



Original Research Article

Impact on Malondialdehyde and Superoxide Dismutase in Smokers of North Indian Punjabi Population

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ABSTRACT

Background: Free radicals released by smoking play a major role for the pathophysiology of many diseases like arthritis, diabetes, cancer cardiovascular disease, etc. In India, smoking is increasing day by day particularly in the young generation. The risk of myocardial infarction is 5 to 6 times higher in people who smoke more than 20 cigarettes per day than non-smokers of similar age.

Aims: To evaluate the effects of cigarette smoking on oxidative stress markers such as malondialdehyde & superoxide dismutase along with lipid levels like total cholesterol, triglycerides, high density lipoprotein-cholesterol, low density lipoprotein-cholesterol and very low density lipoprotein-cholesterol as the study in cigarette smoking in North Indian Punjabi population is scant.

Materials and Methods: A case control study comprising of 100 subjects (50 cigarette smokers and 50 healthy non smokers as a control group) from the general population of male in a rural community and the levels of malondialdehyde, antioxidant enzyme superoxide dismutase along with complete lipid profile were evaluated in North Indian rural Punjabi population.

Results: The levels of total cholesterol, triglycerides, low density lipoprotein-cholesterol and very low density lipoprotein-cholesterol were significantly increased in cigarette smokers while the levels of high density lipoprotein-cholesterol were significantly decreased with respect to normal healthy non smokers. A significant increase was observed in malondialdehyde concentrations whereas the superoxide dismutase levels were significantly decreased in cigarette smokers with respect to healthy non smokers.

Conclusions: These observations suggested that smoking induced the hyperlipidemia and oxidative stress by increasing MDA levels and lowering superoxide dismutase enzymes activity, hence might be responsible for the initiation of cardiovascular diseases/atherosclerosis.

Key Words: Smoking, Acute Myocardial Infarction (AMI), Malondialdehyde (MDA), Oxidative stress (OS), Superoxide Dismutase (SOD) and Atherosclerosis.

INTRODUCTION

Smoke emanating from burning cigarette, cigar, or pipe tobacco, inhaled either directly by the individual smoking or passively by those in close contact with smoker, Cigarette smoke includes more than

4,000 chemical substances including polycyclic aromatic hydrocarbons and oxidative gases, most of which exert a cardiotoxic effect. The precise nature and the toxic mechanism of many of those substances have not been fully clarified.

Toxic products from cigarette smoke, in particular nicotine and carbon monoxide, circulate in the bloodstream, interfering with the efficient working of the endothelium and resulting in broad spectrum of adverse health consequences like cardiovascular diseases, respiratory tract, central nervous system, digestive tract and immunological dysfunctions. [1]

Cigarette smoke constitutes organic compounds or metal ions that act as electrophiles, free radicals, reactive anions or metal ions that act as reducing agents, or free radicals or metal ions that act as oxidizing agents. Reactive oxygen species (ROS) like superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^{\bullet}), malondialdehyde (MDA) and nitric oxide (NO) are generated, when mainstream cigarette smoke interacts with aqueous media or physiological fluids directly involved in multi stage process of carcinogenesis by bringing out a continuous endogenous damage to cellular DNA. [1-3] The cell and plasma contain a variety of antioxidants and free radical scavenging molecules and enzymes like superoxide dismutase (SOD), xanthine oxidase (XOD), glutathione peroxidase (GPx) and glutathione-S-Transferase (GST) etc., which under normal circumstances help the cell to maintain a reducing environment by preventing the potentially deleterious effects of free radical on cell membrane and organelles. Any changes in the activity of these enzymes and antioxidants like glutathione enhance oxidative stress and could lead to the development of non communicable diseases. [4]

Cigarette smoking has been responsible for approximately 140,000 premature deaths annually from cardiovascular diseases. In India smoking is increasing particularly in the young generation. Younger people who smoke more than 20 cigarettes per day have a 5.6

times greater risk of AMI than do non-smokers of similar age. [5] So, in the present work we studied the effects of smoking in the individuals by evaluating the oxidative stress markers MDA and SOD along with various fractions of lipids like total cholesterol levels, triglyceride levels, high density lipoprotein, low density lipoprotein and very low density lipoprotein in Smokers and Normal healthy non smoker individuals in North Indian Punjabi population.

