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ORIGINAL ARTICLE

Erythrocyte Lipid Peroxidation, Glutathione, Ascorbic Acid, Vitamin E, Antioxidant Enzymes And Serum Homocysteine Levels In Patients With Coronary Artery Disease

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ABSTRACT

Background: Coronary Artery Disease is the major cause of mortality and morbidity worldwide. It is associated with various risk factors such as age group (41 – 60 years), male gender, smoking habit and hypertension. The exact pro-oxidant and antioxidant status in patients with Coronary Artery Disease is still not clear. To add a new insight to the question, changes in erythrocyte lipid peroxidation products (MDA), glutathione (GSH), ascorbic acid and plasma vitamin E, and activities of antioxidant enzymes like super oxide dismutase (SOD), glutathione peroxidase (GP_X), catalase in erythrocytes, plasma glutathione – S – transferase (GST) and serum homocysteine levels were measured in patients with Coronary Artery Disease.

Aim: This work was undertaken to assess oxidative stress and antioxidant status in patients with Coronary Artery Disease and its contribution to the risk of cardiovascular disease.

Settings and Design: The study was conducted in sixty - five patients and the values were compared to control values. Erythrocyte MDA, GSH, ascorbic acid, plasma vitamin E and activities of antioxidant enzymes SOD, GP_X, catalase in erythrocytes, plasma GST and serum homocysteine were estimated in Coronary Artery Disease patients. These parameters were measured in sixty - five patients and the values were compared to control values.

Statistical Analysis: Statistical analysis between group 1 (controls) and group 2 (patients) was performed by the Mann Whitney U test. The data was expressed as mean $\pm$ SD. $P < 0.05$ was considered to be significant.

Results: It was observed that there was a significant increase in erythrocyte MDA levels, SOD, GP_X and plasma GST activities, and a significant decrease in erythrocyte GSH, ascorbic acid, plasma vitamin E levels and catalase activity in patients with Coronary Artery Disease when compared to controls. Serum homocysteine levels were significantly higher in Coronary Artery Disease patients than in the controls.

Conclusions: The results of our study suggest higher oxygen free radical production which is evidenced by increased MDA and decreased GSH, ascorbic acid, vitamin E and Catalase activity and support to the oxidative stress in coronary artery disease. Increased homocysteine levels and decreased antioxidant capacity may contribute to the increased risk of cardiovascular disease in patients with coronary artery disease, in addition to known risk factors such as insulin resistance, hypertension, central obesity, and dyslipidaemia. The increased activities of antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress.

Key Words: Malondialdehyde,homocysteine, oxidative stress, antioxidants, cardiovascular risk,coronary artery disease.

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Introduction

Coronary Artery Disease is the major cause of mortality and morbidity worldwide [1]. It is associated with various risk factors such as age group (41 – 60 years), male gender, smoking habit and hypertension. Lipid peroxidation which is mediated by free radicals, is considered to be the major mechanism of cell membrane destruction and cell damage. Free radicals are formed in both physiological and pathological conditions in mammalian tissues [2]. The uncontrolled production of free radicals is considered as an important factor in the tissue damage induced by several pathophysiologies [3]. The effects of lipid peroxides i.e. endothelial cell damage, uncontrolled lipid uptake, decreased prostaglandin synthesis and associated thrombogenecity, are strongly implicated in the pathogenesis of atherosclerosis. Alteration in the oxidant - antioxidant profile is known to occur in Coronary Artery Disease [4]. Oxidative stress due to damage, brought about by free radicals, is also known to influence the response of these patients to therapy. Moreover, the body's defense mechanisms would play a role in the form of antioxidants and try to minimize the damage, adapting themselves to the above stressful situation. Antioxidants are compounds that dispose, scavenge, and suppress the formation of free radicals or oppose their actions [5], and two main categories of antioxidants are those whose role is to prevent the generation of free radicals and those that intercept any free radicals that are generated (6). They exist in both the aqueous and membrane compartment of cells, and can be enzymes or non-enzymes. The traditional risk factors of coronary artery disease include age, sex, dyslipidaemia, blood pressure and smoking. A continued focus on newer risk factors is warranted, as they may further improve our ability to predict future risk and determine treatment when they are included with classic risk factors [7]. The study of these risk factors is important, since the ability to accurately predict the risk of coronary artery disease of a specific individual based on his or her conventional risk factor profile, is limited [8]. One of these newer risk factors is homocysteine. Elevated serum homocysteine may be an important cause for atherosclerosis formation [9]. So, the present study was undertaken to assess oxidative stress and antioxidant status in patients with Coronary Artery Disease, and their contribution to the risk of cardiovascular disease.

