Ferritin, a Potent Threat for Acute Myocardial Infarction?

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Abstract

Background: Studies conducted have yielded contradicting results on the role of ferritin as a risk factor for acute myocardial infarction (AMI). The relation of ferritin status to risk of AMI in Indian men, along with other established major risk factors like serum total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol and triglycerides has not been documented previously. The hypothesis that increased serum ferritin was related to increased chances of AMI along with the risk factors was tested.

Methods: Case control study involving 145 men (100 cases and 45 healthy control subjects) in the age group of 30-70 years. Serum ferritin levels were estimated by using ELISA, and other risk factors by enzymatic methods.

Results: Increased serum ferritin levels significantly (p < 0.001) correlated with an increase of other risk factors in Indian male patients with AMI.

Conclusion: Significant direct correlation between serum ferritin levels and risk of AMI was observed.

INTRODUCTION

Free iron as a catalyst for the production of free radicals has been implicated in lipid peroxidation and atherosclerosis leading to myocardial infarction. Several contradicting results exist on the role of high levels of stored iron (ferritin) increasing the incidence of myocardial infarction. A possible association between body iron status and the risk of coronary heart disease was bolstered from a three year Finnish study relating increased levels of both serum levels of ferritin and dietary iron to an increased risk of myocardial infarction.1 Oxygen free radicals that promote the oxidation of LDL, which occurs in the subendothelial layer of arteries have been postulated to be involved in the development of atherosclerosis.2

The association of high iron stores and coronary heart disease was first suggested by Sullivan.3 Several studies have been conducted since then to assess this association of iron and acute MI. Results of some studies have been in favor of ferritin being a risk factor for AMI,4 while others have not.5 Free iron catalyses free radical production that generates a range of potent oxidants that can induce oxidation of lipids.6 Supporting evidence comes from in vitro lipid peroxidation and lipoprotein modification studies7 from cholesterol-fed iron overload animal models8 and from analysis of the composition of human atherosclerotic lesions.9 Thus the question of whether or not body iron is an independent risk factor for AMI is still not clear.

Since serum ferritin concentrations are directly proportional to intracellular ferritin concentration, it is considered to be the best clinical measure of body iron stores10 and the most feasible to use in epidemiologic studies.11 There are however no previous reports concerning the relation of iron status to the risk of CHD in Indian men. Hence the hypothesis whether the excess body iron as estimated by serum ferritin concentration is associated with increase in risk of myocardial infarction in men was tested along with established major risk factors of CHD such as total serum cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol and triglycerides.

MATERIAL AND METHODS

Consecutive patients admitted to the coronary care unit of St. John’s Medical College Hospital with cardiac symptoms were studied. The study group which included men within the age group of 30-70 years with mean age of control and cases were 47 ± 11.97 and 50.23 ± 9.41 respectively, were assessed by clinical examination, serial ECG and measurement of serum total CK and CK-MB. The study was approved by the medical ethics committee of St. John’s Medical College, Rajiv Gandhi University of Health Sciences, Bangalore and written, informed consent was obtained from all participants.
The serum was separated and stored at -20°C for subsequent analysis.

Fasting venous blood samples were collected from the subjects. Subjects with neoplastic and liver disease, primary/secondary haemochromatosis, alcohol abuse, smoking, diabetes and ESR > 20 mm/hr indicating the presence of inflammation/infection, that could potentially lead to elevated ferritin concentration were excluded from analysis. After exclusion of subjects with a verified history of myocardial infarction only 145 subjects (100 cases and 45 healthy control subjects) remained for analysis of serum ferritin, lipid profile and risk of myocardial infarction. Infarction was judged to have occurred in patients with a h/o of chest pain accompanied by the development of Q waves. ST and T wave changes were considered to indicate infarction when serial serum total CK and CK-MB values exceeded the upper reference limit. Non-ischaemic chest pain was diagnosed when a definite alternative source of the chest pain or an atypical history of the negative stress ECG could exclude angina.

Methodology

After thawing the frozen sample, serum ferritin was determined by using Pathozyme - Ferritin kit (Omega Diagnostics Limited, Scotland, UK) by enzyme linked immunosorbent assay (ELISA) using the Robonik, Easy Elisa Reader.

