BRIEF COMMUNICATION

OXIDATIVE STRESS AND ANTIOXIDANT STATUS IN NORMOLIPIDEMIC AMI PATIENTS

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ABSTRACT

The objective of the study was to observe the correlation between oxidative stress and antioxidants in myocardial infarct patients with normal lipid profile. In the present study investigation of lipid peroxidation and antioxidants were done in patients. This study was carried out on 165 AMI patients, (123 males and 42 females). The control group consisted on 165 normal healthy age-sex matched subjects (123 males and 42 females). Levels of Superoxide dismutase, Glutathione Peroxidase, Catalase, Malondialdehyde and Conjugated dienes were measured. The study found Superoxide dismutase, Glutathione Peroxidase, Catalase were significantly lower in activities ($p < 0.001$) in AMI patients compared to controls. The markers of lipid peroxidation namely Malondialdehyde and Conjugated dienes were higher in AMI patients as compared to controls ($p < 0.001$). The present study is clearly suggestive of increased oxidative stress in AMI patients. Oxidative stress appears as an etiological factor for myocardial infarction as a consequence the free radical scavengers levels are lowered in AMI patient.

KEY WORDS

Oxidative stress, Antioxidants, AMI, Normolipidemia.

INTRODUCTION

Free radicals contain one or more unpaired electrons. They play an important role in the pathogenesis of tissue damage in many different clinical disorders (1). Oxygen free radicals (OFRs) are produced continuously. Normally there is a balance between tissue oxidant and antioxidant activity (2). The later is achieved by the antioxidant scavenger system which includes enzymes (superoxide dismutase, catalase, glutathione peroxidase) and antioxidant vitamins (C, A, E and other carotenoids) (3). Imbalance of this reaction either due to excess free radical formation or insufficient removal by antioxidants leads to oxidative stress (4). Increased generation of OFRs can cause atherosclerosis due to oxidative modification of LDL molecules which is one of the causes of Acute Myocardial Infarction (5). Coronary Heart disease (CHD)

is associated with the greatest morbidity and mortality in industrialized countries (6). Due to increased oxidation of LDL, the formation of malondialdehyde (MDA) and conjugated dienes (CD) takes place with increased lipid peroxidation. The increased concentration of CD and extensive lipid peroxidation could also be due to dyslipidemias and aberrations of lipid profiles. This whole process is involved in atherogenesis which is important cause of AMI, but now a trend is emerging that the chances of Acute Myocardial Infarction (AMI) persists even normolipidemic subjects (7). Hence, the present study was planned to evaluate the oxidative stress in terms of lipid peroxidation and antioxidants enzyme status in normolipidemic AMI patients.

MATERIALS AND METHODS

Setting Design and patients: The study consisted of 165 patients (123 males and 42 females) with AMI, admitted in the Intensive Cardiac Care Unit, Faculty of Medicine, University of Peradeniya, Sri Lanka. The diagnosis of AMI was established according to diagnostic criteria: chest pain, which lasted for up to 3 hours, ECG changes (ST elevation of 2 mm or more in at least two leads) and elevation of serum creatine

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phosphokinase (CPK-MB) and aspartate aminotransferase (AST) enzyme elevation. The control group consisted of 165 age-sex matched healthy volunteers, 123 males and 42 females. The study was conducted for a period of four and half years from April 2002 to August 2006. Informed consent was taken from patients and the study was approved by the ethical committee of the institution.

Patients with diagnosis of AMI with normal lipid profile were included in this study. Patients with diabetes mellitus, dyslipidemia, renal insufficiency, current and past smokers, hepatic disease or taking lipid lowering drugs or antioxidant vitamin supplements were excluded from this study.

Criteria for Normolipidemics: Normal lipid profile was defined if LDL was <160mg/dl, HDL > 35 mg/dl, Total cholesterol (TC) <200 mg/dl and Triglycerides (TG) <150 mg/dl (8).

Blood collection and biochemical methods used: 10 ml of blood was collected after overnight fasting in different vials. EDTA vial: 5.0 ml of blood was taken. Red cells were washed 3-4 times with ice-cold normal saline and used for estimation of glutathione peroxidase, superoxide dismutase and catalase. Plain vial: Remaining blood was taken and serum was separated. Serum was used for determination of lipid profile, malondialdehyde and conjugated dienes.

