Research Article

Genetic variation of the Apo CII gene and plasma lipid levels in healthy and patients with acute coronary syndrome

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ABSTRACT

Background: Genetic polymorphisms in apolipoprotein C2 (APOC2) genes maybe relationship with modification in lipid profile and susceptibility to acute coronary syndrome (ACS). The current study aims to investigate some of the causes of ACS through study APOC2 gene polymorphisms in the Babylon Center population and to investigate the relation of these polymorphisms with lipid, and APOC2 levels. **Materials and Methods:** In this study, participants were 120 men of age group between (40-65) years old and BMI of 20-29.9 kg/m^2. Study group (60 men) included smokers with hypertension and no other risk factors for ACS. On the other hand, control group included 60 men who were nonsmokers, no hypertensive, with no systemic diseases. The serum sample was used to measure the concentration of total cholesterol (CL), triglyceride (TG), and high-density lipoprotein (HDL) using colorimetric methods, low-density lipoprotein (LDL) was calculated using the Friedewald equation, APOC2 level by enzyme-linked immunosorbent assay method, and DNA was extracted from leukocytes in the blood samples to study APOC2 gene. **Results:** Revealed the relationship between levels of APOC2 concentration and the polymorphism of the APOC2 gene a significant elevated in A1A2 genotype. In addition, there was a significantly increase in CL and LDL and significantly decrease in HDL with genotype A1A1 and genotype A1A2 showed significantly increase in TG and very-LDL compared with other genotypes. **Conclusion:** APOC2 gene polymorphism contributed to the occurrence of the ACS, where A1A1 genotype has a higher level of total CL and LDL and this increases the incidence of atherosclerosis, increased concentration of APOC2 play a role in the occurrence of ACS by interfering with the action of lipoprotein lipase, and this may increases the level of TGs.

KEY WORDS: Acute coronary syndrome, Apolipoprotein C2, Apolipoprotein C2 gene polymorphism, Lipid profile

INTRODUCTION

The term acute coronary syndrome (ACS) refers to any combination of clinical symptoms consistent with acute myocardial ischemia and includes unstable angina, non-ST-segment elevation myocardial infarction (STEMI), and STEMI.[1] It is characterized by new-onset or rapidly worsening angina, angina on minimal exertion, or angina at rest in the no show myocardial damage. In contradiction, myocardial infarction occurs when symptoms occur at rest and there is evidence of myocardial necrosis, as determined by an elevation in cardiac troponin or creatine kinase-MB isoenzyme.[2] The major difference between stable angina and ACS is the degree of obstruction of the blood flow induced by the coronary plaque. Stable angina is characterized by adequate blood flow in rest.[3] Apolipoprotein C2 (APOC2) is a 79-amino-acid polypeptide[4] and APOC2 gene lies in a cluster with the APOC1 and apolipoprotein E (APOE) at the long arm of chromosome19 (19q13.2). The APOE C1-C2 cluster gene spans approximately 48 kb,[5] and functions of APOC2 as an activator for lipoprotein lipase (LPL), an enzyme catalyzing the hydrolysis of triglyceride (TG) on chylomicron (CM), and very-low-density lipoprotein (VLDL).[4]

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MATERIALS AND METHODS

Subjects

In this study, participants were 120 men of age group between (40-65) years old and BMI of 20-29.9 kg/m^2. Study group (60 men) included smokers with hypertension and no other risk factors for ACS. On the other hand, control group included 60 men who were nonsmokers, no hypertensive, with no systemic diseases. Patients were recruited from coronary care units in Merjan Medical City admitted

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as cases of ACS by expert physicians. Biomarkers were studied in the laboratories of the Biochemistry Department of the College of Medicine.

DNA Typing

DNA was extracted from leukocytes in the blood samples using FAVORGEN kit and APOC2 gene was amplified by polymerase chain reaction (PCR) with primer upstream 5'- CTGCATCCAGG ACCCAGAAGTTC-3' and primer downstream 5'-CCTTGAGTCCTCAGAAAAGCAG-3', amplification was performed for 35 cycles at 94°C for 30 s, at 60°C for 30 s, and at 72°C for 1 min. The PCR product for APOC2 has a length of 530 bp [Figure 1] and in the case of a natural site AvaII restriction the PCR product will split into two fragments one 361 bp and the other 169 bp. Depending on the presence of the restriction site, the genotypes for APOC2 gene polymorphism are regulated by two codominant alleles (A1 and A2) [Figure 2].

