Research Paper

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Correlation Between Lipid Profile, Lipid Peroxidation and Enzymatic Antioxidants in Chronic Smokers - a Case Control Study in Rewa (Madhya Pradesh)

Medical Science

KEYWORDS : Chronic smoker, enzymatic antioxidants, lipid profile, malondialdehyde

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ABSTRACT

The aim of the present study is to assess the association between smoking and the alteration in plasma concentration of lipid profile, lipid peroxides and enzymatic antioxidants. 45 smokers (study group) and 45 age matched non-smoker (control group) were enrolled on the basis of inclusion and exclusion criteria. Smokers (study group) further subdivided according to duration of smoking i.e. smoking 15-20 cigarettes or bidis/day for <5 year, 15-20 cigarettes or bidis/day for 5-10 years and 15-20 cigarettes or bidis/day for >10 years) for study group. Plasma levels of lipid profile (Total Cholesterol, Triglyceride, HDL-C, LDL-C and VLDL-C), malondialdehyde (a lipid peroxidation product) and enzymatic antioxidants (superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase) were estimated. In smokers the levels of total cholesterol, triglyceride, LDL-C, VLDL-C and malondialdehyde were significantly elevated while HDL-C, catalase, glutathione reductase, glutathione peroxidase and superoxide dismutase were significantly reduced when compared with the controls. These findings indicate that smokers are at a pro- atherogenic state and as in other countries; in India smokers require particular attention in terms of public health interventions.

Introduction:

Cigarette smoking is a serious health problem and most important avoidable cause of death in world. Worldwide more than 8 million people die each year from smoking, half of them are of age <60 years. The first major study associated with smoking predicted risk of cardiovascular disease was published in the year 1985. This was subsequently confirmed by numerous epidemiologic studies. Smoking is now considered as one of major risk factor for atherosclerosis.[1] Incidence of myocardial infarction in heavy smokers is three times more common compared to non-smokers. In India the effects of smoking could be even more harmful than western countries because of wide spread practice of smoking bidis which is more harmful than cigarettes.[1,2,3] More recent concern has been raised about the possible hazardous effects of passive smoking. Approximately 30% increase in risk of death from ischemic heart disease or myocardial infarction is noted in passive smokers.[4,5]

Several steps have been taken all over the world through an extensive mass media propaganda hereby public have been warned to possible health hazard of smoking. In USA it has already stared yielding results. Situation in India is not encouraging. The famous slogan **"CIGARETTE SMOKING IS INJURIOUS TO HEALTH"** found on every cigarette pack is only confined to cigarette pack; no one is concerned about it.

The harmful effects of smoking are caused by change in lipid metabolism and serum levels of total cholesterols, LDL cholesterols, triglyceride and decreased level of antiatherogenic HDL cholesterol. These dyslipidaemic changes have been identified as major risk factors for development of atherosclerosis.[1] MDA (malondialdehyde) is considered as biomarker of lipid peroxidation. Superoxide dismutase is a naturally occurring enzyme that protects the body against active oxygen free radicals by scavenging excess superoxide. Superoxide dismutase has particular value as an antioxidant that can help to protect against cell destruction.[6] The glutathione peroxidase enzyme takes part in a system that converts intra cellular free radicals into less reactive or neutral components.[7] **Superoxide dismutase**, catalase and glutathione reductase convert hydrogen peroxide and scavenge lipid hydro-peroxides.[8]

Smoking is very common in Madhya Pradesh, especially in Rewa districts as tobacco is grown extensively in this area. For this reason, present study was conducted to evaluate the association of smoking with levels of malondialdehyde (a product of lipid peroxidation), lipid profile and enzymatic antioxidants in blood.

Materials and Methods:

The study was started after approval of protocol from Institutional Ethical Committee of Shyam Shah Medical College, Rewa (M.P.). Informed consent was obtained from each participant. Informed consent was obtained from each participant.

