A study of Apo lipoprotein ‘E’ polymorphism and lipid profile in coronary artery disease

S. M. Mallakmir¹*, G. R. Kane¹, S. S. Mallakmir², D. A. Vidhate³, S. Deshpande¹, J. Nadkarni⁴, M. David⁴, G. Ravindranathan⁴, J. Shah⁴

¹Department of Cardiology, ²Clinical Geneticist, ³Department of Biochemistry, Pad. Dr. D.Y. Patil Medical College and Research centre, Nerul, Navi Mumbai, INDIA.
⁴Department of Biotechnology and Bioinformatics, CBD Belapur, Navi Mumbai, INDIA.

Email: drmsomnath@gmail.com

Abstract

Apo lipoprotein E (Apo E), a glycoprotein, plays an important role in lipoprotein metabolism. Polymorphism in Apo E genotype affects the clearance of circulatory lipids and leads to dyslipidemia. Association between Apo E polymorphism and risk of coronary artery disease (CAD) has been evident in earlier studies, but its significance has not yet been established. Indian literature in this subject is scant and cultural, ethnic diversity in Indian population can influence the results, hence there is need for more studies in different population groups. We have studied 160 cases during 2009 to 2012, referred to the Department of Cardiology, Dr. D.Y. Patil Hospital and research Centre, Nerul, Navi Mumbai. Lipid profile and Apo E polymorphisms (E2/E3, E3/E3 and E3/E4) were studied in 110 CAD patients and compared them with 50 healthy controls. Conclusions: Our results reported that Apo E3/E4 genotype is one of the most potent genotype associated CAD with abnormal lipid profile.

Keywords: Apo lipoprotein E polymorphism, coronary artery disease and Lipid profile

INTRODUCTION

Coronary artery disease is the leading cause of death worldwide and is rapidly increasing in prevalence in developing countries¹. CAD has been associated with behavioral, genetic and environmental risk factors². Amongst genetic risk factors, Apo E is one of the most thoroughly studied genetic polymorphisms particularly for its effect on lipid profiles and CAD risk³. India is facing a great challenge because of enormous increase in CAD cases in last few years. Indian literature in this subject is scant and cultural, ethnic diversity in Indian population can influence the results, hence there is need for more studies in Indian population. Plasma lipoproteins are composed of nonpolar lipid core, primarily triglycerides and cholesteryl esters with an external layer of phospholipids and Apo lipoproteins. Apo lipoproteins are the only protein components of lipoproteins which combine with free cholesterol, phospholipids, cholesterol esters and triglycerols to form lipoprotein. Like the different types apolipoprotein, apo E helps to stabilize as well as required for clearance of lipoproteins. Apo E is critical in the metabolism of very low density lipoproteins (VLDL) and chylomicrons⁴. The structural gene locus for this lipoprotein is at chromosome 19q 13.2⁵ and consists of four exons and three introns spanning 3.597 nucleotides and produces a 299 amino acid polypeptide with a molecular mass of about 34 k Da, three common all else designated E2, E3 and E4 code at a single locus. The various Apo E is o forms interact differently with specific lipoprotein receptors ultimately altering circulating levels of triglycerides and cholesterol. Apo E binds to specific receptor cells in liver but there is functional difference between Apo E alleles. While Apo E4 and E3 bind with approximately equal affinity to lipoprotein receptors, Apo E2 binds with less than 2% of this strength, the difference in uptake of postprandial lipoprotein particles contributes to the genotypic differences in total and LDL cholesterol levels⁶. A strong


(accessed 13 June 2014).
correlation was observed between Apo E polymorphism, circulating LDL and Apo B-100 which may enhance atherogenesis by affecting their circulating levels. Blood lipids and lipoproteins are the major coronary risk factors and hence Apo E polymorphism has been studied in greater details in last few years.

**AIM**
Our aim is to investigate the association of Apo E polymorphism and Lipid profile in CAD patients defined by coronary angiography.

