ANTIOXIDANTS, OXIDATIVE STRESS STATUS AND WAIST/HIP RATIO IN NORMOLIPIDAEMIC AMI PATIENTS

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ABSTRACT

Objectives: The main objectives were to compare antioxidants and lipid peroxidation markers in normolipidaemic AMI patients to age/sex-matched controls.

Methodology: The study was conducted in Hindustan Institute of Medical Sciences and Research, Sharda Hospital, India on 165 AMI patients, (123 males and 42 females) admitted in ICCU, Sharda Hospital with age/sex-matched 165 controls (123 males and 42 females). Apart lipid profile measurement, the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase were determined. Oxidative stress markers, malondialdehyde (MDA) and conjugated diene (CD) were also determined.

Results: The mean SOD activity in patients were 813.9 (U/gHb) compared to 1826.5 (U/gHb) in controls which was significantly lower (p<0.02). The mean GPx activity in patients was 42.6 (U/gHb) significantly lower than controls (p<0.001), 61.3 (U/gHb). Significant changes (p<0.001) were observed in mean catalase activities in patients, 193.06 (k/gHb) compared to control 256.2 (k/gHb). The mean MDA levels were significantly higher in patients (p<0.02) showing 14.81 (nmol/L), compared to control, 5.71 (nmol/L). Similarly conjugated diene levels in patients were higher, 48.28 (μmol/L) than controls 31.0 (μmol/L) (p<0.001).

Conclusions: Myocardial Infarction is a multifactorial disease in which normolipidaemic subjects could also be a victim even though the risk is higher in dyslipidemic subjects. Apart from lipid profile measurement in health check up package, inclusion of antioxidants studies should also be incorporated as the current studies highlight the facts with special attention to higher waist/ hip ratio subjects.

KEY WORDS: Antioxidants, Malondialdehyde, Conjugated diene, AMI, Waist / Hip ratio, Normolipidemia.

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INTRODUCTION

Cardiovascular disease would be the leading cause of mortality and morbidity in the world by the year 2015[1] and people from Indian subcontinent are at higher risk. It is a multifactorial disease accompanied by factors like hereditary, hyperlipidemia, obesity, hypertension, environmental factors and life style variables like stress, smoking, alcohol consumption, etc.[2] Fatty diet aggravates coronary artery disease (CAD) and dyslipidemia add additive risk especially in Asians. Elevated low-density lipoprotein cholesterol (LDL) stands important

due to its oxidative properties and atherogenic potential however, some patients of CAD are normolipidaemic.[3] Under oxidative stress apart from circulating LDL molecules other lipoproteins also become a victim to structural changes induced due to oxidation including malondialdehyde and conjugated diene formation. Literature survey reveal the risk of myocardial infarction is aggravated in dyslipidemia.[4]

As we came across patients of AMI with normolipidaemia, the current study was planned to evaluate the balance of lipid of antioxidants and lipid peroxidation in these patients, moreover, extensive literature search revealed not much studies was conducted in this area.

METHODOLOGY

Setting Design and Patients: The study was conducted in Hindustan Institute of Medical Sciences and Research, Sharda Hospital, India. The study comprised of 165 normolipidemic AMI patients, male to female ratio was 3:1, with ages ranged from 48-69 years, mean ± SD (61.8 ± 3.8 y). One hundred sixty five age-sex matched subjects, with similar ratio of male: female as in patients were recruited as controls. Their ages ranged from 48-69 years, mean ± SD (60.55 ± 3.98 y). The AMI cases were diagnosed as per diagnostic criteria: chest pain lasting for ≥3 hours, ECG changes (ST elevation of 2 mm or more in at least two leads), increased creatine phosphokinase (CPK-MB) and aspartate aminotransferase enzyme. Informed consent was obtained from patients and controls recruited for the study and the study was approved by the ethical committee of the Institution.

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

Inclusion Criteria: Normolipidemic AMI patients as per the NCEP ATP -111 guidelines.

Criteria for Normolipidemics: Normal lipid pro-

file was defined if LDL was <160mg/dl, HDL ≥35mg/dl, Total cholesterol (TC) <200 mg/dl and Triglycerides (TG) were <150mg/dl.[5] Blood (10 ml) was collected after overnight fasting in different containers.

1. *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.

2. *Plain vial:* Remaining blood was taken and serum was separated by centrifugation for five minutes at 5000 rpm and was used for determination of lipid profile, malondialdehye and conjugated dienes.

Total cholesterol, triglycerides, and HDL-cholesterol were estimated by enzymatic methods using the kits 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:

$$LDL\text{-}c = TC - \frac{TG}{5} - HDL\text{-}c \ (mg/dl)$$

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

Glutathione Peroxidase (GPx): The glutathione peroxidase activity in red cells was determined by the procedure of Paglia and Valentine.[6] Briefly, the oxidized glutathione produced during GPx enzyme reaction was immediately reduced by NADPH and glutathione reductase. Therefore, the rate of NADPH consumption was monitored as a measure of formation of oxidized glutathione. Results were expressed as units of GPx per gram of hemoglobin.

Superoxide dismutase (SOD): Superoxide dismutase enzyme activity in red cells 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 et al[7] using Ransod assay kit SD 125, Randox Lab. One unit of SOD activity was defined as the amount of protein that inhibits the rate of I.N.T. reduction by 50%. Enzyme activity was expressed

as Unit per gram hemoglobin (U/gHb). Hemoglobin was measured by Drabkin's method.

Catalase: Catalase activity in red cells was measured spectrophotometrically as described by Beutler.[8] One Unit of enzyme activity was expressed as micromole hydrogen peroxide decomposed per min per gram hemoglobin.

