RESEARCH PAPER

Association between apolipoprotein E polymorphism and lipid profile in patients of myocardial infarction

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Received: 08.08.2020 Accepted:07.11.2020

Abstract

Objective: To determine the effect of apo E polymorphism on lipid profile in patients of myocardial infarction as well as normal healthy controls.

Subjects and Method: Total 100 acute myocardial infarction patients with age and gender matched controls, within age ranging from 25 to 80 years were included. Lipid profile levels of MI patients and controls were estimated by standard methods. DNA's were extracted by salting out method and Genotypes for Apo-E were determined by Multiplex Amplification Refractory Mutation System PCR.

Results: The total cholesterol, LDL cholesterol, TC/HDL-C ratio, LDL-C/HDL-C ratio level was significantly increased ($P < 0.01$) in E4E4 allele than E3E3 allele. Analysis of variants has significant difference ($P < 0.01$) observed in total cholesterol and LDL cholesterol levels in all Apo E alleles of MI patients.

Conclusion: Our results suggestive that the risk of myocardial infarction with Apo E4E4 alleles.

Keywords: Myocardial Infarction, Apolipoprotein E, Coronary Artery Disease

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Introduction

A myocardial infarction (MI) is constantly due to the formation of occlusive thrombus at the site of rupture or erosion and atherosclerosis plaque in a coronary artery. Sudden death, from ventricular fibrillation or asystole, may occur immediately, and many deaths occur within the first hour. If the patient survives this most critical stage, the liability to dangerous arrhythmias remains, but diminishes as each hour goes by. Thus, it is vital that patients know not to delay calling for help if symptoms occur. The development of cardiac failure reflects the extent of myocardial damage and is the major cause of death in those who survive the first few hours of infarction.[1] The apolipoprotein E (Apo E) gene is localized on 19q 13.2 in an approximately 45 kb gene cluster containing the genes for Apo C-I, C-II, C-III, C-IV and C-V, along with elements controlling their tissue-specific transcription.[2] Apo E plays an overbearing role in the secretion, processing and metabolism of various lipoprotein particles across cell membranes in systemic and cerebrospinal system through reverse cholesterol

transport in the body.[3] Apo E is a 299 amino acid protein found in chylomicrons, VLDL, intermediate- density lipoprotein, and HDL.[4] It plays an important role in the metabolism of these lipoproteins by binding to the LDL receptor in hepatic and extra hepatic tissues and a putative Apo E receptor or LDL receptor-related protein.[5] Two Single Nucleotide Polymorphisms (SNPs) in position 3937 and 4075 in exon 4 result in three common alleles E2, E3 and E4 differing in amino acid substitution of arginine for codon 112 and 158. [6] There are mainly six genotypes - E2E2, E3E3, E4E4, E2E3, E2E4 and E3E4. Each allele possesses a different ability to bind with LDL receptor and absence has been correlated with significant elevation of chylomicrons and VLDL remnants in blood.[7] In lieu of this variation, triglycerides, HDL and LDL metabolism becomes defective and which leads to the conditions for the development of atherosclerosis and coronary heart disease.[8] The Apo E3 isoforms is associated with normal chylomicrons and VLDL metabolism whereas E2 isoforms does not function as an effective ligand for the receptor-mediated uptake remnant particles, having less than 2% of normal Apo E3 binding to the LDL receptor. The E4 isoforms is associated with higher concentration of LDL cholesterol than the E3 isoform which affects the molecular mechanism on lipoprotein fractions.[9,10] The breakthroughs in our understanding of the genetic basis of arteriosclerosis have been enhanced with completion of sequencing the human genome. Detection of genomic discoveries in atherosclerosis now occurs at a rapid pace. Hence in present study we analyzed the apo E alleles in MI patients and controls. These alarming trends have compelled medical practitioners to look for the preventive aspects to curb the increasing mortality.

Methods

Subjects: The present case-control study was performed in the Department of Biochemistry, Government Medical College, Miraj, India. Total 100 acute myocardial infarction patients and age and gender matched controls, out of which 67 were males and 33 were females, within age ranging from 25 to 80 years were included. The diagnosis of acute myocardial infarction was done by the physicians of General Hospital, Sangli and General Hospital Miraj; based on clinical history, electrocardiogram, and relevant biochemical parameters. Fasting blood sample of patients and controls were collected and analyzed. The protocol was approved by the Institutional Ethical committee and the informed consent was obtained from all subjects for participation in the study. Serum samples were analyzed for lipid profile by using standard protocol and EDTA samples were used for extraction of DNA.

