Genotype and allele frequencies of two Vitamin-D receptor gene polymorphisms (ApaI and BsmI) in patients undergoing elective percutaneous coronary intervention in Iranian population

Parisa Sharifi Ardani1, Farzaneh Foroughinia2,*, Mehdi Dianatpour3,4, Iman Jamhiri3

1 Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran.
2 Department of Clinical Pharmacy, School of Pharmacy, Shiraz University of Medical Science, Shiraz, Iran.
3 Stem Cells Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
4 Department of Medical Genetics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.

Abstract

CAD is a major cause of death in worldwide. Both vitamin D (vit D) and Vitamin-D receptor (VDR) gene polymorphisms have been reported to be associated with Coronary artery disease (CAD). Because of high prevalence of vit D deficiency and mortality caused by cardiovascular diseases in our country, Iran, in this study we aimed to determine the frequency of two known VDR gene polymorphisms (BsmI and ApaI) in patients undergoing Percutaneous Coronary Intervention (PCI) in Iranian populations. Blood samples were collected from 150 patients performing elective PCI (102 males and 48 females). VDR genotypes were determined by RFLP method. Serum vit D levels were measured using HPLC method and patients were divided into three groups as follows: subjects with a total vit D concentration 30 ng/ml> were described as normal, 20-30 ng/ml as insufficient and < 20 ng/ml as deficient. Among 150 samples analyzed for ApaI and BsmI polymorphisms the following genotypic frequency was observed: AA 44.67%, AC 44.67%, and CC 10.66% for ApaI and GG 47.33%, GA 37.33%, AA 15.34% for BsmI. Levels of active vitamin D could be influenced by both environmental and genetic factors. Our results also revealed that VDR gene polymorphisms (ApaI and BsmI) may vary across different ethnic groups in CAD patients.

Keywords: Coronary Intervention, Gene polymorphism, Iranian population, Percutaneous, Vitamin D receptor

1. Introduction

Coronary artery disease (CAD) is the most common cause of death in developed countries and the second most common reason of death in developing countries. However, the incidence of risk factors and the morbidity and mortality of CAD have increased in recent decades (1, 2). In our country, Iran, CAD is one of the most common causes of death; and every year about 3.6 million people with cardiovascular diseases are admitted to the hospitals affiliated with the Ministry of Health and Medical Education leading to near 46% of deaths (3, 4).

Vitamin D (vit D) is involved in a wide variety of biological processes such as bone metabolism, cell proliferation, and regulation of immune responses (5, 6). Recent studies have reported the role of vit D deficiency in the pathogenesis of cardiovascular diseases (7). The metabolic consequences of vit D deficiency can cause several damages to the cardiovascular system, including delayed fixation of atherosclerotic plaques that may lead to the enhanced risk of plaque rupture and the
incidence of cardiovascular problems. In addition, activation of the renin-angiotensin-aldosterone system due to vit D deficiency may increase the risk of ventricular failure during acute coronary syndrome (ACS) (8). One study in CAD patients reported that the higher the number of coronary arteries involved, the lower the vit D serum level would be. It is suggested that low levels of vit D have also been linked to structural changes in the heart, including impaired systolic and diastolic function (9).

Few studies have been performed in evaluating the effect of vit D deficiency on clinical outcomes in patients undergoing coronary angiography. A study conducted on 100 Indian patients undergoing coronary angiography reported that about 80% of the under-study population had vit D deficiency (36% were severely vit D deficient defined as vit D levels<10 ng/ml) and only 7% of them were vit D sufficient. Also, the results of this study demonstrated an association between the vit D deficiency and the higher prevalence of double and triple coronary artery involvement (10).

Vit D receptor (VDR) plays an important role in performing vit D effects. VDR gene defined as the steroid hormone family of nuclear receptors that are responsible for the transcriptional regulation of a number of hormone responsive genes. Several polymorphisms have been distinguished in the VDR gene and their functional significance and potential effects on several disease susceptibilities have been estimated (11).

Extensive studies have been performed to identify the gene mutations involved in coronary heart disease, among which the VDR gene has attracted the attention of researchers. VDR gene has been identified as a probable selected gene for cardiovascular diseases (12).

Considering the high prevalence of vit D deficiency and mortality caused by cardiovascular diseases in our country, Iran, in this study we aimed to determine the frequency of two known VDR gene polymorphisms (BsmI and ApaI) in patients undergoing elective percutaneous coronary intervention (PCI) in Iranian population.

### 2. Material and Methods

#### 2.1. Ethics statement

This study was approved by the Ethics Committee of Shiraz University of Medical Sciences (SUMS) with ethical code of IR.SUMS.REC.1398.331. All patients signed written informed consent before participating in the study.

#### 2.2. Subjects

Blood samples were collected from 150 patients referred to two tertiary care centers, Alzahra Heart Hospital and Namazi Hospital affiliated to SUMS, for undergoing elective PCI between September 2018 and March 2019. Patients with inclusion criteria were entered to the study.

