ABSTRACT

Background and Objective: Diabetes mellitus is the most common risk factor for coronary artery disease (CAD). Cholesteryl ester transfer protein (CETP) TaqIB polymorphism is associated with changes in lipid profile and may be a risk factor for CAD in patients with diabetes. This study aimed to evaluate the association of CETP TaqIB polymorphism with CAD in patients with type 2 diabetes.

Methods: In this case-control study, 292 diabetic patients were divided into two groups based on angiography reports (150 participants with normal angiogram as the control group and 142 participants with more than 50% stenosis of at least one coronary artery as the case group). The CETP TaqIB genotypes were determined by PCR-RFLP analysis. Fasting blood glucose was measured using glucose oxidase and lipid profile (triglycerides, total cholesterol, high density lipoprotein-cholesterol and low density lipoprotein-cholesterol) by an enzymatic method.

Results: There was no significant difference in the frequency of genotypes and alleles between case and control group (control group: B1B1, 17.3%; B1B2, 63.3%; and B2B2, 19.3%; case group: B1B1, 18.3%; B1B2, 64.1%; and B2B2, 17.6%) (P=0.92). In the control group, subjects with heterozygous genotype (B1B2 genotype) had higher levels of cholesterol compared with the other genotypes (B1B1 and B2B2 genotypes). Also, in the case group, subjects with B1B2 genotype had significantly higher weight (P=0.013).

Conclusion: There is no significant correlation between CETP TaqIB polymorphism and the increased risk of coronary artery disease in patients with type 2 diabetes.

Keywords: Cholesterol Ester Transfer Protein; Polymorphism; Type 2 Diabetes Mellitus, Coronary Artery Disease.
INTRODUCTION
The risk of coronary artery disease (CAD) is very high in patients with type 2 diabetes mellitus (T2DM) (1). This is partly due to lipoprotein abnormalities associated with insulin resistance, such as increased levels of very low density lipoprotein (VLDL), triglycerides (TGs), and decreased high density lipoprotein (HDL) levels (1, 2). Studies have shown an inverse correlation between plasma HDL-cholesterol (HDL-C) levels and the risk of CAD (3, 4). In general, the cardioprotective role of HDL-C is by its performance in the reverse cholesterol transport (CT), whereby the excessive cholesterol is transferred to liver for metabolism and secretion into the bile, and ultimately preventing atherosclerosis (5-8). The cholesteryl ester transfer protein (CETP) is a glycoprotein with 476 amino acids and a molecular weight of 74 kDa facilitating the transfer of cholesteryl ester and TGs between anti-atherogenic HDL, pro-atherogenic LDL and VLDL particles (5, 9-11). CETP (Gene ID: 1071) is a 25 kb gene located on chromosome 16q12, adjacent to the lecithin cholesterol acyl transferase gene (16q12-16q21) (12, 13). CETP gene is highly polymorphic and contains several single nucleotide polymorphisms in its coding and non-coding regions (14-17). TaqIB polymorphism (rs708272) is one of the most common mutations in the CETP gene involving the substitution of adenine nucleotide for guanine at nucleotide 277 of intron 1. The allele containing the TaqI endonuclease site is called B1, and the allele without the restriction site is called B2. TaqIB polymorphism is associated with the activity of CETP and the HDL levels, particularly the rare allele B2 that is associated with increased HDL-C levels and decreased CETP activity and may have an anti-atherogenic role in human (18-22). However, such associations are not clearly reported and it seems that metabolic and environmental factors are involved in these changes (16,20,23). A meta-analysis study showed a significant difference between the homozygous B1B1 and B2B2 in HDL-C with an approximately 0.12 mmol/l increased HDL-C concentrations in individuals with the B2 allele compared to those with the B1 allele (24). The results of studies on the relationship of TaqIB polymorphisms have been inconsistent with coronary heart disease (25-27). The findings of a meta-analysis demonstrated an increased incidence of cardiovascular disease in spite of the increase of HDL-C levels in homozygous B2B2 (28). There are contradictory results on the relationship between different genotypes of TaqIB polymorphism and HDL-C levels in diabetic patients. Some studies have reported no relationship between the TaqIB polymorphism and HDL-C levels in T2DM patients (29, 30) while Kawasaki observed conversely. Several factors are involved in this relationship including gender, smoking and body mass index (BMI) (31, 32). Considering the key role of CETP in the metabolism of lipoproteins, different polymorphisms in the CETP gene may affect blood lipid parameters. TaqIB polymorphism is highly associated with CETP level and HDL metabolism. Also, there is an inverse relationship between HDL-C level and CAD. Since there is no enough data about the association of TaqIB polymorphism and CAD in diabetic patients in Iranian population, this study aimed to examine the association between CETP TaqIB polymorphism and the risk of CAD in subjects with T2DM in Khuzestan Province, Iran.

