Oxidative stress and endogenous antioxidants in normolipidemic Acute Myocardial Infarction patients

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Abstract

Background: Although studies have demonstrated the role of endogenous antioxidants in the protection from the deleterious effects of oxygen free radicals in ischemia and reperfusion, there are controversial data on the correlation between endogenous antioxidants and ischemia process.

Aim: The present study was planned to evaluate endogenous antioxidants in normolipidemic acute myocardial infarct (AMI) patients.

Setting & Design: The serum lipid profile, albumin, uric acid, total bilirubin, malondialdehyde and conjugated dienes were determined in 165 normolipidemic patients diagnosed of AMI and 165 age-sexes matched healthy volunteers served as control.

Material & Methods: Serum albumin was measured by Bromo cresol green (BCG) dye binding method, uric acid was measured by phosphotungstic acid reduction method and serum total bilirubin by Jendrassik and Grof method, serum MDA was measure by MDA method estimating TBARS and conjugated dienes by Recknagel and Glende method (with little modifications) in AMI patients and controls. Also lipid profile was analyzed enzymatically in these subjects.

Statistics: The values were expressed as means ± standard deviation (SD) and data from patients and control was compared using student's't'-test.

Results And Conclusion: The endogenous antioxidants were significantly decreased ($p<0.001$) in AMI patients compared to controls. Serum malondialdehyde and conjugated dienes were significantly ($p<0.001$) increased in AMI patients compared to controls.
Also total cholesterol, TC: HDL-C ratio, triglycerides, LDL-cholesterol, LDL-C: HDL-C ratio and TG: HDL-C ratio were higher in AMI subjects ($p<0.001$) and HDL-cholesterol were lower in AMI subjects ($p<0.001$).

INTRODUCTION

With the explosive rise in the incidence of Coronary Artery disease (CAD) it is estimated that this will be the leading cause of morbidity and mortality in the developing world by the year 2015. ([1]) People hailing from Indian subcontinent had a higher probability of dying due to CAD. It is a multifactorial disease and some predisposing factors are hereditary, hyperlipidemia, obesity, hypertension, environmental factors and life style variables like stress, smoking, alcohol consumption, etc. ([2]) Diet especially fat plays an important role the development of CAD and the risk further increases in the presence of dyslipidemia.
Lipoprotein profile has been investigated extensively in recent years, which is found to be deranged in large proportion of CAD patients; especially Asians showing a mixed picture of dyslipidemia. Low density lipoprotein cholesterol (LDL) is considered as the most important risk factor of CAD. However, a significant proportion of patients have a normal lipid profile. ([3]) Free radicals play an important role in the pathogenesis of tissue damage in many different clinical disorders ([4]).Oxygen free radicals (OFR's) are produced continuously. Normally there is a balance between tissue oxidant and antioxidant activity ([5]). 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 ([6]). Among the endogenous antioxidant system, includes albumin, uric acid, and total bilirubin. Imbalance of this reaction either due to excess free radical formation or insufficient removal by antioxidants leads to oxidative stress ([7]). The oxidation of LDL is believed to have a central role in atherogenesis. Subendothelial accumulation of foam cells plays a key role in the initiation of atherosclerosis. These foam cells, which may be generated by the uptake of oxidized LDL by macrophages via scavenger receptors, accumulate in fatty streaks that evolve to more complex fibro fatty or atheromatous plaques.([8]) Oxidation of low-density lipoprotein particles and cytotoxic effects of lipid peroxides enhance the formation of foam cells and atherosclerotic lesion. During the formation and development of atherosclerosis, the intensity of lipid peroxidation and activity of the antioxidant defense system significantly change. Under oxidative stress not only LDL, but other serum lipids are exposed to oxidation. Literature survey reveals that the risk of heart attacks is particularly in those groups of individuals who have dyslipidemia. Now a latest trend is emerging that even in normolipidemic subjects the chances of myocardial infarction (MI) persists.

