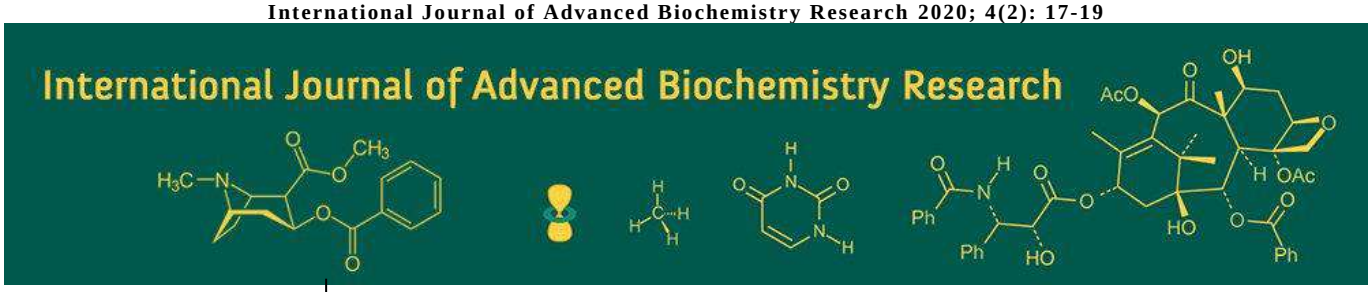

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Lipid peroxidation and antioxidant status in cigarette smokers ischemic heart disease (IHD) patients

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Abstract
Introduction: A close relationship between smoking and atherosclerosis has been proposed by several workers. This analysis was taken up to ascertain the effects of smoking on cholesterol esterification and variations in lipid, lipoprotein profile, lipid peroxidation and antioxidant status leading to cardiac risk which could be explained by proposed mechanism of effect of nicotine on enzymes of lipid metabolism.
Methods: The study group consisted of 50 smokers and 50 healthy individuals aged between 35-65 years. During the course of study, some smokers presented with clinical signs and symptoms of ischemic heart disease. ECG of the individuals of the study group was done and accordingly they were classified as smokers with and without IHD, Lipid peroxidation, antioxidant status, Serum Total Cholesterol and its fractions, Triglyceride, HDL, LDL and VLDL were estimated. Out of the total number of smokers (n=50) involved in the study, 22 individuals presented with changes in their ECG pattern diagnostic of IHD (44% conversion rate). Most of these individuals who presented with changes in ECG pattern of IHD were chain smokers (heavy smokers) or individuals smoking for more than 30 years. All of these individuals were non-diabetic.
Results: The level of Serum Cholesterol, triglycerides, VLDL, LDL, hs-CRP and MDA were found to be significantly raised in smokers while HDL cholesterol, ratio of esterified cholesterol to free cholesterol and Glutathione was significantly decreased (p<0.001). This impact was directly proportional to increase in the number of cigarettes smoked and duration of smoking.

Keywords: smoking, lipid peroxidation, mechanism, IHD

Introduction
In the developed and developing countries like India, Tobacco consumption is on the increase may be as a status symbol in affluent society or because of addition in lower strata. The death rate is increasing day by day. 40 to 60% of individuals below the age of 40 years who suffered from heart disease include chain smokers (heavy smokers). Several epidemiological studies indicated a close relationship between cigarette smoking and atherosclerosis, considering smoking as a potential risk factor for Ischemic heart disease and the one in which cessation has been shown to reduce the risk. High sensitive C-reactive protein (hs-CRP) is one of the major acute phase proteins produced by the liver in many inflammatory conditions [1-5] and is a marker of systemic inflammation. Oxidative stress has been implicated in the pathogenesis of Ischemic Heart Disease. One of the most frequently used biomarkers providing an indication of the overall lipid peroxidation level is malondialdehyde (MDA). There are a number of antioxidants present in the body derived from the diet. Main non-enzymatic cellular antioxidant is reduced glutathione (GSH). Significantly raised Serum Cholesterol, Triglyceride, LDL and VLDL and decreased HDL levels in smokers have been reported by several workers [6-8]. On the contrary few workers have observed no significant rise in Cholesterol or Triglyceride level [9, 10] or decrease in HDL level [11] in smokers.
In view of this, present study was undertaken to ascertain the role of smoking in deranging lipid profile, increased oxidative stress, including direct damage by radical species and the inflammatory response which further leads to cardiac risk in some smokers.

Materials and Methods
The present study was carried out in Govt. Medical College, Jalaun at Department of Biochemistry. The Subjects included for study was 50 smokers and 50 normal individuals

aged between 35-65 years. A detailed history of all the normal individuals and smokers was taken. All the subjects were clinically examined and ECG was done before the start of study of assess cardiac functions.

Duration of smoking was counted from the time when person started smoking regularly. During the course of study, 22/50 smokers showed changes in their electrocardiogram and Clinical Diagnosed as cases of IHD. This prompted us to classify the total smokers (n=50) in to smokers without IHD (n=28) and with IHD (n=22) so as to assess the impending cardiac risk in apparently healthy smokers.

Venous blood samples were collected after overnight fasting. Serum hs-CRP (Immunoturbidimetry), MDA (Thiobarbituric acid reactive substance TARBS), GSH (Ellman's method), Serum Total Cholesterol and its Fractions (Zak *et al*) [12], Serum Triglyceride (McGowan *et al*) [13], Serum HDL (Burstein *et al*) [14], Serum LDL (Bates *et al*) [15] and VLDL (Lowenstein *et al*) [16] were estimated.

