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APOLIPOPROTEIN A5-1131T>C (RS662799) GENE POLYMORPHISM AS A PREDICTOR FOR CORONARY ARTERY DISEASES

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ABSTRACT

Background: Coronary artery disease (CAD) develops when the major blood vessels that supply the heart with blood, oxygen and nutrients (coronary arteries) become damaged. Cholesterol-containing deposits (plaque) in arteries and inflammation are usually to blame for coronary artery disease. The most common type of coronary artery diseases includes: stable and unstable angina and myocardial infarction, a chronic condition that narrows arteries by building fat-filled bulges in the arterial walls. Apolipoprotein A-5 is a protein that in humans is encoded by the *APOA5* gene on chromosome 11. It is significantly expressed in liver. The protein encoded by this gene is an apolipoprotein and an important determinant of plasma triglyceride levels, a major risk factor for coronary artery disease. Considering its association with lipoprotein levels, APOA5 is implicated in metabolic syndrome. The *APOA5* gene also contains one of 27 SNPs associated with increased risk of coronary artery disease. **Aim:** To assess the association between the Apolipoprotein A5 1131T>C (rs662799) gene polymorphism with the coronary artery diseases of Iraqi population. **Materials and Methods:** A case-control study was performed at which 100 patients with coronary artery diseases and another 100 healthy individuals were involved. Genotyping of apolipoprotein A5 gene 1131T>C (rs662799) was performed by polymerase chain reaction- Amplification Refractory Mutation system (PCR-ARMS) method. **Results:** The genotype and allele frequencies of APO-A5 gene polymorphism in CAD and control persons were examined under the co-dominant, dominant and recessive models with the use of multinominal logistic regression analysis. Genotype frequencies of rs662799 were consistent with Hardy-Weinberg equilibrium in both CAD and control. The power of this study to detect a significant difference at level of 0.05 was 91.2%. The results shown that APO-A5 1131T>C gene polymorphism (rs662799) (homozygous CC and heterozygous TC genotype) was significantly associated with CAD patients and the frequency of C allele was higher in CAD patients. **Conclusion:** The 1131T>C (rs662799) SNP of APOA5 gene was significantly associated with coronary artery diseases.

KEYWORDS: Coronary artery diseases (CAD), Apolipoprotein A5, SNP single nucleotide polymorphism 1131T>C.

INTRODUCTION

Coronary artery diseases (CAD) was the first leading cause of death worldwide,[1,2] and responsible for about one-third or more of all deaths in people order over age 35.[3] In 2015, CAD affected 110 million people and caused 8.9 million deaths.[4] Coronary Artery diseases is a chronic condition that narrows arteries by building fat-filled bulges in the arterial walls that develops slowly overtime that includes: angina, myocardial infarction, and sudden cardiac death.[5] Estrogen hormones play a cardio-protective role in women so they have a lower risk and incidence of CAD compared to age-matched men.[6]

There are many risk factor for (CAD) some can modified like diabetes mellitus, high blood pressure, lipoprotein and obesity, some cannot be modified like gender, age and some can use as protective factors like triglyceride.[7,8] On the genetic side there are many genes that associated with CAD risk, apolipoprotein A5 is one of these genes.[9]

The gene for apolipoprotein A5 (APOA5 or APO A-V) was originally found by comparative sequencing of ~200 kbp of human and mice DNA as a last member of the gene cluster of apolipoproteins APOA1/APOC3/ APOA4/APOA5, located on human chromosome 11 at position 11q23.[10] Overall, APOA5 is predicted to have approximately 60% a-helical content. The mature APOA5 protein spans a length of 366 amino acid residues, of which 23 amino acids code for the signal peptide. The molecular mass of the precursor was

calculated to be 41 kDa, while the mature APOA5 protein was calculated to be 39 kDa.

