Study on the association between serum APO-E levels and serum lipid levels in young smokers

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ABSTRACT
Smoking causes cardiovascular deaths which are about 11% of total global death. Cigarette smoking is one of the significant risk factors for cardiovascular disease and atherosclerosis in young age. Apo lipoprotein-E (APO-E) is a glycoprotein which plays an important role in lipid metabolism. However, the relation between serum APO-E levels and serum lipid levels remains controversial. So we aim to find out the association between serum APO-E levels and serum lipid levels in young smokers. The study contains 60 young smokers with CHD aged between 20 to 45 years, and 60 healthy normal individuals were attending in Cardiology OP, Medicine OP and MHC were selected for the study. Serum APO-E levels were measured by Enzyme-linked Immune Sorbent Assay (ELISA), and the lipid profile was measured by the enzymatic method. The mean serum APO-E levels were significantly (p-value <0.0001) higher in smokers with CHD when compared to the controls. A positive correlation was found between the serum APO-E level and BMI (r-value=0.595), FBG (r-value=0.425), TC (r-value=0.650), TGL (r-value=0.449), LDL (r-value=0.456), VLDL (r-value=0.452), No of smoking/day (r-value=0.862), duration of smoking (r-value=0.726) and a negative correlation was found with HDL-C levels. The current study gives a significant correlation between smoking and lipid profile in young smokers. Thus the present study concludes that cigarette smoking produced a significant effect on serum APO-E levels and lipid profile in young smokers which may lead to cardiovascular disease in young smokers.

INTRODUCTION
Smoking is one of the biggest public health threats worldwide (World Health Organization, 2020). Smoking is generally regarded as the environmental factor which is associated with different genetic factors and multifactorial disorders. According to the world health organization, globally, there are about 1 billion people who smoke daily. Out of which 800 million are men and 80% of all these smokers live in middle and low-income countries (Martin, 2020). India is the second-largest consumer and third largest producer of tobacco and the data from WHO suggested that there are about 100 million people are smokers in India, which is about 12% of the total world’s smoker (Pankaj et al., 2017).

The human Apolipoprotein E is a 34-kDa liver-derived multifunctional protein found associated with triglyceride-rich chylomicrons and very-low-density lipoproteins (VLDLs), their remnants and a
subsets of high-density lipoprotein particles (Fagerström, 2002; Pyrgakis, 2009). The human APO-E gene was found on chromosome 19q13.2, which codes 299 amino acids for protein (Mahley, 1988). APO-E gene has two single nucleotide polymorphism variants rs429358 and rs7412 which affects the metabolism of lipid by different protein isoform (Utermann, 1975). APO-E gene has three allelic variants E2, E3 and E4, which arises from 2 single nucleotide polymorphism.

The allelic variants are differentiated based on the interchanging sequence of the amino acid at position 112 and 158, which code for cysteine (Cys) and arginine (Arg) (Mahley, 1988). The E2 variants contain Cys residue at both the positions of the amino acid sequence 112 and 158, E3 variants contain Cys at 112 positions and Arg at 158 position and E4 variants contain Arg at both the positions in the receptor-binding region.

The 3 APO-E variants give six biallelic genotypes E2/E2, E2/E3 and E4/E4 which is homozygous and E2/E3, E2/E4 and E3/E4 which is heterozygous. APO-Es has a significant role in lipid metabolism by mediating high-affinity binding of APO-E-containing lipoproteins to the low-density lipoprotein receptor (LDL-R) and LDL receptor-related protein 1 (LRP1), facilitating clearance of triglyceride-rich lipoproteins from the circulation (Clark et al., 2009).

The report has been found that glycoprotein APO-E causes modulations of lipoprotein concentration in an isoform-independent manner through clearance rate, conversion of lipolytic factor, and very low-density lipoprotein production (Suarez and Schonfeld, 1981). Smoking is one of the main essential risk factors for cardiovascular disease and other vascular diseases. Smoking has a direct association with lipid parameters (Gossett et al., 2009; Campbell et al., 2008).

It generally accelerates the lipid levels, mostly bad cholesterol levels and decreases the concentration of good cholesterol (HDL-C) (Gepner et al., 2011; Austin, 1991). Thus smoking is one of the main essential risk factors which may cause cardiovascular disease (Utermann, 1975; Villeneuve et al., 2015).

Cigarette smoking and its chemical component causes an increase in the low-density lipoprotein and low in HDL concentration and modulates the lipids and proteins, including the APO-E. So we aim to find out the association between serum APO-E levels and serum lipid levels in young smokers (Tamamizu-Kato et al., 2007).

