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Co-relation between xanthine oxidase and ceruloplasmin in acute myocardial infarction

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Abstract

Myocardial ischemia 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. Xanthine oxidoreductase, under normal conditions, exists in dehydrogenase form. Under ischemic conditions it is converted into xanthine oxidase. Ceruloplasmin is α_2 globulin – a glycoprotein, an acute phase protein synthesized by the liver in response to tissue damage. Our aim in this study was to determine levels of xanthine oxidase and ceruloplasmin in acute myocardial infarction.
We found that, xanthine oxidase activity in acute myocardial infarction patients increases significantly ($p < 0.01$) upto 12 hours and then slowly decreases. The maximum activity is found after 12 hours of acute myocardial infarction. While, ceruloplasmin level in the serum of patients increases significantly ($p < 0.01$) upto 48 hours and then slowly decreases. We found xanthine oxidase and ceruloplasmin have no specific co-relation between them. But, still it is found that xanthine oxidase activity reaches to peak more early as compared to ceruloplasmin.
Thus, it is concluded xanthine oxidase is more potent in diagnosis and prognosis of acute myocardial infarction and can be utilized as a marker of oxidative stress developed in acute myocardial infarction.

Keywords: Acute myocardial infarction, ceruloplasmin, Xanthine oxidase, free radicals, ischemia.

1 Introduction

Myocardial ischemia 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 ischemia is prolonged and irreversible then myocardial cell death and necrosis occurs which is defined as acute myocardial infarction (AMI). [1]
Myocardial infarction generally occurs when there is abrupt decrease in coronary blood flow following a thrombotic occlusion of a coronary artery previously narrowed by atherosclerosis. Slowly developing, high grade coronary artery stenosis usually does not precipitate acute infarction because of the development of a rich collateral network over time. Instead infarction occurs when a coronary artery thrombus develops rapidly at a site of a vascular injury. This injury is produced or facilitated by factors such as cigarette smoking, hypertension, and lipid accumulation. In most cases infarction occurs when an atherosclerotic plaque fissures, ruptures or ulcerates and when conditions (local or systemic) favor thrombogenesis, so that mural thrombus forms at the site of rupture and leads to coronary artery occlusion.[2]
Xanthine oxidoreductase, under normal conditions, exists in dehydrogenase form and uses NAD^+ and there is no or very little production of superoxide anion. Under ischemic conditions, there is depletion of ATP and subsequent loss of membrane Ca^{2+} gradient. Increased Ca^{2+} levels activates Ca^{2+} dependent proteases which cause selective proteolysis of the dehydrogenase to convert it into xanthine oxidase which acts both on hypoxanthine and xanthine at the expense of molecular oxygen to produce superoxide ion. Thus xanthine oxidase in ischemic conditions of the heart, as in myocardial infarction, may play an important role in contributing free radical mediated damage.[3]
Ceruloplasmin is α_2 globulin – a glycoprotein carrying six copper atoms per molecule. It is one group of serum protein which rises after any form of tissue injury. Ceruloplasmin synthesis and/or secretion is altered by inflammation, hormones, and copper. Physiological factors like cancer, exercise, chronic inflammation, pregnancy increase its level. It also acts as a host defense mechanism by its radical scavenging and copper donor activity.[4] Ceruloplasmin functions as ferroxidase by catalyzing the Oxidation of Fe^{2+} to Fe^{3+}, and correlates well with its level and antioxidant activity. Ceruloplasmin is an important intravascular antioxidant and it protects tunica intima against free radical injury. Ceruloplasmin is an acute phase protein and is synthesized by the liver in response to tissue damage\ and inflammation. This phenomenon is the basis for constantly observed sudden increase in serum copper and ceruloplasmin levels which

decreases slowly and reaches to baseline within a month.[5,6] Ceruloplasmin exhibits a cardio protective effect and prevents oxygen free radical induced release of nor-adrenaline, a powerful vasoconstrictor.[7,8]
The diagnosis of AMI is made from symptoms and signs of patients at presentation, along with supportive ECG findings and raised creatine kinase (total) and creatine kinase –MB enzyme levels in blood. However, electrocardiogram (ECG) and/or creatine kinase does not co-relates with production of free radicals in the body. Thus, in this research we were concentrated on serum ceruloplasmin and Xanthine oxidase , as they are related with production of free radicals in the body. This study was an attempt to look for the diagnostic and prognostic importance of serum ceruloplasmin and xanthine oxidase in patients of AMI.

