Indian J Physiol Pharmacol 2007; 51 (2) : 141–146

STUDY OF OXIDATIVE STRESS AND TRACE ELEMENT LEVELS IN PATIENTS WITH ALCOHOLIC AND NON-ALCOHOLIC CORONARY ARTERY DISEASE

PURNIMA DEY SARKAR*, N. RAMPRASAD**,
INDRANIL DEY SARKAR*** AND
T. M. SHIVAPRAKASH****

*Department of Biochemistry, M.G.M. Medical College, Indore – 452 001 and **Department of Biochemistry, Holker Science College, Indore – 452 001,

***Medicaps Institute of Technology and Management, Indore – 452 001 ****Department of Biochemistry, Index Medical College Hospital and Research Centre, Indore – 452 001

(Received on March 14, 2007)

Abstract : Alcohol consumption induces oxidative stress, and leads to lipid peroxidation. These effects have been linked to alcohol-related toxicity and diseases are considered relevant to alcohol-atherosclerosis interrelationship. Deficiency of many antioxidants and trace elements may impair the antioxidant defense leading to ethanol induced oxidative stress. In the present study, our aim was to investigate the lipid peroxidation, lipid profile, antioxidant enzymes and trace elements in patients with and without alcoholic coronary artery disease (CAD). Our study included 61 patients suffering from CAD, 124 patients suffering from alcoholic CAD with high to moderate alcohol intake, 75 controls were randomly selected for our study. Increased serum lipid peroxidation, total cholesterol, triglycerides, LDL cholesterol and copper levels were high while levels of HDL cholesterol, glutathione peroxdiase, superoxide dismutase, trace elements like Selenium and Zinc were low in high alcoholic CAD patients compared with moderate and non alcoholic CAD patients. The results obtained from present study indicate that high alcohol intake predicts low antioxidant enzyme and that trace element may contribute to the increased susceptibility for the development of CAD.

Key words : lipid peroxidation antioxidant enzymes trace elements coronary artery disease and alcohol

INTRODUCTION

Coronary Artery Disease (CAD) is a rising problem in developing countries like India. Epidemiological surveys indicate that there could be 15–20 million cases of CAD in India. The incidence is higher in urban than rural population (1).

*Corresponding Author : Dr. Purnima Dey Sarkar, #126, Durga Sadan, Srinagar Extension, Indore – 452 001; Cell : +91-94250-77653; E-mail : pipu47@yahoo.co.in

Disparity in the relation of alcohol consumption to various cardiovascular conditions has become evident. Heavier drinking is related to higher prevalence of cardiomyopathy, hypertension (HTN), hemorrhagic stroke, and cardiac arrhythmias. Moderate drinking is related to lower prevalence of CAD, ischemic stroke, and sudden cardiac death (2). Furthermore, ingested alcohol produces striking metabolic imbalances in the liver and leads to the formation of reactive oxygen species (3). Lipid peroxidation is a free radical related process, which is potentially harmful because it is uncontrolled, self enhancing process causes disruption of membranes, lipids and other cell components. A lot of oxygenated compounds, particularly aldehyde such as malondialdehyde (MDA) are produced during the attack of free radicals to membranes lipoproteins and polyunsaturated fatty acids (4).

A good antioxidant status may be important for human health and especially for the prevention of chronic diseases such as cancer and CAD. Low plasma levels of antioxidant as well as low intake of dietary antioxidants have been associated with an increased risk of atherosclerotic heart disease (5). Trace elements are being increasingly recognized as essential mediators for the development and progression of heart diseases. It is well known that Selenium (Se), Zinc (Zn) and Copper (Cu) in serum can affect certain heart diseases such as keshan disease (i.e. where soil Se deficiency is very low and it is although often associated with a cardiomyopathy it is demonstrated in China) heart failure, cardiomyopathy and atherosclerosis. Therefore, trace elements may play an important role in the etiopathogenesis of the diseases (6). Trace elements may also play an important role in alleviating tissue damage due to formation of free radicals. Thus it is possible that alcoholic CAD which is very common in low socio-economic strata due to heavy drinking habits is associated with changes in life style and associated multinutritional deficiencies.