MATERIALS AND METHODS

The present study was carried out in 100 subjects in the Department of Biochemistry, Govt. Medical College Amritsar in collaboration with Cardiology Ward of Medicine Department, Sri Guru Nanak Dev Hospital, Govt. Medical College Amritsar after obtaining the approval of the institutional thesis committee and ethics committee. The subjects for the present study were randomly selected from a population of male in a rural community. These subjects after obtaining the informed consent were interviewed for tobacco use and question on number of cigarettes smoked on average per day and when they started smoking and the subjects were divided in the following two groups:

Group-1: 50 Normal healthy non-smokers subjects (age range 20-50 years).

Group-2: 50 Smokers (age range 20-50 years).

50 normal healthy young male subjects, who smoked more than 10-20 cigarettes per day continuously for 5 years were recruited in Group-2 and 50 normal healthy subjects, who reporting no previous use of smoking/illegal drugs experience in the age range of 20-50 will recruited in Group-1.

Ethical Issues: The study protocol was approved by the institutional ethic committee. Study details & potential risks and benefits were explained to individuals taking part in the study and at least one

attendant. A written informed consent was obtained voluntarily from the subjects before entering into the study.

Inclusion and exclusion criteria:

Inclusion criteria for smokers (Group-2):

The individuals, who smoked regularly more than 10 cigarettes/beedis per day for 5 years were included in the present study.

Exclusion criteria for smokers (Group-2):

Subjects with hypertension or any Systemic disease like hypothyroidism, diabetes, renal failure etc. those on Drugs like β -blockers, Thiazides, Statins etc. and on diet restriction are excluded from the study.

Inclusion criteria for normal healthy non smokers (Group-1):

The subjects who never smoked or any major illness and non obese healthy individuals.

Exclusion criteria for normal healthy non smokers (Group-1):

The subjects on diet restriction, taking any drugs e.g. β -blockers, Thiazides, Statins etc. and the subjects with IHD, hypertension or any systemic disease e.g. diabetes, renal failure, hypothyroidism, hypertension etc. were excluded.

Collection and processing of blood sample:

Fasting blood sample were collected from both the groups in a dry disposable syringe under aseptic conditions by vein puncture in anticubital vein in a sterile dry, acid washed plain and sodium oxalate vials. The blood samples were centrifuged at 3000 rpm for 10 minutes for the separation of serum and plasma respectively for the estimation of various biochemical assays.

1. Total Cholesterol: Serum total cholesterol level was assayed by the method of Allain *et al.* 1974. [6]
2. Triglycerides: Serum TG level was estimated by using the method of McGowan *et al.*, 1983. [7]
3. HDL-Cholesterol – HDL-cholesterol was estimated by the method of Grillo and Izzo, 1985. [8]
4. Low-Density Lipoprotein (LDL)-Cholesterol: Serum LDL-cholesterol

was estimated from the primary measurements by using the empirical equation of Friedewald *et al.* 1972. [9]

LDL-cholesterol = Total cholesterol – (HDLcholesterol+ TG/5).

5. Malondialdehyde (MDA): Serum MDA levels were estimated by using the method of Satoh, 1978. [10]
6. Superoxide Dismutase (EC 1.5.1.1): Serum SOD levels were estimated by using method Marklund and Marklund, 1974 [11] modified by Nandi and Chatterjee, 1988. [12]

Statistical Analysis: The data was expressed as Mean \pm SD and analyzed with the SPSS 16.0.7 statistical software package. Differences between the smokers and non smokers as a control subjects were evaluated using the Student's independent samples *t* test. Differences were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