In the present study, the following parameters were assessed in the erythrocytes and plasma to elucidate the oxidant-antioxidant status in patients with Coronary artery disease. Erythrocyte malondialdehyde (MDA) levels were measured as thiobarbituric acid reacting substances (TBARS), which serve as an index of extent of lipid peroxidation. Erythrocyte glutathione (GSH), ascorbic acid and plasma vitamin E serve as non enzymatic antioxidant parameters. The activities of antioxidant enzymes, superoxide dismutase (SOD), catalase, glutathione peroxidase (GP_X) in erythrocytes, glutathione-S -transferase (GST) in plasma and serum homocysteine levels were estimated. GST is an enzyme involved in antioxidant defense, and is also involved in detoxication. The present work is an attempt to determine alteration in oxidant – antioxidant status and its contribution to the risk of

cardiovascular disease in Coronary Artery Disease patients.

Materials And Methods

Sixty - Five clinically diagnosed patients of acute myocardial infarction, admitted to the Intensive Cardiac Care Unit (ICCU), were chosen for the study. The patients were randomly selected, and had come for cardiac evaluation during September 2006 and December 2007. An equal number of age and sex matched healthy subjects with a similar socio economic status was also investigated**.** The females of the study group were matched with females of the control group.

Diagnostic Criteria Of Patients

The diagnosis of Acute MI was based on the WHO criteria, which required the presence of at least 2 of the following three elements: i) Ischaemic type of chest pain ii) changes on serial ECG tracings iii) Increase in serum cardiac marker (CKMB) [10].

Exclusion Criteria

The patients with associated renal failure, liver disease, lung disease, pregnancy, thyroid disease, gastro-intestinal disease and those taking methotrexate, carbamazepine or phenytoin, that could alter the required parameters, were excluded from the study. No patient had sustained myocardial infarction within 6 months before taking part in the study. Written consents were also taken from the patients prior to the study, and the objectives of the study were fully explained.

There were two study groups. The controls and patients were divided into two groups.

Group 1: Sixty - Five healthy age and sex matched controls.

Group 2: Sixty - Five patients with clinically diagnosed coronary artery disease.

Blood samples drawn from all the subjects within an hour of admission were processed for the cardiac enzymes (CK, CK-MB, LDH). The heparinised venous blood samples obtained under asceptic conditions from these subjects in the fasting state (after overnight fasting), were used for the analysis of homocysteine. Plasma was separated by centrifugation at 1,000 g for 15 minutes. Separated plasma was used for the estimation of vitamin E, and for the measurement of the activity of GST. All the cases were subjected to routine investigations like haematocrit, urine analysis and blood chemistry (electrolytes, lipids, blood glucose, urea and creatinine). Elevated serum cholesterol and triglycerides along with low HDL levels, is observed in these patients. The buffy coat was removed, and the packed cells were washed three times with physiological saline. The erythrocyte suspension was prepared by the method of Dodge et al. [11], modified by Quist [12]. The packed cells were used for the analysis of GSH, ascorbic acid, MDA, SOD, Catalase and GP_X. Erythrocyte GSH was estimated by the method of Beutler et al [13], using Di Thio Bis Nitro Benzoic acid (DTNB). Ascorbic acid levels were estimated by the method of Tietz [14]. Plasma vitamin E levels were estimated by the method of Baker H et al [15]. MDA was determined as the measure of TBARS [16]. SOD (EC 1.15.1.1) activity was determined in the haemolysate by the method of Misra and Fridovich, based on the inhibition of auto oxidation of epinephrine to adenochrome at Ph 10.2 [17]. Catalase (EC 1.11.1.6) activity was measured by the method of Beers and Sizer [18]. The activity of Glutathione Peroxidase (GP_X, EC 1.11.1.9) was measured as described by Paglia and Valentine [19] in erythrocytes, and activity of GST (EC 2.5.1.18) was measured by using 1-Chloro-2, 4-Dinitro Benzene (CDNB) [20]. Homocysteine was measured using a solid phase immunoassay system, which measures total homocysteine in plasma or serum using a BioRad kit [21].