Plasma Lipids Measurements

Lipid levels were determined from blood samples collected after a fasting (12–16) h. TG, cholesterol (CL), and high-density lipoprotein (HDL)-C levels were determined by enzymatic colorimetric method.[6] LDL was calculated using the Friedewald equation.[7] Individuals with TG levels >400 mg/dL were excluded from the analysis.

Plasma APOC2 Measurement

Fasting APOC2 was measured in plasma by enzyme-linked immunosorbent assay (ELISA), using ELISA Kits Elabscience.[8]

Statistical Analysis

The group of patients and control was compared by use analysis of variance (ANOVA) test, with $P \leq 0.05$ was considered the change as significant. Allelic and genotypic frequencies were calculated using the direct gene count method. Chi-square tests were used to test Hardy–Weinberg equilibrium of all gene variants. The relationship between patients' genetic patterns and controls was examined using the odds ratio (OR). OR with 95% confidence interval, the one way ANOVA test has been implemented for compare the means of lipids, APOC1, and APOC2 levels among different genotypes.

Study Design

This study design was a case-control study.

RESULTS

Lipid Profile

The result of the present study exhibits that the levels of CL P = <0.0001, TG P value (<0.0001), VLDL P value (<0.0001), and LDL P value (<0.0001) are

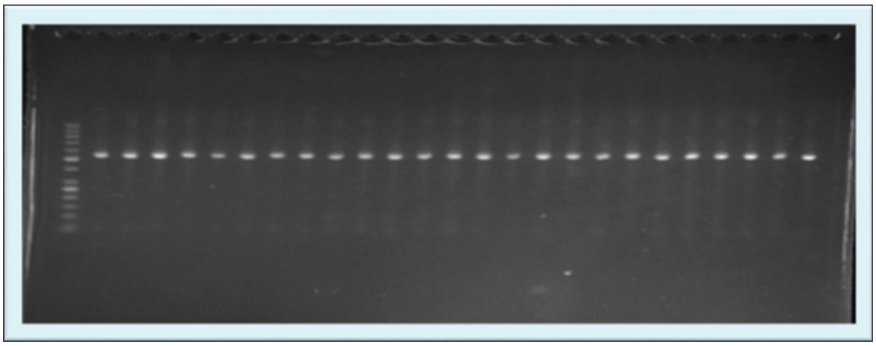

Figure 1: Amplification and polymerase chain reaction product (530 bp) picture of apolipoprotein C2 gene in 1.5% agarose gel. M: DNA ladder 50 bp

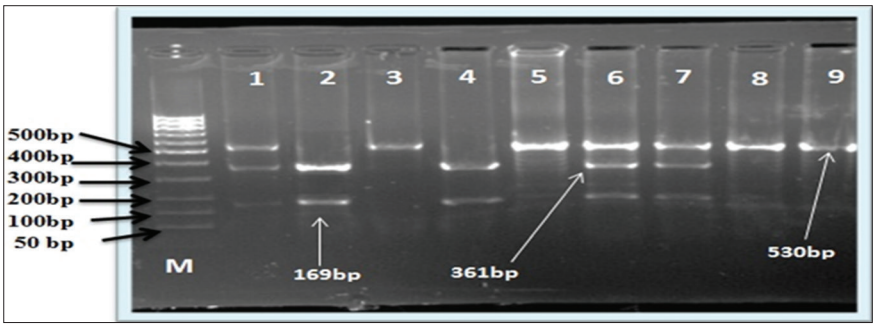

Figure 2: Electrophophoresis pattern of polymerase chain reaction product was digested with AvaII restriction enzyme and separated on 2% agarose gel. Lane: 3,5,8,9 homozygosity for absence, band size (530 bp). Lanes: 1,6,7 heterozygosity bands size (361 bp, 169 bp, and 350 bp). Lanes: 2,4, homozygosity for presence of restriction enzyme bands size (361-bp and 169-bp), M: 50+100-bp DNA molecular weight marker, and identification by red safe staining

significantly increased and HDL decreased in patients group when compared with the control group as shown in Table 1.

APOC2

The result of the present study showed that APOC2 levels are significantly increased in patient group when compared with the control group ($P < 0.0001$) as shown in Table 2.

