hsCRP AND FIBRINOGEN IN THE EARLY DIAGNOSIS OF ATEROGENESIS IN MALE SMOKERS.

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ABSTRACT

Cigarette smoking is one of the major health hazards of the world population as it is contributing to the cardiovascular diseases and to the increasing proportion of the sudden deaths. hsCRP and Fibrinogen are independent & signifigant markers of cardiovascular mortality. The aim of the study is to identify the earlier markers of atherogenesis in healthy adult male smokers for better risk prediction and screening purpose. 60 adult healthy men of age group 19-49 years were enrolled. 30 smokers & 30 non-smoker. Smokers were compared with the non-smokers by doing the blood tests which included blood glucose, lipid profile, hsCRP & Fibrinogen. Smokers had significantly higher levels of total serum cholesterol [245.03±72.82 vs 160.07±21.39 p<0.000], LDL levels [149.17±48.87 vs 98.67±14.93 p<0.000] and triglyceride levels [152.43± 52.40 vs. 94.83±17.75 p<0.000]. HDL levels were low in smokers. hsCRP & Fibrinogen levels were higher in smokers [6.8±3.04 vs 1.38±0.67 p<0.00 & 644.20±231.38 vs 300.20± 49.91 p<0.000] respectively. There is a positive correlation between hsCRP & Fibrinogen levels (r=0.882; p<0.000). There is a positive correlation between the duration of smoking and hsCRP & Fibrinogen levels [r=0.871, p<0.000 & r=0.917 p<0.000]. This case control study shows significantly higher hsCRP & Fibrinogen levels in smokers in proportion to the duration & amount of cigarettes smoked pre day, as compared to the non-smokers. Thus hsCRP & Fibrinogen can be used as the bio markers of sub-clinical atherogenesis screening in healthy adult male smokers.

KEYWORDS: Fibrinogen, highly Sensitive Crp [hscrp], Atherosclerosis, Smokers.
INTRODUCTION
Cigarette smoking is a serious health problem and the most avoidable cause of sudden deaths worldwide. WHO has named tobacco as one of the greatest public health threats of the twenty first century. Tobacco smoking is an escalating public health problem especially in the developing country like India. The prevalence of smoking in India varies from about 15% to over 50% among men with physical and/or psychological dependence. (prevalence of tobacco use New Delhi 2004). Nicotine causes increase in triglycerides, cholesterol, LDL & VLDL levels and decrease in HDL levels, Augestin later on (Clutte –Brown J,&Hugan.S et al 1986), also studied that long term consumption of oral nicotine increased LDL cholesterol and decreased HDL cholesterol. Nicotine increases the circulatory pool of atherogenic LDL via accelerated transfer of lipids from HDL and impaired clearance of LDL from plasma compartment. Therefore it increases the deposition of LDL cholesterol in the arterial wall. Honjack (Hojnack, J.Mulligan et al 1986)  Endothelial dysfunction and inflammation are strongly associated with coronary artery disease (CAD). Inflammation plays a crucial role in the formation of atheromatous plaque, as well as its progress (Ramsdale, D.R.& Bened D. Et al 1985). The secretion of pro – inflammatory cytokines from the vascular endothelium as well as from macrophages induces the production of inflammatory molecules that are measured in the circulation, such as High sensitive C-reactive protein (hsCRP), and fibrinogen (Kampoli A-M, Tousoulis D et al 2009). CRP is a marker of endothelial activation and inflammation (Kampoli A-M, Tousolis D et al 2009). Fibrinogen infiltration of the vessel wall increases blood viscosity, platelet aggregation and thrombus formation. Plasma fibrinogen is also a prominent acute-phase reactant. It augments the degranulation of platelets in response to adenosine-di-phosphate (ADP), when taken up by the granules (Ridker PM, et al 2007). Fibrinogen and products of its decomposition mediate the transportation of adhesion molecules in the surface of endothelium and their further migration to the intima (Schneider DJ, Taatjes DJ et al 1999). Fibrin participates in the close linkage of low density lipoprotein (LDL) and lipid accumulation, leading to the creation of the lipid nucleus of atherosclerotic lipid nucleus of atherosclerotic lesions. (Miyao Y, Yasue H et al 1993) Fibrinogen participates in the formation of atherosclerotic plaque during the first stages of CAD, suggesting that it is a causative factor rather than a result (Smith EB, Fibrinogen et al 1986).
MATERIALS AND METHODS
The study was conducted in Narayana Medical college hospital. Participants included males of 19-49 years in generally good health. Smoker status was defined as the self reported smoking of atleast 5 years and no use of any other nicotine containing products and alcohol intake. Diabetes mellitus, Hypertension, Tuberculosis, Malnutrition, Cancer, chronic disease, and Females were all excluded. Written informed consent was obtained from each subject before entering the study. At visit 1, details of the subjects were documented and advised about the fasting condition. At visit 2, the next day morning after 8 hours of fasting, blood samples were collected, centrifuged and stored at -20 cc. Blood glucose measured by Glucose oxidase method [GOD-POD]. Cholesterol by cholesterol oxidase method and triglycerides by direct enzymatic method. The fibrinogen levels (Normal range: 200-400 mg/dl) were measured by using ACL 7000 auto analyser. hsCRP levels were measured by an immune- turbidometric method using CRP-turbilatex kit. Statistical analysis of the values done using spss 16 version and obtained mean, SD and p values. Pearson correlation coefficient was obtained between the variables.

