Original Article

Study of oxidants and antioxidants in patients of acute myocardial infarction

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Abstract

Background: Oxygen free radicals have become attractive candidates to explain injuries in ischemic heart. An association between raised serum uric acid concentration and increased cardiovascular risk has been recognized, however its role in acute myocardial infarction (AMI) is still unclear. Recently, zinc is also trying to establish its role in tissue injury and oxidative stress.

Materials and Methods: This cross-sectional study was carried on 75 AMI patients. 5 ml of blood was drawn from each patient within 6 h of AMI, to estimate plasma malondialdehyde (MDA), serum zinc, whole blood superoxide desmutase, serum uric acid, and whole blood glutathione peroxidase (GPx). The same biochemicals were also determined in 50 age and gender matched controls for comparison.

Results: We found significantly increased level of plasma MDA (5.649 ± 0.1780 vs. 2.757 ± 0.1623), serum uric acid (4.533 ± 0.1526 vs. 3.200 ± 0.1616) and significantly decreased levels of serum zinc (104.5 ± 1.874 vs. 115.3 ± 3.077), whole blood GPx (4599 ± 101.1 vs. 5519 ± 81.63) and superoxide desmutase (166.8 ± 1.896 vs. 188.3 ± 4.120). All the parameters studied also showed similar significant changes in male and female cases separately.

Conclusion: Raised MDA and decreased zinc, glutathione peroxidase, and superoxide desmutase levels denote the increased oxidative stress. Even being a defense, uric acid is raised as it is abundantly present in our body. Thus, AMI exhibits oxidative stress dependent changes irrespective of gender.

Key Words: Acute myocardial infarction, antioxidants, oxidants, uric acid, zinc

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INTRODUCTION

While oxidative stress has been shown to be related with age and living habit, it plays an important role in the development of vascular diseases that affect vital organs, in particular, brain and heart. Acute myocardial infarction (AMI) is the most critical event in cardiovascular disorders and arises as a consequence of myocardial ischemia due to coronary occlusion.[1]

Oxygen free radicals have become attractive candidates to explain injuries in the heart with growing appreciation that free radicals such as malondialdehyde (MDA) which is the end product of

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lipid peroxidation may accumulate during ischemia at low oxygen tensions.[2]

In AMI, not only the oxidative stress is greater but antioxidant defense system which includes enzymes such as superoxide desmutase (SOD), glutathione peroxidase (GPx) to combat free radical is also altered.[3] An association between raised serum uric acid concentration and increased cardiovascular risk has been recognized for over 50 years. However, its role in AMI is still unclear.[4] similarly, past two decades have seen the flood of information on the potential role of zinc metal as a cellular antioxidant and its role in various diseases and tissue injury.[5]

After searching through various internet search engines, we found that very few attempts were made to correlate levels of serum uric acid, serum zinc, and oxidative stress with AMI, particularly in the Indian population. The novelty of our research lies in finding the levels of serum zinc and uric acid in the Indian AMI patients, particularly central India. Thus we took present work with an aim to investigate status of oxidants and antioxidants in AMI and to substantiate correlation between the levels of MDA, SOD, GPx, uric acid, and zinc in the study population.

MATERIALS AND METHODS

This cross-sectional study was carried out on randomly selected 75 patients of AMI, hospitalized in the Intensive Cardiac Care Unit (ICCU) of Medicine Department, Government Medical College, Nagpur from August 2009 to February 2010, only after taking informed consent from the study participants. The approval from the Institutional Ethics Committee was duly taken before the start of this research. The diagnosis of AMI was carried out by on duty ICCU physician based on clinical presentation, electrocardiography (ECG), and biochemical parameters. Fifty, voluntarily willing, consent giving, age (30–75 years), and gender matched apparently healthy controls were selected based on following criteria.

Inclusion criteria (different for cases and controls):
- For cases
  - Hospitalized in ICCU with chest pain of at least 20 min duration
  - ST-segment elevation of at least 2 mm in two or more consecutive leads of ECG
  - CPK-MB level above 25 IU/L
  - Blood samples collected before the start of treatment and any medication.
- For controls
  - Age and sex matched with cases
  - No history or clinical/laboratory evidence of myocardial infarction
  - CPK-MB level within physiological limits.

