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EFFECT OF SMOKING ON LIPID PROFILE AND LIPID PEROXIDATION IN NORMAL SUBJECTS

A. VENKATESAN, A. HEMALATHA, ZACHARIAH BOBBY*, N. SELVARAJ AND V. SATHIYAPRIYA

[a]*Department of Biochemistry, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry – 605 006*

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Abstract : The aim of the present study was to assess the association between smoking and the alteration in plasma concentration of lipid profile and lipid peroxides. Fourteen smokers and 11 age matched control were enrolled. Plasma levels of fasting cholesterol, triglycerides, lipoprotein cholesterol and malondialdehyde were estimated. In smokers the levels of total cholesterol, LDL cholesterol, Non-HDL cholesterol and MDA were significantly elevated when compared with the controls. The atherogenic index as indicated by various risk ratios were also found to be increased in smokers as compared to controls. These findings indicate that current smokers are at a pro- atherogenic state and as in other countries, in India smokers require particular attention in terms of public health interventions.

Key words : lipid profile, malondialdehyde, atherogenic index, non-HDL cholesterol

INTRODUCTION

Smoking is an escalating public health problem especially in a developing country like India (1). Cigarette smoke is a dominant risk factor for premature or accelerated peripheral, coronary, and cerebral atherosclerotic vascular diseases (2–4). A one to threefold increase in risk of myocardial infraction (MI) has generally been noted among current cigarette smokers (5). The mechanism by which cigarette smoking causes MI remains obscure, but cigarette smoking have been found to alter the levels of lipoproteins (6–9).

Plasma lipoprotein abnormalities are said to be the underlying major risk factors and may even be essential for the common occurrence of atherosclerotic vascular diseases (10). Clinical, genetic, and epidemiological evidence indicates that elevated levels of low density lipoprotein (LDL) are one important risk factor for the disorder (11).

Oxidative pathway appears to be one important mechanism for modifying LDL, because a wide variety of structurally unrelated antioxidants inhibit atherosclerosis in animal models of hypercholesterolemia

*Corresponding Author : E-mail : zacbobby@yahoo.com; Tel.: +91-413-2273078, Fax : +91-413-2372067

(12, 13). *In vitro* and *in vivo* studies have implicated a number of potential oxidative pathways, including those depending on redox-active metal ions, ceruloplasmin, 15-lipoxygenase, and nitric oxide synthase (14–17). The physiological relevance of these pathways to the pathogenesis of human vascular disease remains poorly understood. However recently, there has been intense interest in the notion that lipid peroxidation is one of the pathological processes impairing the function of arterial endothelial cells (18).

Oxidative damage to unsaturated lipids is a well established general mechanism for oxidant mediated cellular injury (19). In addition to extensive experimental studies, increased lipid peroxidation has been reported in a wide variety of clinical and toxicological conditions (20). Cigarette smoke contains oxidizing substances among its > 4000 identified constituents (21), along with several compounds such as polyphenols that are potent antioxidants (22). Because oxidative modification dominates current ideology concerning the pathogenesis of atherosclerosis, many studies have focused on oxidative stress as a probable clinically relevant factor in cigarette smoke-related atherogenesis and cancer (23).

Present work is an attempt to determine the alterations in plasma fasting triglyceride, total cholesterol, HDL, LDL, VLDL, non-HDL cholesterol and the atherogenic indices as indicated by various risk ratios in a group of smokers. In addition, we have examined the effect of smoking on lipid peroxidation and the possible interaction of lipid peroxides with lipid profile.

MATERIAL AND METHODS

Fourteen healthy smokers (mean age 38.86 ± 4.35 years) were recruited in the study. Eleven healthy subjects (mean age 38.18 ± 3.37 years) were enrolled for the comparative assessment. Smokers were included if they had a history of smoking 10 or more cigarettes per day. No participant had a history of hypertension, diabetes mellitus or any other systemic disease predisposing them to endothelial dysfunction. Further exclusion criteria were, current use of antioxidants or vasoactive medications. All participants gave written informed consent and this protocol was approved by the Institutional research committee and human ethics committee.