Hence a systemic approach has been made in the present study to focus on the cardiovascular disease. Oxidative stress was measured by the serum levels of MDA, enzymic glutathione peroxidase (GPx), superoxide dismutase (SOD) and trace elements like Se, Zn, and Cu in angiographically proven alcoholic with CAD and non alcoholic CAD patients along with normal subjects and compared.

MATERIALS AND METHODS

The study was case-controlled in design. We have selected the patient as they have presented. The study was only male oriented because, the incidence of female alcoholism is very low in local population, and hence the female subjects have been excluded from the study. Patients included in the present study were all admitted to the Intensive Coronary Care Unit (ICCU) or attending the out patient department of medicine of Maharaja Yashvantrao Hospital attached to Mahatma Gandhi Memorial Medical College Indore (M.P)

The study group consisted of 185 patients with CAD and they were undergoing admission to hospital and they were between 40–65 years. They were further classified

into three groups. 59 patients were moderate alcoholic, 65 patients were high alcoholic and 61 patients were nonalcoholic. The criteria for the diagnosis of CAD was made on the basis of clinical history, history of myocardial infraction, 12 leads electrocardiogram (ECG) and coronary angiography findings. The patients in high alcoholic CAD group had been drinking more than 80 gm alcohol per day at least for one year and moderate alcoholic CAD group had been drinking less than 80 gm alcohol per day based on CAGE questionnaire and interview. All patients were having CAD but non-cirrhotics. None of the patient was on any antioxidant therapy or lipid lowering drugs. Controls had 75 healthy age matched, non smoking and non alcoholic healthy individuals. All participants gave written informed consent and this protocol was approved by the ethical and research committee of Mahatma Gandhi. Memorial Medical College, Indore. Table-I gives the details of the profiles of the subjects.

Venous blood samples were collected from all the study subjects after an overnight fast. The concentrations of serum total cholestrerol, triglycerides, high density cholesterol (HDL-C) levels were estimated by Human Diagnostics Reagent (Max-Planck-Ring 21 D-65205 Wiesbaden Germany) adapted to 550 expressplus auto analyzer (ciba corning diagnostics corporation, 63 north street, Medfield, MA 02052-9990, USA). Low density cholesterol (LDL-C) was measured by enzymatic method (7). Serum levels of MDA, a marker of lipid peroxidation were measured by thiobarbutric acid (TBA) method (8). The haemolysate prepared from the red cells was used for the estimation of antioxidant enzyme activities. GPx was measured by the method of Paglia and Valentine (9). SOD estimation was based on the reaction between superoxide radicals and 2–4 iodophenyl 3–4 nitrophenol 5-phenyl tetrazolium chloride (10). Serum levels of Se were determined by Zeeman graphite furnace atomic absorption analysis with nickel nitrate or reduced palladium as matrix modifier is the recommended analysis method (11). Serum Zn and Cu measurement was performed by flame atomic absorption spectrophotometery with deuterium background correction (Perkin-Elmer model 5000) (12).

Statistical analysis

All values are presented as means±SD. Statistical significance was analysed by Student t-test and correlation between variables were studied by using Pearson's correlation coefficient test. The level of significance was set at $P<0.05$.

RESULTS

The clinical characteristics of three groups of CAD patients and control subjects are presented in Table I. In our study, number of smokers and hypertensives are more in alcoholic CAD patients compared to non-alcoholics and controls.

Serum levels of total cholesterol, triglycerides, LDL-C and MDA increased in alcoholic CAD and non alcoholic CAD patients as compared to controls ($P<0.001$), whereas decreased HDL-C ($P<0.001$) levels in alcoholic CAD and non alcoholic CAD patients as compared to controls (Table II). Antioxidant enzyme activities of GPx and

TABLE I: Baseline characteristics of study subjects.

