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RESEARCH ARTICLE

ROLE OF LIPID PEROXIDATION, MYELOPEROXIDASE, NITRIC OXIDE AND CATALASE IN PATHOGENESIS OF ACUTE MYOCARDIAL INFARCTION

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ABSTRACT

Acute myocardial infarction (AMI) is mainly related to atherosclerosis, tissue destruction, and inflammation. Reactive oxygen species are involved in the pathophysiology of inflammation and atherosclerosis. In this study, activity of (Myelopeoxidase) MPO, Catalase and levels of Malondialdehyde (MDA) and Nitric oxide (NO) were determined in 50 AMI patients and 50 healthy controls. Unit of MDA as nmol/mL (99.42 ± 52.84, 35.72 ± 11.63) and MDA levels (5.6 ± 1.5, 3.88 ± 1.81) were significantly increased in AMI patients. Unit of NO as µmol/L (20.57 ± 6.05, 36.17 ± 7.02) and activity of the antioxidant enzyme Catalase (20.28 ± 10.35, 35.3 ± 7.7) were found to be significantly decreased in AMI when compared to controls. The present study indicates that there is increased activity of MPO, reduced activity of Catalase and reduced nitric oxide levels may lead to oxidative stress in AMI. MPO generated free radicals may induce damage in the form of endothelial dysfunction, plaque formation and plaque rupture. This may leads to progression of atherosclerosis and finally develops into AMI. Thus the study concluded that MPO, Catalase and nitric oxide have an important role in pathogenesis of AMI.

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INTRODUCTION

Acute Myocardial infarction is a major cause of death and disability worldwide (Nawar *et al*., 2007). The current knowledge of pathophysiology of AMI is that AMI arises due to thrombotic occlusion of coronary artery (Siddharth *et al*., 2006). Reactive oxygen species (ROS) play an important role in the pathogenesis of AMI (Leoper *et al*., 1991). ROS react with polyunsaturated fatty acids (PUFA) and causes lipid peroxidation in memebranes (Salvemini D, Cozzocreas 2003). Accumulation of ROS leads to 'cardiac contractile dysfunction', potentially leading to arrhythmia and myocardial infarction (Fauci Braunwald *et al*., 2008). Myeloperoxidase (MPO) is a neutrophilic enzyme which is involved in propagation of atherosclerosis. It is also present in monocytes and tissue macrophages (Bos *et al*., 1978). MPO is associated with inflammation, LDL oxidation and oxidative stress. Endothelium-derived NO is the most potent vasodilator and is a critical modulator of blood flow, platelet aggregation, oxidative modification of LDL-cholesterol, proliferation of vascular smooth muscle cell, and leucocyte adherence (Stephane Cook, 2006). Free radical oxygen species such as superoxide anion can rapidly react with and inactivate nitric oxide, enhancing proatherogenic mechanisms (leukocyte adherence, impaired vasorelaxation, platelet aggregation) (Landmesser and Harrison, 2001). Catalase plays a major role in the first line of enzymatic antioxidant defense (Yosri *et al*., 2013). In the present study activities of MPO, Catalase and levels of Malondialdehyde (MDA), nitric oxide were determined in AMI patients and compared with healthy controls.

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MATERIALS AND METHODS

The study group included 50 male subjects between 45-75 years of age, with confirmed diagnosis of AMI. Clinical history regarding symptoms, past, personal and family history of concerned risk factors was taken. Criteria for AMI were defined according to the European Society of Cardiology/American College of Cardiology redefinition of MI guidelines (Thygesen *et al*., 2007). 50 age matched healthy male participants from general population comprised the

