Oxidative stress and total antioxidant status in myocardial infarction

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ABSTRACT

Introduction: Coronary artery disease is caused by the additive and interactive effects of inherited and environmental factors. Substantial evidence shows that reactive oxygen species (ROS) play a vital role in the aetiopathogenesis of atherosclerosis. Our study has been designed to evaluate the oxidative stress due to ROS and assess the antioxidant protection against ROS, in addition to the major risk factors, like lipid profiles, habit of smoking and conditions such as diabetes mellitus and hypertension, in myocardial infarction (MI) patients.

Methods: World Health Organisation criteria were followed in the selection of the subjects. 150 patients with MI were included in the study along with equal number of age- and gender-matched controls. Malondialdehyde (MDA) and nitrite/nitrate levels were measured as markers of oxidative stress of free radical induced injury, and total antioxidant status was determined to assess the antioxidant protection against ROS, along with the lipid profiles.

Results: The levels of total cholesterol, low density lipoprotein cholesterol, triglycerides, MDA and nitrite/nitrate were found to be significantly high, while high density lipoprotein cholesterol and total antioxidant capacity were significantly low in MI patients compared to controls.

Conclusion: Our study revealed the importance of determining the total antioxidant status in MI, in addition to the markers of oxidative stress and lipid profiles to enable the formulation of specific antioxidant therapies for an early intervention and better management of the disease. The study also suggests initiating lifestyle modifications as a preventive measure to reduce the burden of the disease.

Keywords: lipid peroxidation, lipid profiles, myocardial infarction, oxidative stress, total antioxidant status

INTRODUCTION

Cardiovascular diseases are the most alarming of the many health predictions for the new millennium worldwide. Current projections suggest that by the year 2020, India will have the largest cardiovascular disease burden in the world. Coronary artery disease (CAD) is the most common form of heart disease. Fatty deposits called plaques, composed of cholesterol and fats, build upon the inner wall of the arteries. The rupture of these lipid-laden plaques and exposure of substances that promote platelet activation and thrombin generation result in thrombus, which interrupts blood flow. This condition leads to an imbalance between oxygen supply and demand, and if this imbalance exceeds, it results in myocardial infarction (MI) or heart attack.(1,2)

The aetiopathogenesis leading to an atherosclerotic artery is still unknown. However, a number of risk factors for CAD in Indians have been identified, such as serum lipids, lipoproteins, hypertension, diabetes mellitus, smoking, and positive family history. It has been shown that oxidative stress due to the disturbance in the balance between the production of reactive oxygen species (ROS) and antioxidant defence plays a vital role in the pathogenesis of coronary atherosclerosis and its complications. Enhanced formation of ROS may affect four fundamental mechanisms that contribute to atherogenesisism, namely: oxidation of low density lipoprotein (LDL), endothelial dysfunction, vascular smooth muscle cells growth, and monocytes migration.(3-5)
It has also been postulated that ROS would play a dual role in removing anti-atherogenic nitric oxide (NO) and producing proatherogenic peroxynitrite anion (ONOO\textsuperscript{−}). Thus an imbalance in the pro- and anti-atherogenic effects of NO may lead to the pathogenesis of atherosclerotic vascular disease.\textsuperscript{(6)} Antioxidants that are effective against ROS could play a major role in limiting atherosclerosis and its clinical manifestations such as MI or stroke. To assess the antioxidant protection against free radical injury, measurement of total antioxidant status (TAS) in body fluids is very important.\textsuperscript{(5)} Studies on the TAS in MI are very rare and have not been reported in India to date. The present study has been undertaken to estimate the oxidative stress by measuring the levels of malondialdehyde (an end product of lipid peroxidation) and NO along with the lipid profiles, and assess the antioxidant defence in the body by measuring the TAS of MI patients to understand the ongoing oxidative process in the arterial walls.