Statistical Analysis

The statistical analysis performed using SPSS. The results are shown as mean ±SD (Standard Deviation) and median (range). The data was analyzed using the student's t-test. The p value of <0.05 was denoted as statistically significant.

Results and Discussions

The levels of hs-CRP, MDA and GSH in various groups have been presented in (Table I). Serum Cholesterol, Triglyceride, and ratio of Cholesterol ester to free Cholesterol, HDL, LDL and VLDL in various groups have been presented in (Table II).

hs-CRP levels were significantly elevated across the all groups (Table I) hs-CRP was significantly elevated in both the group of smokers with and without, when compared to controls. Moreover, hs-CRP elevation in smokers with IHD was significant when compared to smokers without IHD.

These findings suggest that increase in hs-CRP levels was more pronounced in smokers with IHD patients when compared to smokers without IHD and controls. (Table I)

Moreover, significantly elevated MDA were observed in smokers with IHD when compared to smokers without IHD. (Table I)

GSH were significantly lower in both the groups of smokers with and without IHD when compared to controls. (Table I).

Smoking as a risk factor for CAD is closely associated with increased oxidative stress, and the number of cigarettes smoked plays an important role in increasing the level of oxidative damage and reducing antioxidant defence. [21]

Raised, but not very significant, levels of cholesterol were observed in smokers without IHD as compared to non-smokers (p<0.05, table II), while smokers having IHD had significantly increased cholesterol levels as compared to smokers without IHD (p<0.001, table III). Ratio of Cholesterol ester to free cholesterol was significantly decreased in all groups of smokers (p<0.001, table II) as compared to normal individuals indicating the effect of nicotine inhalation on Esterification of Cholesterol by LCAT enzyme in Plasma. These findings are consistent to those of Kulkarni *et al* [17].

Serum Triglyceride levels were found to be raised, very significantly, in each category of smokers (p<0.001, table II) may be due to the fact that nicotine delays the gastric emptying time and also causes and increase in the secretion of nor-adrenaline which activates lipoprotein lipase of adipose tissue resulting in lipolysis and more production of free Triglyceride, Supporting the proposed mechanism of action of inhaled nicotine by Elkeles *et al* [18], Muscat *et al* [19], Simon LA [20] and NS Neki [8]. Pattern of VLDL levels were found to be similar to that of Triglyceride in all groups of smokers as compared to Non-smokers (Table I).

A Significant increase in LDL levels had also been observed in all categories (p<0.001, Table II) of smokers as compared to Non-smokers. The cause of raised Serum LDL in smokers may be that virtually all VLDL in human is converted to LDL by the process in which IDL is the intermediate product. The process is catalyzed by lipoprotein lipase, the action of which is enhanced by nicotine inhalation, as suggested earlier. A very significant decrease in HDL levels virtually in all categories of smokers (p<0.001, table II) makes it the most important parameter to check deranged lipid profile and hence diagnosing the impending cardiac risk to smokers [22-23]. A significant decrease in HDL even in case of mild smokers is really alarming and indicates towards the need of getting lipid profile checked regularly. The decrease in HDL may be due to improper handling of oral fat by nicotine inhalation, which affects gastric emptying time and due to effect of nicotine on LCAT activity.

As suggested, normally LCAT bound to HDL-3 converts' free cholesterol to esterified cholesterol which creates a concentration gradient and free cholesterol is drawn from tissues for transport to excretory system in the liver by anti-atherogenic HDL-2.

Since nicotine hampers LCAT activity, HDL-3 decreases with a concomitant decrease in HDL-2. So scavenging action of HDL is disturbed, resulting in an increased risk of atherosclerosis as proved by significantly decreased levels of HDL-C also in smokers without IHD in our study.

Table 1: hs-CRP, MDA and GSH level in non-smokers, total smokers with and without IHD

Individuals	hs-CRP (mg/L)	MDA (µmol/L)	GSH (mg/dl)
A Normal n=50	0.4 (0.2-0.6)	0.9 ± 0.4	13 (11.6-18.8)
B Total n= 50 smokers	0.8 (0.7-1.0)*	3.3 ± 1.0*	7.7 (6.5-8.4)*
C Smokers without IHD n=28	0.7 (0.6-1.1)*	3.5 ± 1.1*	8.1 (7.1-9.6)*
Smokers with IHD n=22	1.1 (0.7-1.8)*	3.9 ± 1.0*	7.5 (6.4-8.7)*

The data were expressed as mean ±SD. The data was analyzed using the student's, t-test.* indicates p<0.05 and statistically significant.

Table 2: Lipid and lipoprotein level in non-smokers, total smokers with and without IHD

Individuals	Total Cholesterol	CHOL. Ester/Free CHOL.	TRI-GLYCERIDE	Lipoprotein		
				VLDL	LDL	HDL
A Normal n=50	191.4 ± 29.34	7.0 ± 0.55 3.0 ± 0.55	121.19 ± 25.6	24.24 ± 5.12	115.95±23.57	51.26±8.31
B Total n= 50 smokers	211.34±58.02	5.7 ± 0.55: 4.3 ± 0.55	167.5 ± 84.13	33.5 ± 16.82	141.41 ± 53.1	36.99±12.04
C Smokers without IHD n=28	200.6±51.24*	5.9 ± 0.55: 4.1 ± 0.55	143.3 ± 47.81	28.66±9.56	131.94±54.6	40.9±10.28
Smokers with IHD n=22	234.61±67.35*	5.5 ± 0.43: 4.5 ± 0.43	220.37± 119.73	44.06±23.94	162.08±45.37	26.26±8.42*

The data were expressed as mean ±SD. The data was analyzed using the student's, t- test. * indicates $p<0.05$ and statistically significant.

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