

## Short Communication

# Serum Free Iron as a Risk Factor for Acute Myocardial Infarction

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### ABSTRACT

Elevated serum iron levels have been proposed as a risk factor for coronary artery disease. The study is aimed at investigating the role of serum iron in the pathogenesis of acute myocardial infarction (AMI). The study was performed in 100 Acute Myocardial Infarction (AMI) patients sub-grouped as smokers, hypertensives and diabetics. Twenty-five normal healthy subjects were included in the study as controls. Both the patients and the control subjects were evaluated using clinical history, dietary history and biochemical examinations. Serum malondialdehyde (MDA), which is a measure of the extent of lipid peroxidation, serum iron and total iron

binding capacity (TIBC), were determined by standard methods. We found elevated serum iron in all the three risk groups with acute myocardial infarction and among the groups, the smokers were found to have higher values compared to the other two groups. Hence, it may be used as a marker for the diagnosis of AMI. However, the TIBC values were not altered in any of the three risk groups with AMI. The lipid peroxidation, measured as the endogenous MDA levels were found to be more in smokers when compared to the other two risk groups with AMI.

KEY WORDS: acute myocardial infarction, lipid peroxidation, total iron binding-capacity

### INTRODUCTION

Cardiovascular disease continues to be the principal cause of death in the United States and much of Asia<sup>[1,2]</sup>. The exact reason why South Asians have a higher incidence of coronary artery disease is not yet known. The World Bank estimates that the death rate from coronary heart diseases will increase dramatically, and is expected to contribute to loss of more quality-adjusted life years over the next 20 years. A higher incidence of diabetes, low HDL concentration, high triglycerides levels, high levels of lipoprotein (a) and small dense lipoprotein particles and thinner arterial walls have been proposed as possible contributing factors. Indians have one of the highest incidences of coronary heart disease and conventional risk factors fail to explain most of it<sup>[3,4]</sup>. In search of factors that could explain this greater event-rate, newer risk factors are being sought<sup>[5]</sup>.

Epidemiological data have shown a strong relationship between iron levels and cardiovascular disease. Experiments with animal models during the past decade have revealed a strong relationship between iron and lipid

metabolism. Iron is supposed to be involved in the process of lipid peroxidation. Active iron molecules modify LDL to interact with oxidized LDL receptors found in the macrophages. Exposure of membrane lipids to oxygen radicals in the presence of iron salts stimulates the process of lipid peroxidation<sup>[6]</sup>. Atherosclerotic lesions have recently been shown to be rich in both iron and copper and were found to induce lipid peroxidation that was inhibited by iron ion chelator desferrioxamine<sup>[7]</sup>. Transferrin accounts for about 90% of the serum iron binding capacity, and is a major serum antioxidant and thus might have a protective role in the development of atherosclerosis.

A prospective human study concluded that increased iron levels are associated with hypertension and excess risk of heart attack<sup>[8]</sup>. It has also been found recently that NIDDM patients had higher levels of plasma thiobarbituric acid (TBA) reactivity and lipid peroxides than normal individuals<sup>[9]</sup>. Thus diabetes mellitus may, in part, result from oxidative stress catalyzed by transition metals<sup>[10]</sup>. The aim of the present study is to estimate serum free iron levels in AMI patients and

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to determine whether there is any change in serum iron with respect to the risk factors smoking, diabetes mellitus and hypertension. This may enable us to investigate the role of serum iron in the pathogenesis of acute myocardial infarction.

## PATIENTS AND METHODS

### Patients:

Patients admitted to the Intensive Coronary Care Unit of Amala Cancer Hospital, Thrissur, with a diagnosis of acute myocardial infarction within 24 hours of onset of chest pain were included in the study. A total of 100 patients were included in the present study (92 males and 8 females ranging in age from 42 to 60). In addition, 25 sex and age matched subjects were included in the study as controls. The normal volunteers had no past history or evidence of cardiovascular disease, hypertension or diabetes mellitus. The present study does not include control subjects with a history of neoplastic, hepatic, infectious or autoimmune diseases or any surgical procedure in the proceeding six months. The institutional ethics committee approved the study.

Myocardial infarction was diagnosed by at least 0.1 mv ST segment elevation in two or more contiguous limb leads or 0.2 mv ST segment elevation in two or more chest leads associated with typical chest pain. Patients with cardiogenic shock, cerebrovascular accident and significant hepatic or renal disease were excluded. Patients with clear evidence of infection anywhere in the body were also excluded.

In patients included in the study, a detailed history was taken and complete physical examination was carried out by the cardiologist. A 12 lead ECG with V3R and V4R was recorded immediately upon admission and repeated after 2 hrs, 6 hrs, 12 hrs, 24 hrs, 48 hrs and pre-discharge. Chest X-ray was done at the time of discharge from the ICU. An echocardiographic examination was performed at the time of discharge. All patients were seen in the cardiology out-patient department 4 to 6 weeks after discharge, when a detailed history was taken and complete physical examination was carried out. A symptom limited treadmill test was done as per the Bruce protocol and maximum heart rate, blood pressure, double product, time to 1 mm ST depression, METs achieved, duration of exercise, angina, dyspnea and arrhythmias were recorded along with ST segment changes.

