Comparative study of Lipid Profile and level of Antioxidant enzymes in Cigarette smokers with Non-Cigarette smokers

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Abstract:
Cigarette smoking is the serious health problems and most important avoidable cause of death in world. Worldwide more than 8 million people currently die each year from smoking half of them before of the age of 60. Every cigarette reduces the life span by about 5 minutes. Smoke contains oxidising agents and the oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions that damage cells. In the present study 40 male subjects were divided into four different groups and their lipid profile have been estimated by various tests i.e. Cholesterol, Triglyceride, HDL-C, LDL-C, VLDL-C. It was observed that in cigarette smokers HDL-C level decreased and cholesterol, triglyceride, LDL-C, VLDL-C level increased as compared to the control i.e. non-cigarette smokers. In case of MDA and Antioxidant enzymes test, the value of MDA increases and antioxidant enzymes decreases in cigarette smokers as compared to the control i.e. non-cigarette smokers. The variation in the level of lipid profile and antioxidant enzymes from normal values causes several diseases such as Lung cancer, other cancers, heart disease, and stroke and has numerous immediate health effects on the brain and on the respiratory, cardiovascular, gastrointestinal, immune systems.

Key words: Oxidising agents, HDL-cholesterol, MDA, Triglycerides.

INTRODUCTION
A lipid profile is a direct measure of three blood components: cholesterol, triglycerides, and high-density lipoproteins (HDLs). Cholesterol is a vital substance that your body uses to produce such things as digestion-aiding material, hormones, and cell membranes. Cholesterol and triglycerides are transported in the blood by combinations of lipids and proteins called lipoproteins. HDLs, the so-called “good” or “healthy” cholesterol, are lipoproteins made mostly of protein and little cholesterol. HDLs can help to clear cholesterol deposits in blood vessels left by another blood component called low-density lipoproteins, or LDLs. LDL levels calculated from the three directly measured lipids. LDLs and very-low-density lipoproteins (VLDLs) are the so-called “bad” cholesterol. Unlike HDLs, LDLs and VLDLs are high-cholesterol particles. While cholesterol is necessary for various bodily functions, too much cholesterol is harmful,
since excess cholesterol can be deposited in blood vessel walls. These fat deposits can lead to atherosclerosis, or hardening of the arteries, and cardiovascular disease. High levels of triglycerides are also associated with an increased risk of heart disease. Smoking leads to increased serum levels of total cholesterol, LDL cholesterol, triglycerides and decreased levels of HDL-cholesterol. When levels of these lipids are abnormal, there is an increased risk of heart attack and stroke. These dyslipidemic changes also have been identified as major risk factors for development of atherosclerosis (Yasue H et al., 2006). Cigarette smoking may give rise to oxidative stress by formation of reactive species or the initiation of radical chain reactions (Orhan et al., 2005). A one to threefold increase in risk of myocardial infarction (MI) has generally been noted among current cigarette smokers. Oxidants and free radicals present in the cigarette smoke cause lipid peroxidation and oxidative damage to biological substances leading to a significant increase of malondialdehyde (a lipid peroxidation product) level in smokers. Enzymatic antioxidants such as superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase give good protection against such oxidative damage. Cigarette smoking has been found to alter the levels of lipoproteins. Clinical, genetic, and epidemiological evidence indicates that elevated levels of low density lipoprotein (LDL) are one important risk factor for the disorder. Oxidative pathway appears to be an oxidative damage to unsaturated lipids is a well established general mechanism for oxidant mediated cellular injury (Yagi et al., 1994). Multiple studies have reported that all forms of tobacco use (smoked, smokeless and other forms) are highly prevalent in youth and adult and in both men and women in India (Ray et al., 2003). Globally, smoking kills more than 4 million people every year and by 2020 it is likely to cause more premature deaths and disability than single diseases. Cigarette smoke also includes heavy metals, radioactive products, poisons and at least 48 known cancer-producing substances. Among the gases produced by tobacco, CO (Carbon monoxide) is found to be more toxic. It binds to haemoglobin in red blood cells and interferes with the body’s ability to transport and utilize oxygen.

MATERIALS AND METHODS
I) Study population
The study population will consist of 40 male subjects divided into four groups viz. 10 smokers and age- and sex matched 10 non-smokers (healthy volunteers) were selected. Participants in this study were selected from local areas of Paonta Sahib, H.P. India.

