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Evaluation of Antioxidant Status in Myocardial Infarction in Diabetic and Non-diabetic Subjects : A Comparative Study

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Abstract Cellular oxidative stress is the leading cause of the worst outcome of myocardial infarction (MI) in diabetes. Diabetes Mellitus is one of the important risk factors for heart pathology, because of increased production of Reactive oxygen Species(ROS).The present study was designed to find out the antioxidant status in diabetic and non-diabetic MI Patients.100 MI patients were grouped into 50 Diabetic MI(group 2) and 50 Non diabetic MI(group 3) and was compared with age matched 50 controls (group1). Malondialdehyde(MDA) or Thiobarbituric acid reactive substances (TBARs), antioxidant enzymes Superoxide Dismutase(SOD) &Catalase were measured in erythrocytes and Vitamin C in plasma was measured .The result showed that there is a significant decrease in the antioxidant status in diabetic and non-diabetic MI patients and a simultaneous significant increase in the lipid peroxidation .Thus there is an imbalance between oxidant and antioxidant molecules in MI patients, and magnitude of imbalance is greater in diabetic MI patients, possibly because of greater oxidative stress in diabetic patients. Potentially antioxidant therapy may play a critical role in reducing morbidity and mortality in MI.

Keywords Myocardial Infarction, Antioxidants , SOD, Catalase, Vitamin C, TBARs , Oxidative Stress, Reactive Oxygen Species

1. Introduction

Acute myocardial infarction (MI) is defined as death or necrosis of myocardial cells. It is a diagnosis at the end of the spectrum of myocardial ischemia or acute coronary syndromes. [1]. MI can occur at any age, but its incidence rises with age. The actual incidence is dependent upon predisposing risk factors for atherosclerosis [2]. Most MIs are caused by a disruption in the vascular endothelium associated with an unstable atherosclerotic plaque that stimulates the formation of an intracoronary thrombus, which results in coronary artery blood flow occlusion.[3] Six primary risk factors have been identified with the development of atherosclerotic coronary artery disease and MI: hyperlipidemia, diabetes mellitus, hypertension, smoking, male gender, and family history of atherosclerotic arterial disease. The presence of any risk factor is associated with doubling the relative risk of developing atherosclerotic coronary artery disease[1,4-5] Diabetes Mellitus Patients have a substantially greater risk of atherosclerotic vascular disease in the heart as well as in other areas of the vasculature. Diabetes increases the risk of MI because it increases the rate of atherosclerotic progression and adversely affects blood cholesterol levels. This accelerated form of atherosclerosis occurs regardless of whether a patient has insulin-dependent or noninsulin-dependent diabetes. Also Diabetes mellitus act as a source of vascular dysfunction through the formation of REACTIVE OXYGEN SPECIES and increase the risk of Oxidative stress is caused by an imbalance between the production of reactive oxygen Species and a biological system's ability to readily detoxify the reactive Oxygen Species or easily repair the resulting damage. Reactive oxygen species (ROS) include oxygen ions, free radicals and peroxides both inorganic and organic. They are generally very small molecules and are highly reactive due to the presence of unpaired valence shell electrons. Cells are normally able to defend themselves against ROS damage through the use of antioxidants. An Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase(SOD) and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes causes oxidative stress and may damage or kill cells.

In this study we have included antioxidants like SOD, catalase and Vitamin C.As diabetes mellitus itself increases the risk of oxidative stress the use of these antioxidant will be much more when compared to a normal so obviously their level in plasma should decrease due to increase oxidative

stress. Since the antioxidant level decreases the oxidation increases in such patients .Lipid under such condition undergo peroxidation by ROS to for TBARs or lipid peroxidation Product .As the oxidative stress increases the TBARS level increases ,so it can be considered as marker of extend of Oxidative stress. MI or myocardial infarction is one of such diseases where lipid peroxidation is an inevitable phenomenon.