The assay is based on noncompetitive sandwich method, by using monoclonal antibodies in immobilized and enzyme labeled forms against the human ferritin. Specific anti-ferritin antibodies are coated on to microtitration wells. Test sera were applied. Then monoclonal antiferritin labeled with Horseradish peroxidase enzyme (conjugate) was added. If human ferritin is present in the sample, it will combine with the antibody on the well and the enzyme conjugate, resulting in the ferritin molecules being sandwiched between the solid phase and the enzyme-linked antibodies. After 45 minutes of incubation at room temperature, the wells were washed with water to remove unbound labeled antibodies. On addition to the substrate (TMB), a colour developed only in those wells in which enzyme was present, indicating the presence of ferritin.

The reaction was stopped by the addition of dilute hydrochloric acid and the absorbance is then measured at 450 nm. The concentration of ferritin is directly proportional to the colour intensity of the test sample.

Total cholesterol, HDL cholesterol, triglycerides were estimated by enzymatic methods by using autoanalyzer. LDL cholesterol and VLDL cholesterol calculated by using indirect method of empirical equation of Friedewald et al. 12

Data analysis

Association between serum ferritin and risk factors for myocardial infarction in Indian men were investigated by using Pearson’s correlation coefficient, those for categorical variables were investigated by using Chi-square test. Analysis of variance was used to test differences in baseline characteristics between cases and control. All analysis was adjusted for age and sex. Serum ferritin was categorized as being ≥ 200 µg/L and the risk for MI was investigated by odds ratio (OR) with 95% CI. Two-sided “p” values were calculated. Statistical analysis was performed by using SPSS package version 9.0

RESULTS

Serum ferritin concentration, in the case control population ranged from 60 µg/L to 400 µg/L and averaged 230 µg/L. Median concentration of serum ferritin was 220 µg/L.

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. The distribution of serum ferritin for cases and control subjects indicated a shift toward higher concentration in patients with AMI (Fig. 1). Correspondingly, more patients with MI (77.0%) than control subjects (31.1%) had concentrations above the cutoff of 200 µg/L. The mean value of controls and cases for haemoglobin (g/dl) 12.91 ± 0.30 and 14.22 ± 02.36, cholesterol (mg/dl) 186.9 ± 36.55 and 242.81 ± 40.60, LDL cholesterol (mg/dl) 117.58 ± 38.35 and 170.96 ± 39.75, VLDL cholesterol (mg/dl) 28.36 ± 11.77 and 38.41 ± 19.53, triglycerides (mg/dl) 143.49 ± 57.11 and 190.68 ± 97.67, HDL cholesterol (mg/dl) 41.51 ± 11.36 and 33.34 ± 10.50 respectively (Table 1).

Serum ferritin was significantly directly associated with haemoglobin (r=0.586, p < 0.01), serum cholesterol (r=0.439, p < 0.01), serum LDL cholesterol (r=0.381, p < 0.01), serum triglycerides (r=0.280, p < 0.01) and serum VLDL cholesterol (r=0.286, p < 0.01). Serum ferritin was significantly inversely correlated with serum HDL cholesterol (r=-0.210, p < 0.05).

Table 2 shows the comparison between cases and control for each parameter analyzed by ‘t’ test in this study.

When adjusted for age and sex, subjects with serum ferritin concentration of ≥ 200 µg/L tended to have a risk of 7.4 (95% CI (3.3-16.2) : p=0.05) for AMI compared to those with serum

Fig. 1 : Percentage of control and cases in comparison with serum ferritin concentration.
blood donation was associated with decreased risk of CV between whole blood donors and non-donors, showed that examined the relation between sonographically et al. project in middle-aged eastern Finnish men.14 concentrations came from the prospective MONICA AMI risk of myocardial infarction and elevated serum ferritin presented conflicting results. 13 status can be considered as cardiovascular risk factor myocardial infarction. Studies investigating whether iron ferritin concentration was associated with increased risk of subjects of Indian population showed that elevated serum concentration < 200 µg/L.

