Clinical Study

# Association of serum iron and ferritin levels in patients with ischemic heart disease: A matched case-control study

**S. Aklujkar [1], A.B. Aklujkar*[1], Sridharan Kannan[2]**

[1]*Department of Cardiology, Bhakti Vedanta Hospital and Research Centre, Shristi Complex, Mira Road, Thane, Mumbai – 401107. Maharashtra. India.*

[2]*Department of Health Sciences, College of Medicine, Nursing and Health Sciences, Fiji National University, Suva, Fiji.*

Received: 5 December 2016 Revised: 19 December 2016 Accepted: 10 January 2017

## Abstract

**Background:** Contradictory evidence exists for the association of serum iron levels and body iron stores with risk of coronary artery disease (CAD). The present study was designed as a case control study evaluating the plausible association of iron excess and CAD. **Methods:** A case control study in 50 patients diagnosed as CAD and 20 controls was conducted after obtaining their informed consent. Standard methods were used for estimation of serum iron and ferritin levels and total iron binding capacity (TIBC). Additionally, the same parameters were estimated in CAD group on day 8. Descriptive statistics was used for analysis of demographic characteristics, paired/un-paired 't' test was used for analysis of difference in the iron, ferritin and TIBC levels between the study participants. **Results:** Mean (SD) iron levels (in microgram/dl) in participants who have been diagnosed as having CAD was 108.75 (25) while 93.6 (22.2) statistically significant difference in the serum iron and ferritin levels, and TIBC was observed between the study groups. Also, a statistically significant difference was observed in the serum levels of iron in the case group on day 8. No statistically significant difference was observed in any of the sub-group analyses like fatality, CPK-MB and LDL/HDL ratio. **Conclusion:** To conclude, we found out significantly higher levels of serum iron and ferritin in patients with CAD in comparison to age and sex matched controls.

***Keywords***: Myocardial infarction, iron, ferritin

## Introduction

There is a recent surge of incidence of coronary artery disease (CAD) worldwide as well its contribution to global mortality wherein 14.4 million deaths in 1990 to 17.5 million in 2005 attributed to CAD (Fuster, 2010). CAD is the numero one contributor for mortality in South Asia leading to death of 13.6% of the diagnosed cases (Celermajer et al, 2012). In India a four-fold rise in the incidence has been noted for CAD in the past 40 years with a prevalence of 7-13% in urban and 2-7% in rural populations (Mohan et al, 2001; Gupta et al, 2002; Kamili et al, 2007; Gupta et al, 2002; Kumar et al, 2006).

Prevention measures for CAD include reducing the burden of risk factors. Well established risk factors include raised blood pressure, high low density cholesterol cholesterol (LDL-C), diabetes mellitus (DM), tobacco and alcohol use, as well as low vegetable and fruit intake (Shokeen et al, 2015). Scare data exists that evaluated the role of micronutrient levels such as lead, zinc and iron in patients with CAD. In fact as early as 1989, Sullivan proposed a theory of free iron inducing the production of free radicals thereby leading to peroxidation and cell membrane damage leading to ischemic heart disease (Sullivan, 1989). On the contrary, few authors either found out no association or a negative association of serum iron levels as a risk factor for CAD (Bozzini et al, 2002; Auer et al, 2002). Considering the dearth of clear-cut association of serum iron levels and body iron stores with the risk of CAD, present study was conducted.

*Address for Corresponding Author:
Dr. Abhijit Aklujkar
Consultant Cardiologist
Bhakti Vedanta Hospital and Research Centre
Shirsti Complex, Mira Road, Thane, Mumbai – 401107.
Maharashtra. India.
Email: aaklujkar@gmail.com

## Methods

*Study ethics and participants*

The study was conducted after obtaining permission from

**Table 1.** Distribution of risk factors and other blood investigation parameters between the study participants

| Risk factors | Cases (n=50) [n (%)] | Controls (n=20) [n (%)] |
|---|---|---|
| Hypertension* | 17 (34) | 2 (10) |
| Diabetes mellitus | 7 (14) | 1 (5) |
| Tobacco use | 16 (32) | 2 (10) |
| Alcohol use | 13 (26) | 7 (35) |
| Total cholesterol in mg/dl [Mean (SD)] | 216.1 (27.1) | 206.7 (54.2) |
| LDL cholesterol in mg/dl [Mean (SD)] | 144.5 (25.3) | 143.0 (47.3) |
| LDL/HDL ratio | 3.8 (1.4) | 3.8 (1.1) |

