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Xanthine oxidase -oxidase stress marker in acute MI patient: a study at a teaching hospital in Telangana

Sowmya Gayatri[1,*], Routhu Kathyaini[2]

[1]Assistant Professor, RVM Institute of Medical Sciences, Medak, Telangana, [2]Associate Professor, Dept. of Biochemistry, KMC

***Corresponding Author:**
Email: resdoc555@gmail.com

Abstract
Introduction: Despite impressive strides in diagnosis and management over the past three decades, acute myocardial infarction (AMI) continues to be a major public health problem in the industrialized world and is becoming an increasingly important problem in developing countries. Xanthine oxidase in ischemic conditions of the heart may play an important role in generating free radical mediated damage.
Aim of the Study: To measure the levels of xanthine oxidase activity in myocardial infarction patients.
Materials and Method: The study was conducted from the month of June 2011 to May 2012, at MGM Hospital, Warangal blood samples of 30 patients diagnosed as acute myocardial infarction were collected and subjected to plasma xanthine oxidase activity.
Results: 60% of AMI patients belonged to 51-60 years age group. There was significant increase in mean values in Xanthine oxidase activity levels in ($p < 0.001$) in the study group as compared to controls.
Conclusion: Xanthine oxidase is good markers in identifying AMI. They all can be used together as multiple markers to increase their efficiency as markers of AMI. The AST & LDH though exhibited good sensitivity, lack specificity. Hence they should not be used as individual markers.

Keywords: Acute myocardial infarction, Male patients, Xanthine oxidase, Mycocardial injury, Free radicals.

Introduction
Acute myocardial infarction (AMI), the leading cause of deaths in both developed and developing countries. Despite impressive strides in diagnosis and management over the past three decades, acute myocardial infarction (AMI) continues to be a major public health problem in the industrialized world and is becoming an increasingly important problem in developing countries.(1) It is due to the impairment of heart function due to inadequate blood flow to the heart compared to its needs, caused by obstructive changes in the coronary circulation to the heart.(2) It is the cause of 25-30 per cent of deaths in most industrialized countries. The WHO has drawn attention to the fact that AMI is our modern epidemic. Large body of data exists on the occurrence of AMI in hospital patients.(3)

The prevalence was found to be 65.4 and 47.8 per 1000 males and females respectively in urban population and 22.8 and 17.8 per 1000 males and females respectively in rural population.(4,5) AMI most commonly occurs from a sudden thrombotic occlusion at the site of a ruptured or fissured atherosclerotic plaque.(6)

Myocardial ischaemia results from the reduction of coronary flow to such an extent that supply of oxygen to the myocardium does not meet the oxygen demand of myocardial tissue. When this ischaemia is prolonged and irreversible then the myocardial cell death and necrosis occurs which is defined as acute myocardial infarction (AMI). The diagnosis of AMI is based on clinical symptoms, electrocardiographic findings (ECG), characteristic pattern of changes in some serum enzymes such as creatine kinase MB(CKMB), lactate dehydrogenase and its isoenzymes (LDH), aspartate Transaminase (AST), cardiac troponins etc.,(7) ECG is the most widely used method for the diagnosis of myocardial infarction, but many times ECG shows inconclusive pattern.(8)

The older markers like AST, creatine kinase, LDH etc. lost their utility due to lack of specificity and limited sensitivities.(9) However, among the currently available markers the troponin test appears to be the most promising as far as its sensitivity, specificity, turnaround time and cost is concerned. Nevertheless, there are reports to show that abnormal levels of troponin are found in various conditions which are not related to acute coronary diseases.(9,10) It is clear from the foregoing review that none of the markers available so far meet all the criteria required for an ideal biochemical marker of myocardial injury.

Oxygen free radical generation has been shown to be an important mechanism of cellular injury in ischemic myocardium.(11) Several mechanisms have been proposed to be involved in the generation of oxygen free radicals. Xanthine oxidase has been shown to be a major source of free radical generation under ischemic conditions.(12)

Xanthine oxidase (XO) is an enzyme that catalyzes the chain reactions of hypoxanthine oxidizing to xanthine and xanthine oxidizing to uric acid and hydrogen peroxide. Oxidation requires the addition of oxygen and water.(13) Under ischemic conditions, there is depletion of ATP and subsequent loss of membrane Ca^{2+} gradient. Increased Ca^{2+} levels activate Ca^{2+} dependent proteases which are converted into xanthine oxidase.(14) Thus xanthine oxidase in ischemic conditions of the

heart may play an important role in generating free radical mediated damage.[8]

Aim of the study

To measure the levels of xanthine oxidase activity in myocardial infarction patients. A similar analysis would be carried out in apparently healthy human subjects serving as controls.