METHODS

The study population consisted of 150 patients with angiographically-documented MI admitted to the cardiology unit of Durga Bai Deshmukh Hospital and Research Centre, Hyderabad, India. The control group consisted of 150 healthy individuals with no known history of any disease. The study has the approval of the institutional ethical committee for biomedical research. All the patients were examined clinically and information pertaining to age, gender, habits and health status was recorded in a special case proforma. Clinical examination was followed by a series of laboratory investigations to carry out biochemical studies. After obtaining informed consent, blood samples were drawn following an overnight fast by venipuncture into two tubes, with and without anticoagulant. Each blood sample was centrifuged for 10 minutes at 1,000 rpm to collect plasma and serum. Total cholesterol, high density lipoprotein (HDL) cholesterol and triglycerides were measured by an enzymatic method using commercially-available kits (Qualigens Fine Chemical, Glaxosmithkline Pharmaceuticals, Worli, Mumbai, India). LDL cholesterol was calculated by Friedewald’s formula.

Estimation of plasma malondialdehyde (MDA) levels was carried out using the method of Gavino et al.\textsuperscript{[10]} MDA is formed as an end-product of lipid peroxidation which reacts with thiobarbituric acid (TBA) to form a faint pink product. 0.5 ml of plasma was made up to 1 ml with saline, and an equal volume of trichloroacetic acid (TCA) was added and incubated at 37 °C for 20 minutes and centrifuged at 500 g. To 1 ml of TCA extract (the supernatant), 0.25 ml TBA was added and heated in a water bath at 95 °C for one hour till a faint pink appeared. After cooling, it was extracted in 1 ml butanol and the intensity was read at 532 nm. 1,1,3,3-tetraethoxypropane (1–100 nmol/ml) was used as the standard.

Nitrite/nitrate concentrations present in the reaction mixture were determined by using Griess reagent (a 1:1 mixture of 1% sulphanilamide in 5% H\textsubscript{3}PO\textsubscript{4} and 0.1 % N-(1-naphthyl)-ethylene-diamine) using the method of Lepoivre et al.\textsuperscript{[9]} 0.5 ml of serum was precipitated with 50 μL of 70% sulphasalicylic acid, mixed well for five minutes, vortexed and then centrifuged at 3,000 rpm for 20 minutes. 200 μL of supernatant was taken and 30 μL of 10% NaOH, 300 μL of 50 mM Tris buffer and 530 μL of Greiss reagent were added and incubated in the dark for 10 minutes. The absorbance was read against blank (double distilled H\textsubscript{2}O) at 540 nm using Shimadzu UV-240 spectrophotometer (Shimadzu Europa GmbH, Duisburg, Germany). The concentration of nitrite/nitrate in serum was determined using the standard curve.

Total serum antioxidant levels were determined using the method of Re et al.\textsuperscript{[10]} This improved technique involved the direct production of the blue/green ABTS\textsuperscript{+} \[2,2’-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)] radical chromophore through the reaction between ABTS and potassium persulphate. 100 μL of 70 mM potassium persulphate was added to 25 μL of 2.5 mM ABTS. After overnight incubation, the coloured solution was diluted with phosphate-buffered saline to read an absorbance of 0.680–0.820 at 734 nm using a Shimadzu UV-240 spectrophotometer. The initial optical density of ABTS and final optical density after addition of 10 μL of standard/serum were recorded. The extent of decolourisation as percentage inhibition of ABTS\textsuperscript{+} radical cation was determined as a function of concentration and time, and calculated relative to the reactivity of Trolax (a vitamin E analogue (6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid) as a standard under the same conditions.