Exclusion criteria (same for cases as well as controls):
- Presence of valvular heart diseases,
- Presence of liver diseases, pulmonary diseases, renal diseases, neoplastic diseases,
- Diagnosed with any acute infections,
- Diagnosed case of gout,
- Known case of diabetes mellitus,
- History of smoking,
- Known case of hypertension
- Obesity with body mass index >32 kg/m^2.

A 5 ml of blood sample was withdrawn from patients within 6 h of an acute episode of AMI, under all aseptic precautions. Hemolyzed samples were excluded from the study.
- 3 ml of blood in plain bulb for estimation of plasma MDA, serum uric acid and zinc
- 2 ml of blood in heparin bulb for estimation of GPx and SOD.

Samples were analyzed by Semi Auto analyzer, Erba Chem-Pro, Transasia using following methods.
- Plasma MDA: Draper and Hadley 1990[6]
- Zinc in serum: Homster and Zak, 1985[7]
- Uric acid in serum: Henry 1974[8]
- Whole blood SOD: Arthur and Boyne 1985[9]
- Whole blood GPx: Ultraviolet method.[10]

Statistical analysis

Statistical analysis for gender distribution in cases and control groups was done by Fisher's exact test using GraphPad Instat software, version 3.05. published by GraphPad Software, Inc., a privately owned California corporation. Age wise distribution of cases and controls and all the parameters of the study were carried out by unpaired *t*-test using GraphPad prism software, version 5.

RESULTS

Out of 75 case of AMI, 61 (81.33%) were males and 14 (18.67%) were females, and the mean age of cases were 52.68 ± 1.174 years. Out of 50 controls, 39 (78%) were males and 11 (22%) were females, and the mean age of controls were 51.44 ± 1.310 years.

We found significantly increased level of plasma MDA (5.649 ± 0.1780 vs. 2.757 ± 0.1623), serum uric acid (4.533 ± 0.1526 vs. 3.200 ± 0.1616) and significantly decreased levels of serum zinc (104.5 ± 1.874 vs. 115.3 ± 3.077), whole

blood GPx (4599 ± 101.1 vs. 5519 ± 81.63), and SOD (166.8 ± 1.896 vs. 188.3 ± 4.120).

DISCUSSION

In this research, we analyzed for the levels of MDA, serum zinc, serum uric acid, SOD, and GPx. For this, we collected samples from 75 patients of AMI and 50 age and sex matched apparently healthy controls. As shown in Table 1, we found significantly increased level of MDA, serum uric acid, and significantly decreased levels of serum zinc, whole blood GPx and SOD. Following is a short discussion on the individual parameter.

Plasma malondialdehyde

In healthy controls mean MDA level in this research was 2.757 ± 0.1623 nmol/ml, which was in good agreement with those reported by Dey Sarkar *et al*. and Shinde *et al*.[11,12] In AMI patients, increased level of MDA in AMI patients was highly significant as compared to controls [Table 1]. The present study had a fair correlation with findings of many workers.[3,11,13-16]

Plasma MDA values in both male and female cases were significantly elevated as compared to male and female controls, respectively [Table 2]. This clearly shows that AMI patients, irrespective of the gender, were exposed to increased oxidative stress.

Table 1: Comparison of parameters between cases and controls

Parameter	Mean±SEM		*P*
	Cases	Controls	
Plasma MDA (nmol/ml)	5.649±0.1780	2.757±0.1623	<0.0001*
Zinc (μg/dl)	104.5±1.874	115.3±3.077	0.0018*
Uric acid (mg/dl)	4.533±0.1526	3.200±0.1616	<0.0001*
SOD (U/ml)	166.8±1.896	188.3±4.120	<0.0001*
GPx (U/L)	4599±101.1	5519±81.63	<0.0001*

*P<0.05 was considered as statistically significant. SEM: Standard error of mean, MDA: Malondialdehyde, SOD: Superoxide desmutase, GPx: Glutathione peroxidase

Table 2: Gender wise comparison of parameters between cases and controls

Parameters	Gender	Mean±SEM		*P*
		Cases	Controls	
Plasma MDA	Male	5.728±0.2047	2.840±0.1968	<0.0001*
	Female	5.307±0.3359	2.464±0.2327	<0.0001*
Serum zinc	Male	106.9±1.944	116.8±3.507	0.0089*
	Female	93.68±4.493	110.0±6.438	0.043*
Serum uric acid	Male	4.607±0.1722	3.205±0.1853	<0.0001*
	Female	4.214±0.3219	3.182±0.3456	0.0404*
Whole blood SOD	Male	167.2±2.132	188.3±4.120	<0.0001*
	Female	164.9±4.222	188.2±9.846	0.0275*
Whole blood GPx	Male	4631±111.5	5492±90.73	<0.0001*
	Female	4460±244.7	5613±190.7	0.0017*