Methods: Serum Total Cholesterol and HDL cholesterol levels was estimated by enzymatic method.[11] Serum triglyceride was determined by Glycerol phosphate oxidase-peroxidase method. [12] LDL cholesterol level was calculated by using Friedewald formula.[13]

Genetic Analysis: The well-established salting out method were applied on EDTA blood samples for DNA extraction. [14] Genotypes for Apo-E isoforms (E2, E3, and E4) for all the above subjects and controls were determined by Multiplex Amplification Refractory Mutation System (ARMS) PCR. [15] Results were expressed as mean ± SD. Independent samples ‘t’ test was applied to compare difference between means and P value of < 0.01 was interpreted as indicating a statistically significant difference.

All statistical analysis was carried out with SPSS 20.0 software.

Results

Total 100 acute patients of myocardial infarction and age and gender matched controls, among these 67 were males and 33 were females, within age ranging from 25 to 80 years were included.

Table 1. Frequency Distribution of apo E alleles in MI patients and controls.

Genotypes	Patients (n=100)(%)	Controls (n=100)(%)
E2E2	04	04
E3E3	39	52
E4E4	27	18
E3E2	08	13
E4E2	04	02
E4E3	18	11

***Significant**

Table 2. Comparison of lipid profile levels of E4E4 allele in MI patients and controls.

Biochemical parameter	MI patients E4E4 allele (n=27)	Controls E4E4 allele (n=18)	P-Value
Total Cholesterol mg/dl	248.89 ± 62.16	194.11 ± 22.09	P < 0.01*
HDL Cholesterol mg/dl	39.71 ± 15.64	46.84 ± 11.54	P > 0.01
LDL Cholesterol mg/dl	182.39 ± 64.77	128.94 ± 23.65	P < 0.01*
TC/HDL-C ratio	7.20 ± 3.41	4.38 ± 1.16	P < 0.01*
LDL-C/HDL-C ratio	5.42 ± 3.14	2.98 ± 1.06	P < 0.01*
VLDL Cholesterol mg/dl	26.78 ± 10.22	18.32 ± 4.82	P < 0.01*
Triglycerides mg/dl	133.93 ± 51.10	91.61 ± 24.13	P < 0.01*

Table 3. Lipid profile levels of E3E3 allele with E4E4 allele in MI patients and controls.

Biochemical parameter	MI patients E3E3 allele (n=39)	MI patients E4E4 allele (n=27)	P Value	Controls E3E3 allele (n=52)	Controls E4E4 allele (n=18)	P Value
Total Cholesterol mg/dl	212.46 ± 54.28	248.89 ± 62.17	P < 0.01*	162.40 ± 17.54	194.11 ± 22.09	P < 0.01*
HDL Cholesterol mg/dl	35.18 ± 9.85	39.71 ± 15.64	P > 0.01	51.21 ± 10.82	46.84 ± 11.54	P > 0.01
LDL Cholesterol mg/dl	155.08 ± 58.37	182.39 ± 64.78	P > 0.01	93.18 ± 20.22	128.94 ± 23.65	P < 0.01*
TC/HDL-C ratio	6.63 ± 2.83	7.20 ± 3.41	P > 0.01	3.32 ± 0.81	4.38 ± 1.16	P < 0.01*
LDL-C/HDL-C ratio	4.94 ± 2.72	5.42 ± 3.14	P > 0.01	1.96 ± 0.76	2.98 ± 1.06	P < 0.01*
VLDL Cholesterol mg/dl	22.19 ± 14.03	26.79 ± 10.22	P > 0.01	18.01 ± 5.88	18.32 ± 4.82	P > 0.01
Triglycerides mg/dl	110.96 ± 70.13	133.93 ± 51.11	P < 0.01	90.04 ± 29.41	91.61 ± 24.13	P < 0.01

***Significant**

Table 4. Analysis of variance of lipid profile levels for all Apo E allele in MI patients.