RESULTS
Our study revealed significantly higher levels of cholesterol, LDL, VLDL, and triglycerides in the smokers population & low levels of HDL levels. The levels of hsCRP and fibrinogen were significantly high. There was a positive correlation between the hsCRP and fibrinogen levels. The mean serum total cholesterol in non-smokers was cholesterol 160.07±21.39 mg/dl while it was significantly higher in smokers, i.e 245.03±72.82 mg/dl. There is a significant rise in total cholesterol in smokers with p value <0.000. This finding is similar to the study done by Wendy Y Craig, Glenn E Palomaki, James E Haddow, they concluded that serum cholesterol concentrations were higher in smokers, leading to a significant overall increase of 3.0% (p<0.001) (Nikolaos Dimitris tousoulis et al 2010). The mean serum triglycerides levels in smokers and non-smokers were [152.43±52.40 vs 94.83±17.75] respectively. There is a significant raise in triglycerides in smokers with p value <0.000. The mean LDL-C and VLDL-C values in non-smokers were 98.67±14.93 mg/dl and 19.23±4.91 mg/dl respectively. But these values were significantly higher in smokers (LDL-C = 149.17±48.87 mg/dl, VLDL-C =29.70±10.61 mg/dl). The mean HDL-C in non-smokers was 64.27±20.96 mg/dl and was 42.17±6.35 mg/dl in smokers respectively [p value <0.00]. This finding is similar to that of Rosenson (Rosenson RS et al 1993) who reported that there is fall in HDL-C level by 3-5 mg/dl in smokers. A recent meta -analysis has demonstrated accordingly that HDL
cholesterol is about 6% lower in smokers. Similar findings have been reported by (Brischetto et al 1983). This finding is similar to the study done by Wendy Y Craig, Glenn E Palomaki, James E Haddow, they concluded that smoking was associated with significantly higher cholesterol, triglyceride, very low density lipoprotein cholesterol and low density lipoprotein cholesterol concentrations and significantly lower high density lipoprotein cholesterol (all p <0001). The mean hsCRP levels in smokers and non-smokers were 6.88±3.04 mg/dl & 1.38±0.67mg/dl respectively. The mean plasma fibrinogen levels in non-smokers was [300.20±49.91mg/dl] while it was significantly higher in smokers [644.20±231.28mg/dl]. There is a significant rise in plasma fibrinogen level in smokers with p value <0.000. This study is similar to the Framingham study, in this study results showed that plasma fibrinogen values were significantly higher in smokers than in non-smokers. Results show that lipid profile levels and fibrinogen levels are directly proportional to the increase in the duration of smoking. Cases with smoking history of 5-10, 11-20 & 21-30 years had mean total cholesterol levels of 180.17±25.64 mg/dl, 275.64±52.20 mg/dl & 332.50±68.53mg/dl respectively.

Mean TGL levels of 5-10, 11-20 &21-30 years of smoking were 123.83±34.07 mg/dl, 163.93±52.42mg/dl & 183.00±69.28mg/dl respectively. Mean HDL levels of 5-10, 11-20 & 21-30 years of smoking were 44.02±1.7mg/dl, 41±2.45mg/dl, 38±1.31mg/dl respectively.

Mean LDL levels of 5-10, 11-20 &21-30 years of smoking were 106.08±15.10mg/dl, 168.71±35.21mg/dl & 210.00±52.32mg/dl respectively.

Mean VLDL levels of 5-10, 11-20 & 21-30 years of smoking were 23.50±6.55mg/dl, 34.36±10.46mg/dl &32.00±13.92mg/dl respectively.

Mean hsCRP levels of 5-10, 11-20 & 21-30 years of smoking were 4.58±2.26,8.37±2.32 &8.52±3.50 respectively. Mean Fibrinogen levels of 5-10, 11-20 & 21-30 years of smoking were 400.83±65.53mg/dl, 783.07±126.97mg/dl & 888.25±159.99g/dl respectively.
Figure: 1 shows the comparison between Fibrinogen & smoking duration.

Figure: 2 shows the comparison between hsCRP & smoking duration.