RESULTS

Among 150 patients presenting with MI, 104 patients were men, with mean age of 51.5 (± 14.6) years, and 46 were women, with mean age of 56.8 (± 10.0) years. Among 150 healthy controls, 90 were men, with mean age of 50.6 (± 8.5) years, and 60 were women, with mean age of 54.6 (± 12.9) years, respectively (Table I). Out of 104 male patients, 50 (48.1%) were smokers and 33 (31.7%) were alcoholics. All the female patients and healthy controls were non-smokers and non-alcoholics. Out of 150 patients, 98 (65.3%) had diabetes mellitus and 48 (32%) had hypertension and 48 (32%) had diabetes mellitus. The habits and health status of the subjects are shown in Table II.

Serum lipid profiles of MI patients and controls are presented in Table III. The estimated mean (± standard deviation (SD)) levels of cholesterol, LDL cholesterol and triglycerides in MI were 220.18 (± 52.69) mg%, 168.25 (± 32.28) mg/dL and 194.36 (± 32.62) mg/dL,
respectively, and that of the controls were 135.56 (± 32.24) mg/dL, 85.65 (± 18.26) mg/dL and 110.12 (± 20.29) mg/dL, respectively. The values in MI patients were significantly higher compared to controls (p < 0.01). The mean (± SD) levels of HDL cholesterol in MI patients were significantly lower (25.86 ± 11.24 mg/dL) compared to controls (43.56 ± 12.56 mg/dL) at p < 0.01.

MDA levels estimated in MI patients and controls are presented in Table IV. The mean (± SD) of MDA levels (nmoles/ml) in the plasma were found to be 6.78 (± 1.80) in MI patients and 1.92 (± 0.56) in controls. The MDA levels in patients were significantly higher at p < 0.01, compared to controls. Nitrite/nitrate levels estimated in MI patients and controls are presented in Table IV. The mean (± SD) of nitrite/nitrate levels (μmoles/ml) in the serum were found to be 7.42 (± 1.87) in MI patients and 1.56 (± 0.34) in controls. The nitrite/nitrate levels in patients were significantly higher, when compared to controls at p < 0.01.

The TAS estimated in MI patients and controls are presented in Table IV. The mean (± SD) of TAS (μmoles/ml) in the serum were found to be 2.40 (± 0.65) in MI patients and 4.08 (± 0.26) in controls. The TAS levels in the patients were significantly lower compared to controls (p < 0.01). The mean and SD of lipid profiles, MDA, nitrite/nitrate and TAS were estimated in the MI patients and controls, and t-test was employed for statistical significance.

**DISCUSSION**

Coronary heart disease is a multifactorial disease. Elevated levels of serum and LDL cholesterol and low levels of HDL cholesterol have been reported as most important risk factors for CAD. Risk factors are not only additive in their effects but can also be interactive. In the present study, in addition to the major risk factors such as cholesterol, the levels of MDA, nitrite/nitrate and TAS were also determined in patients with MI along with age- and gender-matched healthy controls. The percentage of MI patients with habits like smoking and alcoholic use and health status such as diabetes mellitus and hypertension were also determined.

The Framingham Heart Study demonstrated the concept of low HDL cholesterol as a major risk factor for CAD. The levels of HDL cholesterol are inversely

| Table I. Age and gender distribution of subjects in the study. |
|---|---|---|---|
| Groups | Men | Women |
| | No. (%) | Age (years) mean ± SD | No. (%) | Age (years) mean ± SD |
| Myocardial infarction (n=150) | 104 (69.3) | 51.48 ± 14.64 | 46 (30.6) | 56.82 ± 10.06 |
| Controls (n=150) | 90 (60) | 50.46 ± 8.45 | 60 (40) | 54.64 ± 12.96 |

| Table II. Proportion of MI patients with risk factors. |
|---|---|---|
| Risk factors | MI patients (n=150) | Percentage (%) |
| Smoking | 50/104 | 48.1 |
| Alcoholic use | 33/104 | 31.7 |
| Hypertension | 98/150 | 65.3 |
| Diabetes mellitus | 48/150 | 32.0 |

| Table III. Serum lipid profiles of patients and controls. |
|---|---|---|---|
| Subjects | Total cholesterol (mg%) mean ± SD | LDL cholesterol (mg/dL) mean ± SD | HDL cholesterol (mg/dL) mean ± SD | Triglycerides (mg/dL) mean ± SD |
| Myocardial infarction (n=150) | 220.18 ± 52.69* | 168.25 ± 32.28* | 25.86 ± 11.24** | 194.36 ± 32.62* |
| Controls (n=150) | 135.56 ± 32.24 | 85.65 ± 18.26 | 43.56 ± 12.56 | 110.12 ± 20.29 |