The present study was planned to evaluate the endogenous antioxidants status in MI patients with normal lipid profile. The present study was undertaken due to varied reports on these endogenous parameters and also due to lacunae of data available on normolipidemic patients with myocardial infarction.

MATERIALS AND METHODS

Setting Design and patients: The study consisted of 165 patients (123 men and 42 women) with AMI, admitted to 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 phosphokinase (CPK-MB) and aspartate aminotransferase enzyme elevation. The control group consisted of 165 age-sex matched healthy volunteers, 123 men and 42 women. The study was ethical cleared by the ethical committee of the institution. Informed consent was taken from the patients and subjects participated in the present study.

Inclusion criteria: Patients with diagnosis of AMI with normal lipid profile.

Exclusion criteria: Patients with diabetes mellitus, renal insufficiency, current and past smokers, hepatic disease or taking lipid lowering drugs or antioxidant vitamin supplements.

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. ([9])

Blood collection and biochemical methods used: 10 ml of blood was collected after overnight fasting and serum was separated. Serum was used for determination of lipid profile, serum albumin, serum uric acid, serum total bilirubin, serum 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 from the values of total cholesterol and HDL-cholesterol using the following formulae:

Figure 1

$$\text{LDL-cholesterol} = \text{Total cholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL-cholesterol (mg/dl)}$$

All chemicals of analytical grade were obtained from Sigma chemicals, India.

Serum Albumin: Serum Albumin was measured by Bromocresol green dye binding method using assay kit. This method is based on Doumas et al., 1972 in which albumin bind with BCG causing a shift in the absorption spectra of the dye. Albumin present in the serum binds with bromocresol green dye and the colored complex formed is read at 625 nm which is proportional to the concentration of albumin in the serum sample. Serum albumin was expressed as g/dl.

Serum Uric acid: The serum uric acid was measured spectrophotometrically using phosphotungstic acid reduction method. The method is based on the reduction of uric acid present in the serum to tungsten blue which is measured photometrically at 700 nm. The Serum uric acid was expressed as milligrams/deciliters.

Serum Total Bilirubin: Serum total Bilirubin was estimated by Jendrassik and Grof method. The serum Bilirubin reacts with diazotized sulphanilic acid to form an azo compound, the color of which is measured at 546 nm and is proportional

to the concentration of Bilirubin. The Bilirubin concentrations were expressed in mg/dl.

MDA Method: MDA levels were estimated by thiobarbituric acid (TBA) reaction ($_{10}$). Using 40% tricholoroacetic acid, proteins were precipitated from 0.5 ml serum, and precipitated proteins were incubated with TBA reagent in a boiling water bath for one hour. After bringing down to room temperature, the colored complex formed was measured using spectrophotometer at 532 nm.1, 1, 2, 3-tetraethoxypropane (1 nmol/l) was used as a standard for MDA estimation. Concentrations were expressed in nmol/l.

Conjugated dienes (CD): CD levels were measured by Recknagel and Glende method ($_{11}$) with little modification. Briefly, the principle of the assay is based on with the rearrangement of double bonds in polyunsaturated fatty acids leading to the formation of DC, which absorb light at 233 nm. The oxidation index of the lipid sample at 233 nm and 215 nm is computed which reflect the diene content and the extent of peroxidation. The Lipid Peroxidation (LP) products measured in serum were treated with antioxidant Butylated hydroxytoluene (BHT) twice, immediately after obtaining and before adding the test reagents to suppress artefactual changes during handling and assay procedures. The first stage of LP consists of the molecular rearrangement of the double bonds in polyunsaturated fatty acids residues of lipids, which leads to conjugated dienes (CD) formation and conversion of CD in hydroperoxide (LOOH). Serum was chosen to avoid possible influences of substances required for plasma preparation. Serum sample (150 µl) and (150µl) of 0.9% NaCl (reagent blank contains only isotonic saline) were incubated at 37°C for 25 minutes. 0.25% Butylated hydroxyl Toluene (BHT) (150µl) was added and the lipids were extracted by heptane/isopropanol (1:1). Then samples were acidified by 5 mol/L HCl and extracted by cold heptane (1600µl). After centrifugation for 5 minutes at 3000 rpm the absorbance of heptane fraction were measured spectrophotometrically at absorbance maximum between 220 nm and 250 nm. The amount of hydroperoxides produced was calculated using Molar Coefficient of 2.52 X 10^{4} m^{-1} .

