Relationship between Body Iron Status and Cardiovascular Risk Factors in Patients with Coronary Artery Disease

Sunny Chopra[1], Ashok Kumar Lal[2], Anju Huria[3], Meenakshi Dhar[4]

[1]Assistant professor, Department of biochemistry, Gian sagar medical college and hospital, Banur, Rajpura(Patiala), India

[2]Prof and Head, Department of biochemistry, HIMS, Dehradun.India

[3]Prof and head, Department of Obst & gynae, GMCH Sec-32, Chandigarh, India

[4]Associate professor, Department of medicine, HIMS, Dehradun, India.

ABSTRACT

Background: Iron is an essential trace element. It has a pivotal role in maintaining various cellular functions and enzyme reactions; whereas, iron overload has been known as a risk factor in progression of atherosclerosis. **Objectives:** The aim of this study was to investigate the role of the serum iron, serum ferritin and total iron binding capacity (TIBC) in the causation of coronary artery disease (CAD) and their relationship with other risk factors of CAD. **Material and Methods:** The study group consisted of 40 angiographically confirmed cases of CAD and 40 healthy controls. Serum lipids, serum lipoproteins, serum iron and TIBC were estimated by autoanalyzer (Dxc 900 Beckman coulter). Serum ferritin was measured on Mini VIDAS and malondialdehyde (MDA) was being done by Thiobarbituric acid method. **Results:** Significant difference was found among the controls and CAD patients regarding the occurrence of weight, height, BMI, Hip circumference and Waist circumference and FBS. The ratio of Total cholesterol and HDL was significantly raised in CAD patients (4.80) than controls (3.48). Serum iron and Serum ferritin levels were significantly elevated in patients with CAD when compared with control groups (118.2 ± 22.7mg/dl versus 105 ± 19.6mg/dl, $p<0.001$) and (218.3 ± 58.6mg/dl versus 139.8 ± 66 mg/dl, $p<0.0001$) respectively. TIBC levels were lower in patients than controls (211.5 + 61.2 versus 309.8 + 79.2, $p<0.0001$). When body iron was compared with other risk factors (like smoking, hypertension, diabetes mellitus, tobacco etc) of the disease it was found to be significantly raised. **Conclusion:** Study concluded that increased levels of serum iron, ferritin might consider as risk factor for CAD in conjunction with other risk factors.

Key words: CAD, Ferritin, Iron, MDA,TIBC.

INTRODUCTION

Coronary artery disease (CAD) is an epidemic at present and by 2020, it will be the single most important disease in the world in the terms of mortality, morbidity, disability, and economic loss.[1] The cause behind this disease is deposition of atheromatous plaque in medium and large sized arteries supplying the myocardium. Major risk factors that instigate or accelerate the process of atherosclerosis are dyslipidemia, hypertension, diabetes mellitus, obesity, alcohol consumption, smoking, stress and oxidized low density lipoprotein [Ox-LDL].[2] Over the past decades, it was being observed that these risk factors account for only 50% of the incidence of the cardiac disease.[3] So, aggressive approaches to correct and modifying these risk factors are not enough. With this in mind, recently researches have taken an initiative to identify, the other unconventional risk factors for CAD, such as chronic infections and serum iron levels.

Iron is an essential trace element. It has a pivotal role in maintaining various cellular functions and mediates a

***Corresponding address:**
Dr. Sunny Chopra MBBS, MD
Department of Biochemistry,
224, Phase XI, Sector-65, Mohali,
Punjab-160055, India.
Phone no : 8283886629
E-mail: sunnybhagat16@gmail.com