*-P< 0.05 (Chi-square test)

institutional ethics committee and informed consent from all the study participants. The study was conducted in accordance with the ethical principles laid down in World Medical Association Declaration of Helsinki. The study was a case-control study that included 50 consecutive patients diagnosed to have CAD as per American College of Cardiology/American Heart Association consensus (O'Rourke et al, 2000). A total of 20 age and sex matched controls were recruited and the following investigations were performed for both the groups: Electrocardiogram, hemoglobin, total and differential leukocyte count, serum iron, serum total iron binding capacity (TIBC), serum ferritin, serum creatine phosphokinase-MB (CPK-MB), blood sugar, urea, total lipid profile and chest radiograph. Blood was analyzed for serum iron, TIBC and ferritin on day 8 for CAD patients only.

*Laboratory and statistical analysis*

Serum ferritin was assessed using ADVIA Centaur Ferritin immunoassay which is based on using direct chemiluminometric technology. Serum iron was measured using Nitro-Paps method and a commercially available kit by Pinnacles marketing was used for measuring TIBC.

Descriptive statistics was used to represent the demographic details of the study participants. The numerical variables were assessed for normality and accordingly either a parametric or non-parametric statistical test was used. Chi-square test was used to assess the significance of difference in proportions between the study participants. All the statistical analysis were done by SPSS version 17.0 (IBM Corp. Released 2012. IBM SPSS statistics for Windows, version 21.0. Armonk, NY: IBM Corp.). A p-value of $\leq$ 0.05 was considered significant.

## Results

*Demographic details*

Mean (SD) age in years of the study participants in the case group was 56.08 (±13.93) and in the control group was 51.90 (±16.68) (P>0.05). Male:Female distribution in the case and control groups were 42:8 and 17:3 respectively (P>0.05). Table 1 describes the proportion of known risk factors between the study participants and the various blood parameters.

*Serum iron, TIBC and ferritin concentrations:*

Table 2 compares the iron status in controls and patients and a statistically significant difference in the serum iron and ferritin levels, and TIBC was observed between the study groups. Also, a significant difference was observed in the serum levels of iron in the case group on day 8. Sub-group analyses were conducted to find out the difference in all these parameters fatality, CPK-MB and LDL/HDL ratio and were not statistically significant (Table 3).

## Discussion

The present study was conducted to explore the association of serum levels of iron and iron stores in patients with established coronary artery disease in comparison with age

**Table 2.** Serum levels of iron, TIBC and ferritin between the study groups

| Study groups (n) | Ferritin ng/ml [Mean SD)] | | Iron mcg/dl [Mean (SD)] | | TIBC mcg/dl [Mean (SD)] | |
|---|---|---|---|---|---|---|
| | $D_0$ | $D_8$ | $D_0$ | $D_8$ | $D_0$ | $D_8$ |
| **Controls (20)** | 164.2 (50.8) | NA | 93.6 (22.2) | NA | 354.4 (35) | NA |
| **Cases (50)** | 221.3*(85.9) | 226 (85.6) | 108.75* (25) | 110.2** (25.3) | 329.1* (41.1) | 327.7 (24.3) |

*-P<0.05 (statistically significant) by Unpaired 't' test

** - P<0.05 (statistically significant) by Paired 't' test between day 8 and baseline values

**Table 3.** Sub-group analyses of serum levels of iron and ferritin and TIBC in the case group

| Sub-groups (n) | Ferritin ng/ml [Mean (SD)] | Iron mcg/dl [Mean (SD)] | TIBC mcg/dl [Mean (SD)] |
|---|---|---|---|
| **A. Fatality** | | | |
| Non–Fatal (43) | 221.3 (85.9) | 108.8 (25) | 329.1 (41.1) |
| Fatal (7) | 187.5 (101.9) | 96.6 (26.2) | 335.5 (41.5) |
| **B. CPK-MB levels** | | | |
| CPK– MB > 60U/L (12) | 213.1 (112.9) | 98.2 (±27.7) | 343.3 (34.4) |
| CPK– MB < 60 U/L (38) | 217.3 (80.5) | 109.8 (24.5) | 325.4 (42.1) |
| **C. LDL/HDL ratio** | | | |
| LDL/HDL >5 (10) | 244.6 (96.9) | 116.8 (26.1) | 313.7 (27.9) |
| LDL/HDL <5 (40) | 209.2 (86.1) | 104.6 (25.1) | 333.69 (42.7) |