Criteria for selection of participants:

Inclusion criteria:

1. Smokers/non-smoker individual

Exclusion criteria:

1. Malnourished individual
2. Pulmonary disorder
3. Coronary artery diseases
4. Anemia
5. Diabetes mellitus
6. Hypertension
7. Any other systematic disorders

Participants were enrolled on the basis of inclusion and exclusion criteria. The study comprised 90 participants, of which 45 healthy non smokers (control group) and 45 smokers (study group). Smokers further subdivided according to duration of smoking i.e. smoking 15-20 cigarettes or bidis/day for <5 year, 15-20 cigarettes or bidis/day for 5-10 years and 15-20 cigarettes or bidis/day for >10 years) for study group. All participants were in the age group of 30–60 years.

Biochemical parameters used for analysis are: Malondialdehyde, Superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase, total cholesterol, triglycerides, HDL, LDL and VLDL. Results are analyzed by "unpaired student t-test" to compare the mean blood level of control group with the study group.

Observations:

Table – 1: Effect on Lipid Profile - Control and Study group

Groups → Lipid Profile ↓	Controls (n = 45)	Smokers (<5 years) (n = 15)	Smokers (5–10 years) (n = 15)	Smokers (>10 years) (n = 15)
Cholesterol (mg %)	180.02±24.78	184.18±25.22 (p=NS)	204.70±24.77 (p<0.005)	217.05±17.24 (p<0.0001)
Triglyceride (mg %)	122.82±25.49	124.8±26.50 (p=NS)	128.8±21.27 (p=NS)	145.2±17.27 (p=NS)

HDL-C (mg%)	42.57±5.74	38.42±5.56 (p=NS)	35.15±7.69 (p<0.001)	32.72±6.64 (p<0.0001)
LDL-C (mg %)	112.50±26.53	118.91±28.39 (p=NS)	143.68±27.39 (p<0.002)	158.26±30.52 (p<0.0001)
VLDL-C (mg %)	24.59±5.09	24.96±5.03 (p=NS)	25.83±4.25 (p=NS)	29.04±3.45 (p=NS)

Values in mean±SD

NS= non significant

Table – 2: Effect on Lipid Profile - Control and Study Group (Total)

Groups → Lipid Profile ↓	Control group (n = 45)	Study Group (n = 45)	p-value
Cholesterol (mg %)	180.02±24.78	201.98±26.08	(p<0.0001)
Triglyceride (mg %)	122.82±25.49	132.93±23.28	(p<0.05)
HDL-C (mg%)	42.57±5.74	35.43±6.95	(p<0.0001)
LDL-C (mg %)	112.50±26.53	140.28±32.58	(p<0.0001)
VLDL-C (mg %)	24.59±5.09	26.61±4.65	(p<0.05)

Table – 3: Effect on Malondialdehyde (MDA) and Enzymatic Antioxidants - Control and Study Group

Groups → MDA and enzymatic antioxidants ↓	Controls (n = 45)	Smokers (<5 years) (n = 15)	Smokers (5–10 years) (n = 15)	Smokers (>10 years) (n = 15)
Malondialdehyde (nmol/ml)	4.42±0.35	6.25±1.05 (p<0.0001)	7.99±0.79 (p<0.0001)	11.86±1.02 (p<0.0001)
Catalase (IU/gm of Hb)	8.49±2.27	9.59±1.70 (p<0.0001)	5.05±1.87 (p<0.0001)	2.58±0.62 (p<0.0001)
Glutathione reductase (IU/gm of Hb)	9.12±0.82	7.80±1.15 (p<0.0001)	5.32±1.60 (p<0.0001)	3.41±0.92 (p<0.0001)
Superoxide Dismutase (IU/gm of Hb)	845.32±84.67	671±88.37 (p<0.0001)	492±76.98 (p<0.0001)	402.93±109.36 (p<0.0001)
Glutathione peroxidase (IU/gm of Hb)	23.15±1.82	15.82±2.40 (p<0.0001)	15.16±0.88 (p<0.0001)	12.54±1.53 (p<0.0001)

Table – 4: Effect on MDA and Enzymatic Antioxidants level: Control and Study group (Total)

Groups → MDA and enzymatic antioxidants ↓	Control group (n = 45)	Study Group (n = 45)	p-value
MDA (nmol/ml)	4.42±0.35	8.70±2.55	(p<0.0001)
Catalase (IU/gm of Hb)	8.49±2.27	5.74±3.28	(p<0.0001)
Glutathione reductase (IU/gm of Hb)	9.12±0.82	5.51±2.19	(p<0.0001)
Superoxide Dismutase (IU/gm of Hb)	845.32±84.67	522.25±144.55	(p<0.0001)
Glutathione peroxidase (IU/gm of Hb)	23.15±1.82	14.51±2.21	(p<0.0001)

STATISTICAL ANALYSIS:

Values of the biochemical assay were given as mean ± standard deviation (SD) and were statistically analyzed using "unpaired student t-test". P-value is calculated with help of statistic software. $P < 0.01$ was considered to be significant.