DOI: 10.5530/jcdr.2015.1.3

variety of enzyme reactions. Two main forms of iron in the body are: transferrin and ferritin. Transferrin is a transport glycoprotein with two binding sites for free iron and ferritin is the partial measure of body iron stores. All the serum iron in the human body is bound to proteins except when it is increased. Free or excess iron has an ability to accept and donate electrons by exchanging between ferrous and ferric forms. This exchanging may generate reactive oxygen species (ROS) such as hydroxyl radical through Fenton and Haber-Weiss reactions, causing oxidative stress and oxidation of organic bio-molecules.[4] Iron overload would elevate the risk of CAD by promoting the lipid peroxidation, which is being measured by malondialdehyde (MDA).[5] This relationship between body iron and CAD was first observed by Jerome Sullivan, in 1981. According to his 'iron hypothesis', iron overload produces free radicals, which were subsequently, modify low density lipoprotein cholesterol (LDL) into oxidized LDL (ox-LDL). This ox-LDL is important in the pathogenesis of atherosclerosis and dysfunction of vascular endothelium.[6] This study was supported by various other studies done *in vitro* and *in vivo*.[7,8] The 17-year follow-up study done by Knuiman *et al*., in Australia, did not show any evidence in relation to ferritin level as a risk factor for cardiovascular disease.[9]

Increased body weight or obesity, is considered as the largest public health problem worldwide. It has been associated with an increased risk of morbidity and mortality from CAD, diabetes and other health problems. Obesity, measured by body mass index (BMI, kg/m^2), plays a key role in atherosclerosis mainly through oxidative stress. Evidences indicated that obesity may increase vulnerability of the atheromatous plaque to rupture through hyperlipidemia and inflammation.[10]

The medical literature studied so far did not show any clear consensus regarding the relationship between serum iron and CAD. So, the present study was planned to evaluate the role of body iron, ferritin and TIBC, if any, in causation of disease and whether they are considered as risk factors in the causation of CAD. We also evaluated the relationship between serum ferritin and MDA, a validated marker of lipid peroxidation.

MATERIAL AND METHODS

Study design

The study sample comprised of 124 individuals, attending medicine outpatient department and admitted in the Cardiac care unit of Himalayan institute of Medical Sciences, Dehradun, for coronary angiography. Coronary angiograms were assessed by an experience cardiologist. Finally, forty subjects who fulfilled all the inclusion criteria were chosen for the study. The criteria's for case inclusions were; a) the age of all subjects (case and control) between 30-60 years; b) angiographically confirmed cases of coronary artery disease. The criteria's for case exclusion were; a) any acute or chronic illness (severe kidney disease requiring dialysis, thalassemia, hemochromatosis or malignancy); b) vitamins, iron or antioxidant supplements. The control group comprised of forty inpatients without coronary artery disease and they were matched according to age, sex and same socioeconomic status.

The diagnosis of CAD was based on history of prolonged ischemic chest pain, positive troponin-I test, characteristic electrocardiogram [ECG] changes and was confirmed by angiography. Diabetes mellitus was diagnosed if fasting plasma glucose was >126 mg/dl. Patients were considered to be hypertensive if systolic blood pressure was >140 mm Hg and diastolic blood pressure was >90 mm Hg or self reported use of antihypertensive drugs. Demographic data, any concurrent illness history, and information of medication, smoking, diabetes, hypertension etc were collected by interviews or from the case notes of the patients. Anthropometric assessments included measurement of weight and height. Body weight was measured to the nearest 0.1 kg using the Seca 713 scales. Height of the participants without shoes was determined using measuring tape, and subsequently body mass index (BMI) was calculated by dividing weight (kg) by squared height (m^2). Waist circumference (WC) was measured using an inelastic tape over light clothing at the point midway between the iliac crest and the last floating rib at the end of a normal inhalation. Hip circumference (HC) was measured at the maximal gluteal protrusion or at the most prominent area of the buttocks at the level of symphysis pubis in a horizontal plane. The patients who had total cholesterol [TC] level of >220 mg/dl or triglyceride [TG] >200 mg/dl, high density lipoprotein [HDL-C] levels <45 mg/dl in males and <50 mg/dl in females were considered to be dyslipidemic.

All the participants (patients and controls) were given an oral and written explanation about the study, including its procedures, and were asked to read and sign an informed consent document. The study protocol and ethical aspects were approved by the ethics committee of the Himalayan institute of Medical Sciences, Dehradun.