2 Materials and methods

5 ml of venous blood were collected in a plain bulb from 30 patients of acute myocardial infarction for the study from various hospitals in the solapur city. The sample were collected after arrival of patient in hospital (0 hr). Then, samples were collected at time 3 hrs, 6 hrs, 12 hrs, 24 hrs, 48 hrs and 96 hrs from admission of patient. The blood samples for the controls (n = 30) were collected from the volunteers. The obtained blood was centrifuged at 3000 rpm for 15 minutes. The serum were collected and used for assay. As erythrocytes contains high amount of lactate dehydrogenase (LDH), hemolysis should be avoided. The biochemical parameters and methods to be used for their estimations were as follows:
Xanthine oxidase: Assay of xanthine oxidase was carried out essentially according to the method described by Roussos. 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 $CuSO_4$, 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 . Change in absorbance was recorded at 290 nm at 15 seconds interval for one minute. Suitable control was run simultaneously. One unit of activity has been defined as change in absorbance at 290 nm in 1 minute by 1 ml enzyme preparation. [9]
Ceruloplasmin : Ceruloplasmin was determined using its copper oxidase activity by method of Ravin. In this method, action of ceruloplasmin on p-phenylenediamine is used to measure the amount of ceruloplasmin present in the serum. Dark lavender color was read at 530 nm using control tube as blank. Concentration of ceruloplasmin in mg/dl is absorbance X 87.5. [10]

3 Result and discussion

Acute myocardial infarction (AMI) is defined as a part of acute coronary syndrome characterized by a typical clinical syndrome consisting of chest pain, dyspnoea with rise and fall in troponin or creatine kinase – MB to values greater than 99% of a normal reference population. Oxidative modification of low density lipoprotein (LDL) by lipid peroxidation leads to enhanced uptake of LDL by macrophages and cellular accumulation of cholesterol in the arterial walls, leading to atherosclerosis. Occlusion of coronary artery deprives the myocardium of oxygen, causing reduced fatty acid utilization, increased lactate, and reduction in pH with free radical formation which damage the myocardium further. [11]
Xanthine oxidase is an important source of free radical generation. During ischemic conditions, the adenosine nucleotide pool is degraded to hypoxanthine and xanthine, along with conversion of xanthine dehydrogenase to xanthine oxidase. Xanthine oxidase acts on xanthine and hypoxanthine with the resultant production of oxygen free radicals. Thus, xanthine oxidase activity is found to be increased in acute myocardial infarction. [12,13]
Increased plasma ceruloplasmin levels are associated with the generation of oxidation products, i.e., $\bullet O^{2-}$ and H_2O_2. Oxidation of ferrous ion leads to superoxide ion and leads to peroxidative damage. Ceruloplasmin – due to its ferroxidase activity can catalyze the oxidation of Fe^{2+} with concomitant production of H_2O from H_2O_2 and acts as an acute phase reactant. Antioxidants play an important role in preventing free radical damage. Ceruloplasmin is an important extracellular antioxidant. Ceruloplasmin being an acute phase reactant protein, its level rises immediately after cellular damage in AMI. Ceruloplasmin acts as an antioxidant through ferroxidase activity, and it also scavenges superoxide anion radical($\bullet O^{2-}$). [14]
According to our study, it is found that activity of xanthine oxidase is significantly increases ($p < 0.01$) in patients suffering from acute myocardial infarction. In control subject, no significant change is found in activity of xanthine oxidase when blood was drawn with time intervals. But in case of patients suffering from acute myocardial infarction, there is found a significant change. The xanthine oxidase activity in AMI patients increases significantly ($p < 0.01$) upto 12 hours and then slowly decreases. The maximum activity is found after 12 hours of acute myocardial infarction. (Table 1)

Further, serum ceruloplasmin level is not altered with time intervals in control subjects. However in case of patients suffering from acute myocardial infarction, the ceruloplasmin level in the serum increases significantly ($p < 0.01$) upto 48 hours and then slowly decreases. (Table 2) It was believed that at least one month is required to decrease the ceruloplasmin level in the serum to normal.

Table 1: Showing comparative values of xanthine oxidase (XO) activities (U/L) in control and test (AMI patients)

Time	Controls	AMI Patients	p value as compared to controls at respective time
	XO activity in U/L	XO activity in U/L	
O hrs	5.13 ± 0.76	18.26 ± 1.35	$p < 0.01$
3 hrs	5.11 ± 0.63	22.45 ± 1.42	$p < 0.01$
6 hrs	5.10 ± 0.43	25.68 ± 1.13	$p < 0.01$
12 hrs	5.14 ± 0.77	28.09 ± 1.04	$p < 0.01$
24 hrs	5.13 ± 0.92	27.85 ± 0.99	$p < 0.01$
48 hrs	5.12 ± 0.69	26.63 ± 0.78	$p < 0.01$
96 hrs	5.13 ± 0.46	22.87 ± 0.62	$p < 0.01$