MATERIAL AND METHODS
In this case-control study, 292 T2DM patients referred to Imam Khomeini Hospital in Ahvaz underwent angiography. A total of 150 participants without coronary arteries stenosis were selected as the control group and 142 ones with more than 50% blockage of one of the coronary arteries as the case group. Inclusion and exclusion criteria for the participants and the criteria for determining the stenosis of the coronary arteries have already been published in another article (33). After fasting for 12 hours, 5 ml blood sample was obtained in non-EDTA tubes from each person for plasma preparation and 2 ml was taken in EDTA tube for DNA extraction. To prepare the serum, blood samples were centrifuged for 10 minutes at 2000 g. Biochemical parameters including TGs, total cholesterol, HDL-C, and LDL-C were measured using commercial kits (Pars Azmoon Company, Iran) and an enzymatic method. HDL-C levels were measured using sedimentation technique and the concentration of blood glucose by glucose oxidase method using Pars Azmoon Company Kits , Iran. The fasting blood glucose of ≥126 mg/dl was considered as the indicator of
diabetes. Genomic DNA was extracted from leukocytes using kits (Cinnagen Company) and then stored at -20 °C. A 528 bp fragment was amplified by PCR method using the following primers:

TaqIB Forward: 5’ -CAC TAG CCC AGA GAG AGG AGT GCC- 3’
TaqIB Reverse: 5’- CTG AGC CCA GCC GCA CAC TAA C- 3’

PCR-based genotyping of the gene segments was performed through restriction fragment length polymorphism method. The materials used in each PCR process included: 1.5 mM of MgCl₂, 0.2 mM dNTP mix, 0.025 U/µl Taq polymerase, 2 µl of PCR Buffer 10 X, 0.2 µM of both forward and reverse primers, and 100 ng of DNA in a total volume of 25 µl. Temperature program for the replication of fragment containing the TaqIB polymorphism was as follows: Initial denaturation at 95 °C for 5 minutes, 30 cycles of denaturation at 95 °C for 30 seconds, annealing at 65 °C for 30 seconds and extension at 72 °C for 30 seconds, and final extension at 72 °C for 5 minutes. After the PCR process and ensuring the production of the desired products, 10 microliters of the PCR product was mixed with 1 unit of TaqI enzyme (Fermentas) and 2 ml of buffer provided within the kit. Sterile water was added to the mixture until a volume of 31 µl was reached. Then 10 µl mineral oil was added to prevent evaporation of the materials and the mixture was kept at 65 °C for 16 hours for digestion. The product of enzymatic digestion was electrophoresed for 45 minutes at 90 voltson 2% agarose gel to observe 174 bp and 354 bp fragments following stained with ethidium bromide. The data were analyzed using SPSS 20 (SPSS Inc. Chicago). Independent t-test was used to compare the mean of quantitative variables between groups and the chi-square to compare the frequency of alleles and genotypes between groups. The mean of lipid profile was compared among different genotypes using ANOVA. The effect of different genotypes on the risk of CAD was estimated using logistic regression and odds ratio (OR) with 95% confidence interval (CI). The quantitative data were stated as mean ± standard deviation and the qualitative data were expressed as percentages. In all statistical analyses, P-value of <0.05 was considered as statistically significant.

RESULTS

By comparing the clinical and biochemical characteristics, a significant difference was observed between the case and control in terms of age. By examining the anthropometric characteristics (weight, height and BMI), a significant difference was observed between two groups in weight and BMI. The mean blood glucose level was significantly higher in the case group compared to that of controls (P=0.014). In the lipid profiles, the levels of total cholesterol and TG were higher in case than in control while the HDL-C level was higher in control in comparison with the case (Table 1).