Inclusion criteria were as follows: successful elective PCI, age range from 18 to 80 years, patient's willingness to participate in the study and filling out a personal consent form. Exclusion criteria includes: administration of Vit D supplements during the previous month, ages over 80 or under 18 years, patients with life expectancy of less than one year, patient's unwillingness to continue studying and failed PCI.

#### 2.3. Vit D measurement

Patients' blood samples were taken before angioplasty. Serum was separated by centrifuging the blood at 2500 rpm for 10 min. 25-hydroxyvitamin D (25(OH) D) serum level measured by HPLC method. Classification of overall vit D status was as follows: subjects with a total 25(OH) D concentration >30 ng/ml were described as normal, 20-30 ng/ml as insufficient and <20 ng/ml as deficient.

#### 2.4. Genotyping

The genomic DNA was isolated from peripheral blood using QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany). DNA yield and purity was determined by measuring absorbance at 260/280.

VDR gene polymorphisms were detected by Polymerase chain reaction-restriction fragmentlength polymorphism (PCR-RFLP). PCR reactions were performed using the Applied Biosystems™ Veriti™ HID 96-Well Thermal Cycler (Applied Biosystems, Foster City, CA, USA). The primers for Apal and BsmI designed by primer3 and ordered from Metabion Company (Metabion,
Germany). Detailed primer sequences are shown in table 1.

2.5. ApaI polymorphism

The PCR reaction program for ApaI was as follows: initial denaturation for 4 min at 94°C, followed by 35 cycles of 94°C for 50 sec, 66°C for 50 sec, followed by 72°C for 50 sec and a final elongation at 72°C for 7 min. The reaction mixture consisted of 5 µl genomic DNA, 10 µl of PCR Master Mix, 1 µl of each primer (Metabion, Germany) and 3 µl DDW. Following amplification, the translation initiation site of the VDR gene was detected by RFLP (Restriction Fragment Length Polymorphism) using the restriction endonuclease ApaI (Fermentas, USA) at 37°C for 16 hours. Digested restriction fragments were separated on 1.5% (w/v) agarose (Fermentas, USA) gels. Bands were visualized on an UV Trans illuminator imaging system. Depending on the digestion pattern, genotypes were assigned as follows: AA homozygous for the absence of the ApaI site with an undigested 740 bp band; CC homozygous for the presence of the ApaI site with complete digestion into 530 bp and 210 bp bands and AC in case of heterozygosity all three bands (740 bp, 530 bp and 210 bp) were observed (Figure 1).

2.6. BsmI polymorphism

The PCR reaction program for BsmI was as follows: initial denaturation for 4 min at 94°C, followed by 35 cycles of 94°C for 50 sec, 60°C for 50 s, followed by 72°C for 50 sec and a final extension at 72°C for 7 min. The reaction mixture consisted of 5 µl genomic DNA, 10 µl of PCR Master Mix, 1 µl of each primer (Metabion, Germany) and 3 µl DDW. Following amplification, the translation initiation site of the VDR gene was detected by RFLP (Restriction Fragment Length Polymorphism) using the restriction endonuclease BsmI (Fermentas, USA) at 37°C for 16 hours. Digested restriction fragments were separated on 1.5% (w/v) agarose (Fermentas, USA) gels. Bands were visualized on an UV Trans illuminator imaging system. Depending on the digestion pattern, genotypes were assigned as follows: GG homozygous for the absence of the BsmI site with an undigested 825 bp band; AA homozygous for the presence of the BsmI site with complete digestion into 650 bp and 175 bp bands and GA in case of heterozygosity all three bands (825 bp, 650 bp and 175 bp) were observed (Figure 2).

2.7. Statistical analyses

The results of this study were analyzed by SPSS software version 21 (SPSS Inc, Chicago, USA). Categorical variables were described with absolute and relative (percentage) frequencies. Differences in proportions were tested by Pearson chi-square when assumptions were met; if not, the

Table 1. Forward (F) and Reverse (R) primers.

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Primers</th>
<th>Product length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApaI (rs7975232)</td>
<td>(F) 5’-CAGAGCATGGACAGGGAGCAA-3’ (R) 5’- GCAACTCCTCATGGCTAGGTTC-3’</td>
<td>745</td>
</tr>
<tr>
<td>BsmI (rs1544410)</td>
<td>(F) 5’-CAACCAAGACTACAAGTACCGGTCAGTG-3’ (R) 5’- AACCCGAGGGAAGAGGTCAAGG-3’</td>
<td>825</td>
</tr>
</tbody>
</table>

Figure 1. Restriction Endonuclease digestion for ApaI polymorphism.

Figure 2. Restriction Endonuclease digestion for BsmI polymorphism.
Fisher’s exact test was used. Allele frequency was measured as the number of occurrences of the test allele in the study cases divided by the total number of alleles. A P value < 0.05 is considered a significant level.