MATERIALS AND METHODS

The current cross-sectional study was done in SRM Medical College Hospital and Research Centre, Chennai, Tamil Nadu, India. Sixty young smokers with CHD aged between 20 to 45 years, and 60 healthy normal individuals (non-smokers) were attending in Cardiology OP, Medicine OP and MHC was selected for the study. The patients were recruited between October 2019 and February 2020 at SRM Medical College Hospital. During the health check-up, a questionnaire was given to the subjects which were filed by the subjects based on history and lifestyle characteristics. A physical examination was done in which the subject’s anthropometric measurements were done. A 5ml of fasting blood samples was taken from each subject for further biochemical analysis. The patients with the acute coronary syndrome, cardiomypathy, chronic disease like liver failure, cancer patient, heart failure, pregnancy, cardiovascular accidents, severe systemic illness, and systemic inflammatory disease are excluded from the study. The status of smoking is those who smoked regularly during the study period. The current study was approved by the institutional Ethics Committee, SRM Medical College Hospital (IEC-1779, September 2019).

Assays

The levels of serum Apo lipoprotein-E were measured using a sandwich enzyme-linked immunosorbent assay (ELISA). Plasma glucose level, Serum Total cholesterol, serum triglyceride level, serum High-density lipoprotein cholesterol (HDL-C) levels, serum low-density cholesterol level and serum very low-density lipoprotein level were measured enzymatically in the AU480 automatic analyzer (back man coulter).

Statistical analysis

The current statistical data were analyzed using (SPSS) software version 22. The results were presented by using Student’s t-test, which is used to analyze the difference between the mean levels of various parameters. Correlation between various variables was assessed using Pearson’s correlation equation. The value of “p” <0.05 was considered statistically significant.

RESULTS

Table 1 and Table 2 show the characteristics of the participants according to the CHD and smoking status. Hypertension and dyslipidaemia were significantly more prevalent in the CHD with smoking group compared with the control group. The fasting blood glucose and lipid profile were significantly
Table 1: Anthropometric measurements of smokers with CHD and normal controls. *(p<0.05 is statistically significant)*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Smoker (n=60)</th>
<th>Non Smoker (n=60)</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>41.64 ± 8.17</td>
<td>29.59 ± 10.56</td>
<td>5.6926</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Height(cm)</td>
<td>170.81 ± 3.44</td>
<td>170.5 ± 2.46</td>
<td>0.3993</td>
<td>&lt;0.6906</td>
</tr>
<tr>
<td>Weight(kg)</td>
<td>71.75 ± 5.73</td>
<td>65.09 ± 3.26</td>
<td>5.2876</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.47 ± 1.61</td>
<td>22.53 ± 0.71</td>
<td>5.5870</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>WC(cm)</td>
<td>90.51 ± 4.45</td>
<td>84.77 ± 3.26</td>
<td>5.6953</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HC (cm)</td>
<td>98.8 ± 5.23</td>
<td>99.54 ± 3.11</td>
<td>0.6409</td>
<td>&lt;0.5232</td>
</tr>
<tr>
<td>W/H ratio</td>
<td>0.90 ± 0.04</td>
<td>0.84 ± 0.02</td>
<td>6.8977</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BP(S)</td>
<td>125.6 ± 4.2</td>
<td>117.5 ± 3.3</td>
<td>4.8234</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>No of Smoking /Day</td>
<td>7 ± 1.4</td>
<td>0</td>
<td>23.8912</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Duration of Smoking (years)</td>
<td>9.74 ± 3.8</td>
<td>0</td>
<td>12.2474</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 2: Biochemical parameters of smokers with CHD and normal controls (non-smokers). *(p<0.05 is statistically significant)*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Smoker (n=60)</th>
<th>Non smoker (n=60)</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBG (mg/dl)</td>
<td>140.89 ± 42.40</td>
<td>93.86 ± 6.46</td>
<td>5.2805</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>226.93 ± 29.72</td>
<td>161.72 ± 25.74</td>
<td>9.4182</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TGL (mg/dl)</td>
<td>175.04 ± 49.71</td>
<td>91.72 ± 33.37</td>
<td>7.4889</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>40.63 ± 6.70</td>
<td>42.5 ± 7.13</td>
<td>1.1431</td>
<td>&lt;0.2560</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>152.12 ± 21.02</td>
<td>105.63 ± 18.06</td>
<td>9.5082</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>33.73 ± 12.01</td>
<td>18.18 ± 6.59</td>
<td>5.9095</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>5.61 ± 0.92</td>
<td>3.82 ± 0.61</td>
<td>8.7066</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL/HDL-C</td>
<td>3.78 ± 0.70</td>
<td>2.49 ± 0.46</td>
<td>8.2556</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>APO-E (ng/ml)</td>
<td>51.36 ± 10.38</td>
<td>38.15 ± 7.82</td>
<td>6.1462</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 3: Co-relation of APO-E levels with biochemical parameters in a smoker. *(P <0.05 is statistically significant)*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>BMI</th>
<th>FBG</th>
<th>TC</th>
<th>TGL</th>
<th>HDL</th>
<th>LDL</th>
<th>VLDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>APO-E</td>
<td>0.595</td>
<td>0.425</td>
<td>0.650</td>
<td>0.449</td>
<td>-0.607</td>
<td>0.456</td>
<td>0.452</td>
</tr>
<tr>
<td>p-values</td>
<td>&lt;.00001</td>
<td>&lt;.0007</td>
<td>&lt;.00001</td>
<td>&lt;.0003</td>
<td>&lt;.0001</td>
<td>&lt;.0002</td>
<td>&lt;.0003</td>
</tr>
</tbody>
</table>