**METHODOLOGY**
We studied total 160 subjects. Out of which 110 CAD patients were from Indoor patient department (IPD) and 50 normal controls visiting Outpatient department (OPD) of Cardiology department, Pad. Dr. D. Y. Patil Hospital and research center, Nerul, Navi Mumbai, during the period of 3 years. The CAD was diagnosed on basis of clinical symptoms and signs, electrocardiography (ECG) and echocardiography and coronary angiogram (CAG). Lipid profile was assessed and Apo E polymorphism was evaluated in all study participants. Written informed consent was obtained from each participant before inclusion in the study. This study was approved by the University ethical committee. Fasting blood samples were collected from all the study participants. Triglyceride, Total cholesterol (TC), LDL cholesterol, HDL cholesterol and VLDL levels were measured by auto analyzer. Fasting venous blood collected in EDTA sample tube for the detection of Apo E genotypes. DNA extraction was carried out by using Qi Amp Blood Mini Kit. Extracted DNA samples were checked for quality and quantity analysis. DNA was amplified by PCR in a thermal cycler using specially designed oligonucleotide primers (8). Restriction enzyme digestion of the amplification products was carried out. Finally separation of the digested products and identification of genotype was done by agarose gel electrophoresis. Data is presented as mean and standard deviation (S.D.). SPSS version 16.0 statistical package was used to analyze the data. Independent test was applied to determine the statistical significance of the variable in the study groups.

**RESULTS**

The study data revealed that 27% CAD patients and 90% controls were below age of 45 and 72.7% CAD cases and 10% controls were above age of 45. Gender wise (M/F) ratio in the present study is CAD (4:1) and control (3:1). In CAD group 80% patients were hypertensive, 50.9% were Diabetic. It was observed that in CAD group 71.8% subjects were consuming nicotine in any form while 13.6% subjects were alcoholic.

![Figure1: Comparison of lipid profile parameters in CAD and control groups](image)

**Table 2: CAD and lipid profile**

<table>
<thead>
<tr>
<th>Variables</th>
<th>CAD (110)</th>
<th>Normal (50)</th>
<th>‘p’ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>209.95±29.50</td>
<td>166±34.68</td>
<td>.000</td>
</tr>
<tr>
<td>HDL</td>
<td>37.79±6.57</td>
<td>46.08±9.01</td>
<td>.000</td>
</tr>
<tr>
<td>TAG</td>
<td>182.30±45.85</td>
<td>142.44±102.58</td>
<td>.000</td>
</tr>
<tr>
<td>LDL</td>
<td>135.43±25.79</td>
<td>92.8±25.79</td>
<td>.000</td>
</tr>
<tr>
<td>VLDL</td>
<td>36.43±9.24</td>
<td>27.24±16.82</td>
<td>.000</td>
</tr>
<tr>
<td>Non HDL chol</td>
<td>172.68±28.89</td>
<td>121.24±36.27</td>
<td>.000</td>
</tr>
<tr>
<td>TC/HDL</td>
<td>5.62±1.21</td>
<td>3.67±10.95</td>
<td>.006</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>3.66±0.99</td>
<td>2.07±0.66</td>
<td>.000</td>
</tr>
</tbody>
</table>

The present study observed a significant difference in lipid profile of CAD and normal controls. Circulatory levels of various parameters of lipid profile showed a significant variations as Total Cholesterol (209.95±29.50/166±34.68), HDL (37.79±6.57/46.08±9.01), Triglycerides (182.30±45.85/142.44±102.58), LDL (135.43±25.79/92.8±25.79), VLDL (36.43±9.24/27.24±16.82), Non HDL chol (172.68±28.89/121.24±36.27), TC/HDL (5.62±1.21/3.67±0.95) and LDL/HDL (3.66±0.99/2.07±0.66) between CAD and control group.