Table 2: Showing comparative values of serum ceruloplasmin levels (mg/dl) in control and test (AMI patients)

Time	Controls	AMI Patients	p value as compared to controls at respective time
	Ceruloplasmin in mg/dl	Ceruloplasmin in mg/dl	
O hrs	45.62 ± 8.33	82.01 ± 21.59	$p < 0.01$
3 hrs	46.02 ± 8.25	85.26 ± 23.57	$p < 0.01$
6 hrs	45.19 ± 8.03	89.37 ± 19.17	$p < 0.01$
12 hrs	45.56 ± 8.28	91.63 ± 13.54	$p < 0.01$
24 hrs	44.98 ± 9.68	95.26 ± 14.69	$p < 0.01$
48 hrs	46.31 ± 7.21	98.76 ± 20.98	$p < 0.01$
96 hrs	45.65 ± 6.89	81.29 ± 23.87	$p < 0.01$

Thus, we found xanthine oxidase and ceruloplasmin have no specific co-relation between them. But, still it is found that xanthine oxidase activity reaches to peak more early as compared to ceruloplasmin. Thus, xanthine oxidase is more potent in diagnosis and prognosis of acute myocardial infarction and can be utilized as a marker of oxidative stress developed in acute myocardial infarction.

4 Conclusion

The diagnosis of AMI is made from symptoms and signs of patients at presentation, along with supportive ECG findings and raised CK (total) and CK –MB enzyme levels in blood. However, ECG and/or CK does not co-relates with production of free radicals in the body. Thus, in this research we were concentrated on serum ceruloplasmin and Xanthine oxidase , as they are related with production of free radicals in the body. From the study, it is found that xanthine oxidase activity reaches to peak more early as compared to ceruloplasmin. Thus, xanthine oxidase is more potent in diagnosis and prognosis of acute myocardial infarction and can be utilized as a marker of oxidative stress developed in acute myocardial infarction.

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References

[1] P.K. Nigam. Biochemical markers of myocardial injury. Indian Journal of Clinical Biochemistry, 2007,22 (1): 10-17.
[2] Muhammad Shabbir, Azhar M Kayani, Omarah Qureshi, Muhammad Mujahid Mughal. Predictors of fatal outcome in acute myocardial Infarction. J Ayub Med Coll Abbottabad, 2008, 20(3): 14-7.
[3] Rashmi Raghuvanshi, Aiki Kaul, Pushpa Bhakuni et.al. Xanthine Oxidase As A Marker Of Myocardial Infarction. Indian Journal of Clinical Biochemistry, 2007, 22 (2): 90-92.
[4] Cousins RJ. Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. Physiol Rev 1985; 65 (2): 238-309.

[5] Osaki S, Johnson DA, Frieden E. The possible significance of the ferrous oxidase activity of ceruloplasmin in normal human serum. J Biol Chem 1966; 241: 2746-51.
[6] Suciu A, Chirulescu Z, Zeana C, Pirvulescu R. Study of serum ceruloplasmin and of the copper/zinc ratio in cardiovascular diseases. Rom J Intern Med 1992; 30 (3): 193-200.
[7] Mateescu MA, Chahine R, Roger S et al. Protection of myocardial tissue against deleterious effects of oxygen free radicals by ceruloplasmin. Arzneimittelforschung 1995; 45(4): 476-80.
[8] Zakirova AM. The clinico-hemodynamic effects of the antioxidant ceruloplasmin in IHD patients. Ter Arkh 1995; 67(4): 33-5.
[9] Roussos GG. Xanthine oxidase from bovine small intestine. In: Grossman L, Moldave K, editors. New York (NY): Academic Press; 1967. p. 5-16. (Methods in Enzymology; vol XII A).
[10] Ravin HA. An improved colorimetric enzymatic assay of ceruloplasmin. J Lab Clin Med 1961; 58(1): 161-8.
[11] Alpert JS , Thygesen K, Antman E etal. Myocardial infarction redefined – a consensus document of Joint European Society of Cardiology/American College of Cardiology Commitee for redefinition of myocardial infarction. Jam Coll Cardiol 2000; 36: 959-69.
[12] Bhakuni P, Chandra M, Misra MK. Oxidative stress parameters in erythrocytes of post reperfused patients with myocardial infarction. J Enz Inhib Med Chem 2005; 20:377-81.
[13] Raghuvanshi R, Chandra M, Misra PC, Misra MK. Effect of vitamin E on the platelet xanthine oxidase and lipid peroxidation in the patients of myocardial infarction. Ind J Clin Biochem 2005;20:26-9.
[14] Fridovich I. Oxygen free radicals and tissue damage: Chairman's introduction. Ciba Found Symp 1978; 65: 1-4.