Research Journal of Medicine and Medical Sciences, 4(2): 151-159, 2009

Effect of Cigarette Smoking on Lipids and Oxidative Stress Biomarkers in Patients with Acute Myocardial Infarction

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Abstract: Cigarette smoke may promote atherogenesis by producing oxygen-derived free radicals that damage lipids. The present study was conducted to determine the effect of cigarette smoking on changes in lipid profile, lipid peroxidation and antioxidant status in stable ischemic heart disease (IHD) and acute myocardial infarction (MI). The study population contained 80 patients [MI (n=40) and IHD (n=40)] and 40 healthy subjects. Biochemical parameters such as cardiac markers creatin kinase (CK), creatine kinase isoenzyme MB (CK-MB), troponins T and I, lipid profile, lipid peroxidation thiobarbituric acid reactive substances (TBARS) and antioxidants (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), vitamin A, vitamin C and vitamin E) were measured. A highly significant increase in the levels of cardiac markers was found in MI patients when compared with stable IHD and control subjects. Enhanced lipid peroxidation with concomitant depletion of antioxidants was observed MI patients as compared to stable IHD and control subjects. The levels of serum total cholesterol, triglycerides, LDL and VLDL were found to be significantly high, while HDL was significantly low in MI patients compared to control subjects. In addition, the aforesaid biochemical parameters (cardiac markers, lipids, lipid peroxidation, antioxidants) were more significantly altered in smokers than non-smokers in the MI group. These results suggest that the atherogenic effects of smoking are mediated in part by free radical damage to lipids and possible breakdown of antioxidant status. The study also supports notion that cigarette smoking contributes significantly to cardiovascular morbidity and mortality.

Key words: Smoking- Myocardial infarction- Lipids- Lipid peroxidation- Antioxidant status

INTRODUCTION

Acute myocardial infarction (MI) is one of the major causes of mortality and morbidity in the world [1]. The most common cause of MI is atherosclerotic coronary artery disease with erosion or rupture of a plaque causing transient, partial or complete arterial occlusion. Heart cannot continue to function without adequate blood flow, and if it is severely compromised, death is inevitable. Several risk factors for coronary heart disease have been well documented, including hypertension, hyperlipidemia, diabetes, a positive family story, smoking, obesity and inactivity [2].

Evidence suggests that reactive oxygen species (ROS) may play important roles in the pathogenesis in myocardial infarction. Following ischemia, ROS are produced during reperfusion phase [3]. ROS are capable of reacting with unsaturated lipids and of initiating the self-perpetuating chain reactions of lipid peroxidation in the membranes [4,2]. Numerous reports have demonstrated the increased risk of coronary problems in smokers [5,6]. Smoking is thought to have an influence on the prevalence of myocardial infarction by means of several mechanisms, including atherosclerotic injury, increase in platelet aggregation, increase in the levels of adhesion molecules and fibrinogen and vasoconstriction [7]. Cigarette smoking leads to the uptake of many hazardous compounds. Such compounds or their metabolites may be electrophilic and thereby able to react with biological macromolecules, or they may give rise to oxidative stress by formation of reactive species or the initiation of radical chain reactions [8].

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Free radicals are highly reactive molecules generated by biochemical redox reactions that occur as a part of normal cell metabolism. The human body has an inherent synergistic and multilevel defense mechanism, which comprise of two major classes of cellular protection against ROS [9]. Free radical scavenger enzymes namely SOD, CAT and GPx represent the enzymatic part. The non-enzymatic part includes a large number of natural and synthetic antioxidant compounds (GSH and vitamins) that have the ability to inhibit oxidative stress by scavenging the highly destructive free radicals [10]. The deleterious effects of the free radicals are kept under check by a delicate balance between the rate of their production and the rate of their elimination by these defense systems. When there is an excessive addition of free radicals from exogenous sources added to the endogenous production, the available tissue defense system becomes overwhelmed resulting in oxidative damage to the tissues.

It has been postulated that many of the adverse effects of smoking may result from oxidative damage to critical biological substances [11]. Smokers are extra vulnerable for free-radical-induced damage of the cardiovascular system. They are exposed to free radicals from cigarette smoke and in general they have lower antioxidant vitamin intake and plasma levels [12]. A major exogenous source of free radicals is cigarette smoke which is a heterogeneous aerosol consisting of more than 4000 compounds including high concentrations of free radicals, and reactive oxygen and nitrogen species. The obligatory use of the body's reserve of antioxidants to detoxify the tremendous level of these free radicals in smokers therefore results in severe antioxidant deficiency, thereby predisposing them to the development of life threatening diseases [13].

