

Article type: Original Article

Title: Correlate the relationship of Serum Ferritin Levels with Conventional Risk Factors of Acute MI Like Hypertension, Smoking, Diabetes Mellitus and Body Mass Index

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ABSTRACT

Background: The most common cause of death worldwide is cardiovascular disease that accounts for roughly 12 million deaths a year. **Objective:** To study the relationship of serum ferritin levels with conventional risk factors like diabetes mellitus, body mass index, hypertension and smoking

METHODS: This case control study was conducted among 75 patients with acute MI and 75 age and sex matched controls, who fulfilled the inclusion and exclusion criteria. Study was conducted at Karnataka Institute of Medical Sciences, Hubli. Institutional Ethics Committee approval was taken before starting the study. **RESULTS:** Majority of patients were smokers accounting for 37% and alcohol intake was seen in 22% of cases. Hypertension was seen in 64% of patients while diabetes mellitus was seen in 49% of cases and had mean HbA1c levels of 6.57. The majority of patients in this study were having increased waist-hip Ratio and mean waist hip ratio was 0.96 in cases. Most of the patients were having abnormal BMI, where 56% of patients belonged to pre-obese, obese-I & obese-II category. The present study results showed higher levels of serum ferritin among patients with acute MI compared to age and sex matched control subjects. The mean level of serum ferritin was found to be 252.85ng/mL among cases, whereas it was 112.59ng/mL among controls. This difference was found to be statistically significant with p value of <0.001.

CONCLUSION: Serum ferritin is found to be significantly increased in patients with acute MI compared to age and sex matched controls. Serum ferritin >200ng/ml may be considered as an independent risk factor for myocardial infarction.

KEYWORDS: Serum ferritin, risk factors, hypertension, smoking, diabetes mellitus, body mass index

INTRODUCTION: In the past 3 decades we have seen a shifting pattern in coronary artery disease (CAD) globally, showing declining trend in industrialized and western countries and rising trend in incidence of atherosclerotic disease in developing countries. This growth is driven by the industrialization, urbanization and lifestyle changes known as epidemiological transition. As mentioned by India's registrar general, CAD in 2001-2003, resulted in 17 % of total deaths

and 26% of adult deaths and that increased to 23% of total and 32% of adult deaths in 2010-2013. The prevalence of CAD varies from 1%-2% in rural populations and 2%-4% in urban populations^[1]

CAD risk factors are generally categorized into modifiable and non-modifiable. Classically defined eight risk factors are hypertension, dyslipidemia, diabetes, physical inactivity, smoking, sedentary lifestyle, central obesity, stress and type A-Personality, CAD family history. INTERHEART study found abnormal lipids, smoking, hypertension, Diabetes, abdominal obesity, psychosocial factors, decreased consumption of fruits, vegetables, excess intake of alcohol and physical inactivity account for most of the risk of myocardial infarction worldwide.^[2]

In most cases, CAD occurs due to inadequate coronary perfusion, relative to myocardial demand. Pre existing atherosclerotic occlusion of coronary arteries and new superimposed thrombosis, and/or vasospasm may result in demand supply mismatch. Myocardial infarction (MI) develops due to sudden plaque modification accompanied by thrombosis. The precipitating event is plaque breakdown due to rupture, cracking, or ulceration that expose highly thrombogenic underlying plaque constituents or Sub endothelial basement membrane. Hemorrhage into the center of plaques with plaque volume expansion and enhancement of the luminal occlusion is a well known process by which MI occurs

The contemporary viewpoint of atherogenesis is conveyed through the Hypothesis of “response to injury”. This model treats atherosclerosis as a chronic condition and inflammatory response to endothelial damage by the arterial wall. Inflammation plays an important role throughout atherosclerosis, from the beginning to the rupture of plaque. Sullivan et al^[3] originally proposed that iron depletion would guard against cardiac ischemia. The Framingham study found that the risk of cardiac disease in women increased similarly due to natural menopause or surgical menopause. Uterine factors, especially frequent menstrual blood loss, may be responsible for shielding premenopausal women from ischemic heart disease^[4,5] Lauffer et al^[6] studied about correlation of iron stores and cardiovascular mortality

The hypothesis that increased body iron stores have been associated with increased risk of cardiovascular disease and mortality has been under debate and in research over the past 20 years. There are many trials done in different centers to search for acute myocardial infarction risk factors. And most of these studies have been conducted for typical risk factors. Serum ferritin is not well known as a risk factor for the acute myocardial infarction as per literature. This research was therefore considered as to study the association of serum ferritin and its role in myocardial infarction in Karnataka Institute of Medical Sciences, Hubli.

