A STUDY OF NON-ENZYMATIC ANTIOXIDANT STATUS IN ACUTE MYOCARDIAL INFARCTION PATIENTS WITH METABOLIC SYNDROME ABNORMALITIES

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ABSTRACT

BACKGROUND

The present study was conducted to know the changes in markers of non-enzymatic antioxidant capacity in Acute Myocardial Infarction (AMI) patients with metabolic syndrome abnormalities.

MATERIALS AND METHODS

The study included 50 diagnosed cases of AMI with metabolic syndrome abnormalities and 50 healthy controls. Fasting blood samples were collected and plasma sugar, serum triglycerides, serum HDL-C and non-enzymatic antioxidants -serum vitamin E, vitamin C, blood pressure and waist-circumference were estimated in all the subjects.

Statistical Analysis-It was performed using the unpaired student 't' test to evaluate the significance of differences between the mean values. P-values less than 0.001 were considered as highly significant.

RESULTS

A highly significant increase was observed in levels of plasma sugar and triglycerides, blood pressure and waist-circumference whereas a highly significant decrease levels were observed in serum HDL-C, vitamin E and vitamin C in patients as compared with control group.

CONCLUSION

Oxidative stress is a potential contributor to AMI besides metabolic syndrome. On the basis of our results, it may be concluded that the non-enzymatic antioxidants -vitamin E and vitamin C decreased in the patients.

KEYWORDS

Acute Myocardial Infarction, Metabolic Syndrome, Oxidative Stress, Non-enzymatic Antioxidants, Vitamin C, Vitamin E.

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BACKGROUND

In 2001, the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III provided a new definition for metabolic syndrome.^[1] according to which a person must have three or more of the following five abnormalities:

1. Abdominal adiposity (central obesity).
2. Elevated fasting glucose (insulin resistance).
3. Hypertension.
4. Hypertriglyceridaemia.
5. Low high-density lipoprotein cholesterol.

Metabolic syndrome is also associated with an increased risk of cardiovascular disease.^[2] More advanced vascular damage has been associated with the presence of metabolic syndrome in patients with manifest vascular disease, which may worsen the prognosis.^[3] The metabolic syndrome (MS)

has been shown to be a powerful and potentially modifiable risk factor for coronary artery disease.^[4-6]

Myocardial infarction (MI) or acute myocardial infarction, commonly known as a heart attack, occurs when blood flow stops to a part of the heart causing damage to the heart muscle. Myocardial infarction occurs when an atherosclerotic plaque slowly builds up in the inner lining of a coronary artery and then suddenly ruptures, causing catastrophic thrombus formation, totally occluding the coronary artery and preventing blood flow downstream. When a severe enough plaque rupture occurs in the coronary arteries, it leads to MI (necrosis of downstream myocardium).^[7,8]

Coronary artery disease is the single most important disease entity in terms of both mortality and morbidity in the entire world population.

Risk factors of AMI include diabetes mellitus (Type 1 or 2),^[9] high blood pressure,^[10] dyslipidaemia/high levels of blood cholesterol (abnormal levels of lipoproteins in the blood), particularly high low-density lipoprotein, low high-density lipoprotein, high triglycerides,^[10] and obesity^[11] (defined by a body mass index of more than 30 kg/m² or alternatively by waist circumference or waist-hip ratio) and lack of exercise among others.^[12,13]

Free radicals play an important role in the pathogenesis of tissue damage in many clinical disorders including atherosclerosis. Normally, these free radicals are effectively kept in check by the various levels of antioxidant defences.

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Imbalance of this reaction either due to excess free radical formation or insufficient removal by antioxidants leads to oxidative stress (OS).^[14] Oxidative stress plays a significant role in the pathogenesis of coronary atherosclerosis and its complications. Oxidative stress, characterised by increased generation of oxygen free radicals results in generation of lipid peroxides like malondialdehyde (MDA). Enzymatic and non-enzymatic antioxidants including superoxide dismutase, catalase, and glutathione peroxidase, vitamin E or ascorbic acid are part of intracellular protection mechanisms to overcome oxidative stress.