* Significantly higher at p < 0.01; ** Significantly lower at p < 0.01

| Table IV. Markers of oxidative stress and antioxidant status of patients and controls. |
|---|---|---|---|
| Subjects | MDA levels (nmoles/ml) mean ± SD | Nitrite/nitrate levels (μmoles/ml) mean ± SD | Total antioxidant levels (μmoles/ml) mean ± SD |
| Myocardial infarction (n=150) | 6.78 ± 1.80* | 7.42 ± 1.87* | 2.40 ± 0.65** |
| Controls (n=150) | 1.92 ± 0.56 | 1.56 ± 0.34 | 4.08 ± 0.26 |

* Significantly higher at p < 0.01; ** Significantly lower at p < 0.01
related to CAD incidence, consistent with its putative role in cholesterol removal. In our study, significantly lower levels of HDL cholesterol were found in patients when compared to controls. Low levels of HDL are associated with an increased risk of coronary heart disease and MI. High levels of HDL have also demonstrated dose-dependent protection against peroxidation of LDL. The results of the present study also indicated high levels of cholesterol and LDL cholesterol in the MI patients, when compared to controls similar to the observations of earlier reports. Elevated levels of total cholesterol and LDL have also been found to be directly related to the incidence of coronary heart disease.

Although elevated levels of LDL appear to play a central role in the risk for atherosclerosis, it has been suggested that LDL itself is not atherogenic in vitro but it must be modified to promote atherogenesis. LDL, which may be modified by oxidation, glycation, aggregation, association with proteoglycans, or incorporation into immune complexes, is a major cause of injury to the endothelium and underlying smooth muscle. When LDL particles become trapped in an artery, they can undergo progressive oxidation and be internalised by macrophages by means of the scavenger receptors on the surfaces of these cells. The internalisation leads to the formation of lipid peroxides and facilitates the accumulation of cholesterol esters, resulting in the formation of foam cell.

Lipid peroxidation, thus initiated in the polyunsaturated fatty acids in LDL-surface phospholipids, then propagates to core lipids, resulting in oxidative modification of polyunsaturated fatty acids, cholesterol moiety and phospholipids. Among the compounds with terminal carbonyl groups that result from lipid peroxidation, MDA is widely used as an index of oxidative damage for its ability to interact with lipoproteins. These modified lipoproteins are taken up by macrophages and transformed into foam cells leading to the development of atherosclerotic plaques and progression of atherogenesis. Therefore, to assess the peroxidation process, which determines the extent of peroxide-forming free radical mechanism and the peroxide-removing antioxidative system, MDA, an end-product of lipid peroxidation, was estimated.

The results of the present study revealed that the mean values of MDA of patients of MI were significantly higher than that of the controls. Cavalea et al has suggested that lipid peroxidation is involved in CAD as shown by their observation of significantly increased plasma free and total MDA concentrations compared with controls. Similarly, Tamer et al has also observed high levels of MDA in atherosclerotic patients compared to controls. Ledwozyw et al found significantly increased concentrations of plasma triglycerides, cholesterol, total lipids and lipid peroxides in patients with atherosclerotic lesions compared with controls, and suggested a positive correlation between lipid peroxide level in plasma and in the arterial wall.