A significant increase was observed in the levels of total cholesterol, triglycerides, LDL-cholesterol and VLDL cholesterol from 177.60 \pm 8.78 to 233.54 \pm 10.24mg/dl ($P < 0.001$), from 110.48 \pm 14.98 to 167.34 \pm 9.14mg/dl ($P < 0.001$), from 110.0 \pm 9.05 to 149.0 \pm 12.35 mg/dl ($P < 0.001$) and from 22.09 \pm 2.99 to 33.46 \pm 1.82mg/dl respectively while the levels of HDL-cholesterol was significantly decreased from 51.50 \pm 4.87 to 40.98 \pm 5.00 mg/dl ($P < 0.05$) in smokers with respect to healthy non smoker (Table-2). All these observations suggested that hyperlipidemia observed in the present study. In 2002, Sacks and their colleges reported that 30 percent change in plasma lipid concentrations corresponds to a change in coronary risk of 7 percent for triglycerides versus 30 percent for LDL-cholesterol or HDL- cholesterol. In the present study, we observed 31.49 percent increase in serum total cholesterol, 51.46%

in triglycerides, 35.45% in LDL- cholesterol and 51.47% increase in VLDL- cholesterol while we observed 20.42% decrease in HDL- cholesterol in smokers with respect to healthy non smokers (Table-2), which suggested that ingestion of cigarettes for long time could induce hyperlipidemia.

Table- 1 Anthropometric analysis of smokers and non smokers

Anthropometric and Biochemical Assays	Group- 1 (Healthy non smokers)	Group- 2 (Smokers)
Height (cm)	158.12 ± 0.16 ^a	161.60 ± 0.11 (+2.20) ^{bNS}
Weight (kg)	68.00 ± 14	71.00 ± 10 (+4.41) ^{NS}
Age (years)	31.00 ± 5.00	33.00 ± 6.00 (+6.45) ^{NS}
Body mass index (Kg/m ²)	26.80 ± 0.48 ^a	27.39 ± 0.40 (+2.20) ^{NS}
Blood pressure systolic (mmHg)	128.15 ± 10.53	136.45 ± 12.53 (+6.47) ^{NS}
Blood pressure diastolic (mmHg)	85.01 ± 7.32	89.29 ± 8.74 (+5.04) ^{NS}
Hemoglobin (g/dl)	14.81 ± 1.16	13.89 ± 2.91 (-6.18) ^{NS}

a- Values are expressed as Mean ± S.D of 50 observations
 b- Values in parentheses represent percentage changes compared to normal healthy non smokers
 NS: Not significant

Hyperlipidemia observed in present work might be due to hyperinsulinemia, which caused a significant increase in VLDL levels in cigarette smokers. Hyperglycemia has been shown to increase the activity of lipoxygenase and lipid peroxidation products. Lipoxygenase metabolizes arachidonic acid to produce leukotriene and products that play an important role for initiating atherosclerosis by inducing oxidation of LDL and stimulating growth and migration of vascular smooth muscle cells. [13-15] Most of the cholesterol in the mature lesion originates from circulating LDL particles, the circulating LDL particles cross the endothelium into the intimal of blood vessels. In their native form they are unfavorable for uptake into intimal

macrophages and most return to the circulation. However, some particles may be oxidized by local cells possibly facilitated by the presences of transition metal ions and binding to proteoglycans. After oxidative modification the LDL particles are rapidly taken up into macrophages via the scavenger receptor. Subsequent loading with cholesteryl esters forms so called foam cells, [16] which could be responsible for the initiation of atherosclerosis.

Table-2 Alterations in serum lipid profile in smokers and healthy non smoker individuals.