Materials and Method

The study was conducted from the month of June 2011 to May 2012, at MGM Hospital, Warangal. Ethical committee clearance and approval were taken before starting the study. Prior informed consent was taken from the groups included in the study. The subjects were based on the following inclusion and exclusion criteria:

Inclusion Criteria:

1. Male patients between the ages of 40-65 years were taken into consideration.
2. Patient clinically diagnosed as having acute myocardial infarction with the help of serum CKMB>10 IU/L along with the other diagnostic criteria for MI were included in the study.

Exclusion Criteria:

1. Patients with liver and intestinal pathologies were excluded from the study.
2. Kidney diseases were excluded from the study.
3. Patients with gout.
4. Patients who developed cerebral complications during the course of study.

Subjects: Selection of subjects for the study was done as follows:

Controls: Age matched 30 controls were healthy male subjects in the age group of 40 to65 years, attending the blood banks, and also from other sources. The samples from these patients were collected prior to blood donation. The samples were also obtained from other sources. Prior informed consent was taken from all the patients.

Study groups: Blood samples of 30 patients diagnosed as acute myocardial infarction (admitted within 12 hrs after onset of ischemic pain) were collected.

Sampling: 5 ml of blood samples were taken and equally distributed in to plain tubes and citrated vials. The blood was suitably diluted during analysis wherever required. The plasma formed in the citrated vials was separated and used for Xanthine oxidase estimation, while clotted blood in the plain tubes was subjected to centrifugation. Care was taken to prevent hemolysis of the blood samples. The clear serum was separated and used for the remaining biochemical investigations.

Materials and Method

Plasma Xanthine Oxidase Activity Assay: XO catalyzes the oxidation of hypoxanthine to xanthine and then catalyzes the oxidation of xanthine to uric acid. It was estimated by the method of ROUSSOS.[15,16] The assay mixture, in final volume of 3.0 ml, consisted of 0.30 ml Tris-HCl buffer, 50 mM pH 7.4; 0.30 ml CuSO4, 10 mM; 0.05 ml. Xanthine, 2.58 mM per ml. in 0.05 M glycine buffer, pH 7.4; 0.1 ml. of diluted blood and water to make up the volume The method depends on the enzymatic oxidation of xanthine which if followed by continuous spectrophotometric measurement of the uric acid formed at 293 nm.[15,16] A unit of activity is that forming one µmol of uric acid/min.

XANTHINE OXIDASE ⇨ XANTHINE + H_2O_2 + O URIC ACID + H_2O

Statistical analysis

Statistical analysis was carried out using Student's t-test by statistical packages for social science software (SPSS). Values are expressed as mean±SD and values of $p<0.05$ were considered statistically significant.

Results

This study was conducted at ICCU, MGM Hospital, Warangal. Study group comprised of males in the age range of 40-65 years and diagnosed with AMI, while the control group comprised of healthy age matched males from various sources. Majority (60%) of AMI patients belonged to 51-60 years. (Table 1)

Table 1: Age-wise distribution of cases among study group

Age Group (yrs)	No.	Percentage %
40-45	3	10
46-50	5	16.7
51-55	9	30
56-60	9	30
61-65	4	13.3

Table 2: Age distribution among control group

Age Group (yrs)	No.	Percentage %
40-45	4	13.3
46-50	5	16.7
51-55	9	30
56-60	9	30
61-65	3	10

Table 3: Comparison of xo activity levels between the control group and the study group

Parameter	Control Group	Study Group	Comparision p
Xanthine Oxidase(U/mg protein)	0.0121±0.0028	0.0278±0.0049	**Significant** p <0.001

- Mean values of Xanthine oxidase activity levels in ($p < 0.001$) in the study group as compared to controls as seen in the Table 3.

Discussion

AMI is a major cause of mortality in developed as well as developing countries. It is a disease of multifactorial origin, in which a gross necrosis of

myocardium occurs due to interruption of blood supply to the tissues. ECG is the most widely used method for the diagnosis of myocardial infarction, but many times ECG shows inconclusive pattern.[8] Estimation of serum biochemical markers of myocardial injury arises to confirm the diagnosis of myocardial injury

A range of biomarkers are available for use in clinical investigations. These include molecules of intermediary metabolism, cell signaling molecules, enzymes, or proteins whose concentrations change significantly in response to a disease state and so can be used to monitor the onset or progress of a disease, or predict the outcome in response to its treatment.