Leoper [6] in their study showed that there is increase in TBARs level with MI and simultaneous decrease in SOD level. also Yet another similar study that shows an increasing risk of oxidation in MI by Senthil[7] showing that cardiogenic shocks is associated with increase in lipid peroxidation and simultaneous decrease in the antioxidant status. In his study SOD, Catalase, Glutathione peroxidase, Vitamin C, Vitamin E and beta-carotene were low in patients with MI due to increase utilization to scavenge the lipid peroxides. Diabetes mellitus is a chronic disease in its own right and is also regarded as a cardiovascular risk factor as well as a cardiovascular disease, due to its ability to progress to a stage of cardiovascular co-morbidity. The pathophysiology of cardiovascular complications in diabetes is reported to involve hyperglycaemia-induced oxidative stress .Effects of hyperglycemia (both diabetes and experimental galactosemia) on cardiac metabolism have been determined [8]. In addition, the effect of supplemental antioxidants on these hyperglycemia-induced abnormalities of cardiac metabolism has been investigated. Diabetes or experimental galactosemia of 2 months duration in rats significantly increased oxidative stress in myocardium, as demonstrated by elevation of thiobarbituric acid reactive substances (TBARs) and lipid fluorescent products in left ventricle. Administration of supplemental antioxidants containing a mixture of ascorbic acid, Trolox; alpha-tocopherol acetate, N-acetyl cysteine, beta-carotene, and selenium prevented both the diabetes-induced and galactosemia-induced elevation of oxidative stress.

Di Filippo [9] did useful work on oxidative stress as the leading cause of acute myocardial infarction in diabetics. Hyperglycemia is viewed in this article as the primary mediator of a cascade of heart damaging events, starting from ROS formation and leading to myocardial ischemia, inflammation and death of myocytes.

The risk of hyperglycemia as a precipitating factor in acute coronary complications has also been implicated by Haidara [10].Hyperglycemia and protein glycation, increased inflammation, a prothrombotic state and endothelial dysfunction have all been implicated as possible mechanisms for such complications.

As most of the studies show that the diabetic MI has an increase oxidative stress, in our study we are planning to compare the oxidative stress in diabetic MI and Non diabetic MI. As in diabetic MI the extent of risk for oxidative stress is more when compared with Non diabetic MI so probably the antioxidant level will be much less in diabetic MI and Lipid peroxidation will be more so level of TBARs will be much high when compared to Non diabetic MI.

2. Materials and Methods

The study was carried out at Amrita Institute of Medical Sciences, Cochin. Blood sample for the study was collected from the clinical biochemistry laboratory attached to the hospital .Study consisted of 50 healthy age matched controls(group 1) and 100 myocardial infarction(Based on Troponin I, CK-MB,ECG and History) patient samples further divided into diabetic,(based on their HbA1C ,FBS, Patient history-group 2) and non-diabetic(group 3).The age group was between 40-75.Both male and female patients were involved

Malondialdehyde, antioxidant enzymes (SOD &Catalase) were measured in erythrocytes and Vitamin C in plasma was measured in plasma

Sample collection

The blood samples were collected in Heparinized vacuettainers(2 ml each). The plasma from the blood sample tube was transferred for performing the Vitamin C estimation and blood cells were used for hemolysate preparation for the rest of the estimations.(SOD,CAT,TBARs)

Plasma separation

The blood sample is centrifuged at an RPM of 3000 for 5mts.The plasma is separated and transferred to subsequent labelled vials.

Hemolysate preparation

The blood cells, after separation of the plasma, was washed with 3 ml of cold normal saline(0.9% NaCl), centrifuged and supernatant discarded. This was repeated for 2 more times. The cells were made up to 30 ml with ice cold distilled water. Refrigerated.