**DISCUSSION**

This study including 145 men, (100 cases, 45 controls) subjects of Indian population showed that elevated serum ferritin concentration was associated with increased risk of myocardial infarction. Studies investigating whether iron status can be considered as cardiovascular risk factor presented conflicting results.13 Previous evidence of an association between increased risk of myocardial infarction and elevated serum ferritin concentrations came from the prospective MONICA AMI project in middle-aged eastern Finnish men.14 In 847 Austrian men and women aged 40-79 years, Kiechl et al15 examined the relation between sonographically assessed carotid atherosclerosis and body iron stores. Ferritin was observed to be one of the strongest indicators of the presence of carotid artery disease. Their results were compatible with the hypothesis that iron-induced lipid peroxidation is crucially involved in the early steps of human atherogenesis both ferritin and LDL cholesterol levels are necessary to accurately estimate the risk of progressive atherosclerosis in the subjects. Again from the Rotterdam study Klipstein et al16 concluded that elevated serum ferritin concentration was associated with increased risk of myocardial infarction in the elderly population of Ommoord, Netherlands. Subjects, age and sex adjusted with serum ferritin concentration ≥ 200 µg/L tended to have a risk of 7.41 for Myocardial infarction compared with those with serum ferritin concentration < 200 µg/L.

Meyer et al16 compared cardiovascular (CV) event rates between whole blood donors and non-donors, showed that blood donation was associated with decreased risk of CV events after 5-8 yrs of follow up.

A modified form of LDL is rapidly taken up by macrophages to convert them into foam cells. It is found that chemical acetylation converted LDL to a form recognized specifically by the monocytes/macrophages and the uptake rate is manifold faster than native LDL. Smooth muscle cells, monocytes, macrophages can themselves effect similar modifications in LDL.18 Thus all three of the major cell types in the arterial wall can convert LDL to a form recognized by the acetyl LDL receptor.

All these changes, however, depend on a common initiating step - the oxidation of polyunsaturated fatty acids in LDL. During the oxidative modification there is an extensive conversion of LDL lecithin to lysolecithin. Cell-induced oxidative modification can be mimicked by simply incubating LDL in serum-free medium in the presence of a sufficiently high concentration of copper/iron.19 This suggests that in vivo the process must occur extravascularly in microenvironments.

The cytotoxicity of oxidised LDL could conceivably induce functional changes in the endothelial cells that favour the penetration of circulating monocytes or the movement of LDL into subendothelial space and thus accelerates the formation of fatty streak. The cytotoxicity of oxidised LDL may be sufficient to lead to such denudation. In this way it may help to account for the progression of the relatively benign fatty streak lesions to more complicated ones associated with clinical disease.

We observed that the elevated serum ferritin concentrations to be associated with increased risk of myocardial infarction in an Indian population, also ferritin may adversely affect MI risk in the presence of other risk factors. It may be possible that these factors in interaction with elevated body iron stores may accelerate atherogenesis by stimulating oxidation of LDLs.20

More knowledge about the role of ferritin as the predisposing factor in post-secretory modification of LDL is required.

**REFERENCES**

6. McCord JM. Is iron sufficiency a risk factor in ischaemic heart

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**Table 1 : Characteristics of control subjects and cases**

<table>
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<th>Parameters</th>
<th>Control subjects (n=45)</th>
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<td>Ferritin (µg/L)</td>
<td>155.42 ± 74.10</td>
<td>257.35 ± 76.34</td>
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<td>Cholesterol (mg/dl)</td>
<td>186.9 ± 36.55</td>
<td>242.81 ± 40.60</td>
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<td>HDL Cholesterol (mg/dl)</td>
<td>41.51 ± 11.36</td>
<td>33.34 ± 10.50</td>
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<td>LDL Cholesterol (mg/dl)</td>
<td>117.58 ± 38.35</td>
<td>170.96 ± 39.75</td>
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<td>VLDL Cholesterol (mg/dl)</td>
<td>28.36 ± 11.77</td>
<td>38.41 ± 19.53</td>
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<tr>
<td>Triglycerides (mg/dl)</td>
<td>143.49 ± 57.11</td>
<td>190.68 ± 97.67</td>
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**Table 2 : Analysis by t-test**

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Announcement

India International Forum of Cardiology
Cardiology Summit 2004

India International Forum of Cardiology is organizing Cardiology Summit - 2004 from 13th to 15th February 2004 at Hotel Taj Lands End, Mumbai.

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