MDA Method: MDA levels in serum were estimated by thiobarbituric acid (TBA) reaction.[9] Using 40% trichloroacetic 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. The concentration was expressed in terms of (nmol/l).

Conjugated dienes (CD): CD levels in serum were measured by Recknagel and Glende method [10] 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 CD, 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 CD formation and conversion of CD in hydroperoxide. 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% BHT (150µl) was added and the lipids were extracted by heptane/isopropanol (1:1). Then samples were acidified by 5mol/L HCl and extracted by cold heptane (1600µl). After centrifugation for five minutes at 3000 rpm the absorbance of heptane fraction were measured spectrophotometrically at absorbance maximum between 220nm and 250nm. 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

The baseline variables, antioxidant status and lipid peroxidation in AMI patients compared to control are shown in Table-I&II. Body mass index (BMI), waist / hip ratio, blood pressure,

Table-I: Baseline variables in Controls and AMI patients (mean ±SD)

Variables	Control(n=165)	Case(n=165)	P value
Age (years)	60.55 ± 3.98	61.84 ± 3.80	>0.001
BMI (kg/m^2)	25.40 ± 1.20	26.16 ± 1.45	<0.01
Waist: Hip*	0.93 ± 0.01	0.95 ± 0.01	<0.02
Systolic blood pressure(mm of Hg)	113 ± 8	136 ± 23	<0.05
Diastolic blood pressure(mm of Hg)	85 ± 7	95 ± 10	<0.05
Total Cholesterol †	168.58 ± 12.16	186.44 ± 13.95	<0.001
HDL-Cholesterol †	50.51 ± 6.78	41.27 ± 4.62	<0.001
Triglycerides †	107.84 ± 11.51	128.96 ± 12.19	<0.001
LDL-Cholesterol †	83.59 ± 11.95	119.37 ± 14.05	<0.001
LDL:HDL-C*	1.90 ± 0.31	2.93 ± 0.51	<0.001

* ratio † (mg%)

Table-II: Antioxidant Status and Lipid Peroxidation in Controls and AMI patients (mean ± SD)

Variables	Control(n=165)	Case(n=165)	P value
Superoxide dismutase (U/gHb)	1826.47 ± 31.86	813.95 ± 208.98	<0.02
Glutathione Peroxidase(U/gHb)	61.29 ± 3.94	42.56 ± 6.36	<0.001
Catalase (k/gHb)	256.15 ± 26.65	193.06 ± 35.87	<0.001
TBARS (nmol/L)	5.71 ± 0.97	14.81 ± 1.66	<0.02
Conjugated dienes (µmol/L)	31.04 ± 2.68	48.28 ± 5.50	<0.001

and lipid profile parameters were significantly higher in AMI patients (Table-I).

The antioxidant enzymes and lipid peroxidation are shown in Table-II. Serum SOD, GPx and catalase were significantly lower in AMI patients compared to control. The MDA and CD levels were significantly higher in patients compared with controls (Table-II).

DISCUSSION

Atherosclerosis is the root cause of AMI. Antioxidants play an important role by inhibiting LDL oxidation further minimizing the progression of atherosclerosis. It is a chronic inflammatory condition followed by acute clinical event, by plaque rupture leading to thrombosis. There fore inflammation occupies a central place, in all phases of atherosclerosis before emerging as a clinical event like AMI.[11] Involvement of oxygen free radicals in inflammation, ischemia and reperfusion damage have been reported earlier.[12] Demonstration of elevated serum lipid peroxides, justifies the claim.

Significant rise in MDA (p<0.02) and CD levels (p<0.001) in the current study is suggestive of increased oxidative stress in AMI patients. The current study concurs with the work of Dubois Rande et al,[13] Mc Murray[14] and Senthil et al[15] reported similar findings.

OFRs are generated in early phase of MI and due to involvement of GPx to reduce hydrogen peroxide radicals, their level decreases. The current study observed similar pattern of reduction in GPx activity as reported by Kharb,[16] Simic[17] and Blaustein et al.[18] Perfect negative correlation was established between GPx and MDA levels in AMI patients

(r = -0.62). This suggests GPx being antioxidant gets succumbed to peroxidised products during oxidative damage. Ondregjickova et al.,[19] demonstrated increased glutathione disulfide with concomitant decrease in GPx in myocardium during coronary occlusion. Their study confirms the abnormal balance between the oxidative and protective mechanisms in MI patients which is further supported by the findings of the current study.

The SOD activity was significantly lower in patients (p<0.02) compared to controls in current study. Similar findings were reported by Kumar and Das.[20]

Even catalase activities were significantly lowered in AMI patients (p<0.001) compared to control. Senthil et al[21] reported the reduction of catalase in cardiogenic shock patients. The results of these studies are suggestive of increased utilization of free radical scavenging system to combat the toxic radicals. It could also be due to decreased catalase activities, the lipid peroxides are increased. Studies by Dusinovic et al,[22] Pandey et al[23] and Bhakuni et al[24] on antioxidants defense system in MI patients, also observed similar findings. With the observations of all the past studies along with the current study findings indicates the asynchrony between oxidants and antioxidants in AMI patients.

CONCLUSION

Myocardial Infarction is a multifactorial disease in which normolipidaemic subjects could also be a victim even though risk is higher in dyslipidemic subjects. Apart from lipid profile measurement in health check up package, inclusion of antioxidants studies should also be incorporated as the current studies high-

lights the facts with special attention to higher waist/ hip ratio subjects.

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