Lipid profile (Total cholesterol, triglycerides, and HDL-cholesterol) were analyzed enzymatically using kit obtained from (Randox Laboratories Limited, Crumlin, UK). Plasma LDL-cholesterol was determined (9) from the values of total cholesterol and HDL-cholesterol using formulae: LDL-cholesterol = Total cholesterol - [(Triglycerides/5) + HDL-cholesterol (mg/dl)]. All chemicals of analytical grade were obtained from Sigma chemicals, India.

Glutathione peroxidase activity was determined by the procedure of Paglia and Valentine (10). Superoxide dismutase enzyme activity was measured by SOD assay kit using rate of inhibition of 2-(4-indophenyl)-(4-Nitrophenol)-5-phenyltetrazolium chloride (I.N.T) reduction method modified method of Sun Y Oberly (11) using assay Ransod kit SD 125, Randox Lab. Catalase activity was measured spectrophotometrically as described by Beutler (12, 13). MDA levels were estimated by thiobarbituric acid (TBA) reaction (14). Conjugated dienes levels were measured by Recknagel and Glende method (15) with little modification.

Table 1: Antioxidant Status and Lipid Peroxidation in Control and AMI patients (mean $\pm$ SD)

Variables	Control(n=165)	Case(n=165)
<i>Superoxide dismutase (U/gHb)</i>	1826.47 $\pm$ 31.86	813.95 $\pm$ 208.98 *
<i>Glutathione Peroxidase(U/gHb)</i>	61.29 $\pm$ 3.94	42.56 $\pm$ 6.36 †
<i>Catalase (k/gHb)</i>	256.15 $\pm$ 26.65	193.06 $\pm$ 35.87 †
<i>TBARS (nmol/L)</i>	5.71 $\pm$ 0.97	14.81 $\pm$ 1.66 *
<i>Conjugated dienes (μmol/L)</i>	31.04 $\pm$ 2.68	48.28 $\pm$ 5.50 †

* (p<0.02); † (p<0.001)

RESULTS AND DISCUSSION

Involvement of oxygen free radicals in the pathophysiology of inflammation, ischemia and in reperfusion damage in a number of organs and tissues have been reported in literature (2, 16). Indirect evidence of OFR generation in patients with AMI has been observed by measuring a variety of by products of lipid peroxidation, such as pentane, conjugated dienes and malondialdehyde (MDA). In the present study, a significant increase in malondialdehyde and conjugated dienes in patients with AMI was observed as compared to controls. The plasma concentration of these substances has been reported to be increased in AMI patients in many studies (17). Reduction in infarct size in animal models of temporary coronary artery occlusion and reperfusion has been reported by means of several different anti-free radical interventions (18). OFRs are generated particularly in early stage of AMI and antioxidants are involved in the reduction of hydrogen peroxide radicals, resulting in a decrease in their activities during that period (18, 19). In the present study, low activities of superoxide dismutase, glutathione peroxidase and catalase were observed in AMI patients as compared to control. Despite data linking OFR generation and reperfusion injury, there are reports that fail to show a beneficial effect of oxygen radical scavengers or other agents on infarct size reduction (20). Thus, the present study is suggestive of imbalance between oxidant and antioxidants in AMI patients which is mainly due to increased oxidative stress. Future research including measurement of parameters of oxidative stress and inflammatory markers should be carried out as the role of inflammatory markers like C-reactive proteins, Ceruloplasmin are emerging which could be possibly be a causative factor for atherosclerosis.

Myocardial Infarction is a multifactorial disease which could be caused even in normo-lipaedemic subjects. The earlier concept of keeping in check with lipid profile has become obsolete as the present study unearths fact. It is advisable to keep a check on serum antioxidant status and peroxidative

markers as the present study highlight the fact. Oxidative stress appears to be an etiological factor for myocardial infarction as a consequence the free radical scavengers namely antioxidants levels tends to lower in patients with myocardial infarction.

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