Abnormalities lead to the increased OS due to excess free radical activity.^[15] and these ROS can stimulate oxidation of low density lipoprotein (LDL), cholesterol, cholesterol derived species, and protein modifications which can lead to foam cell formation and atherosclerotic plaques.^[16]

An association between increased oxidative stress and diabetes, hypertension, cigarette smoking and dyslipidaemia, which are well known risk factors for atherosclerosis is seen. The aim of this study was therefore to measure changes in markers of non-enzymatic antioxidant capacity in AMI patients with metabolic syndrome abnormalities.

MATERIALS AND METHODS

The study was conducted in the Department of Biochemistry, GSL Medical College and General Hospital, Rajahmundry, AP, India. The study was carried out from March 2015 to June 2016.

The current study comprised of 50 patients with acute myocardial infarction with metabolic syndrome abnormalities and along with equal number of age and gender matched healthy controls. 31–70 years age group was included in the study with their consent.

Inclusion Criteria of Patients

Patients 31-70 years of age with freshly diagnosed cases of AMI with metabolic syndrome abnormalities and free from any kidney or liver diseases.

Exclusion Criteria of Patients

Patients below 31 years and above 70 years of age with any kidney or liver diseases, malignancy, severe medical or psychiatric illness, renal failure, alcoholics, subjects on lipid lowering drugs, acutely ill patients, subjects with previous history of heart disease and patients who were taking antioxidant vitamins were excluded from the study.

Inclusion Criteria of Controls

Healthy controls aged between 31 to 70 years.

Exclusion Criteria of Controls

Hypertensive, smoker, diabetic, persons with obesity and alcoholics.

Patients were diagnosed as having the metabolic syndrome if they had any three or more of the following five components: abdominal obesity (waist circumference >40 inches in men and >35 inches in women), high triglyceride levels (≥ 150 mg/dL), low HDL-C levels (<40 mg/dL in men and <50 mg/dL in women), elevated fasting glucose levels (≥ 110 mg/dL), and high blood pressure (treated hypertension and

systolic blood pressure/diastolic blood pressure $\geq 130/85$ mm Hg).

Diagnosis of MI was made based on clinical history, echocardiogram and in cases with presence of serial increases in serum biochemical markers of cardiac necrosis including blood tests, troponin and creatine kinase MB (cardiac enzymes), associated with typical electrocardiographic changes,^[17] coronary angiography, history of hypertension, smoking, diabetes mellitus and physical activity were recorded.

Sample Collection

8 mL fasting blood samples were collected. 4 mL blood was collected in EDTA vials (for plasma) for estimation of plasma glucose level and Vitamin C. 4 mL in plain tubes (for serum) for estimation of triglycerides, serum HDL and serum and antioxidant (serum Vitamin E) level.

Waist-circumference was measured and blood pressure was recorded in all subjects. Waist circumference was measured midway between the last rib and iliac crest.

Glucose was determined by using the Glucose Oxidase/Peroxidase (GOD/POD) method. Triglyceride was determined by using enzymatic method (Glycerol Phosphate Oxidase). HDL-C was determined by using Precipitation-Enzymatic method (Cholesterol Oxidase Peroxidase).

Determination of plasma ascorbate was done by 2,6-Dichlorophenolindophenol titration method as given in "Varley's Practical Clinical Biochemistry".^[18] Vitamin E was done by method Baker H, Frank O as given in "Varley's Practical Clinical Biochemistry".^[19]

Statistical Analysis

It was performed using the unpaired student 't' test to evaluate the significance of differences between the mean values. Results were expressed as mean $\pm$ SD. P-values less than 0.001 were considered as highly significant.

RESULTS

50 patients with a confirmed diagnosis of AMI with metabolic syndrome abnormalities and with equal number of age and gender matched healthy controls were enrolled with their consent.