Sample collection: All subjects (patients and controls) were instructed to observe an overnight fast for 12 hours prior to the venepuncture. All blood samples were drawn under

Table 1: Demographic, Para-Clinical and Clinical data of the subjects

Characteristic	Control group (n=40) (Mean±SD)	Cases group (n=40) (Mean±SD)	p-value
Age (Years)	51±4.6	55±3.8	NS
Weight (Kg)	68.5±1.8	71.8±1.0	<0.01
Height (cm)	174.8±1.2	176.4±1.4	NS
BMI (Kg/m2)	24.4±1.3	28.9±3.0	<0.001
Waist Circum ference (cm)	90.7±1.6	110.5±2.1	<0.001
Hip Circum ference (cm)	101.7±2	103.4±2.9	NS
Systolic blood pressure (mmHg)	118±8.5	148±7	<0.001
Diasystolic blood pressure (mmHg)	75±5	94±4.2	<0.001
FBS (mg/dl)	110±10.7	129±8.9	<0.001
LDL-C (mg/dl)	108.2±19.3	144±14.6	<0.0001
HDL-C (mg/dl)	44.8±8.6	39±6.7	<0.0001
Hemoglobin (g/dL)	10.9	13.7	<0.01

Table 2: An association of Iron, Ferritin and TIBC in controls and CAD patients

Parameters	Control group (Mean±SD)	Case group (Mean±SD)	p-value
Serum iron (mg/dl)	105±19.6	118.2±22.7	<0.001
Serum Ferritin (mg/dl)	139.8±66	218.3±58.6	<0.0001
TIBC (mg/dl)	309.8±79.2	211.5±61.2	<0.0001
MDA (mol/l)	4.9±1.0	19.9±1.7	<0.0001

aseptic conditions. Blood was being drawn in a vial or the syringe containing heparin to estimate the TIBC. Plain or yellow top vaccutainers were used for collecting blood samples to estimate iron and ferritn. For the estimation of MDA and hemoglobin, blood samples were collected in EDTA vaccutainer [purple top].

Serum ferritn was done on Mini VIDAS by automated quantitative test kit using an ELFA (Enzyme Linked Fluorescence Assay) technique. Iron and TIBC were estimated by using commercial kits from Weldon Biotec on fully automated analyzer (Beckman coulter DxC900). Serum total cholesterol (TC) and Serum high density lipoprotein- Cholesterol (HDL-C) were estimated by commercially available enzymatic reagents on auto-analyzer [Beckman culter, DxC900]. Serum low density lipoprotein cholesterol [LDL-C] was calculated using Friedwald's formula for samples with TG value less than 350 mg/dl.[11] MDA is a secondary product of lipid peroxidation. It was estimated by measurement of thiobarbituric acid [TBA] in plasma. Proteins were precipitated from plasma with 40% trichloroacetic acid. Precipitated proteins were incubated with TBA reagent in boiling water bath for 60 minutes. The pink chromogen formed was estimated against blank at 535 nm. Results were expressed as µmol/l[12]

Statistical analysis: The data analysis was carried out by using Statistical Package for the Social Sciences [SPSS] Version 17.0. Results were expressed as Mean (± SD). The

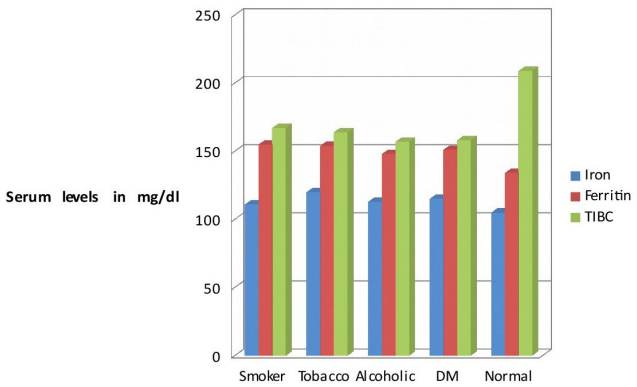

Figure 1: Comparison of Serum iron, Ferritin and TIBC in normal controls(without CAD) with different risk factors of CAD

statistical significance of difference between the various groups was determined by using the student's test; $p>0.05$ not significant, $p<0.05$ was significant, $p<0.01$was highly significant and $p<0.001$ was extremely significant.