This mature apoA5 (343 amino acids), is composed of 4 exons (start codon is localized within the second exon) and 3 introns and codes for the 366 amino acid protein is a highly hydrophobic protein that possesses considerable a-helix secondary structure and is largely insoluble as a lipid-free protein in aqueous solution.[11,12] Apolipoprotein A5 polymorphisms have long been reported to be associated with cardiovascular disease and plasma lipid levels,[13-15] Several lines of evidence indicate that increased plasma triglyceride (TG) levels are associated with CAD,[16] Minor alleles (C1131) is primarily associated with the elevation of plasma triglyceride levels.[17] Furthermore, mutations in the APOA5 gene leading to apoA5 deficiency are related to severe hypertriglyceridemia in humans.[15,18]

MATERIALS AND METHODS

A case-control study include 200 subjects 100 CAD (the criteria for CAD were a ≥70% organic stenosis of at least one segment of a major coronary artery or their main branches confirmed by coronary angiography) and another 100 control was conducted to study the association of 1131T>C SNP in APOA5 gene with coronary artery disease . The patient population included (60 men and 40 women) with coronary artery disease who attended the cardiology center in Kerbala governorate from Dec., 2017 to March, 2018.The Inclusion criteria were:(1) Those patients who were diagnosed by physicians as having (CAD);(2) Age of subjects was >40 years old. The exclusion criteria were:(1) patient with liver disease;(2) patient with renal dysfunction (3) patients with diabetes mellitus or abnormal glucose tolerance test. The control group included 100 apparently healthy subjects (50 men and 50 women) randomly selected from the general population The inclusion criteria were: (1) No past medical history of CAD ;(2) No family history of CAD ; (3) Matched to patients with regard to age, sex, and geographical distribution , (4) BMI < 30 kg/m2 and more than 18.5 kg/m2. All cases completes a detailed questionnaire that included information about age, sex, family history ,drug history , medical history and other relevant information, for all subject weight, height, BMI were measured . Informed consent has been taken from all subject. Karbala Medical College Ethical Committee has approved the study protocol.

Peripheral blood samples of (CAD) and control group were collected in EDTA-anticoagulant tube ,and DNA was extracted from whole-blood samples using the Reliaprep genomic DNA extraction Kit (Promega,U.S.A).Then DNA concentration and purity were measured by UV absorption at 260 and 280 nm (Bio Drop,U.K.). Genotyping for SNP 1131T>C (rs662799) in the Apolipoprotein A5 gene was performed by the polymerase chain reaction-Amplification Refractory Mutation System (PCR-ARMS) method. for APOA5 gene using thermocycler (Biometra, Germany). The primer sequence of ApoA5 gene was used according to Ward et al.[19]

Outer Forward ApoA5 5'-CAAGGTGACAGACAACTGGTGCAATGAT-3',
Outer Reverse ApoA5 5'-AGCCCCTGAAAGCTTCACTACAGGTTCC-3',
Inner Forward ApoA5 5'-TTCAGCTTTTCCTCATGGGGCAAATATC-3', and
Inner Reverse ApoA5 5'-GAGCCCCAGGAACTGGAGCGAAATTA-3'.

Amplification was performed in a total volume of 25 µl which contained 12.5 µl of Go *Taq* Green Master Mix, (Promega Corporation, Madison, WI), 1µl of each primer (One Alpha, U.S.A.), 3.5 µl of nuclease free water, and 5 µl of DNA template. The PCR program for ApoA5 gene show in Table (1).

Table (1): PCR Program for ApoA5 gene.[18]

Type of Cycle	Temperature (C)	Time	No. of cycles
Initial Denaturation	95 C°	2 min	1
Denaturation	95 C°	1min	35
Annealing	59 C°	1 min	
Extension	72 C°	1 min	
Final Extension	72 C°	2 min	1
Total time:1 hour & 45 minutes			

Statistical analysis

Mean and standard deviation (M ± SD) are described. Student T test and ANOVA test were used to compare phenotypic data between control and CAD groups using SPSS windows software (SPSS Inc., Chicago, IL). Genotype frequencies were tested for Hardy-Weinberg equilibrium by X2 test using online software web-Assotest. Genetic power was calculated using the online software OSSE Genotype and allele frequencies in CAD and control group were tested by multino-mial logistic regression analysis with and without adjustment for age, sex and (BMI) using SPSS.

