OXIDATIVE STRESS AND ENZYMATIC ANTIOXIDANTS IN ACUTE MYOCARDIAL INFARCTION

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ABSTRACT

Background: Myocardial infarction (MI) is the single largest killer disease in the world. Oxidative stress is one of the hallmarks in patients with acute myocardial infarction as observed in recent years.

Aims and objectives: The objective of the study was to evaluate oxidative stress and antioxidant enzyme activity in patients of acute MI.

Materials and Methods: The present study comprised of 50 clinically diagnosed and ECG confirmed cases of acute MI and 50 healthy controls. Oxidative stress marker malondialdehyde (MDA) and enzymatic antioxidants like superoxide dismutase (SOD), catalase and glutathione reductase were estimated in the cases and controls.

Statistical analysis: Statistical analysis was carried out by using students't' test (unpaired). Correlation analysis was done by using SPSS software version 16.

Results: The MDA level was significantly increased (P< 0.0001) in the cases (11.5 ± 0.72 nmol/ml) as compared to controls (6.3 ± 0.92 nmol/ml). The activity of SOD, glutathione reductase and catalase were significantly low (P< 0.0001) in acute MI (450.0 ± 116.76 IU/g Hb, 3.1 ± 0.79 IU/g of Hb and 2.6 ± 0.53 IU/g of Hb respectively) as compared to controls (940.4 ± 93.9 IU/g of Hb, 9 ± 1.02 IU/g of Hb and 7.3 ± 0.70 IU/g of Hb respectively). A significant negative correlation was detected between malondialdehyde and catalase activity in acute MI (r= -0.339; p<0.02).

Conclusion: The study indicates increased oxidative stress and decreased antioxidant defence in patients of acute myocardial infarction. Antioxidant supplements are compounds obtained either by extraction from natural foods (dietary antioxidants) or by chemical synthesis (synthetic antioxidants). Many epidemiological data suggest that synthetic antioxidant supplementation may have a beneficial effect in many chronic diseases. So novel therapeutic approaches like antioxidant supplementation may be useful in the management of persisting oxidative stress in acute MI.

Key words: Acute myocardial infarction, malondialdehyde, antioxidants, superoxide dismutase, catalase, glutathione reductase.

INTRODUCTION

Cardiovascular diseases are the most alarming of the many health predictions for the new millennium worldwide[1]. In recent years there is an explosive rise in the incidence of myocardial infarction. Now India is in a stage of epidemiological transition, with infectious diseases gradually contributing less to morbidity and mortality and the non-communicable diseases like Ischemic Heart Disease (IHD) assuming a more menacing proportion[2].

Acute myocardial infarction (AMI) occurs when the coronary blood flow decreases abruptly after a thrombotic occlusion. Atherosclerosis is the general term for hardening of the arteries, characterized by the deposition of cholesterol and LDL in the arterial wall, which leads to the formation of plaque and results in the endothelial damage and narrowing of the lumen[3].

The prevalence and severity of atherosclerosis and IHD among individuals and groups are related to several risk factors like obesity, diabetes and hypertension. Oxidative stress induced by reactive oxygen species is implicated in the pathogenesis of a variety of vascular diseases, including atherosclerosis, hypertension and coronary artery disease. However, these factors explain part of attributable cardiovascular disease[4]. Oxidative stress has been implicated in various other diseases like glomerulonephritis, chronic renal failure, cancer, pre-eclampsia, Alzheimer's disease, depression, stroke and cataract[5]. Significant changes were seen in the levels of MDA and enzymatic antioxidants in these diseases.

Free radical reactions are ubiquitous in living things. They are produced by enzymatic and non-enzymatic reactions[6]. In biological system, oxidative stress refers to a disturbance in the pro-and antioxidants, balance in favor of the pro-oxidants. Oxidative stress ensues when Reactive Oxygen Species (ROS) evade or overwhelm the

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antioxidant protective mechanisms of cells and tissues. These unstable species may cause damage to DNA, carbohydrates, proteins and lipids that are normally counteracted by protective antioxidants[7].

Oxidative stress and antioxidant defence play a major role in the genesis and protection of AMI. Evidence of ROS generation in patients with AMI has been observed by measuring the lipid peroxidation product malondialdehyde (MDA). Earlier studies in different populations suggest conflicting results of MDA in acute MI[8,9], In this context the present study was undertaken to assess oxidative stress by measuring malondialdehyde and antioxidant defence by assessing the activity of enzymatic antioxidants like superoxide dismutase, glutathione reductase and catalase in patients of AMI.