Statistical analysis: The data from patients and controls were compared using Student's‘t'-test. Values were expressed as mean ± standard deviation (SD). Microsoft excel for windows 2000 was used for statistical analysis. ‘P' value of less than 0.05 was considered to indicate statistical significance.

RESULTS

Figure 2

Table 1: Lipid profile in AMI patients and healthy controls (mean ± SD)

Variables	Controls (n=165)	Patients (n=165)	P value (95%CI)
Age	60.55 ± 3.98	61.84 ± 3.80	0.0037(61.26-62.42)
Total Cholesterol †	168.58 ± 12.16	186.44 ± 13.95	<0.001(184.31-188.56)
HDL-Cholesterol †	50.51 ± 6.78	41.27 ± 4.62	<0.001(40.56-41.97)
TC: HDL-C*	3.39 ± 0.36	4.57 ± 0.58	<0.001(4.48-4.65)
Triglycerides †	107.84 ± 11.51	128.96 ± 12.19	<0.001(127.10-130.82)
LDL-Cholesterol †	83.59 ± 11.95	119.37 ± 14.05	<0.001(17.22-21.51)
LDL:HDL-C*	1.90 ± 0.31	2.93 ± 0.51	<0.001(2.85-3.00)
TG: HDL-C*	2.17 ± 0.35	3.16 ± 0.49	0.3149(3.086-3.234)

* ratio † (mg %)

Figure 3

Table 2: Lipid profile in male AMI patients and healthy controls (mean ± SD)

Variables	Control Male(n=123)	Male Patients (n=123)	P value(95%CI)
Age	60.68 ± 4.14	61.53 ± 3.28	0.0366(60.95-62.10)
Total Cholesterol †	168.09 ± 12.10	183.84 ± 13.65	<0.001(182.41-186.25)
HDL-Cholesterol †	49.90 ± 7.30	41.78 ± 4.88	0.0801(40.91-42.64)
TC: HDL-C*	3.42 ± 0.30	4.45 ± 0.58	<0.001(4.34-4.55)
Triglycerides†	105.02 ± 10.31	126.22 ± 11.74	<0.001(124.14-128.29)
LDL-Cholesterol †	79.88 ± 7.98	116.82 ± 13.76	<0.001(114.38-119.25)
LDL:HDL-C*	1.92 ± 0.25	2.84 ± 0.52	<0.001(2.74-2.93)
TG:HDL-C*	2.15 ± 0.37	3.06 ± 0.47	0.0123(2.97-3.14)

*ratio † (mg %)

Figure 4

Table 3: Lipid profile in female patients and healthy controls (mean ± SD)

Variables	Control Female (n=42)	Patients Female (n=42)	P value(95%CI)
Age	60.52 ± 2.93	62.73 ± 4.97	0.0356(61.22-64.23)
Total Cholesterol †	170.00 ± 12.35	194.03 ± 13.03	<0.001(190.08-197.97)
HDL-Cholesterol †	52.31 ± 4.58	39.77 ± 3.37	<0.001(38.75-40.78)
TC: HDL-C*	3.28 ± 0.47	4.96 ± 0.44	<0.001(4.82-5.09)
Triglycerides †	116.11 ± 10.96	136.99 ± 9.81	<0.001(134.02-139.95)
LDL-Cholesterol †	94.47 ± 14.81	126.86 ± 12.22	0.2044(123.16-130.55)
LDL:HDL-C*	1.83 ± 0.44	3.21 ± 0.40	0.3066(3.08-3.33)
TG:HDL-C*	2.23 ± 0.28	3.47 ± 0.41	<0.001(3.34-3.59)