Table 1 contains the % of males, females, increased waist circumference, diabetes, hypertension, increased levels of serum triglycerides, and decreased levels of serum HDL-C in patients. Table 2 contains the mean, SD and P-values of waist circumference, FPG, systolic blood pressure, diastolic blood pressure, TAG, and HDL-C of AMI and controls. Table 3 contains the mean, SD and P-value of Vitamin C and Vitamin E of AMI and controls.

Characteristics	Number (%)
Male	33(66%)
Female	17(34%)
Increased waist circumference	30(60%)
Diabetes	36(72%)
Hypertension	38(76%)
Hypertriglyceridemia	33(66%)
Decreased HDL-C level	31(62%)
Table 1. Number (%) of Characteristics of Study Group AMI (n=50)	

Parameters	Control (n=50) Mean $\pm$ SD	AMI (n=50) Mean $\pm$ SD	P- Value
Waist circumference (inches)	32.6 $\pm$ 4.1	43.2 $\pm$ 6.1	<0.001
Fasting plasma glucose (mg/dL)	78.4 $\pm$ 8.8	142.3 $\pm$ 36.3	<0.001
Systolic blood pressure(mmHg)	116.6 $\pm$ 6.4	150.1 $\pm$ 22.3	<0.001
Diastolic blood pressure(mmHg)	74.5 $\pm$ 5.8	92.3 $\pm$ 18.4	<0.001
Triglyceride (mg/dL)	103.4 $\pm$ 15.2	182.7 $\pm$ 36.3	<0.001
HDL-C(mg/dL)	48.2 $\pm$ 5.4	35.4 $\pm$ 8.5	<0.001

Table 2. Mean $\pm$ SD of Variables in the Study and Control Groups

Parameters	Control (n=50) Mean $\pm$ SD	AMI (n=50) Mean $\pm$ SD	P-Value
Vitamin C (mg/dL)	1.29 $\pm$ 0.16	0.65 $\pm$ 0.12	<0.001
vitamin E (mg/dL)	1.40 $\pm$ 0.19	0.81 $\pm$ 0.11	<0.001

Table 3. Mean $\pm$ SD of Variables in the Study and Control Groups

Males had higher risk of MI in our study. 33 (66%) were male and 17 (34%) were female patients. 36 patients had diabetes (72%), 38 (76%) had hypertension, 33 (66%) had hypertriglyceridaemia, 30 (60%) had increased waist circumference and 31 (62%) had decreased levels of serum HDL-C.

The mean levels of plasma sugar, serum triglycerides, blood pressure and waist circumference were increased highly significantly in AMI ($p < 0.001$) patients as compared to control group.

The mean levels of serum HDL-C, Vitamin C and Vitamin E were highly significantly decreased in AMI ($p < 0.001$) patients as compared to control group and the mean difference was statistically highly significant.

Graph 1 Show the Number of Characteristics of Study Group

Graph 2

Graph 3

Graph 4

Graph 5

Graph 8

Graph 6

Graph 9

Graphs 8 and 9 Show Means and SD of Vitamin C and Vitamin E of Cases and Control Groups

Graph 7

Graphs 2 to 7 Show Means and SD of Waist Circumference, Fasting Glucose, Systolic BP Diastolic BP, TGL and HDL of Cases and Control Groups

DISCUSSION

A study was carried out in a group consisting of 50 patients with AMI with metabolic syndrome abnormalities, which was compared with 50 healthy controls to evaluate the non-enzymatic antioxidant status in patients.

This is the only study conducted on association and impact of the metabolic syndrome components on AMI cases and the importance of determination of the non-enzymatic antioxidants- Serum vitamin E, vitamin C status in AMI at this hospital and medical institute. Isomaa B et al have also reported in their study that the metabolic syndrome was associated with an increased case fatality rate.^[6] In the present study, there was an enhanced oxidative stress and decreased antioxidant status. Males had higher risk of AMI in our study. 33 (66%) were male and 17 (34%) were female patients.