Free radicals, which are important in lipid peroxidation, also react with NO, are produced in large quantities in the inflamed blood vessels during early atherosclerosis, and form peroxynitrite anions contributing to cytotoxicity and tissue injury. Peroxynitrite has been postulated to accelerate the process by initiating lipid peroxidation in LDL and inflicting massive oxidative injury on the vascular endothelium. In the present study, the levels of nitrite/nitrate were estimated and found to be high when compared to controls. Jain et al also reported elevated levels of nitrite in MI patients and suggested that the increase in nitrite is the result of an ongoing free radical mediated oxidative injury, which has involved in nitrite-producing cells. Elevated levels of MDA and nitrite/nitrate in MI patients compared to the control group indicate the role of ROS in the endothelial damage and pathogenesis of the disease.

Recently, Berg et al indicated the pathophysiological roles of ROS acting both as signal molecules and as mediators of tissue injury in cases with acute MI. The additive and interactive effects of inherited and environmental factors also cause coronary heart disease. In the present study, 48.1% of men with MI had the habit of smoking and 31.7% of men had the habit of alcoholic consumption. 65.3% and 32%, respectively, of MI patients had a previous history of hypertension and diabetes mellitus. Of the common environmental factors associated with coronary heart disease, smoking makes a major contribution. It has been observed that in smokers, the environmental impact is much stronger than the genetic component for the development of CAD. The products of tobacco combustion directly damages vascular endothelium, which leads to increased secretion of adhesion molecules. This reaction enhances binding of platelets and monocytes to vessel walls, thus promoting thrombosis and atherosclerosis. Smoking also disturbs lipoprotein metabolism by raising insulin resistance and lipid intolerance, and is implicated in the production of small dense LDL cholesterol. It has also been observed that increased oxidative stress in active smokers is indicated by the relative lack of protective antioxidant vitamins E and C in plasma and an increase in the adhesion molecules E-selectin and ICAM-1 as systemic markers of endothelial dysfunction.

The effect of alcohol intake on plasma lipids and coronary heart disease has been the subject of extensive research. Both case control and cohort studies have described a J- or U-shaped association between alcohol intake and coronary heart disease and between alcohol intake and mortality. Although moderate alcohol
consumption appears to be protective, heavy consumption of alcohol is associated with subclinical impairment of left ventricular function, and occasionally results in overt cardiomyopathy. This may be a consequence of direct toxic effects of alcohol or its metabolites, coexisting malnutrition, associated hypertension, increased ventricular mass, or rarely, toxic additives to alcoholic beverages.

Several epidemiological studies, including the Framingham study, have established hypertension as a major cardiovascular risk factor. The relation between hypertension and cardiovascular disease is continuous and graded. It does not initiate atherosclerosis but accelerates it in presence of elevated LDL cholesterol. The likelihood of developing a vascular event increases as more and more risk factors are added. Diabetes mellitus is another important risk factor for coronary heart disease. The risk for CAD among subjects with diabetes mellitus is greater by a factor of two to four, compared to non-diabetic subjects. Hyperglycaemia, a hallmark of diabetes mellitus, accelerates atherosclerosis by several mechanisms, such as promoting endothelial cell dysfunction, creation of prothrombotic state and formation of advanced glycosylated end-products which increase inflammation, oxidative damage and atherogenesis. Thus, oxidative stress and endothelial dysfunction involved in early atherosclerotic disease is caused by a variety of stimulatory factors, such as cigarette smoking (environmental factor), hypertension and type 2 diabetes mellitus (disease factors).

Several methods have been proposed for studying the mechanisms of antioxidant protection against free radical-induced injury, including the measurement of the TAS in body fluids. In the present study, the TAS was determined and found to be low in the MI patients, compared to controls. Fazendas et al showed that in a group of 23 MI patients, plasma total antioxidant capacity was decreased significantly and increased with bene

ACKNOWLEDGEMENT

The authors are grateful to the Department of Biotechnology, New Delhi, India for providing financial assistance.

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