1. Estimation of vitamin C [29]

Procedure

0.5ml of plasma was added to 2 ml of freshly prepared 6g/dl metaphosphoric acid, mixed well and centrifuged for 15min.1.2ml of the clear supernatant was mixed with 0.4ml of DTCs reagent and incubated at 37°C for 3 hours. The tube was then chilled for 10 min in ice bath and 2ml cold H_2SO_4 (12 M) was added. Absorbance was read at 520nm against blank

2. Estimation of catalase [11]

Procedure

The rate of decomposition of H_2O_2 (2 microlitre,30%)in 0.05M phosphate buffer1ml,pH 7at 240 nm after addition of heamolysate was noted. The specific gravity was calculated assuming molar extinction coeffiecient 40 $M^{-1}cm^{-1}$ for H_2O_2 at 240 nm.

3. Estimation of superoxide dismutase(SOD) [12]

Procedure

The superoxide dismutase activity was measured by the inhibition of autooxidation of 0.2mM pyrogallol (air equilibriated)in 50 mM Tris-HCl buffer (pH 8.2) containing 1 mM DTPA .the rate of autooxidation was monitered at 420 nm. Percentage inhibition of rate of autooxidation of pyrogallol was initiated byaddition of heamolysate.

4. Estimation of Thiobarbituric acid reactive substances(TBARs)or Malondialdehyde(MDA)[13]

Procedure

To 1ml of sample ,2ml of 10% trichloroacetic acid (TCA) was added, followed by 4ml of 0.67% thiobarbituria acid(TBA).The systems were heated in a water bath at 100 °C for 15 minutes. After cooling and centrifugation, the absorbance of the supernatant was read at 535 nm. reagent blank was prepared using water instead of sample. the extend of lipid peroxidation was calculated using molar extinction coefficeint 1.56X 10^5 $mol^{-1}cm^{-1}$ for MDA

Statistical analysis

All data were analyzed using the statistical package SPSS (version 11.0,SPSS) . Mean (M) and standard deviations (SD) were calculated. The sources of variation for multiple comparisons were assessed by analysis of variance(ANOVA), followed by Post Hoc test with Bonferroni's multiple comparisons test for SOD , CAT , Vitamin C but one variable ,TBARs was assessed by Kruskal – Wallis test, followed by Mann-Whitney U test. The differences were considered significant at $P<0.05$.for comparisons between groups 1 and 2, 1 and 3 and group 2 and 3.

3. Results

1. Vitamin C

50 diabetic (group 2) and 50 non diabetic (group3) myocardial infarction patients were included in this age matched control study. Vitamin C levels were found to be decreased in the diabetic group when compared to the non-diabetic. Both the patient groups showed significant decrease in the vitamin C levels when compared to the control(Figure :1)

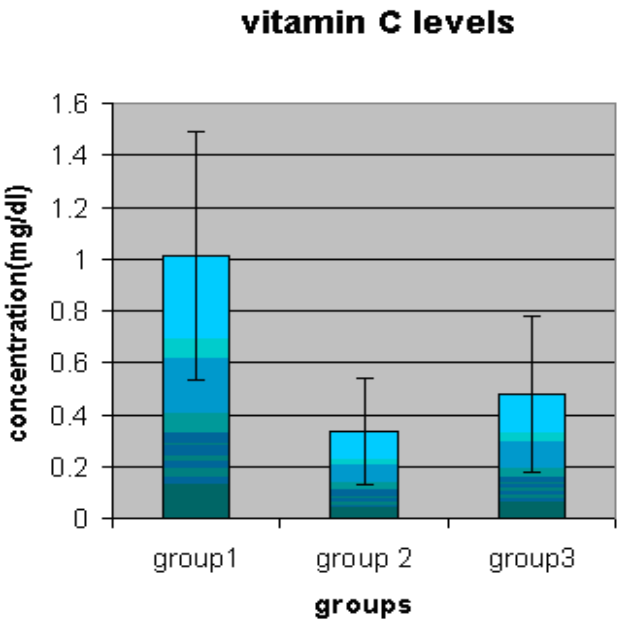

Figure 1

Table 1. Effect on antioxidant status in MI induced oxidative stress between diabetic and non-diabetic when compared with control