Hyperlipidemia is a well-known risk factor for the development of atherosclerosis. Evidence suggests that oxidatively modified LDL contribute to the pathogenesis of atherosclerosis. Increased oxidative stress and the generation of the free oxygen radicals can result in modification of LDL to oxidized LDL that could lead to atherosclerotic lesions [11]. Circulating products of lipid peroxidation and autoantibody titers to oxidized LDL are significantly increased in smokers. It has been reported that exposure to cigarette smoke extract (CSE) caused a modification of LDL and actively taken up by the macrophages to form foam-cells in culture. CSE exposure may also decrease the plasma activity of paraoxonase, an enzyme that protects against LDL oxidation [14,15]. The present study was therefore aimed at evaluating lipid profile, lipid peroxidation and antioxidant status due to smoking in healthy subjects, acute myocardial infarction patients, and in stabilized patients surviving MI.

MATERIALS AND METHODS

Study Population: The study population consisted of 120 male (age-matched) subjects divided into three groups viz. patients admitted to the Intensive Care Unit with MI (n=40; mean age 55.7 ± 10.4 years); patients with stable IHD of more than one year duration (n=40; mean age 53.6 ± 11.2 years) and healthy volunteers who served as control (n=40; mean age 51.7 ± 12.3 years). Participants in this study were selected from Kovai Medical Centre and Hospital (KMCH) & K.G. Hospital and Post Graduate Medical Institute, Coimbatore, Tamil Nadu, India, during the period January 2006 to January 2008. All subjects were medically examined, checked for inclusion and exclusion criteria. The diagnosis of MI was based on a history of prolonged ischemic chest pain, characteristic electrocardiogram (ECG) changes and elevated CK-MB, troponin T and troponin I within 12 h after the onset of pain. Diabetes mellitus was diagnosed if the fasting plasma glucose concentration was >126 mg/dL or if the patient was treated with insulin or oral hypoglycemic agents. Patients with infection, inflammatory diseases, malignancy, and congenital malformations of the heart or vessels or history of MI were excluded. Control subjects were chosen from the same age group and had free from diabetes mellitus and other chronic disease. Patients were excluded from the study if their serum cholesterol levels were >240 mg/ dL or if they were taking any drugs known to affect serum lipids, lipid peroxidation and antioxidant status. Entrance criteria for smoker group included subjects who smoked at least fifteen cigarettes per day for 5–12 years. In spite of medical advice against smoking, some patients continued smoking after infarction and were included in the study. A written informed consent was taken from the subjects or the parents/guardians of the subjects prior to enrolment into the study. The protocol of this study was approved by the Institutional Human Ethics Committee (K.G. Hospital and Post Graduate Medical Institute, Tamil Nadu, India). Study samples were collected in the morning in control and stable IHD groups and samples from MI subjects were drawn soon after admission before starting any medication.

Blood Collection and Hemolysate Preparation: Blood samples were collected by venous puncture in heparinized tubes and the plasma was separated by centrifugation at 1000 g for 15 min. After centrifugation, the buffy coat was removed and the packed cells were washed three times with physiological saline. A known volume of the erythrocytes was lysed with hypotonic phosphate buffer (pH 7.4). The hemolysate was separated by centrifugation at 2500× g for 15 min at 2°C.

Estimation of Lipids and Cardiac Markers: Total cholesterol, triglycerides, HDL, LDL and CK levels were determined by fully automated clinical chemistry analyzer (Hitachi 912, Boehringer Mannheim, Germany). VLDL level was calculated according to Friedewald *et al.* [16]. Both cardiac troponin T and CK-MB mass were measured with highly specific monoclonal antibodies in a sensitive chemiluminescence assay, [Elecsys 2010 instrument Roche Diagnostics, Mannheim, Germany]. Troponin I level was estimated using fully automated immunoassay analyzer (AXSYM-Abbott Laboratories, Abbott Park, USA).

Estimation of Lipid Peroxidation: Lipid peroxides were estimated by measurement of thiobarbituric acid reactive substances (TBARS) in plasma by the method of Yagi [17]. The pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde, a secondary product of lipid peroxidation was estimated. TBARS concentration was expressed as nmoL mL/plasma.