*ratio † (mg %)

Figure 5

Table 4: Endogenous antioxidants and lipid peroxidation in patients and healthy controls (mean ± SD)

Variables	Controls (n=165)	Patients (n=165)	P value (95%CI)
Age	60.55 ± 3.98	61.84 ± 3.80	0.0037(61.26-62.42)
Albumin †	4.43 ± 0.31	4.23 ± 0.34	<0.001(4.17- 4.28)
Total Bilirubin†	0.77 ± 0.16	0.66 ± 0.20	<0.001(0.62 -0.69)
Uric acid †	5.82 ± 1.26	4.32 ± 0.90	<0.01(4.18 -4.45)
MDA (nmol/L)	5.71 ± 0.97	14.81 ± 1.66	<0.02 (11.55-15.06)
Conjugated dienes(µmol/L)	31.04 ± 2.68	48.28 ± 5.50	<0.001 (47.44 - 49.11)

* ratio † (mg %)

Figure 6

Table 5: Endogenous antioxidants and lipid peroxidation in Male patients and healthy controls (mean ± SD)

Variables	Control Male(n=123)	Male Patients (n=123)	P value(95%CI)
Age	60.68 ± 4.14	61.53 ± 3.28	0.0366(60.95-62.10)
Serum Albumin †	4.45 ± 0.31	4.21 ± 0.38	<0.001(4.14 – 4.27)
Serum Total Bilirubin†	0.78 ± 0.15	0.69 ± 0.20	<0.001(0.65 -0.72)
Serum Uric acid †	5.42 ± 1.10	4.34 ± 0.90	<0.001(4.18 – 4.49)
MDA (nmol/L)	5.71 ± 0.97	14.81 ± 1.66	<0.001(14.44 – 15.07)
Conjugated dienes(µmol/L)	31.04 ± 2.68	48.28 ± 5.50	<0.001 (45.88 – 47.77)

*ratio † (mg %)

Figure 7

Table 6: Endogenous antioxidants and lipid peroxidation in Female patients and healthy controls (mean ± SD)

Variables	Control Female (n=42)	Patients Female (n=42)	P value(95%CI)
Age	60.52 ± 2.93	62.73 ± 4.97	0.0356(61.22-64.23)
Serum Albumin †	4.37 ± 0.29	3.60 ± 0.23	<0.001(3.53 – 3.66)
Serum Total Bilirubin†	0.74 ± 0.18	0.58 ± 0.14	<0.001(0.53 – 0.62)
Serum Uric acid †	5.74 ± 1.37	4.26 ± 0.90	<0.001(3.98 -4.53)
MDA (nmol/L)	5.71 ± 0.97	14.81 ± 1.66	<0.001 (14.61 – 15.30)
Conjugated dienes(µmol/L)	31.04 ± 2.68	48.28 ± 5.50	<0.01 (51.36 -53.31)

*ratio † (mg %)

The lipid profile is shown in Table 1, Table 2 and Table 3. Total cholesterol, TC: HDL-C ratio, triglycerides, LDL-cholesterol, LDL: HDL-C ratio were higher in AMI subjects as compared to control (p<0.001). Also, significant differences were seen in HDL-C levels between AMI and controls (p<0.001). Total cholesterol, TC: HDL-C ratio, triglycerides were higher in both genders of AMI subjects as compared to control (p<0.001). Significant differences were seen in HDL-C levels between AMI and control only in female (Table-3) (p<0.001).LDL-cholesterol, LDL: HDL-C ratio were higher in male AMI subjects compared to control (Table-1) (p<0.001)

The endogenous antioxidants status and index of lipid peroxidation are shown in Table 4, Table 5 and Table 6. All the endogenous antioxidant were significantly decreased (p<0.001) in AMI patients compared to controls. Serum malondialdehyde and conjugated dienes were significantly (p<0.001) increased in AMI patients compared to controls.