control group. Written consent of all the participants were obtained and the study was approved by ethical committee of BIMS, Belagavi. Exclusion criteria-Patients with past history of AMI, tobacco smokers, tobacco chewers, participants consuming vitamin and antioxidant supplements and subjects with acute infection and inflammatory disorders were excluded from the study. Sample collection: 5ml of venous blood sample was collected under aseptic precaution from the anticubital vein of all participants. Samples were taken within 6hrs of onset of symptoms from AMI patients.2ml of the sample was transferred to bulb containing anticoagulant EDTA. Rest of the sample was taken in a plain tube. The Serum was used for estimation of MDA, MPO and NO. Haemolysate prepared after washing RBCs with saline was used for estimation of erythrocyte Catalase activity. MDA was estimated by thiobarbituric assay as described by Satoh (1978). The proteins in serum were precipitated by trichloroacetic acid (TCA) the mixture was heated with thiobarbituric acid in 2M sodium sulfate, in a boiling water bath for 30 minutes. The resulting chromogen was extracted with n-butyl alcohol and absorbance of organic phase was determined at 530nm wavelength. MPO activity was estimated by method of Weiss (1982) using commercial kit obtained from sigma aldrich co USA. In this assay, MPO catalyzes the formation of hypochlorous acid, which reacts with taurine to form taurine chloroamine. Taurine chloroamine reacts with the chromophore TNB, resulting in the formation of the colorless product DTNB. One unit of MPO activity is defined as the amount of enzyme that hydrolyzes the substrate and generates taurine chloramine to consume 1.0 mmole of TNB per minute at 25 °C.

For Nitric oxide estimation serum was deproteinized first and then nitrate, the stable product of nitric oxide present in filtrate was reduced to nitrite by cadmium. The nitrite produced was determined by diazotization of sulfanilamide and coupling to naphthylene ethylene diamine as described by Najwa and Cortas (1990). The colour complex was measured at 540nm wavelength using spectrophotometer. Erythrocyte Catalase activity was determined by method of Aebi (1984). Saline washed RBCs were hemolysed. Concentrated hemolysate (5g Hb/100ml) was prepared. Hemolysate diluted with phosphate buffer, was used for determination of Catalase activity. Catalase decomposes H_2O_2 to form water and molecular oxygen. In the ultraviolet range H_2O_2 shows continual increase in the absorption with decreasing wavelength. At 240nm, H_2O_2 absorbsmaximum light, when H_2O_2 was decomposed by catalase then the absorbance decreased. This decrease in absorbance measured at 240nm for every 15sec interval upto 1 minute and the difference in absorbance (A at 240nm) per unit time was measured., All the analysis was carried out on systronics UV-VTS spectrometer 117. Statistical analysis-Continuous variables are expressed as mean ± SD. Categorical data is expressed as percentage frequency. Statistical significance was analyzed by using student's unpaired 't' test. P value of < 0.05 was considered to be significant.

RESULTS

In the present study activity of MPO and Catalase and levels of MDA and NO were determined in 50 AMI patients and 50 healthy controls. Baseline characteristics of all the participants are shown in Table 1. There is no significant difference in the age of controls and AMI patients. 12% of the patients had hypertension and 10% had diabetes mellitus. All the participants were non smokers and non alcoholics. The mean CK-MB levels in patients with STEMI and NSTEMI is shown in Table 2.

Table 1. Baseline characteristics of patients and controls

S.No.	Characteristics	Controls (n=50)	AMI Patients (n=50)
1.	Age (years)	54.34 ± 9.4	59.27 ± 8.6* (p=0.37)
2.	History of Smoking(%)	00	00
3.	History of Hypertension(%)	00	12
4.	History of Diabetes Mellitus(%)	00	10
5.	History of alcohol consumption(%)	00	0

*Age expressed as mean ± SD, no significant difference in the age between AMI and controls. n = number of participants.