RESULTS

The oxygen saturation of all the subjects (cases and controls) was observed to be normal. Table 1, summarizes the demographic data of the study subjects. Significant difference was found among the controls and CAD patients regarding the occurrence of weight, BMI, Hip circumference and Waist circumference, hemoglobin,

serum LDL-C, serum HDL-C and FBS. Both systolic and diastolic blood pressures were slightly higher in the men than in the women (130 versus 126 mm Hg and 110 versus 88 mm Hg, respectively).

Table 2, showed that the serum levels of iron, ferritin and MDA were significantly higher in CAD patients as compared to normal healthy controls. The levels of TIBC were found to be significantly lower in patients when compared to controls.

In the present study from the total forty CAD patients, fifteen were smokers (smokes 1-2 bundles i.e. 15-20 biddies per day), ten were taking tobacco, five with alcoholic and five were suffered from diabetic mellitus and rest with more than one risk factors. It was observed that the levels of iron (mg/dl) and ferritin (mg/dl) were significantly increased in smokers, alcoholics, tobacco chewers and DM patients than normal healthy controls (The values were mentioned along with figure). The levels of TIBC (mg/dl) were found to be lower in patients with different risk factors as compared to healthy subjects Figure 1.

DISCUSSION

The current study showed a significant difference among the study groups, regarding the serum iron and ferritin levels were increased in CAD patients as compared to healthy control group. In addition the levels of TIBC were found to be lower in patients than normal individuals. The results suggest an association of abnormality of body iron store and the development of coronary artery disease.

The hypothesis that body iron stores are associated with risk of CAD has been generated an extensive debate in the literature. In clinical medicine, ferritin is predominantly utilized as a serum marker of total body iron stores. Excessive iron is capable of stimulating the progression of atherosclerotic lesions. It reduces the levels of antioxidants in plasma, increases the production of free radicals, and promotes lipid peroxidation; therefore, it can be associated with the progression of atherosclerosis and increase in the risk of cardiovascular events in the body.[13]

Haidari *et al.* in 2001 conducted a study on 400 CAD patients. This study concluded that high stored iron concentration, as assessed by serum ferritin, is a strong and independent risk factor for premature CAD in the male Iranian population. The results were also showed serum iron and transferrin saturation significantly high, whereas total iron binding capacity was found to be significantly low in coronary heart disease patients as compared to the control subject.[14] The findings of Ralph *et al* (2010) support a biologic rationale for measurement of serial ferritin levels in patients with atherosclerosis. Because iron-induced oxidative stress contributes to inflammatory responses, determination of optimal iron marker levels to be maintained by calibrated phlebotomy is a clinically relevant concept for future outcome studies in ischemic heart disease.[15]

Kraml P *et al* (2004) conducted a case-control study, which enrolled 216 subjects (76 patients of cardio vascular disease and 140 healthy controls). They observed that the plasma ferritn levels were found to be significantly increased while anti-oxLDL antibodies, nitrites/nitrates, tocopherol and HDL levels were significantly decreased in patients, as compared to healthy controls. Study supports the hypothesis that high ferritin levels contributes to oxidative stress and thus elevate the risk for development of cardiovascular disease.[16]

On the other hand, there are many investigations that were inconsistent with our findings. Armaganijan and Batlouni in 2003, done a comparative clinical study between two groups, one group had patients with coronary atherosclerosis and second group had patients without coronary atherosclerosis. Study suggested that serum ferritin and other organic iron indicators were neither risk factors nor risk markers for coronary atherosclerosis and serum iron levels were higher in the group without atherosclerosis.[17] Auer *et al.* conducted a study on 100 white subjects who underwent coronary angiography. They proposed that higher ferritin concentrations and transferrin saturation levels were not associated with an increased extent of coronary atherosclerosis in patients who referred for coronary angiography.[18]