MATERIAL AND METHODS

The present study comprised of 50 clinically diagnosed and ECG confirmed cases of AMI admitted to the intensive care unit of KLE'S Dr Prabhakar Kore Hospital and Research Centre, Belgaum. All the patients were in the age group of 40—60 years. Fifty, age and sex matched healthy individuals served as controls. The study was conducted between—September 2007 to August 2009. Informed consent was taken from all the cases and the study was approved by the ethical and research committee of JNMC Belgaum.

Inclusion criteria: Clinically diagnosed and ECG confirmed cases of AMI.

Exclusion criteria: Angina pectoris, aortic dissection, renal failure, pulmonary tuberculosis, pneumonia, rheumatoid arthritis, chronic smokers, alcoholics, peptic ulcer and pregnancy.

Five ml of blood was collected from the patients with aseptic precautions immediately after admission. One ml of whole blood was used for the estimation of malondialdehyde. Haemolysate was prepared using four ml of blood and the enzymatic antioxidants were analysed within 24hrs of sample collection. Controls Samples were also processed similarly.

A) In the whole blood:-

- Malondialdehyde (Thiobarbituric acid method). [10]

B) In the haemolysate:-

- Haemoglobin (Drabkin's method) [11]
- Enzymatic antioxidants:

Superoxide dismutase (Misra and Fridorich) [12]

Glutathione reductase (Beutler's method)[13]

Catalase (Beutler's method)[13]

Statistical analysis was carried out by using students't' test (unpaired). The significance was defined at P value of 0.05. Correlation analysis was done by using SPSS software version 16.

RESULTS

Table 1 shows the MDA and enzymatic antioxidant levels in AMI patients and healthy controls. The mean level of MDA in controls was 6.3 ± 0.92 nmol/ml and in cases it was 11.5 ± 0.72 nmol/ml (Figure 1). The level was significantly increased (P< 0.0001) in the cases compared to controls. The value of SOD in controls was 940.4 ± 93.9 IU/g of Hb, and in cases was 450.0 ± 116.76 IU/g Hb. The activity of SOD was significantly decreased (P<0.0001) in the cases compared to controls. The mean glutathione reductase level was 9 ± 1.02 IU/g of Hb in controls and in cases it was 3.1 ± 0.79 IU/g of Hb. The activity of glutathione reductase was decreased significantly (P<0.0001) in the cases compared to controls. The mean catalase level in controls was 7.3 ± 0.70 IU/g of Hb while in cases was 2.6 ± 0.53 IU/g of Hb. The activity of catalase was decreased significantly (P<0.0001) in the cases compared to controls. Among the antioxidant enzymes, only catalase showed significant negative correlation (r= -0.339; p<0.02) with MDA (Figure 2).

Table 1. Malondialdehyde (MDA) and enzymatic antioxidants in Acute Myocardial Infarction patients and Controls

Parameters	Controls (n=50)	AMI patients (n=50)	P value
MDA nmol/ml	6.3 ± 0.92	11.5 ± 0.72	<0.0001*
SOD IU/g Hb	940.4 ± 93.9	450.8 ± 116.7	<0.0001*
Glutathione reductase IU/g Hb	9 ± 1.02	3.1 ± 0.79	<0.0001*
Catalase IU/g Hb	7.3 ± 0.7	2.6 ± 0.53	<0.0001*