Information about the intake of iron was collected from the subjects through oral questionnaires. None of the subjects was taking iron orally in pharmaceutical preparation. Intake of

iron through fruits and vegetables was within acceptable limits. The various CAD risk factors encompassed in the study were :

- i) Hypertension
- ii) Diabetes Mellitus
- iii) Smoking

### Methods:

A total of 10 ml of blood was withdrawn for laboratory analysis. Creatine kinase was repeated 2 hrs, 6 hrs, 12 hrs, 24 hrs and 48 hrs after admission. Lipid profiles and random blood glucose was estimated by standard methods<sup>[11]</sup>. Free radical production was measured by estimating malondialdehyde (MDA) levels<sup>[12]</sup>, in which 0.2 ml of serum was mixed with 1 ml of 20% trichloroacetic acid (TCA). To the mixture 0.4 ml of 0.67% thiobarbituric acid (TBA) was added, shaken and kept for 3 minutes in a boiling water bath. After cooling to room temperature, 1.6 ml of butanol was added and the mixture was shaken. The organic mixture was separated by centrifugation and its absorbance was measured at 532 nm. The breakdown product of 1,1,3,3-tetramethoxy propane was used as standard.

The serum free iron and the total iron binding capacity (TIBC) were estimated by using dipyriddy<sup>[13,14]</sup>, in which equal volumes of serum, 0.1M sodium sulphite and dipyriddy reagent were mixed in a glass stoppered tube and centrifuged, heated in boiling water bath for five minutes, cooled and 1 ml of chloroform was added, stoppered and shaken vigorously and again centrifuged and the supernatant was taken for the iron assay. The absorbance was taken at 520 nm. For TIBC estimation, 100 µl of serum was mixed with 200 µl of ferric chloride. After standing for five minutes, 200 µg magnesium carbonate was added, shaken frequently for thirty minutes. The supernatant was used for the TIBC assay by using the same procedure as for iron. Solution containing 100 µg of iron per ml was used as the standard. Percentage saturation of transferrin was derived from the formula: (iron/TIBC) X 100. Statistical analysis was done according to the student's t-test. The values were expressed as mean ± SD. P-value <0.001 was considered as significant.

## RESULTS

Table 1 shows the results of the present study. Lipid peroxidation, which was measured as the endogenous MDA levels in the serum, was found to be elevated in all the three risk groups with AMI, when compared to the normal subjects (p < 0.001). The highest MDA levels were found in smokers with AMI. The serum iron value was also found to be more in all the three risk groups when

**Table 1:**

Serum MDA, serum iron, iron binding capacity and percentage transferrin saturation in normal subjects and AMI patients. Values are expressed as mean  $\pm$  SD

Parameters	Healthy Controls (n = 25)	Patients with AMI (n = 100)	Patients with risk factors (n = 100)		
			Smokers n = 73	Hypertensives n = 58	Diabetics n = 32
MDA (nmol/ml)	4.8 $\pm$ 2.5	14.8 $\pm$ 3.7	15.6 $\pm$ 2.9*	12.9 $\pm$ 2.2*	12.3 $\pm$ 3.1*
serum iron ( $\mu$ gm/dl)	103.8 $\pm$ 19.1	159.5 $\pm$ 28.4	167.2 $\pm$ 20.5*	151.2 $\pm$ 19.7*	155.2 $\pm$ 19.0*
TIBC ( $\mu$ gm/dl)	355.6 $\pm$ 22.6	312.2 $\pm$ 80.8	335.7 $\pm$ 57.1*	306.9 $\pm$ 48.8*	304.2 $\pm$ 73.5*
percentage transferrin saturation	28.3 $\pm$ 15.9	52.1 $\pm$ 16.8	50.2 $\pm$ 18.1*	48.3 $\pm$ 16.1*	50.1 $\pm$ 11.5*

(\*p < 0.001)

compared to the normal healthy subjects (p < 0.001). We could not find much difference in the TIBC values in AMI patients when compared to the controls, but the percentage saturation of transferrin was elevated in all the three risk groups with AMI when compared to the normals (p < 0.001). The value of both serum free iron and MDA, however, was found to be higher in smokers when compared to the other two groups.

## DISCUSSION

Iron is a transitional metal that can catalyze toxic, redox reactions, and has been suggested to be involved in many harmful biological processes and diseases in the human body. Excess iron has been proposed to be a risk factor for coronary heart disease.

The presence of iron in cytochromes, catalase, hydroxylase, peroxidases, saturases, lipoxigenases and cyclooxygenases suggests that iron has an important role in various metabolic events related to lipids, such as the oxidative degradation of fatty acids and the synthesis of unsaturated fatty acids, plasminogens and prostaglandins. However, studies in this area are limited. Oxidation of LDL-cholesterol is catalyzed by iron present in atherosclerotic gruel<sup>[15]</sup>. Serum deficient in iron has minimal oxidative capacity that increases with iron repletion. Several studies have been conducted in developed countries to assess the association of iron with coronary heart disease or acute myocardial infarction. Iron besides promoting lipid peroxidation could increase the risk of acute myocardial infarction through the elevation of blood haematocrit and blood haemoglobin levels. This in turn increases the

viscosity of blood and has a direct thrombogenic effect<sup>[16]</sup>.

The present results clearly show that there is an increased level of MDA in all the subjects with AMI when compared to that of the normal controls. However, within the AMI patients, the value of MDA is found to be the highest in smokers as compared to the other two risk groups. Free radical induced oxidative damage has been implicated as an important mechanism responsible for the toxicity of both active and passive smoking. Cigarette smoke contains short and long-living radicals that can stimulate cellular production of highly reactive oxygen species injurious to the cells and may accentuate the peroxidation events involved in the pathogenesis of coronary artery diseases<sup>[17]</sup>. The increase in serum free iron in smokers clearly indicates their vulnerability towards acute myocardial infarction.

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