Chemicals

All reagents used, were of analytical reagent grade. DTNB, CDNB and Thio Barbituric Acid were obtained from Sigma Chemicals, St.Louis; MO.

Statistical Analysis

The statistical analysis between group 1 (controls) and group 2 (patients) was performed by the Mann Whitney U test. The data was expressed as mean $\pm$ SD. $P < 0.05$ was considered as significant.

Results

The basic characteristics of the coronary artery disease patients and controls are given in [Table/Fig 1].

(Table/Fig 1) Clinical Characteristics Of The Study Groups

Characteristics	Group1 (controls)	Group2 (Patients)
Age in Years (Mean ± SD)	54.95 ± 3.26	58.61 ± 4.01
Total Number	65	65
Males	56 (86.1%)	56 (86.1%)
Females	09(13.8%)	09(13.8%)
Hypertension	22 (33.8%)	49 (75.3%)
Smokers	19 (29.2%)	39 (60%)

The mean ± SD of erythrocyte GSH, ascorbic acid, MDA, SOD, Catalase, GP_x , plasma vitamin E, plasma GST and serum homocysteine in patients with coronary artery disease and controls are indicated in [Table/Fig 2].

(Table/Fig 2) The Mean ± SD Values Of Malondialdehyde (MDA), Glutathione, Ascorbic Acid, Vitamin E, Super Oxide Dismutase (SOD), Catalase, Glutathione Peroxidase (GP_X), Glutathione – S – Transferase And Serum Homocysteine In Controls And Patients With Coronary Artery Disease.

Parameter	Group1 (Controls) (mean ± SD) n = 65	Group2 (Study Subjects) (mean ± SD) n = 65
Glutathione (mg/gm of Hb)	19.96 ± 1.05	19.20 ± 1.01 ***
Ascorbic Acid (mg/dl)	4.39 ± 1.28	3.75 ± 1.15 ***
Vitamin E(µmoles/L)	8.95 ± 2.29	8.39 ± 2.26 ***
MDA(nmoles/gm of Hb)	13.4 ± 1.24	19.8 ± 2.78 ***
SOD(U/gm of Hb)	680.29 ± 35.54	770.19 ± 31.86 *
Catalase(U/gm of Hb)	12.48 ± 1.94	12.05 ± 2.38 *
GP_X (U/gm of Hb)	63.6 ± 1.36	80.8 ± 1.01 ***
GST(micromoles / dl of plasma)	12.46 ± 1.78	15.39 ± 3.46 ***
Serum Homocysteine (µmole/L)	14.3 ± 1.52	20.8 ± 1.75 ***

* P < 0.05 compared to controls *** P < 0.001 compared to controls

There was a statistically significant increase in the erythrocyte MDA and serum homocysteine levels in patients with Coronary Artery Disease, as compared to controls. The activities of erythrocyte antioxidant enzymes, SOD, GP_X and plasma GST were significantly increased in group 2 (study subjects) as compared to group1 (controls). The levels of erythrocyte GSH, ascorbic acid, plasma vitamin E and catalase activity were significantly decreased in patients with Coronary Artery Disease as compared to controls.

