wjpmr, 2019,5(4), 01-07

SJIF Impact Factor: 4.639

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

ASSOCIATION BETWEEN APOLIPOPROTEIN A5-1131T>C (RS662799) GENE POLYMORPHISM AND LIPID PROFILE IN ISCHEMIC HEART DISEASES

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Article Received on 25/01/2019 **Article Revised on 15/02/2019** **Article Accepted on 05/03/2019**

ABSTRACT

Background*:** the most common type of Ischemic Heart Diseases, includes :stable and un stable angina and myocardial infarction, a chronic condition that narrows arteries by building fat-filled bulges in the arterial walls. Apolipoprotein A5 Gen polymorphisms On the genetic side that associated with Ischemic Heart Diseases**.** ***Aim: To assess the association of the Apolipoprotein A5-1131T>C (rs662799) gene with the Ischemic Heart Diseases in the genral population**,** and verify the relationship of the investigated SNPs with the metabolic changes related to IHD, in particular, serum lipid profile. ***Methods:*** A case control study was performed at which 100 patients with Ischemic Heart Diseases and 100 healthy individuals. Genotyping for SNP 1131T>C (rs662799) in the Apolipoprotein A5 gene was performed by the Genotyping of polymerase chain reaction- Amplification Refractory Mutation System (PCR-ARMS) method. Lipid profile (HDL,LDL,TAG,VLDL) were measured by enzymatic methods. ***Results*:** The genotype and allele frequencies of APOA5 gene polymorphism in IHD 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 IHD 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 IHD patients and the frequency of C allele was higher in IHD patients. There is significant increases in the level of triglyceride (P=0.001), VLDL (P=0.001), BMI(P=0.001) and a significant decrease in the level of cholesterol (P=0.03), Low density lipoproteins (P=0.2) in the group of patients with the CT+CC genotypes when they were compared with those of the TT genotype. **Discussion:** The results shown that APOA5-1131T>C (rs662799) gene polymorphism (homozygous CC and heterozygous CT genotype) was significantly associated with IHD subject. ***Conclusion*:** The -1131T>C (rs662799) SNP of APOA5 gene is associated with Ischemic Heart Diseases. in the population of Kerbala. The C allele is seemed to increase serum lipid concentrations so it could be considered as an atherosclerotic parameter.

KEYWORDS: for Ischemic Heart Diseases (IHD), Apolipoprotein A5, SNP single nucleotide polymorphism 1131T>C.

INTRODUCTION

Ischemic Heart diseases (IHD) are the most common type of cardiovascular diseases and 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] The ischemic heart disease (IHD) also is well-known as Coronary arteries disease (CAD), In 2015, IHD affected 110 million people and caused 8.9 million deaths.[4] Ischemic Heart 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 IHD compared to age-matched men.[6] There are many risk factor for (IHD) 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] Lipid profile is regarded as an important factor in the development of Coronary heart disease, There have been numerous studies confirming the association of hyperlipidemias with Coronary Heart Disease in most of the Western and Asian countries of the world.[8,9] The impact of risk factor confluence on Ischemic Heart diseases (CAD) risk by testing whether genetic risk scores (GRSs) associated with these risk factors.[10] The relationship of the disease at the changes in level of the DNA through the analysis of genetic mutations is responsible for the emergence of familial

clustering of coronary risk factors such as high iron, hyperlipidemia and others calculated biomarkers.[11] On the genetic side there are many genes that associated with IHD risk, Apolipoprotein A5 is one of this genes.[12] The gene for apolipoprotein A5 (APOA5 or APO A-V) (APOA5, gene ID 116519, OMIM accession number – 606368) 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.[13] Mature apoA-V (343 amino acids), which lacks the 23 amino acid signal peptide, 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.[14,15] Apolipoprotein A5 polymorphisms have long been reported to be associated with cardiovascular disease and plasma lipid levels.[16,18] Several lines of evidence indicate that increased plasma triglyceride (TG) levels are associated with CAD.[19] Minor alleles (C1131) is primarily associated with the elevation of plasma triglyceride levels.[20] Furthermore, mutations in the APOA5 gene leading to apoA-V deficiency are related to severe hypertriglyceridemia in humans.[18,21]

Study subjects

A case-control study of 200 subjects 100 IHD (The criteria for IHD were a ≥70% organic stenosis of at least one segment of a major coronary artery or their main branches confirmed by coronary angiography) and 100 control was conducted to study the association of 1131T>C SNP in APOA5 with Ischemic Heart disease . The patient population included 100 subjects (60 men and 40 women) with Ischemic Heart Disease who attended the cardiology center in Kerbala governorate from from December, 2017 to March, 2018.The Inclusion criteria were:(1) Those patients who were diagnosed by physicians as having (IHD) ;(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 IHD ;(2) No family history of IHD ;(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.

Sample Collection and processing

For chemical analysis venous blood samples were acquired from patients subject and healthy subject .Blood sample were allowed to clot at room temperature then separate serum by centrifuge and kept at -80 ^{o}C. Blood samples collected in EDTA tube to prevent coagulation for molecular study.