Table 4: Co-relation of APO-E levels with smoking per day and duration of smoking. *(P <0.05 is statistically significant)*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Smoking/day</th>
<th>Duration of smoking(years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APO-E</td>
<td>0.862</td>
<td>0.726</td>
</tr>
<tr>
<td>p-values</td>
<td>&lt;0.00001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
more significant in smoking with CHD compared to controls. CHD patients with smoking were not receiving any kind of lipid-lowering treatment.

Serum APO-E levels were measured in both the groups, smoking with CHD and normal controls. The mean serum APO-E levels were significantly (p-value <0.0001) higher in smokers with CHD when compared to the controls.

Our study also shows an association between serum APO-E levels with smoking and CHD. A positive correlation (Table 3 and Table 4) (Figure 1) was found between the serum APO-E level with BMI (r-value=0.595), FBG (r-value=0.425), TC (r-value=0.650), TGL (r-value=0.449), LDL (r-value=0.456), VLDL (r-value=0.452), No of smoking/day (r-value=0.862), duration of smoking (r-value=0.726) and a negative correlation was found with HDL-C levels.

**DISCUSSION**

APO-E is a glycoprotein which has a significant role in growth and development (Han et al., 2016). The genetic and environmental components determine the APO-E. However, the facts remain controversial and unexplained (Schiele et al., 2000). The risk of cardiovascular disease is more in young cigarette
smokers than normal healthy individuals. This can be understood by various associations like derangement in the serum lipid and lipoprotein concentrations, a rupture in the arterial wall, alternating in the blood coagulation (Singh, 2016).

This present study shows evidence that there is a significant correlation between the lipid levels in young smokers when compared to non-smokers with a significant p-value <0.05. The different study already revealed that there is a positive correlation between serum APO-E levels and plasma LDL-C and TGL (Gómez-Coronado et al., 1999; Boer et al., 1997). Other studies also show that smoking has a significant association with elevated LDL-C and TGL levels and a decline in HDL-C concentration (Freeman and Packard, 1995; Brischetto et al., 1983). On the other hand, some study revealed that there is an inconsistent finding between and lipid profile. Some studies suggested that smoking affects LDL-C through the decrease in lipoprotein lipase (Freeman et al., 1998, 1993). In contrast, some study reported no significant difference in lipoprotein lipase activity in smokers and non-smokers (Moriguchi et al., 1991; Eliasson et al., 1997).

The current study shows increased APO-E concentration in smokers has a perfect association with CHD. Increased APO-E concentration may lead to CHD via one or both of the two hypothesis. First high APO-E level may reflect a determinant lipid profile. We suggested that increase APO-E concentration reflect the increase lipoprotein levels such as LDL-C and VLDL-C levels which are pathogenic (Freeman and Packard, 1995; Brischetto et al., 1983). The second hypothesis by which APO-E leads to CHD is through proinflammatory properties. Plasma APO-E binds lipid antigens and appears to be critical for the presentation of lipid antigens by binding to antigen-presenting cells through the LDL receptor, which is followed by endocytosis (Gossett et al., 2009). The concomitant inflammatory response adequately eliminates the lipid antigen from the circulation. In this model, increased APO-E concentration combines with increased lipid-antigen presentation lead to chronic inflammation and which may lead to atherosclerosis (Mooijaart et al., 2006).

The current study shows that the relation of APO-E with lipid profile is modified by cigarette smoking. So the study is consistent with other reports which indicate that both APO-E and smoking are associated with increased LDL-C, VLDL-C, TC, TGL and decreased in HDL-C concentration, which may contribute at a greater risk of CHD in young smokers.

CONCLUSION

The current study gives evidence that smoking modifies the relation of APO-E with lipid profile. Thus the present study concludes that cigarette smoking produced a significant effect on serum APO-E levels and lipid profile in young smokers which may lead to cardiovascular disease in young smokers. So the study concludes that young smokers with increased APO-E levels are at increased risk of CHD, which may lead to cardiovascular death.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

REFERENCES


