%</th>
<th>OR 95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Met 1 Thr) TC</td>
<td>150</td>
<td>150</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>50</td>
<td>33.3</td>
<td>100</td>
<td>66.7</td>
<td>0.25</td>
<td>0.15–0.40</td>
</tr>
<tr>
<td>TC</td>
<td>70</td>
<td>46.7</td>
<td>45</td>
<td>30</td>
<td>2.04</td>
<td>1.27–3.28</td>
</tr>
<tr>
<td>CC</td>
<td>30</td>
<td>20</td>
<td>5</td>
<td>3.3</td>
<td>7.25</td>
<td>2.74–19.20</td>
</tr>
</tbody>
</table>
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. They are also an agreement with data of studied populations of Han Chinese (HE, 2015), reported the role of vitamin D3 through VDR-FokI rs 2228570 on CAD in Iranian population, but Pan et al. (2009) reported the FokI polymorphism was not found to be associated with CAD incidence in Chinese. Sowjanya et al. reported that there is no significant association of VDR FokI polymorphism with CAD in south Indian population.

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

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No.</th>
<th>Allele frequency</th>
<th>HW—observed frequency</th>
<th>percentage %</th>
<th>HWE—expected frequency</th>
<th>percentage %</th>
<th>X2</th>
<th>P-value HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>50</td>
<td>C: 0.43</td>
<td>50</td>
<td>33.3</td>
<td>48.2</td>
<td>32.1</td>
<td>0.37</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>TC</td>
<td>70</td>
<td></td>
<td>70</td>
<td>46.7</td>
<td>73.7</td>
<td>49.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>30</td>
<td></td>
<td>30</td>
<td>20</td>
<td>28.1</td>
<td>18.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>100</td>
<td>C: 0.18</td>
<td>100</td>
<td>66.7</td>
<td>100.1</td>
<td>66.8</td>
<td>0.0005</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>TC</td>
<td>45</td>
<td></td>
<td>45</td>
<td>30</td>
<td>44.9</td>
<td>29.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>5</td>
<td></td>
<td>5</td>
<td>3.3</td>
<td>5.0</td>
<td>3.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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.

References

1. WHO. 2012. The 10 leading causes of death by broad income group.