Variable	Group 1		Group 2		Group 2	
	M	SD	M	SD	M	SD
Vitamin C(mg/dl)	1.98	0.48	0.34	0.2	0.48	0.3
Superoxide dismutase (SOD) (U/gm Hb)	1526	1001.97	1114.12	892.99	1377	836.39
Catalase (CAT mmol H_2O_2 decomposed /mg protein/.mt)	0.01836	0.01293	0.00332	0.002596	0.00836	0.006785
TBARs (nmols/ ml)	2.6379	0.81035	5.0483	0.68515	4.5312	1.2888

Table 2. P values of different group

Variable	Comparable group and P values		
	Group 1 & 2	Group 1 & 3	Group 2 &3
Vitamin C	0.001	0.001	0.001
Catalase	0.001	0.001	0.001
TBARs	0.001	0.001	0.001

P values of SOD did not show any significance

2. SOD (Super Oxide Dismutase)

In the age matched control study of 50 diabetic (group 2) and 50 non-diabetic (group 3) myocardial infarction patients, SOD levels were found to be slightly decreased in the diabetic group when compared to the non-diabetic. Both the patient groups showed decrease in the Super oxide dismutase levels when compared to the controls. (Group 1) but the values didn't so any statistical significance (Figure :2)

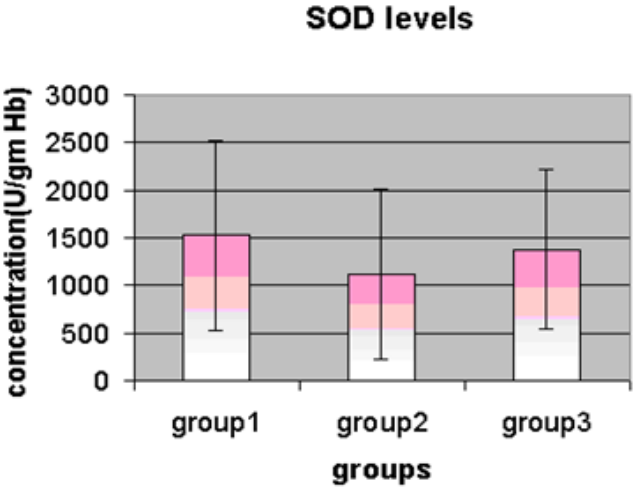

Figure 2

3. Catalase

Catalase levels showed significant changes from group to group. There was a decrease observed in the diabetic group compared to the non-diabetic group. And there was an overall significant decrease observed in the patient group compared to the controls.(Figure:3)

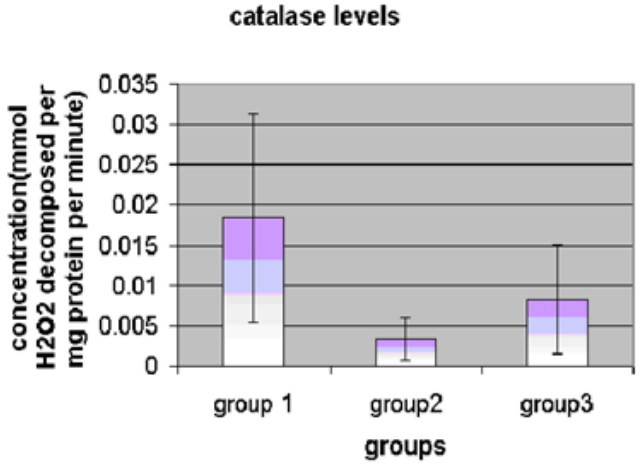

Figure 3

4. TBARs

In the present age matched control study done, the levels of thiobarbituric acid reactive substances or malondialdehyde were found to be significantly increased in patient group than the control group. The oxidative stress associated with the process of inflammation in myocardial infarction causes this significant increase.. The comparison between the patient groups 2 and 3 reveals a significant increase in the diabetic group due to the still more oxidative stress encountered as a result of hyperglycemia.(Figure :4)