Assay of Enzymatic Antioxidants: SOD was assayed utilizing the technique of Kakkar *et al.* [18] based on inhibition of the formation of nicotinamide adenine dinucleotide, phenazine methosulfate and amino blue tetrazolium formazan. A single unit of enzyme was expressed as 50% inhibition of NBT (Nitroblue tetrazolium) reduction min/mg/Hb. CAT was assayed colorimetrically at 620 nm and expressed as µmoL of H_2O_2 consumed min/mg/Hb as described by Sinha [19]. The reaction mixture (1.5 mL) contained 1.0 mL of 0.01 M pH 7.0 phosphate buffer, 0.1 mL of hemolysate and 0.4 mL of 2 M H_2O_2. The reaction was stopped by the addition of 2.0 mL of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio).

GPx activity was measured by the method described by Rotruck *et al.*[20] with modifications. Briefly, reaction mixture contained 0.2 mL of 0.4 M Tris-HCl buffer pH 7.0, 0.1 mL of 10 mM sodium azide, 0.2 mL of hemolysate, 0.2 ml glutathione and, 0.1 mL of 0.2 mM H_2O_2. The contents were incubated at 37°C for 10 min. The reaction was arrested by 0.4 mL of 10% TCA, and centrifuged. The supernatant was assayed for glutathione content by using Ellmans reagent (19.8 mg of 5,5'-dithiobisnitro benzoic acid (DTNB) in 100 mL of 0.1% sodium nitrate). GPx activity was expressed as µmoL of GSH consumed min/g/Hb.

Estimation of Non-enzymatic Antioxidants: Plasma GSH level was determined by the method of Ellman [21]. 1.0 mL of plasma was treated with 0.5 mL of Ellmans reagent and 3.0 mL of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm. GSH level was expressed as mg /dL.

Plasma vitamin A was estimated by the method of Bradly and Hombeck [22]. Proteins were precipitated with ethanol and the carotenes were extracted into light petroleum. The intensity of the yellow color due to carotene was read directly at 450 nm using a violet filter. Vitamin E was measured by the method of Baker *et al.* [23] on the basis of the reduction of ferric ions to ferrous ions by vitamin E and the formation of a red colored complex with 2.2'-dipyridyl at 520 nm. Vitamin C was estimated by the method of Roe and Kuether [24]. This involves oxidation of ascorbic acid by copper followed by treatment with 2,4-dinitrophenylhydrazine that undergoes rearrangement to form a product with absorption maximum at 520 nm.

Statistical Analysis: All data were expressed as mean ± SD. The statistical significance was evaluated by Student's t test using Statistical Package for the Social Sciences (SPSS Cary, NC, USA) version 10.0.

RESULTS AND DISCUSSION

Information about the demographic characteristics of the study population is shown in Table 1. The mean age limit was 53.6 ±11.2 years in MI patients, 55.17 ± 10.4 years in stable IHD patients and 51.70 ±12.3 years in control subjects. The body mass index (BMI) of MI patients (28.25 ± 0.97 kg/m2) was significantly higher ($p<0.05$) when compared with control subjects. However, there was no statistically significant difference in BMI of stable IHD patients when compared with control subjects and MI patients. Entrance criteria for smoker group included subjects who smoked at least fifteen cigarettes per day for 5–12 years.

Table 2 shows the levels of myocardial injury markers in patient groups and control group. Creatine kinase, CK-MB, troponin T and troponin I levels were significantly increased ($p<0.001$) in subjects suffering from MI and IHD compared with control subjects. Besides, the increase in CK, CK-MB, troponin T and troponin I were significantly greater ($p<0.001$) in smokers as compared to non-smokers in the MI group.

Table 3 shows the levels of serum lipids and lipoproteins in patient groups and control group. Total cholesterol, TG, LDL and VLDL levels were significantly increased ($p<0.001$), while the HDL level was significantly decreased in patients suffering from MI and IHD compared with control subjects. A highly significant increase ($p<0.001$) in serum cholesterol, TG, LDL, VLDL and decrease in HDL level was observed in smokers than non-smokers in the MI group.

Table 4 indicates the levels of plasma TBARS and

erythrocyte antioxidant enzyme activities in patient groups and control group. TBARS level was significantly increased ($p<0.001$), whereas the antioxidant enzyme activities were significantly decreased ($p<0.001$) in patients suffering from MI and IHD compared with control subjects. In addition, a significant increase ($p<0.001$) in the extent of lipid peroxidation with concomitant reduction in the erythrocyte enzymatic antioxidants (SOD, CAT, GPx) were observed in smokers than non-smokers in the MI group.