Table1- Comparison of the mean anthropometric and biochemical characteristics between two groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Case group (n=142)</th>
<th>Control group (n=150)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54.5±5.9</td>
<td>49.6±6.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.4±8.2</td>
<td>163.7±8.7</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.9±11.1</td>
<td>70.8±10.7</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.2±4.6</td>
<td>25.8±4.8</td>
<td>0.013</td>
</tr>
<tr>
<td>Blood glucose (dL/mg)</td>
<td>152.8±45.5</td>
<td>139.3±39.3</td>
<td>0.014</td>
</tr>
<tr>
<td>Total cholesterol (dL/mg)</td>
<td>183.9±49.1</td>
<td>172.9±24.3</td>
<td>0.041</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>39.4±10.6</td>
<td>46.2±10.8</td>
<td>0.001</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>113.1±43.3</td>
<td>107.5±34.7</td>
<td>NS</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>168.9±71.4</td>
<td>150.1±53.9</td>
<td>0.038</td>
</tr>
</tbody>
</table>

Table2- Distribution of genotype and allele frequency in two groups and the risk of CAD in the studied subjects

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Case group (n=142)</th>
<th>Control group (n=150)</th>
<th>χ²</th>
<th>P-value</th>
<th>OR(95%CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1B1</td>
<td>26(18.3%)</td>
<td>26(17.3%)</td>
<td>0.163</td>
<td>0.92</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>B1B2</td>
<td>91 (64.1%)</td>
<td>95 (63.3%)</td>
<td>0.014</td>
<td>0.9</td>
<td>1.00</td>
<td>0.9</td>
</tr>
<tr>
<td>B2B2</td>
<td>25 (17.6%)</td>
<td>29 (19.3%)</td>
<td>1.160</td>
<td>0.54-2.48</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>59.2%</td>
<td>58.7%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td>40.8%</td>
<td>41.3%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The logistic regression was used to estimate the odds ratio (OR) for CAD in the studied subjects.
After amplification of the desired fragment in intron 1 of the CEPT gene, the fragments were cut using the TaqIB restriction enzyme. The resulting Pattern were: a single-stranded fragment with a length of 528 bp representing the individuals with genotype B2B2, triple-stranded fragments with lengths of 174 bp, 354 bp and 528 bp indicating the heterozygous individuals with genotype B1B2, and double-stranded fragments with lengths of 174 bp and 354 bp pointing out the individuals with genotype B1B1. The frequency of genotype B1B1, B1B2 and B2B2 among the case group was 18.3%, 64.1% and 17.6%, respectively. However, these frequencies among the control group were 17.3%, 63.3% and 19.3% (p=0.92). The frequency of alleles B1 and B2 in the group of patients was 59.2% and 40.8%, respectively. While the frequency of alleles B1 and B2 among the controls was 58.7% and 41.1%, respectively (P=0.9). No statistically significant difference was observed in the frequency of genotypes and alleles between the two groups. Also, no significant correlation was found between different genotypes and alleles and increased risk of CAD in the subjects. The odds ratios of B1B2 and B2B2 genotypes relative to the B1B1 reference genotype are shown in Table 2.

## DISCUSSION

The results showed no significant difference in the distribution of genotypes and allele frequencies of TaqIB polymorphism between the case and control groups. Also, there was no association between different genotypes or alleles and the increased risk of CAD in the subjects. The weight and BMI were significantly higher in the case group than the controls. The total cholesterol in diabetic patients with genotype B1B2 was greater in the case group compared to patients with homozygous genotypes (P=0.002). The patients in the case group with genotype B1B2 had higher weight. These results are consistent with Hsieh (2007) study on 365 Taiwanese T2DM patients (101 subjects with CAD and

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Table 3: The mean of biochemical characteristics of two groups according to the genotypes of TaqIB polymorphism