II) Collection, storage of blood samples and hemolysate preparation
10ml of blood was collected from chronic smokers, non-smokers under aseptic precautionary measures using sterile disposable syringe. Out of which 5ml was collected in separate heparin containing vial for estimating Malondialdehyde, SOD (Kakkar et al., 1984), Glutathione peroxidise (Rotruck et al., 1973), Glutathione reductase (Ellman et al., 1959) and Catalase (Sinha et al., 1972). The remaining blood was centrifuged at 1000 r.p.m for 15 min, serum separated and analysis was carried out immediately. The hemolysate used for the enzyme
activities. After centrifugation, the buffy coat will be removed and the packed cells will be washed three times with physiological saline. A known volume of the erythrocytes will be lysed with hypotonic phosphate buffer (pH 7.4). The hemolysate will be separated by centrifugation at 2500 r.p.m for 15 min at 2°C.

III) Estimation of lipid profile
Total cholesterol, triglycerides, HDL, LDL, specific levels will be determined by fully automated clinical chemistry analyzer. VLDL level will be calculated according to (Friedewald et al., 1972).

RESULTS AND DISCUSSION
I) Lipid Profile
The present study comprises 10 normal healthy controls and 10 chronic smokers. The age ranges from 25 to 45 years. The results are mentioned in Table No.1. The mean cholesterol level in controls was 180.02±24.78mg%. In smokers 5-10 years, the values were 217.05±17.24 mg%. The level of cholesterol was significantly increased in smokers compared with control (Non-smokers). The mean serum total cholesterol in non-smokers was 164.1 ± 20.26 mg/dl while it was significantly higher in smokers, i.e., 181 ± 28.10 mg/dl. These observations are in tune with the findings of other workers (Rustogi et al., 1989). Cigarette smoking substantially increases the risk of coronary heart disease and ischaemic stroke. The total cholesterol values in subjects smoking 1-10 cigarettes/bidis per day was 176.45 ± 29.17 mg/dl and those smoking 11-20 cigarettes/bidis per day was 186.15 ± 34.19 mg/dl. These findings are in accordance with those of other worker (Muscat JE, 1991). The mean triglyceride level in controls was 122.82±25.49 mg/dl. In smokers 5-10 years, the value was 145.2±17.27mg/dl. Cigarette smoking has been found to increase the concentrations of triglycerides as compared to control (Non-smokers). The mean serum triglycerides levels in non-smokers and smokers were 129.10 ± 31.60 mg/dl and 173 ± 56.65 mg/dl respectively. These findings are similar to those observed by (Wynder et al., 1989). The values of serum triglycerides and total cholesterol were significantly higher in those subjects smoking. The mean HDL-C level in controls was 42.57±5.74mg/dl. In smokers 5-10years the value were 32.72±6.64mg/dl. The level of HDL-C was significantly decreased in all smokers compared with control. The mean HDL-C in non-smokers was 46.65 ± 5.18 and 43.8 ± 5.12 in smokers respectively. This finding is similar to that of (Rosenson et al., 1993) who reported that there is fall in HDL-C level by 3-5 mg/dl in smokers. Further, the subjects smoking 11-20 cigarettes/bidis per day had significantly low HDL-C (41.2 ± 5.80 mg/dl) as compared to those who smoked 1-10 cigarettes/bidis per day (44.6 ± 6.09 mg/dl). Similar findings have been reported by (Brischetto et al., 1983). The mean LDL-C level in controls was 112.50±26.53mg/dl. In smokers 5-10 years, the values were 158.26±30.52mg/dl. The level of LDL-C was significantly raised in smokers compared with control. The mean VLDL-C level in controls was 24.59±5.09mg/dl. In smokers 5-10 years the value was 29.04±3.45mg/dl. There was an increase in VLDL-C level of smokers compared with control. The mean LDL-C and VLDL-C
values in non-smokers were $87 \pm 17.80$ mg/dl and $18.6 \pm 1.5$ mg/dl respectively. But these values were significantly higher in subjects smoking 11-20 cigarettes/bidis per day as compared to those smoking 1-10 cigarettes/bidis per day.