Overweight and physical inactivity are major risk factors in CAD. Weight loss through reduced calorie intake and improved fitness through increased physical activity are central strategies in both primary and secondary prevention. CAD mortality has decreased considerably during the last 30 years due to improved treatment, prevention and rehabilitation. Obesity affects the cardiovascular system directly as well as indirectly in many ways. Numerous studies have shown that anthropometric indices including body mass index (BMI), waist circumference, waist-hip ratio, and waist-height ratio are associated with CHD risk factors or adverse events.[19-22] A growing number of studies suggest a potential link between obesity and altered iron metabolism. It may be a result of obesity-related inflammation and/or related comorbidities.[23]

Study done in 2012, have explored the possible biological interaction of serum ferritin levels and BMI with CAD risk,

and found evidence of additive interaction. It concluded that obesity could elevate serum ferritin levels. This finding provided further evidence that iron overload to the risk of CAD appeared to be most significant among persons with higher BMI.[24] Terry Martin proposed that cigarette smoke does its damage through similar mechanisms. One is oxidative stress that mutates DNA, promotes atherosclerosis, and leads to chronic lung injury. Oxidative stress is thought to be the general mechanism behind the aging process, contributing to the development of cancer, cardiovascular disease.[25] Many studies suggested that elevated serum ferritin increased the risk of atherosclerosis in the presence of other risk factors. Ferritin can act as a catalyzer in the production of oxygen free radicals and lipid peroxidation and play a role in the formation of oxidized LDL.[26]

In conclusion, this study indicated an association between a positive balance of body iron and coronary artery disease, so the measurement of Iron, Ferritin and OX- LDL could be of great assistance in predicating premature coronary artery disease. The present study also gives message to the clinicians that, caution should be exercised in administration of iron supplements to patients with coronary artery disease and in consumption of food rich in iron by them.

CONCLUSION

In conclusion, this study indicated an association between a positive balance of body iron and coronary artery disease, so the measurement of Iron, Ferritin and OX- LDL could be of great assistance in predicating premature coronary artery disease. The present study also gives message to the clinicians that, caution should be exercised in administration of iron supplements to patients with coronary artery disease and in consumption of food rich in iron by them.

CONFLICT OF INTEREST

Authors have no conflict of interests.

ACKNOWLEDGEMENTS

The present study was funded by Himalayan institute hospital trust (HIHT), jolly grant, Dehradun. We thank Dr Anurag Rawat (cardiologist), nurses and staff of cardiology and CTVS wards and laboratory technicians of biochemistry department for their cooperation. We gratefully acknowledge Dr Anju huria for her good cooperation and support.