Overnight fasting blood samples were taken by venipuncture in tubes containing EDTA. The samples were centrifuged (2500 × g 10 minutes at 4°C) and the plasma thus obtained were used for the estimation of lipid profile and MDA.

Malondialdehyde was measured using the established thiobarbituric acid (TBARS) method (24). This assay is based on the formation of red adduct in acidic medium between thiobarbituric acid and malondialdehyde, a colorless product of lipid peroxidation, measured at 532 nm. The MDA values were calculated using the extinction coefficient of MDA-thiobarbituric acid complex 1.56×10^5 $l \times mol^{-1} \times cm^{-1}$ at 532 nm and expressed as nmol/ml.

Plasma HDL cholesterol was estimated using Agappee diagnostic kits adapted to the 550 Express Plus auto analyzer (Ciba Corning Diagnostics, Oberlin, OH). Plasma

triglyceride and total cholesterol were estimated using the kits supplied by Accurex (Thane, India) and Bicon Diagnosemittel Productions (Germany), respectively in 550 Express Plus auto analyzer. LDL cholesterol was calculated by Friedwald's and Fredickson's formula.

Non HDL cholesterol is defined as the difference between total cholesterol and HDL cholesterol and includes all the cholesterol present in lipoprotein particles considered to be atherogenic. It was calculated as total cholesterol minus HDL cholesterol. Risk ratios were calculated as Total cholesterol/ HDL cholesterol, LDL cholesterol/HDL cholesterol, Non-HDL cholesterol/HDL cholesterol and these ratios multiplied by their respective MDA values (25).

Statistical analysis :

All variables are shown as the mean ± SD. The data between control and test groups was compared using unpaired student's *t* test. Correlation was determined by Pearson's correlation coefficient. The level of significance used was p less than 0.05.

RESULTS

The values of lipid peroxides and lipid profile are presented in Table I. Both the groups were comparable in age. There was a significant difference in the MDA, total cholesterol, LDL and Non HDL levels between the two groups. Plasma triglycerides and HDL cholesterol were not statistically different between the two groups.

The atherogenic index as indicated by various risk ratios are shown in Table II. The risk ratio calculated as total cholesterol /HDL cholesterol. LDL cholesterol/HDL cholesterol, Non HDL cholesterol/HDL cholesterol were significantly elevated in smokers when compared to controls.

Since increased lipid peroxidation is also a risk factor for MI, it has been suggested

TABLE I: Plasma lipid peroxides and lipid profiles in smokers and controls.

	Controls (n=11)	*Smokers (n=14)*	*'P' value*
MDA (nmol/ml)	5.48±0.37	6.47±0.35	0.001
Total cholesterol (mg/dl)	189.91±4.13	204.29±20.24	0.021
HDL cholesterol (mg/dl)	42.64±2.54	40.64±3.99	0.143
LDL cholesterol (mg/dl)	122.75±3.25	140.61±20.52	0.006
VLDL cholesterol (mg/dl)	24.71±2.17	23.70±3.27	0.365
Non HDL cholesterol (mg/dl)	147.27±4.29	162.93±20.80	0.016
Triglycerides (mg/dl)	123.55±10.83	118.50±16.35	0.365

n = number of subjects; Values are mean±SD; A 'P'<0.05 was considered significant.

TABLE II: Atherogenic index as indicated by various risk ratios.

	Controls (n=11)	*Smokers (n=14)*	*'P' value*
Total cholesterol/ HDL-C	4.47±0.25	4.98±0.72	0.022
LDL-C/HDL-C	2.88±0.21	3.44±0.69	0.011
Non HDL-C/HDL-C	3.47±0.25	3.89±0.69	0.049
(Total cholesterol/ HDL-C) × MDA	24.49±2.12	32.17±4.29	0.001
(LDL-C/HDL-C) × MDA	15.80±1.53	22.18±4.04	0.001
(Non HDL-C/ HDL-C) × MDA	19.00±1.86	25.07±4.03	0.001

n = number of subjects; Values are mean±SD; A 'P'<0.05 was considered significant.

that MDA values multiplied by risk ratios provides a new index which serves as a better predictor of MI. All the ratios multiplied by MDA values were significantly elevated in smokers when compared with controls.

