



Original Article

Status of Certain Oxidative Stress Markers in Ischemic Heart Disease Patients with and Without Smoking in North Indian Punjabi Population

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ABSTRACT

Background: Oxidative stress alters normal endothelial function, supporting proinflammatory, prothrombotic, proliferative and vasoconstrictor mechanism that favor the development of atherosclerosis and vascular tissue injury.

Aim: The aim of the present study was to investigate the status of oxidative stress in ischemic heart disease patients with smoking and without smoking by measuring malondialdehyde, superoxide dismutase, reduced glutathione and total antioxidant activity in North Indian Punjabi Population.

Material and Methods: A well known 50 ischemic heart disease patients with smoking (n=25) and without smoking (n=25) admitted in the hospital and 50 normal healthy adult subjects who have no previous history of smoking as a control were recruited in the study for the evaluation of oxidative stress markers like malondialdehyde, superoxide dismutase, reduced glutathione and total antioxidant activity.

Results: A highly significant ($p < 0.001$) increase was found in malondialdehyde levels in ischemic heart disease patients with and without smoking in comparison to normal healthy control subjects while a significant reduction in the levels was observed in superoxide dismutase, reduced glutathione and total antioxidant activity in ischemic heart disease patients with and without smoking with respect to control subjects. Further we also observed in the present study that smoking significantly promoting the malondialdehyde levels upon significantly decrease the levels superoxide dismutase, reduced glutathione and total antioxidant activity in ischemic heart disease patients.

Conclusion: All the above observations suggested that oxidative stress was induced in IHD patients and this study also pointed out that smoking accelerate the oxidative stress by promoting malondialdehyde levels while reducing superoxide dismutase,



reduced glutathione and total antioxidant activity in circulation of IHD patients in the early age. Further studies with more sample size are needed to correlate these associations and accordingly future antioxidative therapy for beneficial of IHD patients.

Introduction

India has one of the highest burdens of cardiovascular diseases (CVDs). The prevalence of cardiovascular disease (CVD) in rural India is 7.4% and in urban India is 11%¹. The incidence of ischaemic heart disease (IHD) and coronary heart disease (CHD) is steadily increasing in the Indian subcontinent. According to World Health Report 2002, CVD will be the largest cause of death in India by 2020. It is predicted that 2.6 million Indians will die due to CHD. This number will represent 54.1% of all CVD deaths in the age group of 30–69 years^{1,2}.

IHD is a progressive disease arising when the supply of oxygen in the myocardium is compromised by impeded blood flow in the coronary vasculature caused by luminal occlusion. IHD leads to the development of atherosclerosis and hypertrophy of musculature. The pathogenesis of atherosclerosis involves damage to the capillary endothelium caused by various factors including oxidized low-density lipoprotein^{3,4}. Muscular hypertrophy is caused by elaboration of local elaboration of growth factors from surrounding macrophages, which are primed by free radicals. The plaque formed thereupon is covered by thinned out intima and media. A luminal compromise of nearly 70% is needed to produce symptomatic IHD. Free radicals are chemical species that can be considered as fragments of molecules possessing unpaired electrons. They are generally very reactive and they are produced continuously in cells either as by-products of metabolism or during

phagocytosis^{5,6}. Recently, generation of free radicals particularly reactive oxygen species (ROS) have been implicated in various cardiovascular disorders including ischemia/reperfusion, atherosclerosis, hypertension, cardiotoxicity induced by drugs, cancer etc^{7,8}. The cellular mechanisms involved in the pathogenesis of myocardial ischemia/reperfusion injury are complex and involve the interaction of a number of cell types, including coronary endothelial cells, circulating blood cells (e.g., leukocytes, platelets), and cardiac myocytes, most of which are capable of generating ROS. These ROS have the potential to injure vascular cells and cardiac myocytes directly, and can initiate a series of local chemical reactions and genetic alterations that ultimately result in an amplification of the initial ROS-mediated cardiomyocyte dysfunction and/or cytotoxicity.

Smoking is one of the major cardiovascular risk factors able to cause harmful effects on the heart and blood vessels as well as endothelial dysfunction^{9,10}. The World Health Organization estimates that there are around 1.3 billion smokers in the world, of which almost 1 billion are men. This represents about one third of the global population. Further, Indians have the highest mortality rate due to IHD amongst ethnic groups study so far⁴. The risk of IHD in Indian is 3-4 times higher than white Americans, 6 times higher than Chinese and 20 times higher than Japanese.