APOC2 Gene Polymorphism

In this study, the APOC2 genotype in patients group was (A1A1) genotype found in 20 (33.3%), whereas 33 (55%) patients carried the (A1A2) genotype and 7 (11.7%) patients (A2A2) genotype. Within the control group, the (A2A2) genotype was found in 12 (20%), whereas 38 (63.3%) in control group carried the (A1A2) genotype and 10 (16.7%) individuals carried the (A1A1) genotype.

The distribution of APOC2 genotype showed a positive association between (A1A1) and (A1A2) genotypes among the patients group when compared to (A2A2) genotype [Table 3], these results show there may be a relationship between the A1A1genotype and the occurrence of the ACS where the probability of injury in general is three and a half times for the holders of this genotype compared to the A2A2 genotype.

The Relationship between APOC2 Genotypes, APOC2 Concentration, and Lipid Profile

The relationship between APOC2 and lipid profile concentration with APOC2 genotypes showed a significant relationship [Table 4].

DISCUSSION

Biochemical Markers

In this study, there was significantly increased in the level of serum TG and this result is in accordance with researches established by Aldaham *et al.*[9] The possible explanation of the abnormal lipid levels among smokers is that nicotine can stimulate the release of adrenaline from the cortex of the adrenal gland, leading to increased concentration of serum free fatty acids, which stimulates the synthesis in liver and CL secretion as well as liver secretion of VLDL.[10]

TG levels also increased the potential for TG exchange with the cholesteryl ester (CE) in HDL through the cholesterol ester transfer protein (CETP); a decrease in HDL concentration due to low CE and TG-rich HDL is less stable; this explains the increase in TG and VLDL associated with low of HDL. The decreasing in the HDL level among smokers may be also due to the changing in the critical enzymes that responsible for lipid metabolism and transport. Lowering lecithin cholesterol acyltransferase activity which responsible

Table 1: Mean±standard deviation and *P* value of CL, TG, VLDL, LDL, and HDL concentration in patients and control groups

Parameter	Group	Mean	Std. deviation	*P* value
CL (mg/dl)	Control	158.7500	21.31732	<0.0001
	Patient	179.1500	38.25730	
TG (mg/dl)	Control	114.5667	30.64082	<0.0001
	Patient	158.7333	58.16833	
HDL (mg/dl)	Control	57.8967	14.96570	<0.0001
	Patient	34.9317	16.65593	
VLDL (mg/dl)	Control	22.9133	6.12816	<0.0001
	Patient	31.7467	11.63367	
LDL (mg/dl)	Control	77.9400	22.67795	<0.0001
	Patient	112.4717	35.55635	

CL: Cholesterol, TG: Triglyceride, VLDL: Very-low-density lipoprotein, LDL: Low-density lipoprotein, HDL: High-density lipoprotein

Table 2: Mean±standard deviation and *P* value of APOC2 concentration in patients and control groups

Parameter	Group	*n*	Mean	Std. deviation	*P* value
APOC2 concentration (ng/ml)	Control	60	30.7667	10.69194	<0.0001
	Patients	60	39.1259	13.33834	

APOC2: Apolipoprotein C2

Table 3: Distribution of APOC2 genetic patterns among patient and control groups

Group	APOC2 genotypes	Odds ratio	95%CI	*P* value
Patients and control	A1A2 versus A2A2	1.49	0.53–4.22	0.458
	A1A1 versus A2A2	3.43	1.03–11.41	0.042
	A1A1 and A1A2 versus A2A2	1.42	0.52–3.89	0.680
	A1 versus A2	1.6603	0.9946–2.7715	0.0525

APOC2: Apolipoprotein C2, CI: Confidence interval

Table 4: Association APOC2 genotypes with APOC2 concentration and lipid levels

Parameters	Genotypes	*n*	Mean	Std. deviation	*P* value
TG (mg/dl)	A1/A1	30	128.8000	42.37468	0.048
	A1/A2	71	145.5775	55.38067	
	A2/A2	19	115.6842	41.44615	
Cholesterol (mg/dl)	A1/A1	30	182.9333	34.29882	0.021
	A1/A2	71	165.1408	31.64865	
	A2/A2	19	161.1053	27.23742	
HDL (mg/dl)	A1/A1	30	39.6267	20.61588	<0.0001
	A1/A2	71	45.1930	17.20416	
	A2/A2	19	61.6947	18.90801	
VLDL (mg/dl)	A1/A1	30	25.7600	8.47494	0.048
	A1/A2	71	29.1155	11.07613	
	A2/A2	19	23.1368	8.28923	
LDL (mg/dl)	A1/A1	30	117.5467	37.26516	<0.0001
	A1/A2	71	90.8324	31.49421	
	A2/A2	19	76.2737	20.89872	
APOC2 concentration ng/ml	A1/A1	30	33.8922	12.23575	0.039
	A1/A2	71	37.0196	13.31899	
	A2/A2	19	28.8632	9.24013	