RESULTS

The patients included (60 male and 40 female), ages with mean ± SD (58.59 ± 5.46) and BMI (27 ± 4). The control group (50 male and 50 female) ages with mean ± SD (51.16 ± 4.7) and BMI (23.7 ± 3.61). Results of APOA5 gene rs662799 included 404 and 250 bp band for wild type (TT) genotype, for the heterozygous genotype (TC) three bands 404, 250 and 242 bp and for homozygous genotype (CC) two bands 404 and 242 bp. Genotype and allele frequencies of APOA5 gene are shown in (Table 2). Genotype frequencies of rs662799 were consistent with Hardy-Weinberg equilibrium in both CAD and Control. The power of this study to detect a significant difference at level of 0.05 was 91.2%. The results shown

that APOA5 gene polymorphism rs662799 (homozygous CC and heterozygous TC genotype) was significantly associated with CAD patients and the frequency of C allele was higher in CAD patients.

Table (2): Genotype and allele frequency of rs662799 polymorphism of APOA5 gene and association of this variant in CAD and Control group in the study individuals.

Genotypes	Control N=100	CAD N=100	Unadjusted OR(95% CI)	P Value	Adjusted OR (95%CI)	P value
CC(Reference)	82	22				
TC	10	52	1.84 (0.71-3.66)	0.0001	1.76 (0.67-3.52)	0.0001
CC	8	26	1.51 (0.63-2.59)	0.0001	1.54 (0.56-2.61)	0.0004
Frequency of C allele	0.13	0.52		0.0001		

DISCUSSION

Results of the assessment of genotype distribution of the (rs662799) SNP under various inheritance models exhibited significant increase of the C allele in CAD patients when compared with those of the control group. The difference in the occurrence of the genotypes TT, TC, and CC pointed out a remarkable observation which is the significant variation of lipid profile for patients among the three groups (TT, TC, CC). The highest serum TG level was found in the carriers of the CC genotype and CT genotype. However, the minor C allele frequency in the CAD was also elucidated to be significantly higher than those of the control group. It is evident in the current study that the C allele of the −1131T>C (rs 662799) SNP in APOA5 gene is associated with the occurrence of CAD and could be considered as a risk factor for the development of the disease. To explain the possible reason of the involvement of (rs662799) SNP of APOA5 gene in the development of CAD. The exact mechanism of how APOA5 affects plasma TG is not completely understood. Some possible mechanisms suggest a catalytic role for APOA5 on triglycerides rather than a structural one where APOA5 could increase the lipolysis by lipoprotein lipase, or effect the very low-density lipoprotein secretion, or accelerate the hepatic uptake for the remnants of lipoproteins.[13,20] The presence of the -1131C variant was associated with higher levels of TG and coronary heart disease.[21] Recently, Jang et al.[22] showed that -1131C carriers exhibited reduced clearance of postprandial triglyceride-rich lipoproteins, along with higher oxidative stress with increased serum dense LDL, C-reactive protein and urinary 8-epi-prostaglandin F2 levels. They also exhibit more lymphocyte damage. Overexpression of the APOA5 gene in mice led to decreased plasma triglyceride concentrations, whereas its disruption resulted in hypertriglyceridemia.[9,23] APOA5-deficient mice have shown decreased LPL activity and the accumulation of larger very low density lipoprotein (VLDL) particles.[24] Results of this study are in agreement with the results on Turkish Cypriot,[25,26] as well as populations of Chinese,[27,30] as well as populations of Taiwan.[31] and in populations of Koreans,[32] Ferreira et al, (2013) reported the role of APOA5 1131C allele on coronary artery disease in Brazilian.[33] But other studies are not in agreement with the results,[9,34,35] Talmud et al (2004) reported that ApoA5 polymorphism was not found to be associated with CAD incidence.[36]

CONCLUSIONS

APOA5 gene polymorphism rs662799 was associated with CAD. Carriers of the homozygous genotype (CC) and heterozygous (CT) genotype of rs662799 have strong association and increased risk of development of CAD.

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