DISCUSSION:

Smoking is major risk factor for coronary artery disease. It is preventable cause of morbidity and mortality. Many investigators proved that cigarette smoking is one of the major cause for atherosclerosis. The degree of atherosclerosis is directly associated with number of cigarette smoked and duration of smoking. The multiple chemical constituent present in cigarette smoke have multiple effects on different parameters. In the present study parameters like malondialdehyde (MDA), catalase, glutathione reductase, glutathione peroxidase, superoxide dismutase, serum cholesterol, triglyceride, HDL-C, LDL-C, VLDL-C are being discussed to correlate them with the controls and finding of other studies.

Malondialdehyde:

The mean level of MDA in control group and study group was in the range of 4.42±0.35 nmol/ml and 8.70±2.55 nmol/ml respectively. Among the study subgroups, smokers <5 years, smokers 5-10 years and smokers >10 years, it was in the range of 6.25±1.05 nmol/ml, 7.99±0.79 nmol/ml and 11.86±1.02 nmol/ml respectively.

In the present study, mean levels of MDA significantly higher in all study subgroups as compared to control group. The present findings are in accordance with the study of I. Rahaman and W. MacNEE,[9] MD Stringent et al.,[3] A. Venkateshan et al.[10] and Sharma S. B. et.al.[11]

The rise in MDA level is due to free radicals present in cigarette smoke which leads to increased lipid peroxidation. According to I. Rahaman and MacNEE[9] all tissues are vulnerable to cigarette smoke. Free radical oxidizes 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 aldehyde. The end product of these reactions is malondialdehyde, which is increased in chronic smokers.

Catalase:

The mean catalase level in control and study group is 8.49±2.27 IU/ml and 5.74±3.28 IU/ml respectively.

Among the study subgroup, smokers <5 years, smokers 5-10 years and smokers > 10 years, it was in the range of 9.59±1.70 IU/ml, 5.05±1.87 IU/ml and 2.58±0.62 IU/ml respectively. The level of catalase was significantly decreased (P<0.001) in study group as compared to control, except for the subgroup of smokers <5 years. Our findings are in accordance with the finding of Zhou JF et al.[12], A Hemalatha et. al.[13], Yildiz L et al.[14] and B. Yokus et al.[15].

Toth K M et al.[16] and Garg N. Singh et al.[17] have shown an increase in catalase level in their study. Kocyigit A et al.[18], Durak I. et al.[19], and Alejandro D Bolzen et al.[20] have shown catalase value to be unchanged.

According to N. Garg et al.[17] the increase in catalase activity might be caused the fact that for a given concentration of catalase, the initial rate of hydrogen peroxide removal is directly proportional to the hydrogen, a particular concentration of hydrogen peroxide. Below the threshold concentration, catalase has no role to play. Thus an increase in the catalytic activity in smokers may be caused by high level of hydrogen peroxide formation. B Yokus et al.[17] showed a reduced catalase activity, may be due to oxidative inactivation of enzyme protein.

Glutathione Reductase:

The mean glutathione reductase level in controls and study group was 9.12±0.82 IU/ml and 5.51± 2.19 IU/ml. respectively. Among the study subgroups, smokers <5 years, smokers 5-10 years and smokers >10 years, it was in the range of 7.80 ±1.15 IU/ml. 5.32±1.60 IU/ml. and 3.41±0.92 IU/ml. respectively.