Table 5 shows the levels of plasma GSH, vitamin A, vitamin C and vitamin E in patient groups and control group. The results indicate that all the four non-enzymatic antioxidants studied viz, GSH, vitamin A, C and E were significantly decreased ($P<0.001$) in patients suffering from MI and IHD compared with control subjects. In addition, decrease in plasma GSH, vitamin A, C and E levels were significantly greater in smokers than non-smokers in the MI group.

Discussion: Cigarette smoking seems to be the most important risk factor for myocardial infarction. Smoking can trigger myocardial infarction in individuals with minimal atherosclerosis or even with normal coronary arteries, promoting temporary coronary vessel occlusion, as a result of thrombus formation, coronary artery spasm or both. Additionally, smokers tend to present other lifestyle choices, concerning diet and physical activity that have an independent effect on the risk of coronary disease [25].

Elevated levels of CK-MB, troponin T and troponin I have been regarded as biochemical markers of myocyte necrosis [26]. CK and more particularly its isoenzyme CK-MB still have a formal place in defining myocardial infarction. These enzymes normally exist in cellular compartment and leak out into the plasma during myocardial injury due to disintegration of contractile elements and sarcoplasmic reticulum [27,2]. Troponins T and I are proteins of the troponin regulatory complex involved in cardiac contractility.

Table 1. Demographic characteristics of different study group

	Control (n=40)	Stable IHD (n=40)	MI (n=40)
Total number of subjects (n)	40	40	40
Sex (male)	100 %	100 %	100 %
Mean age (mean ± SD; years)	51.7 ± 12.3	53.6 ± 11.2^{NS}	55.7 ± 10.4^{NS}
Body mass index (mean ± SD; Kg/m^2)	25.12 ± 0.96	26.92 ± 0.96^{NS}	$28.55 \pm 0.97^{\dagger}$
Risk factors Hypertension	-	30.7 %	14.5 %
Diabetes mellitus	-	5.6 %	3.2 %
Smoking status Smokers (n=20)	50 %	50 %	50 %
Non-smokers (n=20)	50 %	50 %	50 %
Past history STK therapy	-	-	5 %
Unstable angina	-	-	7.5 %
Stable angina	-	-	30 %

Values are given as mean ± S.D from forty subjects in each group.
MI patients compared with control subjects.
Stable IHD compared with control subjects ($^{\dagger}p<0.05$; NS-not significant).

Table 2. Comparison of myocardial injury markers patient groups and control group

Parameters	CPK (IU/ L)	CK-MB (IU /L)	Troponin T (ng/mL)	Troponin I (ng/mL)
Control				
Non-smokers (n=20)	90 ± 20.3	15 ± 4.0	0.020 ± 0.007	0.40 ± 0.03
Smokers (n=20)	93 ± 21.6	17 ± 5.1	0.026 ± 0.009	0.61 ± 0.10
Stable IHD				
Non-smokers (n=20)	95 ± 25.2^{NS}	$20 \pm 7.3^{\dagger}$	0.022 ± 0.011^{NS}	0.50 ± 0.04^{NS}
Smokers (n=20)	115 ± 21.5*	32 ± 10.1*	$0.032 \pm 0.032^{\dagger}$	$1.02 \pm 0.21^{\dagger}$
MI				

Table 2: Continue

Non-smokers (n=20)	220 ± 32.2*	37 ± 13.0*	0.187 ± 0.142*	2.07 ± 1.11*
Smokers (n=20)	356 ± 36.5*‡	72 ± 5.3*‡	1.570 ± 0.330*‡	3.85 ± 1.20*‡

Values are given as mean ± S.D from twenty subjects in each group.
Stable IHD patients (non-smokers) compared with control subjects (non-smokers).
Stable IHD patients (smokers) compared with control subjects (non-smokers).
MI patients (non-smokers) compared with control subjects (non-smokers).
MI patients (smokers) compared with control subjects (non-smokers).
MI smokers compared with MI non-smokers (†p<0.05; *p<0.001; ‡ p<0.001; NS-not significant).