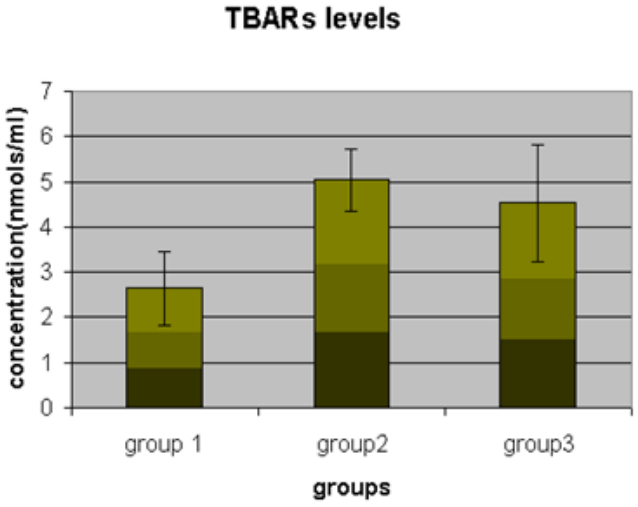

Figure 4

4. Discussion

Main cause for MI is atherosclerosis . Atherosclerosis is a chronic inflammatory condition that is gradually getting converted to an acute clinical event like MI. So inflammation occupies an important part in atherosclerosis, even though inflammation smoulders for decades before resulting in clinical events. Free radicals play an important role in the pathogenesis of tissue damage and inflammation. Antioxidants thus plays a crucial role in preventing free radical induced inflammation and tissue damage

In the present study, it has been observed that there is a significant decrease in the antioxidant status in diabetic and non-diabetic MI patients and a simultaneous significant increase in the lipid per oxidation product when compared with the controls. Oxidative stress is known to accompany most of the chronic and inflammatory disease states including MI.. The condition of the oxidative stress involves an increase in the production of free radicals and a compensatory decrease in the level of antioxidants. There are indications that acute myocardial infarction is a state of enhanced free radical activity, which causes endothelial damage [14]

In Diabetes-associated MI, oxidative stress is a consequence of increased production of free radicals and reduced capacity of anti-oxidative defense along with hyperglycemia which is a major factor in the pathogenesis of atherosclerosis in diabetes, leading to cardiovascular complications. There is reduced anti oxidative defense in type 2 diabetics with prominent cardiovascular complications. This correlates with glucose concentrations and duration of diabetes and cardiovascular complications [15]. In this study erythrocyte malondialdehyde (MDA) was

measured as markers of oxidative stress and activity of erythrocyte super oxide dismutase (SOD) catalase (CAT) and plasma vitamin C were taken as markers of oxidative defense system.

In the present study a comparison of vitamin C level between different groups was performed and the results were found to be statistically significant. There was a significant decrease in vitamin C level between group 1 and 2, group 1 and 3 and group 2 and 3.This indicates a severe damage in the antioxidant system, which is unable to combat the oxidative stress and inflammation in MI. This is in accordance with studies of Singh [16] who demonstrated that there was a significant drop in vitamins C, whereas lipid peroxides were significantly higher in MI patients, compared with controls. Also studies of Rudolf [17] showed that Vitamin C deficiency, is a risk factor for coronary heart disease. Plasma levels of Vitamin C studied was low, remained low or decreased transiently in patients with MI according to the study of Labadarios [18]

Catalase catalyses the dismutation of hydrogen peroxide. Catalase activity decreases with the increase in oxidative stress presumably because of generation of superoxide or increased activity of lipid per oxidation or combination of both [19]. Our data suggested a significant decrease in the erythrocyte CAT activity in MI patients when compared with controls, and within the patients diabetic MI showed still more decrease in CAT activity than that of the non –diabetic. Similar to our observation Abou-Seif f [20] suggested a significant decrease in catalase levels as evaluated in estimation of some biochemical changes in diabetic MI patients. As per the study of Kesavulu [21] there was significant decrease in erythrocyte antioxidant enzyme Catalase(CAT) in diabetic patients and non-diabetic patients with MI. . Our results also showed that, CAT activities were decreased in DM and MI in comparison to the corresponding activities of the control subjects.