As per our results, the measurement of waist circumference was highly significantly raised ($p < 0.001$) in the patients of AMI with metabolic syndrome abnormalities than control group. Waist circumference raised in 30 (60%) patients.

Our findings showed highly significantly raised ($p < 0.001$) level of FPG in the patients than controls. Similar findings were made by Buse JB et al^[9] In our study, diabetes was found in 36 (72%) cases. Elevated fasting blood glucose has such a high predictive value in AMI.

Our study revealed that the levels of both systolic blood pressure and diastolic blood pressure were highly significantly raised ($p < 0.001$) in the patients when compared with controls. These were in accordance with earlier findings.^[10] In our findings, 38 (76%) were hypertensive. As per different studies, hypertension was a major risk factor for atherosclerosis,^[20,21,22,] in coronary arteries leading to MI.^[23,24] In our experiment, we observed highly significantly raised ($p < 0.001$) level of serum triglyceride in the cases when compared with controls. The report of Smith SC et al correlated with our findings.^[10] In our study, we observed raised level of serum triglyceride in 33 (66%) patients.

The level of HDL-C was highly significantly decreased ($p < 0.001$) in cases than in controls. Smith SC et al in their study reported the same.^[10] The level of HDL-C was decreased in 31 (62%) patients. Low HDL-C levels have increased rates for heart disease.^[25] High HDL-C levels correlated with better cardiovascular health.^[26]

According to this study, we found that serum vitamin C and vitamin E concentrations deteriorated highly significantly ($p < 0.001$) in patients compared with control group. This was in accordance with earlier findings.^[27]

The prevalence of the metabolic syndrome is high worldwide and increasing day by day due to sedentary lifestyles. Obesity is the cause of 20% of coronary artery disease.^[28] Lack of exercise has been linked to 7–12% of cases.^[28,29]

Free radicals, cytokines, nitric oxide (NO), and antioxidants play a major role in both atherosclerosis and myocardial damage and preservation.^[30] In the present study, it was found that the patients had reduced Vitamin E and ascorbic acid levels. Chandra et al (1994) demonstrated that there were elevated superoxide anion, malonyldialdehyde, and glutathione reductase levels in unstable angina and acute myocardial infarction cases.^[31] Gupta and Chari (2006) measured the levels of pro-oxidants and antioxidants in patients with ischaemic heart disease (IHD) and type II diabetes mellitus. According to Gupta and Chari, significantly increased levels of malondialdehyde and decreased levels of vitamin C were found in IHD compared with control subjects.^[32]

Vitamin E, a potent chain breaking lipid soluble antioxidant, reacts with lipid peroxy radicals eventually terminating the peroxidation chain reaction and thereby reducing oxidative damage. In the present study, serum vitamin E levels were highly significantly decreased in the patients when compared to controls.

Vitamin C represents the major water-soluble antioxidant in the human body. Vitamin C levels are reduced in associated with increased risk of AMI. In our present study the serum vitamin C levels were decreased highly significantly in the patients. It is due to the exhaustion of this antioxidant in the neutralisation of free radicals which are formed in excess during ischaemia.

In the present study, it was observed that non-enzymatic antioxidants were highly significantly decreased in patients as compared to healthy controls. It indicates that the acute

myocardial infarction patients are unable to produce sufficient amounts of antioxidants to cope up with the increased oxidative stress in them.

CONCLUSION

On the basis of our results, it may be concluded that non-enzymatic antioxidants- vitamin E and vitamin C decreased in the patients. Non-enzymatic antioxidants, serum vitamin E, vitamin C have a role in preventing cellular oxidative damage. The study also suggests initiating lifestyle modifications as a preventive measure to reduce the burden of the disease. There are various strategies to prevent the development of metabolic syndrome such as increased physical activity (walking 30 minutes every day),^[33] a healthy and reduced calorie diet.^[34] The present study suggests that apart from symptomatic treatment there is a necessity for supportive antioxidant therapy to decrease the stress and the muscle damage and for an early intervention and better management of the disease.

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