Table 3. Serum lipids and lipoproteins in patient groups and control group

Parameters	TC	TG	HDL	LDL	VLDL
			(mg /dL)		
Control					
Non-smokers (n=20)	175 ± 15.3	145 ± 17.6	48 ± 7.8	66 ± 15.2	29 ± 10
Smokers (n=20)	189 ± 27.3	150 ± 22.5	45 ± 6.8	79 ± 17.5	30 ± 8.5
Stable IHD					
Non-smokers (n=20)	180 ± 20.6 NS	133 ± 27.0*	45± 5.3†	71 ± 18.5NS	26± 7.5†
Smokers (n=20)	193± 26.3NS	179 ± 25.3*	41 ± 6.8*	83 ± 20.1NS	36 ± 9.6*
MI					
Non-smokers (n=20)	221 ± 23.8*	246 ± 26.8*	37 ± 6.0*	120 ± 20.3*	49 ± 11.8*
Smokers (n=20)	263 ± 28.6*‡	377 ± 32.5*‡	22 ± 8.6*‡	145 ± 21.6*‡	75 ± 17.9*‡

Values are given as mean ± S.D from twenty subjects in each group.
Stable IHD patients (non-smokers) compared with control subjects (non-smokers).
Stable IHD patients (smokers) compared with control subjects (non-smokers).
MI patients (non-smokers) compared with control subjects (non-smokers).
MI patients (smokers) compared with control subjects (non-smokers).
MI smokers compared with MI non-smokers (†p<0.05; *p<0.001; ‡ p<0.001; NS-not significant).

Table 4. Plasma TBARS and erythrocyte antioxidant enzymes in patient groups and control group

Parameters	TBARS (nmoL /mL)	SOD (U^A mg/Hb)	CAT (U^B mg/Hb)	GPX (U^C mg/Hb)
Control				
Non-smokers (n=20)	0.69 ± 0.12	3.71 ± 0.26	69.5 ± 6.60	9.18 ± 1.59
Smokers (n=20)	0.75 ± 0.17	3.45 ± 0.12	64.3 ± 9.03	8.89 ±0.84
Stable IHD				
Non-smokers (n=20)	0.80 ± 0.41NS	3.51 ± 0.11*	70.1 ± 7.70NS	8.83 ± 0.71NS
Smokers (n=20)	1.00 ± 0.51*	3.41 ± 0.10NS	63.2 ± 6.04NS	8.54 ± 0.53NS
MI				
Non-smokers (n=20)	2.83 ± 1.04*	2.14 ± 0.11*	40.7 ± 7.70*	6.03 ± 0.80*
Smokers (n=20)	4.03 ± 1.12*‡	1.14 ± 0.10*‡	30.2 ± 6.04*‡	3.54 ± 0.68*‡

Values are given as mean ± S.D from twenty subjects in each group.
Stable IHD patients (non-smokers) compared with control subjects (non-smokers).
Stable IHD patients (smokers) compared with control subjects (non-smokers).
MI patients (non-smokers) compared with control subjects (non-smokers).
MI patients (smokers) compared with control subjects (non-smokers).
MI smokers compared with MI non-smokers (†p<0.05; *p<0.001; ‡ p<0.001; NS-not significant).
A–One unit of activity was taken as the enzyme reaction, which gave 50% inhibition of NBT reduction in one minute.
B – µmol of H_2O_2 consumed/ min.
C – µg of GSH consumed/ min.

Table 5. Plasma GSH and vitamin A, vitamin C and vitamin E in patient groups and control group

Parameters	GSH (mg /dL)	Vitamin A (mg /dL)	Vitamin C (mg /dL)	Vitamin E (mg /dL)
Control				
Non-smokers (n=20)	38.98 ±1.67	0.87 ± 0.17	1.22 ± 0.28	1.09 ± 0.21
Smokers (n=20)	35.10 ±3.49	0.76 ± 0.14	1.11 ± 0.16	0.99 ± 0.17
Stable IHD				
Non-smokers (n=20)	35.22 ±3.01*	0.83 ± 0.05^{NS}	1.02 ± 0.21*	1.03 ± 0.20^{NS}
Smokers (n=20)	30.22 ±2.95*	0.75 ± 0.05^{NS}	0.85 ± 0.19*	0.90 ± 0.15*
MI				
Non-smokers (n=20)	25.09 ± 3.14*	0.46 ± 0.06*	0.53 ± 0.041*	0.58 ± 0.10*
Smokers (n=20)	17.22 ±3.45*‡	0.27 ±0.06*‡	0.21 ± 0.052 *‡	0.32 ± 0.07*‡

Values are given as mean ± S.D from twenty subjects in each group.
Stable IHD patients (non-smokers) compared with control subjects (non-smokers).
Stable IHD patients (smokers) compared with control subjects (non-smokers).
MI patients (non-smokers) compared with control subjects (non-smokers).
MI patients (smokers) compared with control subjects (non-smokers).
MI smokers compared with MI non-smokers (†p<0.05; *p<0.001; ‡ p<0.001; NS-not significant).