Table 2. Mean CK-MB levels in AMI patients

ECG finding	Number of patients (%)	Mean CK-MB level (U/L)
STEMI	23	118.98
NSTEMI	27	96.3

STEMI-ST elevation Myocardial Infarction, NSTEMI-non ST elevation Myocardial Infarction

Table 3. Biochemical parameters in AMI patients and controls

S.No.	Biochemical parameter	AMI Patient (n=50)	Controls (n=50)	p value
1	MDA (nmol/ml)	5.6 ± 1.5	3.88 ± 1.81	<0.006*
2	MPO activity (mU/ml)	99.42 ± 52.84	35.72 ± 11.63	<0.0001*
3	Catalase activity (mm H_2O_2 decomposed/mg of Hb)	20.28 ± 10.35	35.3 ± 7.7	<0.0001*
4	Nitric oxide (µmol/L)	20.57 ± 6.05	36.17 ± 7.02	<0.0001*

*p value is significant, n=number of participants. All values are expressed as mean ±SD

MPO activity and malondialdehyde levels were increased significantly ($p<0.05$) in AMI patients compared to controls. NO levels and catalase activity was found to be decreased significantly in AMI ($p<0.05$) patients when compared to controls, as shown in Table 3.

DISCUSSION

In the present study MPO activity was increased significantly in AMI in comparison with control participants. Tessa *et al*. found elevated levels of MPO in patients with AMI (Tessa *et al*., 2000). Plasma MPO levels were high in patients with acute ST elevation MI in a study done by Mehmet *et al*. (2012). MPO is found in the leucocytes located in atherosclerotic plaque. It is released into circulation after plaque rupture during AMI. MPO plays an important role in atherogenic process by oxidizing LDL cholesterol, which is present in the core of atherosclerotic plaque. MPO is also involved in other multiple process throughout the atherosclerosis progression like activation of protease cascades and promotion of endothelial cell apoptosis, leading to breakdown of fibrous cap (Nicholls and Hazen, 2005; Podrez *et al*., 2000). In the study MDA levels were higher in AMI patients when compared with control participants. Increased MDA levels indicate lipid peroxidation in AMI patients. In a study done by Lalitha *et al*. (2012), and Gururajan *et al*. (2010) increased levels of MDA was observed. MDA is formed as a secondary product during lipid peroxidation. Lipid peroxidation contibutes to atherosclerosis progression (1998). The cells present in atherosclerotic lesion and the endothelial cells generate excess ROS. Oxidized lipids are also generated by direct action of MPO. Increased oxidative stress can cause of cell death. This leads to formation of necrotic core in the atherosclerotic lesion. This necrotic core is the hallmark of advanced atherosclerotic plaque. In the present study reduced activity of erythrocyte catalase enzyme was observed in AMI patients when compared with controls. In a study by Khaper *et al*. (2003), they found that there was reduced catalase activity compared to other antioxidant enzymes in AMI patients. In a study done by Gupta *et al*. (2009), decreased levels of antioxidants including catalase were observed in patients with coronary artery disease when compared with normal healthy controls. The increased MPO in AMI can generate reactive oxygen species (ROS). These ROS can structurally modify the tyrosine residues present in the active site of catalase. This modified catalase structure might reduce the catalase activity in AMI. Significantly decreased levels of Nitric oxide were observed in AMI patients when compared to controls. Marwan *et al*. (2014) found in their study that there was significant reduction in serum nitric oxide levels in AMI patients compared with healthy participants. Rizk *et al*. (2004) showed that there was significantly reduced levels of nitric oxide in AMI when compared to controls. There is increased ROS in AMI. These ROS may reduce the nitric oxide synthase (NOS) activity might reduce the NO levels in AMI. This reduced NOS activity might reduce the NO levels in AMI. The excess free radicals in atherosclerotic lesion can rapidly convert nitric oxide to toxic perxinitrite. This further enhances oxidative damage.

Limitation

Some of the AMI patients were diagnosed to have diabetes mellitus and hypertension at the time of admission and these factors might affect the levels of measured parameters.

Conclusion

The lipid peroxidation in AMI might be due to raised activity of MPO along with decreased catalase activity and nitric oxide levels.MPO, catalase and nitric oxide have an important role in pathogenesis of AMI.

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