**Table 3: Distribution of Apo genotype among CAD and normal controls**

<table>
<thead>
<tr>
<th>Apo E Genotypes</th>
<th>CAD (n=110)</th>
<th>Normal (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2/E3(3)</td>
<td>2.7%</td>
<td>0%</td>
</tr>
<tr>
<td>E3/E3(77)</td>
<td>70.0%</td>
<td>92.0%</td>
</tr>
<tr>
<td>E3/E4(30)</td>
<td>27.3%</td>
<td>8.0%</td>
</tr>
</tbody>
</table>

The study data revealed that 27% CAD patients and 90% controls were below age of 45 and 72.7% CAD cases and 10% controls were above age of 45. Gender wise (M/F) ratio in the present study is CAD (4:1) and control (3:1). In CAD group 80% patients were hypertensive, 50.9% were Diabetic. It was observed that in CAD group 71.8% subjects were consuming nicotine in any form while 13.6% subjects were alcoholic.
In our study E3/E3 is more frequent genotype [in CAD 70% and in control group 92%]. E3/E4 is genotype is mainly associated with CAD [in CAD (27.3 %) as compared to control group (8.0%)] which is statistically significant (P= 0.008). E2/E3 is less frequent genotype found in our study and also less frequently associated with CAD [CAD group (2.7%) and control group (0%)].

DISCUSSION

In this study we found a significant correlation between various parameters of Lipid profile between CAD patients and healthy normal controls. Further distribution of Apo genotype among CAD and normal controls was evaluated in all study participants. It was observed that E3/E3 is the more frequent genotype in both CAD and normal population. A study carried out by Aceves et al, included various populations and showed that E3/E3 is more frequent genotype as compared to E3/E2 and E3/E4 among different populations. While in 2011, Belkovets et al, studied Apo E genotypes in Siberian females. Then in addition to that, E3/E4 genotype has been found to be associated more with CAD than controls. Our observations were supported by a study carried out by Singh et al, 2008. While it was also observed that E2/E3 genotype is less frequently associated with CAD. Apo E polymorphism E3/E4 influences lipoprotein metabolism which reflects in abnormal lipid profile and confer the risk of CAD. Apo E determines the clearance of VLDL but due to specific polymorphisms, amino acid sequence in Apo E is disturbed which affects the structure of the Apo E protein and ultimately affects lipid clearance from the circulation. The various Apo E isoforms interact differently with specific receptor, ultimately altering circulating levels of cholesterol. Apo E form VLDL, chylomicrons and chylomicrons remnants. Chylomicron remnants bind to specific receptor cell in the liver. Apo E2 is associated with decreased ability to bind the LDL receptor, hence less efficient at making and transferring VLDLs and chylomicrons from blood plasma to the liver. By contrast carriers of E3 and E4 are much more efficient in this process. While Apo E3 A and Apo E4 binds with approximately equal affinity to LDL receptor, Apo E2 binds with less than 2% of this strength. Thus compared with carrier of E3or E4 alleles, carriers of E2 are slower to clear dietary fat from blood. The difference in uptake of postprandial lipoproteins particles result in difference in regulating hepatic LDL receptor which in turn contributes to genotypic difference in total and LDL cholesterol levels.

CONCLUSION

The study result concludes that Apo E3/E4 genotype is one of the most potent genotype associated with CAD due to abnormal lipoprotein metabolism. More cohort studies in India will help in genotyping this entity which will further impact preventive measures.

ACKNOWLEDGEMENT

We express our sincere gratitude to all teaching and technical staff members of Department of Cardiology and Medicine, Dr. D.Y. Patil Medical College, Nerul Navi Mumbai. We would also like to thank the teaching and technical staff of Department of Biotech and Bioinformatics, Dr. D.Y. Patil University, CBD, Navi Mumbai.

DISCLOSURE

No conflicts of interest are declared by the authors.

REFERENCES


Source of Support: None Declared
Conflict of Interest: None Declared