### 3. Results

Among 150 samples analyzed for ApaI and BsmI polymorphisms, the following genotypic frequency was observed AA 44.67%, AC 44.67%, and CC 10.66% for ApaI and GG 47.33%, GA 37.33%, AA 15.34% for BsmI (Table 2). According to our results, polymorphisms of ApaI AA, ApaI AC and BsmI GG are the most frequent ones among our patients doing PCI.

The genotypic distribution of ApaI and BsmI were also estimated based on gender, showed higher frequency for ApaI AC and BsmI GG in males and ApaI AA and BsmI GG in females; however, a significant difference was not observed (Table 3).

Out of 150 patients, 105 (70%) were Vit D deficient, 34 (22.7%) Vit D insufficient and 11 (7.35) Vit D sufficient. No significant relationship was observed between serum levels of Vit D with ApaI and BsmI polymorphisms (P > 0.05) (Table 4).

### 4. Discussion

VDR is a transcription factor that processes its actions via its own ligand action, which is often 1, 25-dihydroxy vit D, in variant tissues (13). Recently, Vit D and VDR gene mutations have got attraction of many researchers in the field of coronary heart diseases (12-14). More than 30 single nucleotide polymorphisms have been discovered for the VDR gene, among which FokI (rs2228570), Apal (rs7975232), BsmI (rs1544410), and TaqI (rs731236) have been of more interest to research-
Genotype and allele frequencies of two VDR gene polymorphisms (ApaI and BsmI)

Genotype and allele frequencies of two VDR gene polymorphisms (ApaI and BsmI) because of their potential effects on predicting a variety of physiological and pathophysiological phenotypes, including cardiovascular diseases, cancer, and diabetes (15, 16).

VDR genotypes and alleles may differ among populations according to the ethnicity. In addition, VDR gene polymorphisms could affect the incidence and severity of some diseases such as CAD. As a result, studies focusing in determination of genotypes and allele frequency of VDR gene polymorphisms in patients and then comparison of it with other different populations may be of special value. Therefore, this study aimed to specify the frequency of two known VDR gene polymorphisms (Bsml and ApaI) in patients undergoing elective PCI in Iranian population. The ApaI AA, ApaI AC, and Bsml GG genotypes were the most frequent ones among the studied population.

In one study, VDR gene (Fok-I, Taq-I, and Apa-I) polymorphisms was studied for frequency determination in healthy individuals from North Indian population. Reported distributions were 44%, 49%, and 7% for FokI genotype (FF, Ff and ff, respectively), 49%, 40%, and 11% for TaqI genotypes (TT, Tt, and tt, respectively), and 36%, 44%, and 20% for ApaI genotypes (AA, Aa and aa, respectively) (17). In view of ApaI genotypes, results of this study were relatively similar to the result of Iranian population studied in our research. Our report on ApaI polymorphisms were also consistent with the results of Jordan (18) and Syrian populations (19).

One research performed on healthy participants and patients with heart failure in Chinese population, reported similar genotype and allele distribution for ApaI and Bsml polymorphisms as our results (more frequency for genotypes AA and Aa for ApaI and BB for Bsml) (20). In contrast, there are studies revealed differences between the Asian and the Caucasian populations in terms of VDR gene distribution. For instance, Bsml B and TaqI t are less common in Asian people (21, 22) while Bsml BB genotypes occurs more frequently in Caucasian populations (23).

The Correlation of ApaI and Bsml genotypes with the vit D levels was not statistically significant in our study ($P<0.05$). The overall frequency of vit D deficiency in our patients were reported to be about 93% that is more prevalent compared to the general population in Iran. In one meta-analysis included 48 studies, the prevalence of vitamin D deficiency among Iranian male and female was estimated to be 45.64% and 61.90%, respectively (24). High prevalence of vit D deficiency in our research is similar to the reports of studies assessed vit D status in CAD patients (18). Vit D deficiency in these patients may come from several reasons including wrong diet programs, non-healthy life style, and more prevalence of habits such as smoking in these categories. Therefore, it is suggested to educate these patients for correction of their life style and also to treat vit D deficient ones with vit D supplements.

5. Conclusion

This study, as previous researches, showed the influence of both environmental and genetic factors on the levels of active vitamin D in humans. It also revealed that VDR gene polymorphisms (ApaI and Bsml) may vary across different ethnic groups and also different diseases.

Acknowledgments

This research, extracted from a thesis written by Parisa Sharifi, was financially supported by Shiraz University of Medical Sciences (grant number: 16465).

Conflict of Interest

None declared.

References


3. Foroughinia F, Salamzadeh J, Namazi MH. Protection from procedural myocardial injury...
20. Hao Y, Chen Y. Vitamin D levels and vita-


Parisa Sharifi Ardani et al.