**Table No.1. Lipid Profile: Control and Smokers**

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n=10)</td>
<td>$180.02 \pm 24.78$</td>
<td>$122.82 \pm 25.49$</td>
<td>$42.57 \pm 5.74$</td>
<td>$112.50 \pm 26.53$</td>
<td>$24.59 \pm 5.09$</td>
</tr>
<tr>
<td>Smokers (5-10 yrs) (n=10)</td>
<td>$217.05 \pm 17.24$</td>
<td>$145.2 \pm 17.27$</td>
<td>$32.72 \pm 6.64$</td>
<td>$158.26 \pm 30.52$</td>
<td>$29.04 \pm 3.45$</td>
</tr>
</tbody>
</table>

**MDA and Enzymatic Antioxidants:**
The mean MDA level in controls was $4.42 \pm 0.35$ nmol/ml. In smokers 5-10 years the value was $11.86 \pm 1.02$ nmol/ml. The level of MDA was significantly increased in smokers compared with control. In the present study it is observed that mean levels of MDA was significantly higher in all group of smokers compared to controls. The present findings are in accordance with the study of (Venkateshan A, et al., 2006). The rise in MDA level is due to free radicals present in cigarette smoke which leads to increased lipid peroxidation. Free radicals generated oxidize the membrane phospholipids. The major site of free radical attack is polyunsaturated fatty acids in cell membranes, producing lipid peroxidation which generates hydro peroxides and long lived aldehydes. The end product of this reaction is malondialdehyde, which is increased in chronic smokers. The results are mentioned in **Table No.2.**

**Table No.2. MDA and Enzymatic Antioxidants: Controls and Smokers**

<table>
<thead>
<tr>
<th></th>
<th>MDA (nmol/ml)</th>
<th>Superoxide dismutase (IU/gm of Hb)</th>
<th>Catalase (IU/gm of Hb)</th>
<th>Glutathione Peroxidase (IU/gm of Hb)</th>
<th>Glutathione Reductase (IU/gm of Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n=10)</td>
<td>$4.42 \pm 0.35$</td>
<td>$845.32 \pm 84.67$</td>
<td>$8.49 \pm 2.27$</td>
<td>$23.15 \pm 1.82$</td>
<td>$9.12 \pm 0.82$</td>
</tr>
<tr>
<td>Smokers (5-10 yrs) (n=10)</td>
<td>$11.86 \pm 1.02$</td>
<td>$402.93 \pm 109.36$</td>
<td>$2.58 \pm 0.62$</td>
<td>$12.54 \pm 1.53$</td>
<td>$3.41 \pm 0.92$</td>
</tr>
</tbody>
</table>
The mean superoxide dismutase level in controls was 845.32±84.67 IU/ml. In total smokers, smokers 5-10 years the value were 402.93±109.36 IU/ml. The level of superoxide dismutase was significantly decreased in smokers compared with control. Our findings are in accordance with the findings of (Sharma SB, et al., 2005. Garg et al., 2006) attributed the cause of the decrease in SOD level to be by the inactivation of SOD by hydrogen peroxide. The mean catalase level in controls was 8.49±2.27IU/ml. In smokers 5-10 years the value was 2.58±0.62 IU/ml. The level of catalase was significantly decreased in smokers compared with control. Our findings are in accordance with the findings of (A Hemalatha et al., 2006) have shown an increase in catalase level in their study. (Durak et al., 1999) has shown catalase value to be unchanged. According to (Garg et al., 2006) the increase in catalase activity might be caused by the fact that for a given concentration of catalase, the initial rate of hydrogen peroxide removal is directly proportional to the hydrogen peroxide concentration. The mean glutathione peroxidase level in controls was 23.15±1.82 IU/ml. In total smokers, smokers 5-10 years the values were 12.54±1.53 IU/ml. The level of glutathione peroxidase was significantly decreased in smokers compared with control. Our findings are in accordance with the findings of (Kocyigit A, et al., 2001). Since glutathione peroxidase reduce hydrogen peroxides, the level falls in chronic smokers in whom there is continuous generation of peroxides. The mean glutathione reductase level in controls was 9.12±0.82 IU/ml. In smokers5-10years, the value was 3.41±0.92 IU/ml. The level of glutathione reductase was significantly decreased in smokers compared with control. Our findings are in contrary to the findings of (Solak ZA, et al., 2005). The variation in the level of lipid profile and antioxidant enzymes from normal values causes several diseases such as Lung cancer, other cancers, heart disease, and stroke and has numerous immediate health effects on the brain and on the respiratory, cardiovascular, gastrointestinal, immune systems. So from the present study it has been concluded that we have to restrict the use of cigarette smoking and alcohol consumption because it is injurious to heath and cause several diseases.

REFERENCES