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 resulting in production of ROS. The literature reports from India and abroad have revealed that IHD is no more confined to the affluent and rich people but has made its inroad into the lower and middle socioeconomic segment of the society might be due to smoking habits. The most worrying part of the whole scenario is that IHD is rapidly spreading in younger people¹¹⁻¹³. So in the present study, we evaluated the changes in certain oxidative stress markers like malondialdehyde (representing lipid peroxidation), superoxide dismutase (SOD), reduced glutathione (GSH) along with total antioxidant activity (TAA) in Ischemic heart disease patients with smoking and without smoking in North Indian Punjabi Population.

Material 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 vide reference No. 14269/D-26, dated 19/6/2013. 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: 25 IHD patients without smoking (age range 20-50 years).

Group-3: 25 IHD patients with smoking (age range 20-50 years).

25 IHD without Smoking (Group-2) and 25 IHD patients with smoking (Group-3) admitted in various wards of Medicine Department, Guru Nanak Dev Hospital attached to Government Medical College-Amritsar were diagnosed on the basis of 12 lead electrocardiogram, raised cardiac enzymes like CPK-MB, SGOT/SGPT and LDH) and altered lipid profile levels in the age range of 20- 50 years were included in group-2 & group-3 respectively.

50 normal healthy subjects, who reporting no previous uses of smoking/illegal drugs experience in the age range of 20-50 were recruited in Group-1 as a control group.

Inclusion and Exclusion Criteria

Inclusion criteria for smokers and non-smoker IHD patients

- (i) Patients with Ischemic heart disease evidenced by ECG criteria along with raised CPK-MB, SGOT, LDH and altered lipid profile.
- (ii) Both smokers and non smokers.

Exclusion criteria for smokers and non-smoker IHD patients

- (i) Subjects with hypertension or any Systemic disease like hypothyroidism, diabetes, renal failure etc.
- (ii) Those on Drugs e.g. β -blockers, Thiazides, Statins etc.
- (iii) Those on diet restriction.

Inclusion criteria for normal healthy non smokers

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

Exclusion criteria for normal healthy non smokers

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.

Ethics

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.

Collection and processing of blood sample

Fasting blood sample were collected from all the three 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 oxidative stress markers.

1. **Estimation of Malondialdehyde (MDA):** Serum MDA levels were estimated by using the method of Satoh, 1978¹⁴.
2. **Estimation of Superoxide Dismutase (EC 1.5.1.1):** Serum SOD levels were estimated by using method Marklund and Marklund, 1974¹⁵ modified by Nandi and Chatterjee, 1988¹⁶.
3. **Estimation of Reduced Glutathione (GSH):** The levels of GSH from whole blood were estimated by applying the method of Beutler et al¹⁷.
4. **Estimation of Total Antioxidant Activity (TAA):** Plasma total antioxidant capacity was estimated in plasma by the

FRAP (Ferric Reducing Ability of Plasma) assay by applying the method of Benzie & Strain, 1996¹⁸.

5. **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 ($p < 0.001$) increase by 143.21% and 264.32% was observed in malondialdehyde levels in IHD patients without smoking and with smoking respectively with respect to normal healthy control subjects (Table-2). Malondialdehyde representing lipid peroxidation is an index of oxidative stress. Lipid peroxidation is a free radical mediated chain reaction and is self perpetuating^{19,20}. Tissue damage is considered proportional to lipid peroxide contents and thus cell membrane damage is evaluated by measuring lipid peroxide contents by various ways. A significant in serum MDA levels, in IHD patients without smoking suffered from oxidative stress might be due to a poor enzymatic and non-enzymatic antioxidant defense system. The biological effects of free radicals are normally controlled in vivo by a wide range of antioxidants like 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 ROS by catalyzing the dismutation of O_2^- radical to H_2O_2 and O_2 ^{21,22}. Three isozymes of SOD that is CuZn-SOD, Mn-SOD and extra cellular-SOD have been recognized in human²³. CuZn-SOD is located primarily in the cytosol. Mn-SOD is located in mitochondrial matrix¹⁸. EC-SOD is present

in plasma, bound to heparin sulfate ion on 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 O_2^- radicals immediately and protects the cells from oxidative damage. A significant inhibition by 38.69% and 61.09% in SOD activity in IHD patients without smoking and with smoking respectively may result in an increased flux of O_2^- radical and hence reflects the tissue damage/injury.

Reduced glutathione (GSH) is an important constituent of the cellular defense mechanism of the body against various exogenous as well as endogenously produced xenobiotics. It plays the role of a sulfhydryl (SH) group provider for direct scavenging reactions. GSH acts both as a substrate in the scavenging reaction catalyzed by glutathione peroxidase (GPx) and as a scavenger of vitamins like vitamin -C and vitamin -E radicals²⁴. In the present study we found a significant depletion in GSH levels by 36.31% and 60.43% in the IHD patients without smoking and with smoking respectively. This observation again suggested that the cardiac tissue has suffered from ischemic damage due to oxidative imbalance thereby decreasing this biomarker, since GSH is a thiol containing non enzymatic antioxidant of the body.