MATERIALS AND METHODS: This case control study was conducted among 75 patients with acute MI and 75 age and sex matched controls, who fulfilled the inclusion and exclusion criteria. Study was conducted at Karnataka Institute of Medical Sciences, Hubli. Institutional Ethics Committee approval was taken before starting the study. Prior informed consent was obtained from all the patients and controls

INCLUSION CRITERIA: The diagnosis of acute myocardial infarction based on fulfilling any

two

of the following criteria:

1. Chest pain of <12 hours duration.
2. ST elevation >1mm in at least two consecutive leads in ECG.
3. Increased cardiac markers (CK-MB and Troponin-T).
4. Presumably new onset bundle branch block.

EXCLUSION CRITERIA: The following patients will be excluded from the study.

1. Haemochromatosis
2. Liver disease
3. Tuberculosis
4. Chronic inflammatory disease
5. Those on iron therapy
6. ESR > 20mm in 1st hour
7. Neoplastic disease.

DATA COLLECTION AND METHODS: Patients admitted for chest pain were evaluated and those who were diagnosed as having acute MI were included for the study according to the inclusion and exclusion criteria. Elaborate clinical history and clinical findings, ECG, cardiac markers and other relevant laboratory findings were documented meticulously. Simultaneously 75 age and sex matched controls were included in the study and identify the study variables like history of smoking, alcohol intake, hypertension, diabetes with further workup. Patients who were known case of hypertension and diabetes mellitus were noted. Those patients without such history were further evaluated by measuring blood pressure, fasting and 2 hours post-prandial blood sugar.

Serum lipid profile was done for all patients to identify abnormalities in lipid metabolism which in turn may serve as a risk factor for acute MI. Waist circumference and hip measurement was taken and finally waist hip ratio was calculated. Assessment of obesity was done by considering the body mass index (BMI) and patients were categorized into following groups.

NUTRITIONAL STATUS BMI RANGE

- 1 Underweight < 18.5
- 2 Normal 18.5 – 22.9
- 3 Overweight 23 – 24.9
- 4 Obese 25 -29.9
- 5 Morbidly obese \geq 30

SPECIMEN COLLECTION AND HANDLING: Blood collection was performed by venipuncture and allowed to Clot. Serum separation was done by centrifugation at 5000rpm for 5 minutes at room temperature. If sera cannot be assayed immediately, they were stored at 2-8oC which were analyzed within a period of 48 hours. Hemorrhagic samples, lipemic samples and

sera from jaundiced patients were not evaluated as it can interfere with assay detection method. Whenever hemorrhagic or lipemic samples were obtained, a repeat venipuncture was performed to obtain fresh sample.

SERUM FERRITIN ASSAY METHOD: Serum ferritin was measured for all cases and controls by using enzyme linked immunosorbent assay (ELISA) method. UBI MAGIWEL FERRITIN

Quantitative test system was used which is a solid phase ELISA system which measures serum ferritin with high sensitivity (Limit of detection = 0.011ng/mL) and high specificity. It has 96 well plate coated with specific antiferritin antibodies. The standards provided along with the kit and serum samples from cases and controls were incubated in the well for 30 minutes and washed with distilled water. Another anti-ferritin antibodies conjugated with horseradish peroxidase was added to each well and incubated for 30 minutes. The wells were washed with distilled water to remove unbound anti-ferritin antibody conjugate. Finally chromogenic substrate was added which will result in formation of coloured product. The intensity of colour developed will be proportional to the amount of ferritin in the standards and samples.