**(n=50),** P>0.05 (not statistically significant) between any of the groups

**Table 4.** Summary of studies conducted with the similar hypothesis

| Study Id: | Study type and participants | Key findings |
|---|---|---|
| Bozzini et al, 2002 | Case control study; 546 CAD patients and 303 controls | A slightly higher mean ferritin levels were observed in CAD patients that disappeared after adjusting for sex and c-reactive protein. The prevalence of high concentrations of stored iron, defined as ferritin concentrations above the sex-specific upper quintiles of the control distribution, was also similar in the two groups. No differences in iron markers were found in CAD patients with or without myocardial infarction. |
| Auer et al, 2002 | Prospective cohort study; 100 CAD patients | No significant association was observed for the risk of CAD with serum ferritin levels relative risk 0.83 (95% CI: 0.63-1.24). Also, sub-group analysis based on categories of severity of CAD showed no difference between the groups. |
| Nough et al, 2006 | Prospective cohort study; 112 patients with CAD and 63 normal healthy individuals | A statistically significant higher levels of serum iron (12.9±4 micromoles/liter and 10.8±5 micromoles/liter in the CAD group and the control group respectively). Also, a statistically significant higher levels of serum ferritin [126±75 microgram/liter and 101±75 microgram/liter in case and control group] were observed |
| Pormoghaddas et al, 2014 | Case control study; 212 CAD patients and 220 controls | No significant difference in the serum iron was observed between the groups (cases – 106.8±46.9 micromoles/liter and controls- 107.6±29.6 micromoles/liter). However, a statistically significant higher level of ferritin was observed in CAD patients (cases – 206.8±156.3 micromoles/liter and controls – 147.3±132.9 micromoles/liter). Also, and odds ratio of 1.006 [95% CI 1-1.01] and 4.49 (95% CI 1.72-11.70) was observed for serum ferritin and serum ferritin ≥ 200 micromoles/liter. |
| Klipstein-Grobusch et al, 1999 | Nested case control study; 60 CAD and 112 age and sex matched controls | The age- and sex-adjusted risk of MI for subjects with serum ferritin concentrations > or = 200 microg/L was 1.82 (95% CI: 0.90, 3.69; P = 0.096). The odds ratio (OR) was 1.26 (95% CI: 0.98, 1.64; P = 0.078) for the highest tertile of serum ferritin and was only slightly altered in a multivariate model. Risk of MI associated with the highest tertile of ferritin was most evident in current or former smokers (OR: 1.68; 95% CI: 1.17, 2.47; P for trend = 0.008) and in subjects with hypercholesterolemia (OR: 1.43; 95% CI: 0.99, 2.11; P for trend = 0.056) or diabetes (OR: 2.41; 95% CI: 1.12, 7.67; P for trend = 0.027). No association with risk of MI was observed for tertiles of serum iron, serum transferrin, or total dietary iron. |

and sex matched controls. We found out significantly high levels of serum iron and ferritin and lower TIBC in the case group.

Many investigators explored to find out the association of iron status and risk of CAD and the results were contradictory and Table 4 summarizes the key findings of some of the major studies. Danesh *et al* (Danesh, 1999) conducted a systematic review of published studies involving a total of 7800 CHD cases and did not find any significant difference in total iron-binding capacity (risk ratio, 1.0; 95% CI, 0.7 to 1.5) or serum iron (0.8; 95% CI, 0.7 to 1.0) and total dietary iron (0.8; 95% CI, 0.7 to 1.1). However, this meta-analysis has been attributed with inclusion of studies that had significant heterogeneity and differing techniques used for measuring the parameters. Plausible mechanisms of excess iron causing myocardial injury include free-radical mediated damage to the myocardial cells. Further, the cut-off level of serum ferritin beyond which the risk of CAD increases has not been clearly established. Although, initial reports emerged from eastern Finnish men, (Mainous, 2004) that serum ferritin concentration higher than 200 microgram/l had a 2.2-fold

risk of CAD, this is an area of interest that needs further exploration.

The study is limited by the fact that it was a case-control study involving few individuals and the measurement of ferritin levels in CAD patients has been recently debated due to the fact that it is an acute phase reactant protein. (Eftekhari et al, 2012). Probably, future studies might focus on evaluating serum soluble transferrin receptor concentration that would best indicate the iron status in human beings.