Both have very high myocardial tissue specificity and offer an improved sensitivity and specificity for MI versus a combination of ECG and traditional biochemical markers. The cardiac-specific troponins are highly sensitive and specific markers of myocardial damage and therefore cardiac troponins are the preferred markers for the diagnosis of myocardial infarction [2,28]. In this study, increased CK, CK-MB, troponin T and troponin I levels were found in patients with MI as compared to healthy controls. Moreover, the levels of myocardial injury markers were significantly greater in smokers as compared to non-smokers in the MI group.

Cigarette smoking could promote atherosclerosis, in part, by its effects on lipid profile. Changes in the concentration of plasma lipids including cholesterol are complications frequently observed in patients with MI and certainly contribute to the development of vascular disease. Cholesterol has been singled out as the primary factor in the development of atherosclerosis. HDL is regarded as one of the most important protective factors against arteriosclerosis. HDL's protective function has been attributed to its active participation in the reverse transport of cholesterol. Numerous cohort studies and clinical trials have confirmed the association between a low HDL and an increased risk of coronary heart disease [29]. The concentration of LDL correlates positively whereas HDL correlates inversely to the development of coronary heart disease. Smokers have significantly higher serum cholesterol, triglyceride, and LDL levels, but HDL is lower in smokers than in non-smokers [15]. Evidence suggests that oxidatively modified LDL contribute to the pathogenesis of atherosclerosis. Increased oxidative stress and the generation of the free oxygen radicals can result in modification of LDL to oxidized LDL that could lead to atherosclerotic lesions [11]. Smokers have high oxidative stress and usually both a lower intake and plasma level of antioxidant vitamins, which may render their LDL cholesterol more susceptible to lipid peroxidation. Oxidation of LDL cholesterol is thought to play an important role in early atherosclerosis by depositing cholesterol in macrophages, leading to the formation of fatty streaks. Increased oxidation of LDL cholesterol in smokers is supported by reported higher levels of lipid peroxides in the plasma of smokers. Moreover, the susceptibility of LDL to oxidation decreased in smokers after they had quit [11,12].

Free radical-mediated oxidative stress is emerging as the pivotal step for the development of atherosclerosis. Several experimental reports demonstrate that ischemia followed by reperfusion elicit a cascade of proinflammatory reactions that are known to lead to the production of ROS, activation of the complement system, leukocyte mediated injury of myocardial cells and production of cytokines [30,31,2]. Cigarette smoke is a complex milieu possessing an array of free radicals and ROS, namely hydroxyl, peroxyl, nitric oxide, and superoxide radicals. The sustained release of reactive free radicals from the tar and gas phases of smoke imposes an oxidant stress, promotes lipid peroxidation and consequently perturbs the antioxidant defense system [15].

Analysis of TBARS in plasma is a widely used method for the evaluation of lipid peroxidation. The concentration of TBARS in plasma was higher in MI patients than in control subjects. A highly significant increase in TBARS level was found in smokers as compared to non-smokers in the MI group. In

ischemia, the ATP is drastically reduced and is converted to hypoxanthine and then to uric acid by xanthine oxidase upon reperfusion. During this process, enormous amounts of superoxide radicals formed which can simulate Haber-Weiss reaction for further generation of ROS, initiating lipid peroxidation [32].

Antioxidants constitute the foremost defense system that limit the toxicity associated with free radicals. It is known that plasma antioxidant capacity decreases and oxidative/antioxidative balance shifts to the oxidative side in patients with MI. A reason for increased lipid peroxidation in plasma of MI patients may be due to a poor enzymatic and non-enzymatic antioxidant defense system. SOD along with CAT and GPx, the preventive antioxidants, plays a very important role in protection against lipid peroxidation. In this study, SOD, CAT and GPx activities were significantly lower in MI and IHD patients than in control subjects. Besides, decrease of SOD, CAT and GPx activity was much more pronounced in smokers than in non-smokers with MI, thus making those individuals more vulnerable to oxidative stress.