*P<0.05 was considered as statistically significant. SEM: Standard error of mean, MDA: Malondialdehyde, SOD: Superoxide desmutase, GPx: Glutathione peroxidase

Peroxidation of lipids is a chain of reactions providing a continuous supply of the radicals that initiate further peroxidation and is responsible for damage to tissues *in vivo* causing atherosclerosis, cancer, inflammatory disease, aging, etc., Peroxidation is also catalyzed *in vivo* by heme compounds and lipoxygenases found in platelets, leucocytes, etc., Lipid peroxides formed in this reaction degraded to form a characteristic product such as MDA.[17] Thus MDA, which is an end product of lipid peroxidation, is commonly used as a marker of oxidative stress.

Zinc

Mean serum zinc concentration in AMI patients was significantly decreased as compared to controls [Table 1]. The results of the present study were well correlated with that of previous studies.[14,18,19]

Our study found significantly decreased level of zinc in males as well as females, as compared to controls. We failed to find any such study showing gender wise significance of serum zinc level in AMI patients. We also found a significant decrease of zinc levels in female cases as compared to male cases.

The mechanism of antioxidation property of zinc can be divided into acute and chronic effects. Chronic effects involve the exposure of an organism to zinc on a long-term basis, resulting in induction of some other substance, which is the ultimate antioxidant. On the other hand, chronic zinc deprivation generally results in increased sensitivity to oxidative stress. The acute effects are generally thought to involve two mechanisms: Protection of protein sulfhydryls or reduction in the formation of $^{\bullet}OH$ from H_2O_2 through the antagonism of redox-active transition metals, such as iron and copper.[5]

Katayama *et al*. described the process known as "Scar healing," in which they proposed that, circulating zinc is taken up by ischemic myocardial tissue for healing of infarcted tissue, so fall in zinc levels in AMI may be reflective of tissue reparative process.[20]

To explain decreased zinc concentration in AMI, Lekakis and Kalofoutis postulated a more credible theory. It describes that stimulus such as infections and microbial endotoxins, as well as tissue injury such as AMI release a factor called "leukocyte endogenous mediator" by polymorphonuclear cells. When this factor is released, there is an increase in both uptakes of zinc by the liver and amino acid flow to the liver, which leads to decrease in the amount of zinc and iron in the serum.[21]

Gender wise significant decrease of zinc levels in female cases may be due to the exaggeration of the

normal pattern of differences of zinc levels between male and female controls. However, further studies are needed to arrive at a definite conclusion.

Zinc has been shown in numerous systems to antagonize the catalytic properties of the redox-active transition metals, iron, and copper with respect to their abilities to promote the formation of OH from H_2O_2 and superoxide and thus prevents the destructive processes initiated by $^{\bullet}OH$.[22]

Uric acid

The present study reproduces the significantly increased concentration of serum uric acid in cases as compared to controls [Table 1].[14,15,23,24]

Xanthine oxidoreductase, under normal condition, exists in dehydrogenase form and uses NAD^+ as the electron acceptor, and there is no production of superoxide anion. Under ischemic conditions, when the blood platelets cannot synthesize requisite amount of ATP, its level drops, and the membrane is no longer capable of maintaining proper ionic gradient, causing a redistribution of Ca^{2+}. Increased Ca^{2+} activates certain proteases that are capable of converting xanthine dehydrogenase into xanthine oxidase (XO). XO acts on xanthine during ischemia and hypoxanthine during reperfusion to produce uric acid and superoxide anions. Serum uric acid causes lipid peroxidation and produces many other reactive oxygen species; therefore elevated serum uric acid levels may act as a marker of underlying tissue ischemia.[24]

Nevertheless, in human uric acid contributes about 60% of free radical scavenging activity. Serum uric acid interacts with peroxynitrite to form a stable nitric oxide donor, thus promoting vasodilatation and reducing the potential for peroxynitrite induced oxidative damage, this property of uric acid could be expected to protect against ischemic stress.[14]

Krishnan *et al*. demonstrated that hyperuricemia was an independent risk factor for AMI in multivariable regression models, with an odds ratio of 1.11 (95% confidence interval: 1.08–1.15, $P < 0.001$).[24]