To conclude, we found out significantly higher levels of serum iron and ferritin in patients with CAD in comparison to age and sex matched controls. However, large robust studies need to confirm the same.

**Conflict of interest:** None

**Acknowledgement:** None

## References

Auer J, Rammer M, Berent R, Weber T, Lassnig E, Eber B. 2002. Body iron stores and coronary atherosclerosis assessed by coronary angiography. Nutrition Metabolism Cardiovascular Disease, 12:285-90.

Bozzini C, Girelli D, Yinazzi E, Olivieri O, Stranieri C, Bassi A, *et al*. 2002. Biochemical and genetic markers of iron status and the risk of coronary artery disease: an angiography-based study. Clinical Chemistry, 48:622-628.

Celermajer DS, Chow CK, Marijon E, Anstey NM, Woo KS. 2012. Cardiovascular disease in the developing world: Prevalences, patterns, and the potential of early disease detection. Journal of the American College of Cardiology, 60:1207-16.

Danesh J. 1999. Coronary heart disease and iron status. Meta-analyses of prospective studies. Circulation, 99:852-54.

Eftekhari MH, Mozaffari-Khosravi H, Shidfar F, Zamani A. 2013. Relation between Body Iron Status and Cardiovascular Risk Factors in Patients with Cardiovascular Disease. International Journal of Preventive Medicine, 4:911-16.

Fuster V, Kelly BB. 2010. Institute of Medicine (US) Committee on Preventing the Global Epidemic of Cardiovascular Disease: Meeting the Challenges in Developing Countries; Fuster V, Kelly BB, eds. Promoting Cardiovascular Health in the Developing World: A Critical Challenge to Achieve Global Health. Washington (DC):) Chapter 2, Epidemiology of Cardiovascular Disease. National Academies Press, US.

Gupta AK, Bharadwaj A, Ashotra S. 2002. Feasibility and training of multipurpose workers in detection, prevention and control of coronary artery disease in apple-belt of Shimla hills. South Asian Journal of Preventive Cardiology, 6:17–22.

Gupta R, Gupta VP, Sarna M. 2002. Prevalence of coronary heart disease and risk factors in an urban Indian population: Jaipur Heart Watch-2. Indian Heart Journal, 54:59–66.

Kamili MA, Dar IH, Ali G. 2007. Prevalence of coronary heart disease in Kashmiris. Indian Heart Journal, 61:44–49.

Klipstein-Grobusch K, Koster JF, Grobbee DE, Lindemans J, Boeing H, Hofman A, *et al*. 1999. Serum ferritin and risk of myocardial infarction in the elderly: the Rotterdam Study. American Journal of Clinical Nutrition, 69:1231-6.

Kumar R, Singh MC, Ahlawat SK. 2006. Urbanization and coronary heart disease: a study of urban–rural differences in northern India. Indian Heart Journal, 58:126–130.

Mainous AG 3rd, Wells BJ, Everett CJ, Gill JM, King DE. 2004. Association of ferritin and lipids with C-reactive protein. American Journal of Cardiology, 93:559-62.

Mohan V, Deepa R, Rani SS. 2001. Prevalence of coronary artery disease and its relationship to lipids in a selected population in South India. Journal of Amerian College of Cardiology, 38:682–687.

Nough H, Sezavar H, Mohebbi A, Noohi F. 2006. Association between body iron stores and coronary artery disease. Iranian Heart Journal, 7:37-41.

O'Rourke RA, Brundage BH, Froelicher VF, Greenland P, Grundy SM, Hachamovitch R, *et al*. 2000. ACC/AHA expert consensus document on elctron beam computed tomography for the diagnosis of coronary artery disease. Circulation, 102:126-40.

Pourmoghaddas A, Sanei H, Garakyaraghi M, Esteki-Ghashghaei F, Gharaati M. 2014. The relation between body iron store and ferritin, and coronary artery disease. ARYA Atherosclerosis, 10:32-36.

Shokeen D, Aeri BT. 2015. Risk Factors Associated with the Increasing Cardiovascular Diseases Prevalence in India: A Review. Journal of Nutrition and Food Sciences, 5:331.

Sullivan J. 1989. The iron paradigm of ischemic heart disease. American Heart Journal, 117:1177-88.