A significant increase in the cardiac enzymes, which included AST, LDH, CK-MB, Cardiac troponins etc., was found in AMI patients which rose in parallel to the extent of myocardial injury. The characteristic pattern of the rise in the serum cardiac enzymes was: they start to increase 4- 6 hours after injury, reach peak concentrations after 12-24 hours and return to the baseline after 48-72 hrs.[17] The cardiac enzymes are released from the necrotic heart muscle after AMI. Cardiac enzyme concentrations become abnormal in the peripheral blood once the capacity of the cardiac lymphatics to clear the intertitium of the infarct zone is exceeded and spill over into the venous circulation occurs. In the present study, the serum CK-MB, AST & LDH activities are significantly increased in AMI patients when compared to the controls, indicating the presence of myocardial damage. This is in agreement with earlier reports.

- The free radicals generated in oxidative stress due to ischemia and subsequent reperfusion can cause vascular endothelial dysfunction. The lipid peroxidation occurs as a damaging reaction consequent to free radical production in cells. Chain reactions can directly damage the structure of membrane and indirectly damage the cell components by superoxide radicals.[18] Oxygen free radical generation has been shown to be an important mechanism of cellular injury in ischemic myocardium.[11] Several mechanisms have been proposed to be involved in the generation of oxygen free radicals. Xanthine oxidase has been shown to be a major source of free radical generation under ischemic conditions.[12]
- Several studies have identified the importance of serum uric acid concentration in young populations in predicting the risk of cardiovascular disease, such as AMI.[19] This study shows that serum uric acid levels are higher in younger patients with AMI on the day of admission and the day of discharge when compared to the control. Serum uric acid has antioxidant properties and contributes to free radical scavenging activity in human serum, thus, uric acid can be protective against oxidative stresses, but it can also lead directly or indirectly to vascular injury.[20] High levels of serum uric acid may induce endothelial dysfunction by decreasing the production of nitric oxide, which is a potent vasodilator in the vascular endothelial cells. In this study, there is a significant increase in serum uric acid concentration. Our results agree with most of the other studies that suggest that elevated serum uric acid may act as a marker for tissue ischemia, cardiac failure and myocardial infarction.[21,22]
- Circulating XO has been suggested to be specifically involved in the mechanism of peripheral endothelial dysfunction and it could play a crucial role in the generation of ROS in the body. Berry C.E et al., observed that MI patients were characterized by increased activity of the XO system. Therefore, it could be established that XO can be regarded to be cardiovascular risk factor for human.[23] In the present study the XO activity levels were increased approximately by two fold in the AMI patients as compared with the controls. We confirm the significance of Xanthine oxidase in the pathophysiology as a major source of free radicals/ ROS for therapeutic intervention. The results of the study were comparable to the similar kind of studies.
- XO catalysis of the reaction that generates oxygen free radicals is considered one of the most significant. The enzyme acts in the metabolism of purine, converting both hypoxanthine and xanthine to uric acid at the expense of molecular oxygen to produce superoxide ions, which oxidize cellular proteins and membranes resulting in myocardial cellular injury.[24,25] In the present study, the finding of high levels of XO activity in the blood of patients with AMI compared to the levels in the control subjects indicate that myocardial ischemia has a definite correlation with XO activity, suggesting that XO is specifically involved in the mechanism of peripheral endothelial dysfunction, and it could play a crucial role in the generation of ROS in the body.[26] XO can be used as a biochemical indicator of AMI, along with Electrocardiography observations.[25] Under normal physiological conditions, ROS production is balanced by an efficient system of antioxidants which are molecules that are capable of scavenging ROS and thereby preventing oxidant damage

Conclusion

The Xanthine oxidase activity is significantly increased in AMI. Thus increased Xanthine oxidase activity plays an important role in the pathophysiology of AMI through contributing to increased ROS.

Xanthine oxidase is a good markers in identifying AMI. Further prospective studies in larger data include different populations are needed to confirm our data.

Scope for Future Study

From the present study Xanthine an oxidase activity level increased in AMI and plays an important role in the

pathophysiology of AMI through contributing to increase in ROS. Hence indicating Xanthine oxidase activity as a potential for therapeutic intervention.

Administration of allopurinol, a Xanthine oxidase inhibitor has been shown to reduce myocardial infarct size elicited by coronary ligation and reperfusion induced arrhythmias.

We are aware of no studies specifically designed to evaluate whether free radical scavenger agents, such as allopurinol, could be beneficial in clinical situations associated with severe tissue damage after significant ischaemia and reperfusion.

As we look back on the advances in the treatment of uric acid related disease, we can only hope that similar progress will be made in the elucidation of intricacies of free radical formation & their interaction with other systems.

Further studies in the area of basic & integrated clinical research should provide fascinating insights into physiological & pathological processes, and would ultimately dictate relevant advances in protecting the body against free radical mediated diseases, such as myocardial ischaemia, inflammatory diseases and rheumatic conditions.

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