	Controls (n=75) Mean±SD	*Non-alcoholic CAD patients (n=61) Mean±SD*	*Moderate-alcoholic CAD patients (n=59) Mean±SD*	*High-alcoholic CAD patients (n=65) Mean±SD*
Age (yrs)	47.9±8.1	50.9±19.8	52.9±11.2	56.2±12.4*
BMI (kg/m^2)	22.8±8.1	24.8±3.8	25.7±3.9	28.7±4.1*
HTN (%)	8	58	60	70
Smokers (%)	10	41	55	74

*Highly significant (P<0.001) vs controls.
HTN=Hypertension, BMI=Body Mass Index, CAD=Coronary Artery Disease

TABLE II: Lipid profile and malondialdehyde concentrations in study subjects.

	Controls (n=75) Mean±SD	*Non-alcoholic CAD patients (n=61) Mean±SD*	*Moderate-alcoholic CAD patients (n=59) Mean±SD*	*High-alcoholic CAD patients (n=65) Mean±SD*
T. Cholesterol (mg/dl)	148.2±17.7	227.2±16.1*	235.0±14.2*	288.1±26.1*
Triglycerides (mg/dl)	119.1±16.2	197.2±17.4*	210.0±16.8*	239.1±19.8*
HDL-C (mg/dl)	49.2±4.8	38.5±4.5*	37.1±5.2*	34.2±5.5*
LDL-C (mg/dl)	70.8±20.1	145.0±18.1*	159.0±14.3*	190.0±27.2*
MDA (nmoles/ml)	3.5±0.69	6.4±0.51*	6.8±0.40*	8.4±1.6*

GPx=Glutathione peroxidase, SOD=Superoxide Dismutase, Se=Selenium, Zn=Zinc, Cu=Copper.
*Highly significant (P<0.001) versus controls.

SOD were significantly decreased (P<0.001) in non alcoholic CAD and further decreased in high alcoholic CAD patients as compared to controls (P<0.001). However in non alcoholic CAD patients as compared to moderate alcoholic CAD, there is no significant difference in GPx and SOD (P<0.21). However, serum levels of trace elements like Se and Zn were significantly lower (P<0.001) in high alcoholic CAD subjects as compared to non alcoholic CAD patients, but there was no significant difference observed between moderate alcoholic and non alcoholic CAD patients. However, serum Cu levels were significantly increased (P<0.001) in high alcoholic CAD subjects as compared to non alcoholic CAD

TABLE III: Activities of erythrocyte antioxidant enzymes and serum trace elements.

	Controls (n=75) Mean±SD	*Non-alcoholic CAD patients (n=61) Mean±SD*	*Moderate-alcoholic CAD patients (n=59) Mean±SD*	*High-alcoholic CAD patients (n=65) Mean±SD*
GPx (U/gmHb)	32.2±11.2	24.8±3.5*	22.0±5.5*	15.6±2.4*
SOD (U/gmHb)	8.81±1.6	7.2±0.91*	7.0±1.3*	5.8±0.40*
Se (µg/dl)	98.5±11.3	78.2±8.5*	76.1±9.1*	62.2±10.1*
Zn (µg/dl)	89.2±7.7	72.2±5.7*	70.0±5.9*	61.8±7.6*
Cu (µg/dl)	104.2±9.4	124.1±11.9*	129.2±11.6*	136.1±10.1*

GPx=Glutathione peroxidase, SOD=Superoxide Dismutase, Se=Selenium, Zn=Zinc, Cu=Copper.
*Highly significant (P<0.001) versus controls.

TABLE IV: Correlation analysis of Malondialdehyde levels between non-alcoholic and high alcoholic CAD patients.