Phenotypic data

Biochemical analysis were performed total cholesterol (TC) triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C), low-density lipoproteien cholesterol (LDL-C) was calculated using the formula of Friedewald et al and BMI.

Genotypic data

Peripheral blood samples of (IHD) and control group were collected in EDTA-anticogulant 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 thermocycle (Biometra, Germany). The primer sequence of ApoA5 gene was used according to Ward et al.[22]

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.[22]

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 IHD 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 (23). 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 (23.76±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 IHD 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 IHD and Control group in the study individuals.

Genotypes	Control n=100	IHD n=100	Unadjusted OR (95% CI)	Pvalue	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		

The current study included 200 subjects (100 IHD and 100 control individuals). The clinical and biochemical characteristics of the recuritued individuals were presented in table 2. It shows significant differences in BMI, Age, Cholesterol, LDL, HDL in the group of IHD patients when compared with those of the control group. However, no significant difference was seen in VLDL and sex.

Table (3): Clinical and biochemical characteristics of study subjects.

Parameter	Control subjects	IHD subjects	P value
No (M/F)	100 (60/40)	100 (50/50)	0.064
Age (y)	49.16±4.6	57.41±6.38	0.001
BMI (kg/m2)	22.76±2.91	28±3	0.001
Cholesterol(mg/dl)	180.87±12.14	162.49±16.45	0.033
Triglycerides(mg/dl)	212.003±45.49	138.99±37.33	0.001
VLDL (mg/dl)	42.40±9.17	25.00±6.00	0.231
LDL (mg/dl)	95.20±9.16	138.99±37.33	0.001
HDL (mg/dl)	40.58±9.17	50.17±5.47	0.001

Table (4): Clinical characteristics of IHD subjects according to ApoA5 Genotypes.

Clinical characteristic	CT	CC	TT	P Value
Cholesterol(mg/dl)	179.71±5.14	180.39±17.92	184.18±15.28	0.3
Triglycerides(mg/d)	215.94±33.1	253.19±34.34	154.005±5.40	0.0001
VLDL (mg/dl)	43.18±6.62	50.63±6.86	30.80±1.08	0.0001
LDL(mg/dl)	94.56±6.47	93.91±6.70	98.21±15.11	0.2
HDL(mg/dl)	41.71±9.54	40.34±9.59	38.21±7.52	0.3

When the data were analyzed under the dominant model, differences being more obvious. There is non-significant with Low density lipoproteins (P=0.2), cholesterol (P=0.3), and a significant with Triglycerides (P=0.001) and VLDL in the group of patients with the CT+CC genotypes when they were compared with those of the TT genotype.

DISCUSSION

Dyslipidemia was an important risk factor for Ischemic Heart disease.[24-26] and it is a major health problem in many countries because of its high prevalence and its causal relationship with serious medical conditions such as Ischemic Heart disease (IHD).[27,28] increased triglycerides, cholesterol, (LDL-C), and decreased HDL cholesterol (HDL-C) in Plasma are associated with increased risk for IHD.[29-31]

Plasma Triglyceride levels are One of the important risk factors for developing IHD.[32-34] TG levels are known to be associated by many factors, and inherited contributions around 40–60% have been reported. Polymorphisms in the apolipoprotein factors have been reported to be only one of the contributing factors.[27,28,35]

Results of the assessment of genotype distribution of the rs (662799) SNP under various inheritance models exhibited significant increase of the C allele in IHD 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 IHD 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 IHD 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 IHD, 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 .[17,36] The presence of the -1131C variant was associated with higher levels of TG and coronary heart disease.[37] Recently, Jang et al.[38] 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.[13,39] APOA5-deficient mice have shown decreased LPL activity and the accumulation of larger very low density lipoprotein (VLDL) particles.[40] Results of this study are in agreement with the results on Turkish Cypriot.[41,42] as well as populations of Chinese.[43-46] as well as populations of Taiwan.[47] and in populations of Koreans.[48] Ferreira et al, (2013) reported the role of APOA5 1131C allele on coronary artery disease in Brazilian.[28] But other studies are not in agreement with the results.[13,49, 50] Talmud et al (2004) reported that ApoA5 polymorphism was not found to be associated with CAD incidence.[51]

This study provides the basis for future studies to establish disease associated APOA5 polymorphisms and develop protocols for prevention strategies. As part of a prevention strategy, these polymorphisms could be offered as a screening option to people who come in for medical check-up and based on the results they could be offered potentially lifesaving lifestyle changes in their eating and exercise habits. The results of this study call for genome-wide association studies in the Karbala population.

CONCLUSIONS

1- APOA5 gene polymorphisms (rs662799) is associated with IHD.
2- The C allele frequency of T>C rs662799 was in an association with an increased risk of development of IHD by affecting TG levels.
3- Serum lipid changes are directed by the genotypes of (rs662799) of APOA5 gene.

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