Discussion

In the present study, the lipid peroxidation product i.e. MDA levels have been increased significantly in erythrocytes of the patients with Coronary Artery Disease as compared to controls. MDA is a decomposition product of autooxidation of poly unsaturated fatty acids, which is used as an index of oxidative damage [22]. The rise in MDA concentration indicates increased membrane lipid peroxidation, characterized by hyperlipidaemia, specifically hypercholesterolaemia, in these patients. Rise in MDA could be due to increased generation of reactive oxygen species (ROS), due to the excessive oxidative damage generated in these patients. These oxygen species in turn, can oxidize many other important biomolecules including membrane lipids. Similar reports of elevated MDA levels have been reported in patients with coronary artery disease [4].

We observed a significant decrease in the levels of erythrocyte glutathione (GSH), ascorbic acid and plasma vitamin E (non enzymatic antioxidant defense system) in patients with coronary artery disease, when compared to controls. The decrease in the levels of these non enzymatic antioxidant parameters may be due to the increased turnover, for preventing oxidative damage in these patients, suggesting an increased defense against oxidant damage in coronary artery disease. Some other groups have also reported decreased GSH, Ascorbic acid and Vitamin E levels in patients with Coronary Artery Disease [23].

In our study, the activities of erythrocyte antioxidant enzymes i.e. SOD and GP, have been increased significantly in patients with coronary artery disease, as compared to controls. SOD is an important antioxidant enzyme having an antitoxic effect against super oxide anion. The overexpression of SOD might be an adaptive response, and it results in increased dismutation of superoxide to hydrogen peroxide. There is an enhanced production of super oxide anions by ischaemic cells. In contrast to our study, decreased concentrations of SOD in the haemolysate of these patients, was reported [4]. GP_X, an oxidative stress inducible enzyme, plays a significant role in the peroxyl scavenging mechanism, and in maintaining functional

integration of the cell membranes. The rise in the activity of GP_X could be due to its induction to counter the effect of increased oxidative stress.

The Glutathione – S – Transferase is a group of multifunctional proteins, which plays a central role in detoxification of electrophilic chemicals and the hepatic removal of potentially harmful hydrophobic compounds from blood [24]. We have observed a significant increase in the GST activity in patients with Coronary Artery Disease, as compared to controls. The rise in the activity of GST could be due to its induction to counter the effect against increased oxidative stress.

In the present study, we have observed a significant decrease in the activity of catalase in patients with coronary artery disease, as compared to controls. Catalase is the enzyme which protects the cells from the accumulation of hydrogen peroxide by dismutating it to form water and oxygen, or by using it as an oxidant in which it works as a peroxidase.

Homocysteine has been recognized recently as a risk factor for vascular diseases. In our study, Serum Homocysteine levels were significantly increased in patients with CAD, as compared to controls. Increased serum homocysteine levels also lead to the formation of atherosclerotic plaques, which ultimately lead to myocardial infarction. The sulfhydryl groups in homocysteine were oxidized to disulfide, catalyzed by the transition metals by which several reactive oxygen species and hydroperoxides were produced, and initiates lipid peroxidation which is responsible for endothelial injury [25]. Similar reports of increased levels of homocysteine in coronary artery disease were reported [26].

Conclusion

In Conclusion, Oxidative stress may be involved in coronary artery disease. The results of our study have shown higher oxygen free radical production and decreased catalase activity, suggesting the incidence of oxidative stress in coronary artery disease. The increased activities of antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress. Increased homocysteine levels and decreased antioxidant capacity may contribute to the increased risk of cardiovascular disease in patients with coronary artery disease, in addition to known risk factors such as age, sex, dyslipidaemia, blood pressure and smoking. So, treatment with antioxidants in the management of the coronary artery disease may be useful as secondary therapy to prevent oxidative damage.

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