<table>
<thead>
<tr>
<th>variables</th>
<th>Case group</th>
<th>P-value</th>
<th>Control group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>53.5±5.5</td>
<td>54.2±6.6</td>
<td>55.8±4.5</td>
<td>NS</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>162.7±8.7</td>
<td>165.2±7.7</td>
<td>167.3±9.2</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69.3±10.4</td>
<td>76.4±11.3</td>
<td>72.7±9.5</td>
<td>0.013</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.1±3.2</td>
<td>27.7±5.3</td>
<td>26.0±2.9</td>
<td>NS</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>179.5±41.5</td>
<td>184.5±54.1</td>
<td>187.7±47.4</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>37.2±9.0</td>
<td>40.0±11.5</td>
<td>41.3±9.9</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>113.4±38.2</td>
<td>113.3±49.5</td>
<td>111.6±34.6</td>
<td>NS</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>154.2±71.7</td>
<td>170.0±70.8</td>
<td>189.1677.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are expressed as mean ±S.D; BMI = Body mass index; TC = Total cholesterol; HDL-C = High density lipoprotein-cholesterol; LDL= Low density lipoprotein-cholesterol; TG = Triglyceride
264 without CAD) indicating similar BMI, duration of diabetes, TG, HDL-C, and LDL-C levels in the two tested groups. In the mentioned study, the patients in the case group were significantly older than the control ones, and the serum cholesterol in the case group was higher than that of controls, which are consistent with our study. The frequency of genotype B1B1 and allele B1 in the case group was significantly higher than that in control group. The HDL-C level in patients with allele B1 (B1B1 and B1B2) was lower than those without this allele (B2B2) (34). Some population-based studies have examined the relationship between TaqIB polymorphism and the risk of CAD in diabetic patients, but the reported results have been inconsistent (19, 25, 29). For example, two previous studies reported a relationship between TaqIB polymorphism and macrovascular complications of diabetes (29, 35), while according to Chaaba (36), there is no correlation between this polymorphism and CAD in type 2 diabetic patients, which is in line with our study. It is still unknown how CETP isoforms could lead to the development of CAD in diabetic individuals. CETP may lead to redistribution of cholesterol by reducing the extraction of cholesterol ester from atherosclerotic lesions, and ultimately result in decreased HDL function. CETP also regulates one of the steps of the reverse CT and modifies the HDL-C concentration (37, 38), and thus it may change the susceptibility to CAD. According to Rahimi’s study (39-42) on the Iranian population, the allele B1, independent of the HDL-C level, was associated with increased risk of CAD and T2DM. In some studies, the genotype B2B2 was related to the increased level of HDL-C (25, 43) and decreased risk of atherosclerosis and CAD (44). TaqIB polymorphism cannot independently change the HDL-C level, and since it is a silent mutation, it cannot directly affect the transcriptional regulation of the CETP and its sequence. A slight increase in the HDL-C level may be due to its relationship with other functional polymorphisms in the promoter region of the gene, which may reduce the CETP activity and consequently increases the HDL-C level. QI YU (2012) in a meta-analysis including 10 case-control studies, assessed the relationship between the TaqIB polymorphism of CETP gene and CAD in the Chinese population. A significant correlation was found between this polymorphism and CAD. People with genotype B1B1 were at higher risk of CAD compared to other genotypes. The allele B2 was associated with a higher level of HDL and decreased activity of CETP. The allele B1 was also subtly associated with increased risk of CAD (45). CAD is a polygenic disease caused not only by the environmental and genetic factors but also through the complex interaction between these two factors (46). In a prospective study conducted on 8141 Caucasian people, the allele B2 of the CETP TaqIB polymorphism was not associated with decreased risk of CAD despite its effect on increased HDL-C level (47). The effect of the TaqIB polymorphism on the blood lipid metabolism and CAD phenotype varies in different studies, the reason for which may be as follows: 1. The plasma HDL-C level cannot really reflect the process of reverse CT. 2. A non-linear relationship may exist between the genotype and phenotype of CAD. 3. The CETP TaqIB gene may be a micro-effect gene for CAD or not be a master gene for the development of CAD. Most of differences and similarities between this study and previous studies may be due to differences in gene pool, migration, mutation, population, ethnicity and number of the participants studied, as well as other factors.

CONCLUSION
The results of this study indicate that there is no significant correlation between the CETP TaqIB polymorphism and increased risk of CAD in type 2 diabetic patients.

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CONFLICT OF INTEREST
There are no conflicts of interest.
REFERENCES