SOD is the first enzyme in antioxidant defense that scavenges superoxide radicals to form H_2O_2 and hence diminishes the toxic effects of the radical. Decreased activity of SOD has been reported in pathological conditions. The quinone–semiquinone radicals from the tar phase of cigarette smoke are capable of reducing molecular oxygen to superoxide radicals whose excessive generation inactivates this enzyme [13]. Hence, a decrease in SOD activity upon smoke exposure could have resulted from its inactivation by tar phase oxidants. CAT is involved in the detoxification of high concentrations of H_2O_2. CAT has been suggested to play an important role in the protection of the erythrocyte against oxidative stress [13,33]. The presence and production of the free radicals from smoke lower this enzyme, leading to accumulation of H_2O_2 and lipid hydroperoxides further worsening the damage. A marked decrease in the activity of CAT in patients suffering from MI in the present study suggests the inability of host antioxidant defense to meet the oxidative stress following chronic exposure to cigarette smoke.

GSH, a widely distributed cellular reductant is a metabolic regulator and putative indicator of health. Blood glutathione levels are believed to be predicators of morbidity and mortality [34]. GSH plays a key role in protecting cells against electrophiles and free radicals. GSH can act directly as a free radical scavenger by neutralizing hydroxyl radicals, or indirectly by repairing initial damage to macromolecules inflicted by hydroxyl radicals. It is essential in the maintenance of protein and non-protein SH group in reduced form [35]. Smoking-induced depletion of GSH level has also been reported. This depletion was directly associated with elevation in lipid peroxidation which could be attributed to its protection against ROS generated by smoke, besides its consumption by the antioxidant enzymes GPx and GST. Acetaldehyde, a major aldehyde from the smoke has been shown to deplete the cells of their GSH by conjugating with it, which further makes the cells more vulnerable to peroxidative damage [13]. GPx catalyses the reduction of H_2O_2 and organic hydroperoxides with simultaneous oxidation of GSH [35]. Absence of an augmentation in GPx activity upon smoke exposure in this study has been hypothesized to arise from a decrease in the levels of GSH that is essential for the conjugation of lipid peroxides. Low GSH levels and the decreased activity of GPx found in patients suffering from MI in the present study supports the hypothesis that smoking leads to a greater oxidative burden and depletion of antioxidant defences.

Vitamin C is the first strong reductant in the aqueous phase that readily reacts with cigarette smoke oxidants and affords considerable protection to the cells. Studies involving different types of oxidative stress have shown that under all types of oxidative stress, ascorbic acid successfully prevents detectable oxidative damage and therefore it would be helpful in prevention of diseases in which oxidative stress plays a causative or exacerbation role [36]. Vitamin E, an important lipophilic antioxidant has an effective role in maintaining the cell structure against oxidative damage through blocking the chain reaction of free radicals. Vitamin E reacts with peroxyl radicals present in the smoke and terminates lipid peroxidation and vitamin A effectively quenches singlet oxygen [37]. Hence, the decrease in GSH levels could possibly be related to the inability of host tissue to synthesize GSH that is reflected from decrease in vitamin C, E and A. GSH and these vitamins are tightly linked to each other in a way that it helps to replenish vitamin C which in turn regenerates vitamin E and A. However, smokers are constantly overexposed to free radicals through inhalation of long-lived carbon- and oxygen-centered radicals that subsequently deplete the plasma and tissue stores of these micronutrients [37,38]. *In vitro* exposure of plasma to cigarette smoke resulted in the destruction of tocopherols, carotenoids and retinal [39]. The present study also revealed depletion in the levels of non-enzymatic antioxidants such as vitamin C, E and A in plasma of MI patients as compared to control subjects. Further, a highly significant decrease in vitamin C, E and A level was observed in smokers than non-smokers in the MI group.

Cigarette smoking is one of the most important exogenous factors, which cause 3-fold higher incidence of myocardia infarction. Free radical-mediated oxidative

stress appears to play a central role in cigarette smoking-mediated athero-thrombotic diseases. The results of present study clearly show that cigarette smoking induces an oxidative stress in MI patients by augmenting lipid peroxidation and diminishing both enzymatic and non-enzymatic antioxidant status. The above findings also support the hypothesis that the atherogenic effects of smoking are mediated in part by free radical damage to lipids. Future studies investigating the potential cigarette smoke-inducible endogenous cellular mechanisms could further our understanding of the complex pathobiology of cigarette smoke and cardiovascular dysfunction.

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