Further, we found that the levels of MDA were significantly ($p < 0.001$) increased by 264.32% in IHD patients with smoking in comparison to control and 49.80% in w.r.t to IHD patients without smoking while a significant fall was recorded in SOD, GSH and TAA levels in by 61.09%, 60.43% and 43.43% in group-3 (IHD patients with smoking) respectively w.r.t. control (Group-1) and a significant reduction by 36.55%, 37.88% and 23.63% was seen in SOD, GSH and TAA levels in IHD patients with smoking (Group-3) in comparison to IHD patients without

smoking (Group-2). Our observation of the present study suggesting that smoking further enhancing the oxidative stress by altering the levels of MDA, SOD, GSH and TAA in IHD patients

Conclusion

A significant alteration in the levels of MDA, SOD, GSH and TAA in the circulation of IHD patients suggested that oxidative stress was induced in IHD patients. Therefore, assessing these biomarkers of oxidative stress may be useful in diagnosis of patients with ischemic heart diseases. We also observed that smoking enhanced the oxidative stress by promoting malondialdehyde and by reducing SOD, GSH & TAA in circulation of IHD patients in the early age. Further studies with more sample size are needed to correlate these associations and accordingly future antioxidative therapy for beneficial of IHD patients.

Abbreviations

CVDs: Cardiovascular disease; GSH: Reduced Glutathione; IHD: ischemic heart disease; LPO: Lipid Peroxidation; MDA: Malondialdehyde; OS: Oxidative stress; SOD: Superoxide dismutase; TAA: Total Antioxidant Activity; H_2O : Water; O_2 : Oxygen; H_2O_2 : Hydrogen Peroxide; O_2^- : Superoxide anion.

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Table 1: Anthropometric analysis of smokers and non smokers

Anthropometric and Biochemical Assays	Group- 1 (Control)	Group- 2 (IHD patients without smoking)	Group-3 (IHD patients with smoking)
Height (cm)	158.12 ± 0.16 ^a	161.01 ± 0.11 ^a	159.19 ± 0.18 ^a
Weight (kg)	68.00 ± 14	67.00 ± 16	65.00 ± 12
Age (years)	35.00 ± 10.00	52.00 ± 13.00	32.00 ± 7.00
Body mass index (Kg/m ²)	26.80 ± 0.48	25.80 ± 0.28	27.14 ± 0.32
Blood pressure systolic (mmHg)	120.15 ± 10.53	122.12 ± 9.51	123.11 ± 8.22
Blood pressure diasystolic (mmHg)	85.01 ± 7.32	86.131 ± 4.14	85.42 ± 6.19
Hemoglobin (g/dl)	14.81 ± 1.16	13.89 ± 1.22	14.07 ± 1.01
Glucose (Fasting)	76.23 ± 7.32	79.22 ± 5.12	81.41 ± 5.22

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

Table 2: Alteration in Malondialdehyde, Superoxide dismutase, Reduced Glutathione and Total Antioxidant Activity in normal healthy individuals, IHD patients with and without smoking.

Oxidative Stress Markers	Group- 1 (Control)	Group- 2 (IHD patients without smoking)	Group-3 (IHD patients with smoking)
Malondialdehyde (nmol/ml)	1.99±0.60 ^a	4.84±1.02	7.25±1.04
Superoxide dismutase (U/ml)	4.73± 0.82	2.90± 0.27	1.84± 0.27
Reduced Glutathione (nmoles/ml)	29.22± 5.82	18.61± 5.82	11.56± 5.82
Total Antioxidant Activity (μ mol/L.)	709.26 ± 12.19	525.33 ± 9.69	401.19 ± 6.37

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

Table 3: Percent change in Malondialdehyde, Superoxide dismutase, Reduced Glutathione and Total Antioxidant Activity in different groups

Groups	Control vs. IHD patients without smoking)	Control vs. IHD patients with smoking	IHD patients without Smoking vs. IHD patients with smoking
Malondialdehyde	+143.21***	+264.32***	+49.80**
Superoxide dismutase	-38.69**	-61.09***	-36.55*
Reduced Glutathione	-36.31**	-60.43***	-37.88**
Total Antioxidant Activity	-25.94*	-43.43**	-23.63*

* P < 0.05, ** P < 0.01, *** P < 0.001