TG: Triglyceride, VLDL: Very-low-density lipoprotein, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, APOC2: Apolipoprotein C2

for esterifying of free CL that leads to increase in HDL size and changing in the CETP as well as hepatic lipase enzyme activity among smokers, results in reduce HDL size.[11]

The decrease HDL concentration in the plasma decreased the transfer pathway of the reverse CL which results in the CL deposition in the peripheral tissue and dyslipidemia that leading to atherosclerotic lesion in the vascular endothelium.[12]

Insulin resistance will increase in smoking and cause hyperactivity of insulin. TG, VLDL, and LDL are high in hyperinsulinemia due to reduced lipoprotein activity, a key enzyme for converting VLDL to LDL.[13]

Cigarette smoking causes sympathetic activation, oxidative stress, and acute vasopressor effects that are associated with increases in markers of inflammation that is linked with hypertension.[14]

APOC2 has a role in the lipoprotein metabolism as an activator of LPL and its presence is required for maximal rates of TG-rich lipoprotein lipolysis.[15]

APOC2 is a component of HDL, VLDL, LDL, and CM.[16]

In normolipidemic, APOC2 was observed to be for the most part distributed in the HDL-C, whereas in hypertriglyceridemia, mostly distributed in the LDL-C and VLDL-C.[17]

APOC2 at high concentrations proved to inhibit LPL rather than stimulate it.[18]

In this study, there was an increase in serum APOC2 level in patients compared with the control group; hence, the high concentration of APOC2 may be related to the occurrence of ACS and this corresponds to Howlett *et al.* study suggesting that atherosclerotic lesions contain a large component of lipids, including oxidized lipid derivatives. The oxidized CL derivative, 3β-hydroxy-5-oxo-5,6-secocholestan-6-al, presents in atherosclerotic plaques and enhances amyloid fibril formation by APOC2.[19]

The increasing of APOC2 may contribute to the increased TG concentration and change in the HDL distribution and thus increase the risk of occurrence ACS.[17]

Polymorphism of APO2 Gene

In this study, there is significant among all genotypes of APOC2 in APOC2 concentration where A1A2 genotype has higher level from APOC2 and A2A2 genotype has lower level from APOC2. This difference in the level of concentration may be result of the substitution of nucleotides from T to C that altered in the form or dysfunction of the protein and amount of production and thus the action of the LPL enzyme will be affected, and this will affect the level of VLDL and CM remnant.

This hypothesis may justify the cause of a significant difference between all genotypes of APOC2 in concentration of TG and VLDL. Where the A1A2 genotype had a high level of VLDL and TG compared with the level of TG and VLDL in A1A1 genotype and this agrees with Wang *et al.* study found that the TG level in subjects carrying with A1A2 was significantly higher than that in subjects with A1A1.[20]

There was also a significant difference in LDL-C, CL, and HDL-C concentration between the genotypes where the A1A1 is associated with elevated levels of

total CL, LDL, and low level in HDL-C concentration, while A2A2 genotype is associated with the decreased levels of LDL-C, total CL, and high level in HDL-C and this result agree with Hong *et al.* study, a rise in CL, LDL, and low HDL-C level was found in the genotype more than other genotypes and suggested that there may be an imbalance in lipid levels due to the imbalance in the binding due to the location of the AvaII polymorphic site in intron 3 of the APOC2 gene. Since the sequential change of the AvaII location does not alter the amino acid, it is possible that the allele of the polyphonic form of AvaII is involved in identifying other functional sequences within or near the APOC2 gene.[21]

CONCLUSION

The study reached a conclusion that a significant association was found between ACS and APOC2 gene polymorphism that contributed to the occurrence of the disease by playing an important role as a modulator of dyslipidemia. The study demonstrates that A1A1 genotype increasing the incidence rate of ACS where A1A1 genotype has a higher level of total CL and LDL than other genotypes and has a lower level of HDL and this contributes to increased incidence of atherosclerosis, and A2A2 genotype may have a protective action against dyslipidemia.

Increasing APOC2 concentration plays a role in the occurrence of ACS which the increased level may inhibit the activity of LPL that contributes by increase TG level.

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