	Non-alcoholic CAD patients r	*High-alcoholic CAD patients r*
MDA/Se	–0.52*	–0.58*
MDA/Zn	–0.62*	–0.65*
MDA/Cu	0.48*	0.53*

*Significant (P<0.05).
r=correlation coefficient.
CAD=Coronary Artery Disease, MDA=Malondialdehyde, Se=Selenium, Zn=Zinc, Cu=Copper.

patients and healthy controls (Table III). As indicated in Table IV, a significantly negative correlation was found between MDA against Se and Zn levels. However, positive correlation occurs between MDA and Cu levels in non alcoholic CAD patients and high alcoholic CAD patients, i.e. as the Se, Zn levels decreases and elevated Cu levels shows increasing trend of MDA levels.

DISCUSSION

Atherosclerosis is a process for which there is substantial evidence of a role for oxidative stress. Hypercholesterolemia and triglyceridemia are independent risk factor that alone or together can accelerate the development of CAD and progression of atherosclerotic lesions. HDL may be protective by reversing cholesterol transport, inhibiting the oxidation of LDL and by neutralizing the atherogenic effects of oxidized LDL. A greater increase of LDL may also cause a greater decrease of HDL as there is reciprocal relation ship between the concentrations of LDL and HDL (13).

Lipid peroxidation of cellular structure, a free radical-induced activity, is thought to play an important role in ageing, atherosclerosis and late complications of diabetes mellitus (14). Few authors have reported increased levels of MDA in CAD patients (14, 15). In our study also, increased levels of MDA in high alcoholic CAD patients as compared to non alcoholic CAD and healthy controls, because alcoholics seem to have still greater degree of oxidative stress. The estimation of lipid peroxidation along with lipid profile in the CAD patients is very useful as it may serve as a useful monitor to judge the prognosis of the patient.

Se is a part of GPx in the cytosol and mitochondria, which protects biomembranes against destruction. Selenium is also a central enzyme for eliminating oxygen free radicals and peroxidase. In contrast, the trace elements deficiency causes cardiomyopathy as a result of the depletion of essential enzymes, which protect cell membrane from damage by free radical due to the trace elements have a key role in essential enzymes such as GPx and SOD. It is therefore not surprising that one of the important biological functions of the trace elements is antioxidation. GPx and SOD reduce the production of hydrogen peroxide and superoxide, therefore diminishing the propagation of free radicals (16, 6).

In the present study we found decreased levels of Se, Zn and decreased activity of GPx and SOD in non alcoholic CAD patients. Previous studies also showed same findings (6, 14, 15). In addition we also observed further decreased levels of above parameters in high alcoholic CAD as compared to moderate alcoholic CAD patients. These data showed that Se deficiency not only increases the risk of CAD, but also increases the risk of acute events of myocardial infraction.

The transition metal copper has been shown to promote the oxidation of LDL-C and inversely associated with risk of CAD. Most of the case-controlled studies have shown high Cu levels in patients with CAD and Myocardial infraction compared to healthy controls (17). In our study also we found significantly increased Cu levels in non alcoholic, moderate alcoholic CAD patients and further increased in high alcoholic CAD patients as compared to controls, However, Mehdi Hassanzadeh et al have been reported that there is no significant difference in Cu levels among CAD patients (18). We suggested

that there are so many risk factors in CAD but among all those alcohol is the main risk factor, so patients who are suffering from alcoholic CAD are becoming more risky as compared to only CAD patients. In constract, men who drink should be warned of the increased risk of CAD associated with heavy alcohol consumption.

In conclusion, high alcohol intake predicts low antioxidant and trace elements levels in the body and they may contribute to the increased susceptibility for the development of CAD. So reduced consumption of alcohol, animal saturated fat and increased consumption of n-3 fatty acids, natural antioxidants, fresh fruits, vegetables and tree nuts, maintenance of body weight and secondary preventive measures like control of hyperglycemia may be suggested to the alcoholic CAD patients to protect them from further cardiovascular detoriation.

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