Results show that fibrinogen levels increase proportionally with the increased quantity of smoking. Cigarettes smoked per day has positive correlation with hsCRP & Fibrinogen ($r=0.817, p<0.000$ & $r=0.814, p<0.000$). The results were similar to the Framingham study where there was a dose-dependent increase with smoking in both sexes; ex-smokers had values as low as those of non-smokers.

### TABLE 1 SHOWING COMPARISON OF BLOOD GLUCOSE, LIPID PROFILE, hsCRP, FIBRINOGEN IN SMOKERS AND NON SMOKERS.

<table>
<thead>
<tr>
<th>Parameters [mg/dl]</th>
<th>Smokers</th>
<th>Non smokers</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose</td>
<td>96.00±9.54</td>
<td>89.53±9.76</td>
<td>0.120</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>245.03±72.82</td>
<td>160.07±21.39</td>
<td>0.000</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>152.43±52.40</td>
<td>94.83±17.75</td>
<td>0.000</td>
</tr>
<tr>
<td>LDL</td>
<td>149.17±48.87</td>
<td>98.67±14.93</td>
<td>0.000</td>
</tr>
<tr>
<td>VLDL</td>
<td>29.70±10.61</td>
<td>19.23±4.91</td>
<td>0.000</td>
</tr>
<tr>
<td>HDL</td>
<td>42.17±6.35</td>
<td>64.27±20.96</td>
<td>0.000</td>
</tr>
<tr>
<td>hsCRP</td>
<td>6.88±3.04</td>
<td>1.33±0.67</td>
<td>0.000</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>644.20±231.38</td>
<td>300.20±49.91</td>
<td>0.000</td>
</tr>
</tbody>
</table>
TABLE 2 SHOWING THE CORRELATION BETWEEN hsCRP AND FIBRINOGEN.

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Fibrinogen</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsCRP</td>
<td>R=0.882</td>
<td>p&lt;0.000</td>
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</table>

DISCUSSION

In our cross sectional study, we found that the levels of lipid profile, hsCRP & Fibrinogen were higher among smokers. The increase in Fibrinogen and hsCRP was proportionate with the smoking duration and cigarettes per day. There was also a positive correlation between hsCRP and Fibrinogen levels. In smokers the levels of total cholesterol, LDL cholesterol, Non-HDL cholesterol were significantly elevated when compared with the controls (Venkatesan, A. Hemalatha et al 2006). It was shown that hsCRP was independently associated with stroke or other vascular events over a 7 year follow up (Soinio M, Marniemi et al 2006). The fibrinogen levels were also found to correlate with a higher risk of ischaemic stroke, which was independent of the blood glucose levels (Scarabin PY, Vissac AM et al 1999). Although hsCRP is a non-specific inflammatory marker it is a strong predictor of cardiovascular risk (Blake GJ, Ridker PM et al 2002). Cigarette smoking is strongly associated with increased plasma fibrinogen levels, and the adverse cardiovascular effects of smoking may partly be mediated through an increase in plasma fibrinogen levels. Indeed, each cigarette smoked per day increases mean plasma fibrinogen by 0.35 g/l.(Vanderwall AC, Becker AE et al 1994). A role in subclinical atherosclerosis has been also attributed to this acute phase protein, as higher levels of fibrinogen during young adulthood were positively associated with prevalence of coronary artery calcification and increased carotid intima-medial thickness in middle age, while the magnitude of the association decreased with aging.(Green D, Chan C, et al 2009) Fibrinogen and CRP levels were strong predictors of subclinical atherosclerosis (associated with an extension of carotid atherosclerosis) in hypertensive postmenopausal women. (Rizzo m, Corrado E et al 2009) Supporting data had previously shown that fibrinogen is involved in the subclinical phase of extra coronary and coronary atherosclerosis and may add to the atherogenic effect of hyperlipidemia(Levenson J, Giral P et al 1997).

This study suggests that smoking potentially increases LDL-C, hsCRP and Fibrinogen levels in healthy adult males. The synergetic increase of hsCRP and fibrinogen level in smokers is dose dependent and is also directly related with duration of smoking. Our study not only suggests that smoking is the individual risk factor for atherogenesis and it further indicates that smoking more than 5 years increases the atherogenic risk in
otherwise healthy males. Moreover our study suggests that hsCRP & Fibrinogen can be used as the sensitive and specific biomarkers to predict the atherogenic risk in sub–clinical stage among healthy adult male smokers.

CONCLUSION
Our present study reveals that the elevation of plasma fibrinogen, hsCRP and dyslipidemia are important cardiovascular risk factors in otherwise healthy smokers. It also unveils the positive correlation between fibrinogen and hsCRP among smokers. Our study concludes smoking may increase the inflammation and dyslipidemia which contributes to atherosclerosis in smokers and suggests fibrinogen as a bio-marker of atherogenic risk prediction among healthy male smokers.

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