The level of glutathione reductase was significantly decreased

(p<0.001) in all study subgroups as compared with control. Our findings are contrary to the finding of Solak ZA et al.[21]

Total Cholesterol:
The mean cholesterol level in control and study group was 180.02±24.78 mg% and 201.98±26.08 mg% respectively. Among the study subgroups, smokers < 5 years, smokers 5-10 years and smokers >10 years, it was in the range of 184.18±25.22 mg%, 204.70±24.77 mg% and 217.05±17.24 respectively. The level of cholesterol was significantly increased (p> 0.001) in all the subgroups of smokers compared with control except the subgroup of smoker <5 years. The association of total cholesterol level with smoking is indeed very prominent. The findings of the present study are in accordance with study of M. Khurana et al.[22], Craig W.Y. et al.[23], Axelson M. et al.[24] and Sharma S.B. et al.[11]. Cholesterol have been shown to be unchanged in the studies of Vani Gupta et al.[25], Cheryl S.B. et al.[26] and Mjos et al [27].

Triglycerides:
The mean triglyceride level in control and study group was 122.82±25.49 mg% and 132.93±23.28 mg% respectively. Among the study subgroups, smokers <5 years, smokers 5-10 years and smokers >10 years, it was in the range of 124.8±26.50 mg%, 128.8±21.27 mg% and 145.2±17.27 mg% respectively.

The level of triglyceride was increased but not significant in smokers compared with control. Our findings are well co-related with Vani Gupta et al.[25], Craig W.Y. et al.[23], Axelson M. et al.[24] and Sharma S. B. et. al.[11]. Cholesterol have been shown to be unchanged in the studies of Cheryl S. B. et al.[26], Mjos et al.[27], Sinha A K et al.[28] and Yasue H et al.[1].

HDL – Cholesterol:
The mean HDL-C level in control and study group was 42.57±5.74 mg% and 35.43±6.95 mg% respectively. Among the study subgroups, smokers <5 years, smokers 5-10 years and smokers >10 years, it was in the range of 38.42±5.56 mg%, 35.15±7.69 mg% and 32.72±6.64 mg% respectively. The level of HDL-C was significantly decreased (P<0.001) in all the study subgroups compared with control, except for the subgroup of smokers <5 years.

Our findings are comparable with studies of M. Khurana et al.[22], Axelson et al.[24], Shinha A. K. et al.[28], Craig W Y et al.[23], Yasue H et al.[1] and Vani Gupta et al.[25].

Sharma SB et al.[11] have shown that there is no change in the level of HDL-C in chronic smokers compared with controls.

LDL – Cholesterol:
The mean LDL–C level in control and study group was 112.50 ± 26.53 mg% and 140.28 ± 32.58 mg% respectively. Among the study subgroup, smokers <5 years, smoker 5-10 years and smokers >10 years, it was in the range of 118.91 ± 28.39 mg%, 143.68 ± 27.39 mg% and 158.26 ± 30.52 mg% respectively. The level of LDL–C was significantly raised (p<0.001) in all the groups of smokers compared with control, except for the group of smokers <5 years.

The level of LDL-C in the present study is found to be accordance with the finding of Zimmerman M et al.[29], Walter W et al.[30], Vani Gupta et al.[25], Craig W.Y. et al.[23], Axelson M. et al. [24], Sharma S.B. et al.[11].

LDL-C have been shown to be unchanged in the studies of Cheryl S.B. et al.[26], Mjos et al.[27], Sinha A K et al.[28], Yasue H et al.[1] in chronic smokers compared with control. LDL-C deposited under endothelial cells of blood vessels which undergo oxidation by free radical release from endothelial cell. This attracts microphages. Microphages are then converted in to foam cells and promote atherogenic plaque formation.

VLDL - Cholesterol:
The mean VLDL–C level in control and study group was 24.59 ± 5.09 mg% and 26.61 ± 4.65 mg% respectively. Among the study subgroup, smokers <5 years, smokers 5-10 years and smokers >10 years, it was in the range of 24.96 ± 5.03 mg%, 25.83 ± 4.25 mg% and 29.04 ± 3.45 mg% respectively. The level of VLDL–C is increased in study group as compared to control group but not significantly.

Our findings are comparable with studies of Mjos O D et al.[27], Cheryl SB et al.[26], Vani gupta et al.[25] and M Khurana et al.[22] have shown that there is an increase in VLDL-C in smokers compare to control.

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