DISCUSSION

Atherosclerosis is the root cause of Acute Myocardial Infarction (AMI). Contrary to earlier belief, research in the last two decades has shown that atherosclerosis is neither a degenerative disease nor inevitable due to ageing but it seems to be a chronic inflammatory condition that is converted to an acute clinical event by the induction of plaque rupture, which in turn leads to thrombosis. Hence inflammation occupies a very important central position in all phases of atherosclerosis, although inflammation must smolder for decades before resulting in a clinical event, like AMI ([16]). Myocardial ischemia occurs oxygen demand exceeds the oxygen supply and if this condition is not reversed, myocardial infarction precipitates. Reperfusion of the ischemic myocardium can restore oxygen supply but sudden massive increase in oxygen supply causes a burst of oxygen consumption with the consequent generation of free radicals, resulting in an imbalance of oxidative– anti-oxidative processes. The excess production of reactive oxygen species may initiate lipid peroxidation in cell membrane. These processes may result in a loss of contractile function of the heart and lead to severe myocardial cell damage, collectively termed as reperfusion injury ([17]). The significant decrease in endogenous antioxidant in the patients could be due to overwhelming production and accumulation of superoxide anion causing inhibition of antioxidant activity. Whatever might be the cause of the decreased endogenous antioxidants, the net result is accumulation of H_2O_2, one of the most damaging products of the free radical metabolism. H_2O_2 can readily react with superoxide anion to produce the highly toxic hydroxyl radical and HOCl. Many findings suggests that antioxidants depletion has relevant impact to the precipitation of myocardial infarction and these findings are consistent with the notion that increased levels of antioxidants are protective([18]).

The present study observed significant lowering of endogenous antioxidants namely albumin, uric acid and total bilirubin and significant higher levels of malondialdehyde and conjugated dienes in patients (p<0.001). The findings of the present study is similar to the observations of the studies conducted by Dubois Rande et al ([19]) and Mc Murray ([20]) where they reported a significant rise in MDA levels (p<0.001), a lipid peroxidation product, with a concomitant decrease in antioxidants in patients of unstable angina and chronic heart failure. The present study is in concurrence with studies of Verma et al ([21]), who demonstrated that there was a significant drop in antioxidant, whereas lipid peroxides were significantly higher in AMI patients, compared with controls. The findings of the present study is also in good agreement with the study conducted by Kharb ([22]) where significantly decreased levels of antioxidants and increased levels of lipid peroxides was reported. This indicates severe depletion in antioxidant system, is unable to combat oxidative stress and inflammation which rather could be an important protective system against oxidative damage, happens to be severely impaired in AMI patients. The findings of the present study indicate the existence of an abnormal balance between the oxidative and protective mechanisms in patients can be a causative factor for then going for AMI. Increased MDA levels in plasma have been used as an index of free radical-mediated damage, which is significantly elevated in AMI patients, is clearly vindicate the extent of damage caused by reperfusion of ischemic myocardium.

To summarize, the present study shows that during reperfusion of ischemic myocardium, there is copious generation of reactive oxygen species, depletion of

antioxidants system. 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, caeruloplasmin are emerging which could be possibly be a causative factor for atherosclerosis leading to myocardial infarction.

CONCLUSION

Myocardial Infarction is a multifactorial disease which could be caused even in normolipidemic subjects. The earlier concept of maintaining lipid profile within normal and safe limits has been overruled as the present study unearths fact. The present study suggests, measuring of serum antioxidants at regular interval of time as the present study highlight the fact. Oxidative stress appears to be an etiological factor for myocardial infraction as a consequence of the free radical scavengers namely antioxidants which tends to lower in AMI patients.

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