Optical density (OD) of both standards and samples were measured by using micro titer plate reader at a wavelength of 450nm. The serum ferritin concentrations of unknown samples were

Statistical analysis: Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. Chi-square test was used as test of significance for qualitative data. p value of <0.05 was considered as statistically significant after assuming all the rules of statistical tests

RESULTS: Patients were seen from 32 years to 88 years of age with mean age of 63.77 ± 12.28 years. Majority of patients were seen in 6th decade of life (32%) and predominantly involved male patients (64%). In Cases, 91.38% had typical anginal type of chest pain, rest 8.6% complained of atypical chest pain or chest discomfort. In Control Group, HTN was observed in 52.00% and 64.00% in Cases. There was no significant difference in HTN distribution between two groups.

smoking and alcohol intake were also evaluated between cases and controls. Majority of patients were smokers accounting for 37% and alcohol intake was seen in 22% of cases. Patients who were already diagnosed, those on treatment and new onset disease (hypertension and diabetes) were enrolled and evaluated in the study. Hypertension was seen in 64% of patients while diabetes mellitus was seen in 49% of cases. Mean HBA1C in Control Group was 5.95 ± 0.84 and in cases was 6.57 ± 1.02 . There was a significant difference in mean HBA1C Comparison between two groups.

Table 1: Mean HBA1C Comparison between two groups

Group	Mean HBA1C	Standard Deviation
Control Group	5.95	0.84
Cases	6.57	1.02

HBA1C

The majority of patients in this study were having increased waist-hip Ratio and mean waist hip ratio was 0.96 in cases.

Table 2: Mean Waist Hip Ratio Comparison between two groups

Waist Hip Ratio

Body mass index was calculated and results were tabulated as normal, pre-obese, obese-I & obese-II. Most of the patients were having abnormal BMI, where 56% of patients belonged to pre-obese, obese-I & obese-II category. Hypercholesterolemia was seen in 49% of patient population.

Table 3: Mean BMI Comparison between two groups

	BMI

The present study results showed higher levels of serum ferritin among patients with acute MI compared to age and sex matched control subjects. The mean level of serum ferritin was found to be 252.85ng/mL among cases, whereas it was 112.59ng/mL among controls. This difference was found to be statistically significant with p value of <0.001.

Table 4: Mean serum ferritin Comparison between two groups

Ferritin

Most common ST-T changes were observed in anterior wall(53%) followed inferior wall(37%) .Most common vessel involved was LAD (36%) followed by DVD(LCX+RCA)-16% and least affected being left main coronary artery

Table 5: Serum Ferritin levels with respect to Number of vessels involved among cases

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Vessel

In the study there was no significant difference in Serum Ferritin levels with respect to number of vessels among cases.

DISCUSSION: The current study including 150 subjects (75 cases and 75 controls) to determine the serum ferritin levels in acute ST segment myocardial infarction and to study and correlate the relationship of serum ferritin levels with conventional risk factors of acute MI like hypertension, smoking, diabetes mellitus and body mass index

In present study, acute MI was commonly seen in age group of 61-70yrs (32%), followed by age >70yrs (30%). Least affected were among age group of 30-40yrs (5%) Majority of affected patients were male population about 64% In our study male female ratio observed as 1.7:1 Similarly, Holey et al, observed male female ratio as being 2.4:1 which can be explained by Sullivan's iron hypothesis, in females due to reduced iron levels have less

incidence of CAD. The mean age of patients with acute MI was 63.77 ± 12.28 years. Zodpey SP et al^[7] who have analyzed the risk factors of acute MI including Indian patients by case control study noted that 6th and 7th decade of life being majorly affected, with males forming majority about 70% of cases which is similar to our study

In the present study, 37% of the cases were smokers and 22% were alcoholics. Odds ratio of 1.3 for smoking and 0.8 for alcohol intake has been estimated as per other studies as risk factors of acute MI Smoking predisposes to atherosclerosis and thrombosis by disruption of endothelial function. Catecholamine released by nicotine causes elevation of blood pressure and platelet aggregation predisposing to atherothrombosis^[8]

64% Of patients presenting with MI in our study were hypertensives whereas in controls 52% of cases were hypertensives.49% cases were diabetic and controls 50% of them were diabetics. Hypertension and diabetes are well known risk factors for atherosclerosis and coronary artery disease^[9]

Sekhri T et al^[10] studied risk factors of CAD among urban Indian population Among the study participants, diabetes and hypertension was seen in significant number of individuals.

In our study ,the mean waist hip ratio was 0.96 ± 0.08 in cases, in comparison to 0.9 ± 0.12 in controls with p value of <0.001 .Increased waist-hip ratio is indicator of abdominal obesity and seen in sedentary lifestyle, excessive alcohol intake ,high fat diet and promote the risk of developing coronary artery disease.