Values are expressed as Mean ± SD
*** Statistically significant change**

Figure 1. Malondialdehyde levels in AMI cases and controls

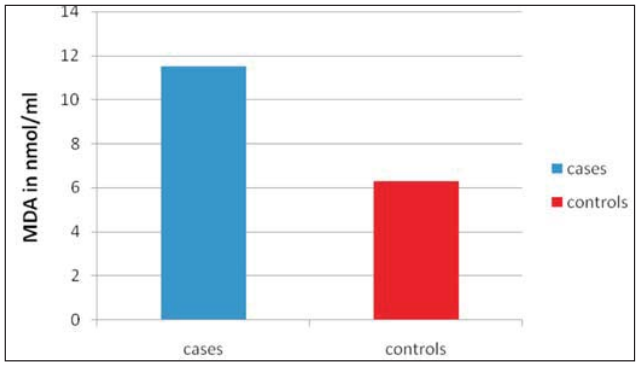
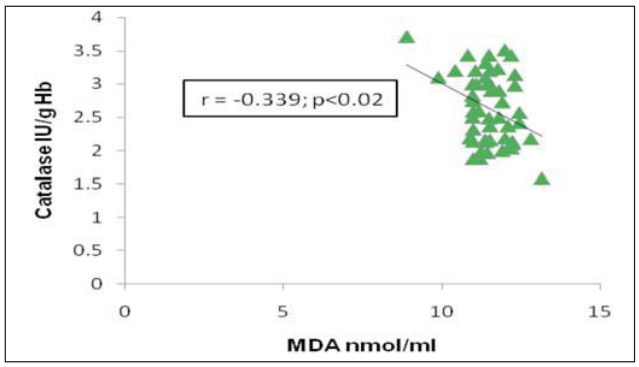


Figure 2. Negative correlation between MDA level and catalase activity in acute Myocardial Infarction patients



DISCUSSION

Acute myocardial infarction is the major cause of mortality and morbidity worldwide. The root cause of AMI is mainly atherosclerosis initiated by oxidative stress. It is associated with various risk factors such as age, gender, diabetes mellitus, hypertension and smoking. Involvement of oxygen free radicals (OFR) in the pathophysiology of inflammation and ischemia in a number of organs and tissues has been reported in literature[14,15]. Evidence of OFR generation in patients with AMI has been observed by measuring the lipid peroxidation product malondialdehyde(MDA) and is used as an index of oxidative damage. The antioxidant defence assessed by measuring superoxide dismutase, catalase and glutathione reductase. Many studies on MI have been done, but the correlation analysis within the parameters has not been highlighted. Our study was also aimed at knowing the correlation between MDA and enzymatic antioxidants. Oxidative stress parameter MDA can be used as an assistant marker to the other conventional markers in the diagnosis of MI as it involves a simple procedure, but the supporting evidence is still ambiguous.

In the present study mean level of MDA was increased significantly in cases compared to controls. Our findings are in accordance with the study of Neela Patil et al[8]. According to K Kaur et al[16] the high concentration of MDA in patients indicates increased membrane lipid peroxidation. Enhanced lipid peroxidation may occur as a result of imbalance between scavenging mechanisms and free radical generation process. Our findings are contradictory to the findings of Kasap Segil et al[9] who showed significant decrease in MDA levels in AMI.

The level of SOD was significantly decreased in cases compared to controls. The findings of our study are in accordance with study of K Kaur et al[16], Arun Kumar et al[17] and Gunasekera et al[18]. According to K. Kaur et al[16] decrease in SOD activity may be attributed to hypoxia due to ischemia. There is an enhanced production of superoxide anions by ischaemic cells. Increased concentration of LDL causes uncoupling of endothelial nitric oxide synthase and consequently increased production of superoxide anions in the vessel wall. In patients with Coronary Artery Disease (CAD), secretion of TNF- α and increased free radical load causes depression of extracellular SOD activity.

The level of glutathione reductase was decreased significantly in patients compared to controls. The findings of the present study are in accordance with the results of Simmi Kharb et al[19]. According to Simmi Kharb et al[19],Oxygen Free Radicals (OFR) are generated particularly in the early stages of MI and glutathione reductase is involved in the detoxification of hydrogen peroxide radicals, resulting in a decrease in glutathione reductase levels. Blaustein et al[20] have demonstrated that reduced glutathione (GSH) is important in protecting the myocardium against OFR injury. Depressed levels may be associated with an enhanced protective mechanism to oxidative stress in AMI.

The level of catalase was decreased significantly in AMI patients compared to controls. Our findings agree with the findings of Arun Kumar A et al[17] and Gunasekara et al[18]. Free radical scavenging enzymes such as SOD and catalase are the first line of defense against oxidative injury, decomposing oxygen and hydrogen peroxide before interacting to form the more reactive hydroxyl radical. Decrease in the levels of catalase could be due to inactivation of the enzymes by cross-linking or due to exhaustion of the enzymes by increased peroxidation.