The study suggested that there was no significant decrease in the SOD activity between any groups compared. This slight decrease in SOD activity may probably be a response towards oxidative stress [22]. This was similar to the study conducted by Muzákova[23] which showed erythrocyte superoxide dismutase (SOD) did not exhibit significant changes during the interval studied in patients with MI, probably due to the stability of erythrocyte metabolism. Also study conducted by Kesavulu [21,24] showed that no significant change was observed in SOD activity in both groups of diabetic patients and non-diabetic patients with MI compared to those in controls. In contrast to our observations, in a study of ischemic heart conditions, Pandey[25] reported a decrease in the activities of free radical scavenging enzymes like superoxide dismutase. In another context, the work done by Senthil [7] on cardiogenic shock complicating to MI, showed greater than normal lipid peroxidation with an imbalance in antioxidants' status. Their results indicated low activities of SOD in the circulation of patients, which may be due to increased utilization to scavenge lipid peroxides.

A significant rise in the MDA level in our patients is indicative of elevated oxidative stress in MI patients. The result showed that the rise was more in diabetic MI. This is similar to work of Dubois Rande[26] and Mc Murray [27] who showed a decrease in antioxidant enzyme activities and increase in lipid per oxidation products (MDA or TBARS) in patients with unstable angina and chronic heart failure. Study by Kesavulu[21] showed diabetes patients with MI had higher levels of TBARS compared to those non- diabetic MI . The result suggest that Lipids peroxides are formed by free radicals and play an important role in the development of atheromatous vascular diseases .It was found that the plasma levels of MDA was significantly elevated in the patients of acute myocardial infarction compared to the control group thereby indicating that oxygen free radicals cause endothelial damage in them. Lipid peroxidation mediated by free radicals is considered to be a primary mechanism of cell membrane destruction and cell damage [28]

Thus the present study clearly shows increased inflammation and oxidative stress in patients with acute myocardial infarction. Depression of antioxidant system in these patients can be the most plausible reason for these observations. Hyperglycemia increased the risk of oxidative stress in MI. Antioxidants play a potential role in preventing atherosclerosis, so that it help in inhibiting some major complication of atherosclerosis such as MI. This study showed that the diabetic subjects with vascular complications have a defective cellular antioxidant level so that they have less antioxidant defence against the oxidative stress caused by hyperglycemia. So the observations from our study suggest the concept that antioxidant therapy may be great benefit in these subjects in reducing the damage caused by free radicals and delay the onset of complications of MI

Limitation of the study is that it is a small study including three antioxidants and one lipid per oxidation product. The number of parameters to evaluate the oxidative stress was less when compared to similar other studies and also one of the parameter SOD didn't show significance. But such insignificance in case of SOD was there for other studies also. But even after having such limitation the study was able to show an increased oxidative stress in MI patients and that too more in diabetic subjects than non – diabetic ones

5. Conclusions

Oxidative stress plays an important role in the pathological processes ongoing in MI. Excessive oxidative stress has adverse effects on myocardial cells survival and function, and accelerates complications in target organ and tissue. Free radical mediated damage is aggravated by a reduction in cytoprotective enzymes and other antioxidants .Our study indicates an imbalance between oxidant and antioxidant molecules in MI patients, and magnitude of imbalance is greater in diabetic MI patients, possibly because of greater oxidative stress in diabetic patients.. Depression of antioxidant system in these patients

confirms this conclusion. So a antioxidant therapeutic intervention may reduce the oxidative stress and inflammation and can delay the onset of complication of MI in diabetic patients. There is a need for continued investigation in this field. So that antioxidants can be used not only for treatment but also for the prevention of complications caused by the oxidative stress in hyperglycemic patients with vascular complications

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