Among cases of acute STEMI , the most common were involving anterior leads-36%AWMI,12%-ALWMI,5% ASWMI TOTAL 53% involving anterior leads followed by IWMI.Most common angiographically involved vessel was LAD(36%) as single most affected, followed by 16% LCX and RCA combined .

Based on Mahmoodzadeh et al^[11] ECG showed sensitivity for detection in LAD(37%).LCX(25%) and RCA(25%) respectively.Serum ferritin were observed to be higher in patients with double vessel disease(307ng/ml) however further studies are required to correlate the same

In our present study, higher serum ferritin levels were seen among patients with acute MI in comparison to age and sex matched control subjects. The mean level of serum ferritin was found to be 252.41 ± 118.94 ng/mL among cases, whereas it was 112.11 ± 47 ng/mL among controls. This difference was statistically significant with p value of <0.001 .This is very similar to Holay MP et al studied 75 cases of AMI and 75 age and gender matched controls age group 30-70 years showed that median serum ferritin levels were significantly higher in cases (220 μ g/L) than controls (155 μ g/L)($P \leq 0.0001$).^[12]

Hoque et al studied 130 subjects 65 patients with acute coronary syndrome and 65 age and sex matched controls ,males 88 in number (67.7%) and females 42(32.3%).Serum ferritin level was significantly higher in ACS patients (46.2% in case group vs 4.6% in control group)($p = <0.001$)^[13]

Ishran et al [studied 100 subjects 50 cases and 50 healthy controls.Mean serum ferritin was higher in MI patients (202 ± 81.2 μ g/L) as compared to controls(135.4 ± 90 μ g/L) and it is

statistically significant^[9]

Tianyu Xiao ,Hongyan Wang studied 126 patients 41 controls, 52 unstable angina, 31 NSTEMI and 43 STEMI patients. Serum ferritin levels with ACS (221.51±86.73ng/ml) is significantly higher than control group(119±92.74ng/ml)(P<0.05)^[14]

Silvia WD, Biswas S, Uttappa S ,Shetty P,et al case control study involving 145 men(100 cases and 45 healthy control subjects) in age group of 30-70 yrs and the mean value of serum ferritin(µg/L) in controls and cases were found to be 155.42±74.10 and 257.35±76.34 respectively very similar to our study^[15]

Bharathi B K, Shrikanth Chandrashekhar et al studied 30 cases and 30 controls median serum ferritin levels were significantly higher in cases (325.5µg/L) of AMI as compared to controls (65.5µg/L)(p<0.001) and there is independent relationship of high SF with AMI and SF was significantly high in diabetics and smokers^[16]

Rotterdam study ,by Grobusch KK^[17] et al studied serum ferritin as a risk factor for acute MI in elderly patients. This was a community based prospective cohort study of 7983 subjects. As per univariate analysis, no association was observed but multivariate analysis shows serum ferritin adversely affecting the occurrence of acute MI in the presence of other well known risk factors like smoking, hypercholesterolemia and diabetes mellitus.The odds ratio for evaluation of risk of MI in elderly was calculated in this study and noted as 1.68 in smokers, 2.41 in Diabetics and 1.43 in patients with hypercholesterolemia in patients with serum ferritin levels (≥200mg/L).

Effect of blood donation on cardiovascular events supports iron hypothesis.^[18] The benefit of blood donation was higher in men with higher levels of LDL cholesterol.

Tuomainen et al studied on 2682 Finnish men by prospective cohort study for duration of 5.5 yrs and concluded as blood donation reducing risk of MI by 86%^[19] Salonen et al^[20] performed 3 phlebotomies by removal of 1.5L of whole blood, which had 700mg of iron , among healthy subjects and observed the reduction of susceptibility for lipid oxidation. Matthews et al^[11] concluded the utility of deferiprone, iron chelator, in prevention of LDL oxidation in rabbits

Conclusion: Serum ferritin is found to be significantly increased in patients with acute MI compared to age and sex matched controls. Serum ferritin measurement can be done in individuals with increased risk of acute MI during monitoring of other conventional risk factors like diabetes and hypertension. Phlebotomies to decrease the iron content can be tried in those with increased serum ferritin levels.

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