Hayden and Tyagi reported that abnormal elevations (>6.5 or 7 mg/dl in men and >6.0 mg/dl in women) in serum uric acid should be definitely considered as one of the multiple injurious stimuli to the arterial vessel wall and capillary, which may contribute to endothelial dysfunction and atherosclerosis.[25]

Regarding increased uric acid concentration there are two schools of thoughts; the first one believes that high uric acid concentration is an important risk marker for AMI, the second one stipulates uric acid to be a powerful free radical scavenger (>50%) in humans and paradoxically these antioxidant properties could be expected to offer number of benefits within the cardiovascular system.[4,14,24,25]

Thus raised level of uric acid in our research indicates increased oxidative stress directly or it may indicate a defense against acute oxidative stress.

Superoxide desmutase

Concentrations of SOD in whole blood was significantly decreased in cases as compared to controls [Table 1].[3,11,14]

SOD is the major antioxidant enzyme in the cell involved in the primary mechanism for clearance of superoxide anions. It catalyzes dismutation of superoxide anions to hydrogen peroxide and molecular oxygen.[2]

Kairamkonda *et al*. proposed that significantly decreased activity of SOD may be due to compensatory efforts generated by SOD to combat oxidative stress to which patients were chronically exposed. SOD acts as a scavenger for toxic superoxide radical which is implicated in lipid peroxidation. Reduced activity of SOD could be the result of intermolecular and intramolecular cross-linking of proteins and thereby causing conformational changes in SOD, which leads to accumulation of reactive oxygen species such as H_2O_2, which induces further lipid peroxidation leading toward atherosclerosis and cardiovascular complication.[14]

We found nonsignificant increased level of SOD in male cases and control group as compared to females. More studies need to be carried out regarding the significance of gender in the correlation of SOD level and AMI.

It is clearly evident that patients with AMI are exposed to enhanced oxidative stress in the form of excessive superoxide anion formation on day 1.[26]

As plasma SOD represents a readily mobilizable form which contributes to buffering of oxidative stress by clearance of superoxide anion, there may be decreased concentration of SOD in AMI.[27]

Glutathione peroxidase

Results of the present study show significantly decreased levels of GPx levels in AMI as compared to controls.[3,11,12,28-31]

Our study shows there was a significant decrease in GPx concentration of male and female cases as compared to male and female controls, respectively.

GPx (EC1.11.1.9 GPx) is a tetrameric enzyme, which utilizes reduced glutathione as a hydrogen donor to remove hydrogen peroxides and other lipid hydroperoxides. This inactivating role prevents the formation of free radical products and lipid peroxides that could alter arterial endothelium.[32]

It is one of the major antioxidant enzyme involved in the protection of cell against peroxidation particularly in the heart muscle. Most of the GPx activity of blood is within the erythrocytes and only 1–2% in plasma.[33] The previous study postulated the prognostic value of GPx for future cardiovascular events. Lower the GPx, more the risk for AMI and vice versa. Thus GPx, the antioxidant enzyme seems to protect against adverse oxidative effects in AMI.[29]

There is a growing evidence of information that antioxidant GPx plays a major role in the prevention of oxidative stress induced by cardiovascular risk factor which renders it as an important antiatherogenic enzyme.[11] This is further supported by an experimental study of deficiency of GPx in mice. It was found that endothelial dysfunction and structural abnormalities such as periadventitial inflammation, neo intimal formation, and collagen deposition around coronary arteries due to GPx deficiency leads to atherosclerosis and AMI.[34] So, decreased the concentration of GPx found in our study and number of other studies may be due to the compensatory mechanism of the body against increased oxidative stress generated in the AMI.[3,11,29,35]

CONCLUSION

AMI exhibits disturbances in oxidants and antioxidant metabolism irrespective of the gender.

Quantitative estimation of free radicals such as lipid peroxide (MDA) and antioxidants such as serum uric acid, GPx, zinc, and SOD are simple, feasible, and economical. Quantitative estimation of MDA can be one of the excellent parameters to judge the oxidative damage taking place in the body in AMI. Measurement of GPx, SOD, and nonenzymatic compounds such as serum uric acid, zinc can judge the status of antioxidant defense system which may help in the treatment, prognosis, and prevention of AMI for which further studies are needed.

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Conflicts of interest
There are no conflicts of interest.

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