Biochemical parameter	All apo E allele Mean ± SD	Test statistic (F)	P Value
Total Cholesterol mg/dl	220.21 ± 55.70	4.79	P < 0.01*
HDL Cholesterol mg/dl	37.83 ± 12.58	2.09	P > 0.01
LDL Cholesterol mg/dl	158.22 ± 58.22	3.94	P < 0.01*
TC/HDL-C ratio	6.48 ± 2.89	1.42	P > 0.01
LDL-C/HDL-C ratio	4.7 8 ± 2.72	2.51	P > 0.01
VLDL Cholesterol mg/dl	24.15 ± 12.03	0.62	P > 0.01
Triglycerides mg/dl	120.76 ± 60.16	0.62	P > 0.01

***Significant**

Discussion

PP Singh, et al [16] and his co-workers illustrated that E3 allele (> 80%) and E3E3 genotype was the most common in coronary heart disease patients and in healthy controls. E4 carriers were observed to be more common in patients than in controls. They also showed disease association analysis, that the carrier of E4E4 allele was portending higher risks of coronary heart disease. Different populations exhibit variable frequencies in the distribution of Apo E isoforms and so far, the most frequent allele in all populations examined is E3. Several studies have shown that E2 allele is associated with low levels of total cholesterol, LDL cholesterol and Apo B, whereas for E4 allele oppositely observed.[17] Hence, we have studied a relationship between Apo E polymorphism with lipid profile. The lipid profile levels were compared in patients as well as controls on the basis of the genotype between E4E4 allele demonstrated in (Table-2). These results indicate that MI patients having E4E4 allele had higher lipid profile values than in controls. Investigation showed that approximately 50% of the variability in normal serum cholesterol levels is due to genetic differences among individuals. [17] It was estimated that as much as 16% of the genetic variance of LDL cholesterol was due to allelic differences at the apo E gene locus. [18] Utermann G 18[19] observed that subjects with the E3;12 phenotype have about 20% lower, and E3;14 subjects have on average 10% higher levels of LDL cholesterol than subjects possessing the E3;13 phenotype. Due to the association between LDL cholesterol levels and atherosclerosis, it has been suggested that Apo E polymorphism may play a role in determining the risk of coronary artery disease (CAD). [20] Studies have demonstrated the impact of Apo E polymorphisms in cardiovascular diseases in a reproducible fashion. The Apo E isoforms have shown to be associated with variation in plasma LDL cholesterol and Apo B level, with the E4 allele exerting a greater influence than E3. Apo-E has consistently shown significant gene environment interactions modulating its association with plasma lipid parameters as well as cardiovascular disease risk.[21,22] Genetic polymorphisms in Apo E have been studied for association with plasma LDL cholesterol levels and Apo E polymorphisms have shown consistent associations. [23, 24] Dallongeville J et al[8] observed the persons with the E2 and E4 alleles had about 5% lower and 5% higher levels of plasma cholesterol, respectively, than did E3E3 homozygotes. Their study also showed that those with the E2 and E4 alleles had higher levels of plasma triglycerides than did E3E3

homozygotes. Hence, we also compared lipid levels in patients as well as in controls on the basis of the genotypes between E3E3 and E4E4 allele and it is shown in (Table-3). Manttari M et al [25] and Tikkanen MJ et al [26] showed that Apo E variation affects the serum cholesterol response to diet with the E4 allele may be more sensitive to dietary manipulation of LDL cholesterol levels. It has been reported that the biochemical mechanism is related to dysfunction of the E4 isoform in lipoprotein metabolism due to the E4 allele may affect the development and severity of CAD indirectly via influencing the lipid levels where increased concentration of serum cholesterol and triglycerides leads to atherosclerosis so Apo-E levels were demonstrated to be associated with CAD risk. [27-29] The study supports the fact that variability in Apo E gene locus is associated with CAD complication by influencing the plasma lipid levels that are important risk factors for CAD and other disorders. Alterations of lipid profile could be associated with acceleration of atherosclerosis and higher risk of incident CVD.[30] Moreover, obesity, smoking, etc. conditions associated with high oxidative stress, can aggravate the progression of disease and genetic studies can thus provide information that may help to improve the ability to identify individuals, families and populations at increased risk, and to improve the clinical management of patients of MI. This information may be useful in developing public health programs reinforcing primary and secondary prevention for CAD and patients identified with high risk genotype or allele should be treated aggressively to prevent the progression of disease. [31]

Null Hypothesis: There is no significant difference of lipid profile levels in all Apo E alleles of MI patients after analysis of variants was checked and it is shown in (Table-4).
As per the analysis of variance the null hypothesis is rejected.

Conclusion

The study suggested that the E4 homozygous allele increases plasma total cholesterol, LDL cholesterol, TC/HDL-C ratio and LDL-C/HDL-C ratio; hence it is an independent risk factor for the development of myocardial infarction. The study proposed the link between Apo E4E4 alleles and high lipid parameters in our population.

Acknowledgments

Authors of this manuscript disclose no any financial and personal relationships with other organizations / people that inappropriately could influence their work. All authors disclose no any financial support for this original research article preparation. The authors declare that there is no conflict of interests.

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