No significant correlation was obtained between MDA and lipid profile in smokers.

DISCUSSION

Free radical mediated lipid peroxidation has been associated with the pathogenesis of many diseases and clinical conditions (26). Malondialdehyde (MDA) an end product of lipid peroxidation in plasma may come from three different sources: (a) circulating endogenous lipid peroxides, (b) MDA produced in platelets during prostaglandin H_2 and thromboxane (TXA_2) synthesis and (c) from other sources (27–29).

The increased level of MDA demonstrated by us is an evidence of intensification of lipid peroxidation processes in smokers which may cause chronic stress for endothelial cells (29). On the other hand, it can also reorientate enzymatic systems of the arachidonic acid cascade towards intensified TXA_2 synthesis (30). This increased lipid peroxidation in smokers are consistent with that reported by several authors (31, 32). An increased level of MDA has also been documented in smokers by Kharb et al (33).

Ample evidence exists to suggest that smoking influences plasma lipoprotein lipid concentrations and may thus mediate an important role in the development of atherosclerotic vascular disease (6–9).

In the present study, increased LDL cholesterol levels were found in smokers when compared with controls. Low density lipoprotein has a positive relationship to the risk of coronary heart disease. A plethora of evidence suggests that oxidation of low density lipoprotein generates potent pro-atherogenic mediators (12–17). Lipoprotein oxidation is presumed to occur in the artery and the specific cell type(s) or mechanism(s) that may generate superoxide radicals, hydrogen peroxide or lipid peroxides outside the cell may contribute to the oxidation of LDL (34). This result is in accordance to the report of Khavrana et al and Whig et al (35, 36). They found that smokers had higher values of LDL cholesterol compared with non-smokers. Contrary report to this has also been documented by Sirisali et al who found that total and LDL cholesterol did not vary between smokers and non smokers (37). This difference in observation can be due to ethnic differences in the study population. Tai et al have reported that in Asia the epidemiology of atherosclerosis has increased tremendously, with few ethnic groups being affected at particularly high rate (38). This difference in susceptibility has been attributed to the variation in genetic and epigenetic factors which can influence the lipid profile levels.

Non-HDL cholesterol has been found to be a better tool for screening and assessing the risk for atherosclerosis (10). In the present study, the level of non-HDL cholesterol level was significantly elevated in smokers when compared to controls. The atherogenic ratios that indicate the risk for the development of atherosclerosis were found to be significantly elevated in smokers when compared to controls.

The constellation of these altered lipoproteins along with lipid peroxides suggests that smokers are at a high risk for the development of coronary heart disease.

Cigarette smoking has been found to increase the concentrations of triglycerides and lowers the concentration of HDL cholesterol (39, 40). These changes were found to contribute towards the atherogenic potential of cigarette smoking. But in the present study we found no significant difference between smokers and controls with regard to the triglyceride and HDL cholesterol values. We also did not find any correlation between lipid peroxides with lipid profile. Previous study has shown a correlation between lipid peroxides with both total cholesterol and triglycerides in patients suffering from cardiovascular disorders (41). The limited number of subjects analyzed in the present study could be the reason for the lack of correlation between lipid peroxides with HDL cholesterol and triglycerides in smokers.

In summary the findings from the present study have both clinical as well as public health implications. For clinicians, our finding reemphasizes the point that individual lipid profile parameters may not provide the true picture of the risk of atherosclerosis. Antioxidant supplementation almost certainly has beneficial effects on modifying the apparently reversible events of atherosclerosis; yet smoking cessation should be the primary goal of risk factor modification in smokers. Future study with more number of samples would through more light to the relationship between MDA and other lipid profile, yet the results from the present study points to the fact that current smokers have an elevated risk for heart disease.

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