Biochemical Assays	Group- 1 (Healthy non smokers)	Group- 2 (Smokers)
Total-Cholesterol (mg/dl)	177.60 ± 8.78 ^a	233.54 ± 10.24 (+31.49) ^{b**}
Triglyceride (mg/dl)	110.48 ± 14.98	167.34 ± 9.14 (+51.46) ^{***}
LDL-Cholesterol (mg/dl)	110.0 ± 9.05	149.0 ± 12.35 (+35.45) ^{**}
VLDL-Cholesterol (mg/dl)	22.09 ± 2.99	33.46 ± 1.82 (+51.47) ^{***}
HDL-Cholesterol (mg/dl)	51.50 ± 4.87	40.98 ± 5.00 (-20.42) [*]

a- Values are expressed as Mean ± S.D of 50 observations
 b- Values in parentheses represent percentage changes compared to normal healthy non smokers
 * P < 0.05, ** P < 0.01, *** P < 0.001

The levels of malondialdehyde, representing lipid peroxidation was found to be significantly increased in smokers by 63.81%, P<0.001 (from 1.99 ± 0.60 to 3.26 ± 0.89 nmol/ml) in comparison to healthy non smokers (Table- 3). Cigarette smoking might lead to an increased production of free radicals. The oxygen free radical thus produced would induce endothelial cell damage by causing peroxidation of membrane phospholipids. The first step in lipid peroxidation is the initiation reaction, which begins by taking out hydrogen atom from polyunsaturated fatty acids (PUFAs) by oxygen radicals. The second step is the propagation and the final step is termination. The extent of lipid peroxidation has often been determined by the thiobarbituric acid test, which has also been considered for the determination of malondialdehyde. A

significant increase in MDA levels in smokers observed in the present study might lead to susceptibility of the biomembrane, which finally leads to tissue injury/ damage. SOD, a superoxide radical scavenging enzyme is considered the first line of defense against the deleterious effect of oxygen radicals in the cells and it scavenges reactive oxygen radical species by catalyzing the dismutation of O₂⁻ radical to H₂O₂ and O₂. In mammals, three isozymes of SOD that is CuZn-SOD, Mn-SOD and extra cellular-SOD exists. [17] CuZn-SOD is located primarily in the cytosol. CuZn-SOD consists of two protein sub units each has an active site containing one Cu ion and one Zn ion. Cu ion serves as active redox site and Zn ion maintain the protein structure. Mn-SOD is located in mitochondrial matrix. [18] It has four subunits each with Mn ion. EC-SOD is present in plasma, bound to heparin sulfate ion the surface of endothelial cells. EC-SOD is tetrameric glycoprotein, which contains Cu and Zn ion. The presence of SOD in various compartments of our body enables it to dismutate O₂⁻ radicals immediately and protects the cells from oxidative damage. In 2009, Jain et al [19] reported that smoking of cigarettes lowers the levels of SOD as compared to non-smokers due to the utilization of these antioxidants for the scavenging of free radical generation, could be due to accounted on the basis of excess of carbon monoxide, tar and other toxic constituents present in the smoke of the cigarette/beedi. Many studies also explained that there was initial up-regulation and then rapid down-regulation of Mn SOD expression in the initially low-expressing bronchiolar epithelial cells in response to cigarette smoke. This could represent an acute protective mechanism against oxidant damage. [20-22] A significant reduction in SOD activity by 23.46% in cigarette smokers in the present study (Table-3) may

results in an increased flux of O₂⁻ radical and hence reflects the tissue damage/injury.

Table -3 Alterations in serum MDA and SOD levels in smokers and healthy non smoker individuals.

Biochemical Assays	Group- 1 (Healthy non smokers)	Group- 2 (Smokers)
Malondialdehyde (nmol/ml)	1.99 ± 0.60 ^a	3.26 ± 0.89 (+63.81%) ^{b***}
Superoxide Dismutase (U/ml)	4.73 ± 0.82	3.62 ± 0.70 (-23.46%)*

a- Values are expressed as Mean ± S.D of 50 observations

b- Values in parentheses represent percentage changes compared to normal healthy non smokers

* P < 0.05, *** P < 0.001

CONCLUSION

In conclusion, aforementioned observation suggested that smoking of cigarettes induced hyperlipidemia by altering the levels of lipids and also oxidative stress by further increasing MDA levels while lowering SOD activities in rural population of North Indian hence could act as initiator of cardiovascular diseases /atherosclerosis.

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