CONCLUSION

Increased oxidative stress and decreased antioxidant defence may be the root cause of atherosclerosis in myocardial infarction. Antioxidant supplements are compounds obtained either by extraction from natural foods (dietary antioxidants) or by chemical synthesis (synthetic antioxidants). Of course, they do not have the same composition as natural antioxidants in foods. Many epidemiological data suggest that synthetic antioxidant supplementation may have a beneficial effect in many chronic diseases. Exogenously supplied antioxidants play an important role in helping endogenous antioxidants for the neutralization of oxidative stress. Hence antioxidant therapy with supplementation of natural antioxidants like β-carotene, vitamin E and vitamin C could be a promising avenue in the management of persisting oxidative stress in acute MI.

LIMITATIONS OF THE STUDY

1) Follow up study after supplementation with antioxidants is not done.
2) A random blood sample was used in the study rather than a fasting sample and parameters like MDA may be influenced by the type of blood sample.
3) The age of the study group is 40—60 years and the results may not be representing the younger population.

REFERENCES

1. Surekha RH, Srikant BBMV, Jharna P, Ramachandra RV et al. Singapore Medical Journal 2007; 48 (2): 137-142.
2. Braunwald Zipes Libby. Heart Disease. In: A textbook of cardiovascular medicine, 6th Edition, pp 1114-1115.
3. Berliner JA, Navab M, Fogelman AM, Frank JS, Demer LL, Edwards PA, Watson AD, Lusis AJ. Atherosclerosis: basic mechanisms. Oxidation, inflammation, and genetics. Circulation 1995; 91: 2488-2496.
4. Palaniswamy P, Y.Yagneswara Rao, Jawahar Farook et al. Oxidative stress and cardiac biomarkers in patients with acute myocardial infarction. European Journal of Scientific Research 2009; 27(2): 275-285.
5. Lien Ai Pham- Huy et al. Free radicals, antioxidants in disease and health. International Journal of Biomedical Science 2008; 4(2): 89-96.
6. Carrol CE. Oxygen free radical and human disease. Annals of Internal Medicine 1987; 107: 526-45.
7. Hanachi P, Rashid et al. Investigation of lipid profiles and lipid peroxidation in patients with type 2 diabetes. European Journal of Scientific Research 2009; 28(1): 6-13.
8. Neela Patil, Vishwas Chavan, Karnik ND. Antioxidant status in patients with acute myocardial infarction. Ind J of Cli Biochem 2007; 22 (1): 45-51.
9. Kasap Segil, Gonenc Amylek. Serum cardiac markers in patients with acute MI: Oxidative stress, CRP & N-terminal probrain natriuretic peptide. J of Clin Biochem & Nutrition 2007; 41(1): 50-71.
10. Placer ZA, Linda L, Crushman JBC. Estimation of Product of lipid peroxidation in biochemical System. Annal Biochem 1966; 16: 359-64.
11. Drabkin DL, Austin JH. Spectrophotometric constants for common hemoglobin derivatives in human, dog and rabbit blood. J Biol Chem 1932; 98: 719-733.
12. Misra HP, Fridorich I. The role of superoxide anion in the auto oxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972; 247: 3170.
13. Beutler E. Red cell metabolism. In: A manual of biochemical methods. London Edinburgh Churchill Livingstone Publication, Vol-16, 1986.
14. Frei B, Stocker R, Ames BN. Antioxidant defenses & lipid peroxidation in human blood plasma. Proc Natl Acad Sci USA 1988; 85: 9748-52.
15. Uhling S, Wendel A. The Physiological consequences of glutathione variations. Life Sci 1992; 51: 1083-94.
16. K Kaur, G Bedi, M Jaur, Anil Vij and I Kaur. Lipid peroxidation and the levels of antioxidant enzymes in coronary artery disease. Ind J of Clin Biochem 2008; 23(1): 33-37.
17. Arun Kumar, Ramaih Sivakanesan & Sunil Gunasekera. Oxidative Stress and antioxidant status in normolipidemic AMI patients. Ind J of Clin Biochem 2008; 23(3): 296-298.
18. Gunasekara, Kumar A, Sivakanesan R. Antioxidants, Oxidative Stress status and waist / hip ratio in normolipidaemic AMI patients. Pak J Med Sci 2008; 24 (5): 689-93.
19. Simmi Kharb. Lab blood glutathione levels in acute myocardial infarction. Ind J of Med Sci 2003; 57: 335-37.
20. Blaustein A, Dencke SM, Stolz R. Myocardial glutathione impairs recovery after short periods of ischemia. Circulation 1989; 80: 1449-57.