REFERENCES

1. Gazino JM. Global burden of cardio vascular disease. In Braunwald Eugene, Heart disease a text book of cardiovascular medicine. edition, editor. Philadelphia, Pensylvania: WB Saunders Company; 2005.
2. Kasap S, Gonenc A, Sener DE, Hisar I. Serum cardiac markers in patients with acute myocardial infarction: Oxidative stress, C-reactive protein and NT erminal Probrain Natriuretic Peptide. J Clin Biochem Nutr. 2007; 4(1): 50-7.
3. Rehema A, Zilmer M, Zilmer K, Kullisaar T, Vihalemm T. Could longterm alimentary iron overload have an impact on the parameters of oxidative stress? A study on the basis of a village in southern Estonia. Ann Nutr Metab. 1998; 42(1): 403.
4. Halliwell B, Gutteridge JMC. Role of free radicals and catalytic metal ions in human disease: an overview. Meth Enzymol. 1990; 186: 1– 85.
5. Yesilbursa D, Serdar Z, Serdar A, Dirican M, Gemici K, Ozdermir A, *et al.* The relationship of serum ferritin with malondialdehyde concentration in patients with coronary artery disease: Ferritin and oxidative stress in CAD. International journal of Angiology 2001; 10(2): 88-91.
6. Sullivan JL. Iron and the sex difference in heart disease risk. Lancet 1981; 1(8233): 1293-4.
7. Baykan M, Celik U, Orem A, Malkoc M, Erdol C, Baykan EC, *et al.* Iron status and its relationship with lipid peroxidation in patients with acute myocardial infarction. Acta Cardiol. 2001; 56(5): 277- 81.
8. Klipstein-Grobusch K, Koster JF, Grobbee DE, Lindemans J, Boeing H, Hofman A, *et al.* Serum ferritin and risk of myocardial infarction in the elderly: the Rotterdam Study. Am J Clin Nutr. 1999; 69(6): 1231–6.
9. Knuiman MW, Divitini ML, Olynyk JK, Cullen DJ, Bartholomew HC. Serum ferritin and cardiovascular disease: a 17-year follow-up study in Busselton, Western Australia. Am J Epidemiol. 2003; 158(2): 144-9.
10. Visser M, Bouter LM, McQuillan GM, *et al.* Elevated C-reactive protein levels in overweight and obese adults. JAMA. 1999; 282(22): 2131–2.
11. Friedwald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preoperative ultracentrifuge. Clin Chem.1972; 18(1): 499-502.
12. Yagi K. Lipid peroxides and human diseases. Chem Phys Lipids. 1978; 45(2): 337-51.
13. Zheng H, Cable R, Spencer B, Votto N, Katz SD. Iron stores and vascular function in voluntary blood donors. Arterioscler Thromb Vasc Biol. 2005; 25(8): 1577–83.
14. Haidari M, Javadi E, Sanati A, Hajilooi M, Ghanbili J. Association of increased ferritin with premature coronary stenosis in men. Clin Chem. 2001; 47(9): 1666–72.
15. Ralph GD, Hayes VW, Chow BK, Shamayeva G, May PE, Zacharski LR. Ferritin levels, inflammatory biomarkers, and mortality in peripheral arterial disease: A substudy of the Iron (Fe) and Atherosclerosis Study (FeAST) Trial. Journal of Vascular Surgery 2010; 51(6): 1498– 503.
16. Kraml P, Potockova J, Koprivova H, Stipek S, Crkovska J, Zima T, *et al.* Ferritin, oxidative stress and coronary atherosclerosis. Vnitr Lek. 2004; 50(3): 197–202.
17. Armaganijan D, Batlouni M. Serum ferritin levels and other indicators of organic iron as risk factors or markers in coronary artery disease. Rev Port Cardiol. 2003; 22(2): 185–95.
18. Auer J, Rammer M, Berent R, Weber T, Lassnig E, Eber B. Body iron stores and coronary atherosclerosis assessed by coronary angiography. Nutr Metab Cardiovasc Dis. 2002; 12(5): 285–90.
19. Sowers JR. Obesity as a cardiovascular risk factor. *Am J Med.*2003; 115 (suppl 8A): 37S-41.
20. S.Kanaya AM, Vittinghoff E, Shlipak MG. et al. Association of total and central obesity with mortality in postmenopausal women with coronary heart disease. Am J Epidemiol. 2003; 158(12): 1161-70.
21. Pradhan AD, Skerrett PJ, Manson JE. Obesity, diabetes, and coronary risk in women. J Cardiovasc Risk. 2002; 9(6): 323-330.
22. Johansson S, Wilhelmsen L, Lappas G, Rosengren A. High lipid levels and coronary disease in women in Goteborg—outcome and secular trends: a prospective 19 year follow-up in the BEDA study. *Eur Heart J.* 2003; 24(8): 704-16.

23. Heeringa J, Kors JA, Hofman A, van Rooij FJ, Witteman JC. Cigarette smoking and the risk of atrial fibrillation: the Rotterdam study. Am Heart J. 2008; 156(6): 1163- 9.
24. Cheng HL, Bryant C, Cook R, *et al*. The relationship between obesity and hypoferraemia in adults: a systematic review. Obes Rev. 2012; 13(2): 150–61.
25. Terry Martin, About.com Guide Updated July 20, 2008 .About.com Health's Disease and Condition content is reviewed by our Medical Review Board.
26. Liao X, Lv C, Zhang X, Masuda T, Li M, Zhao G. A novel strategy of natural plant ferritin to protect DNA from oxidative damage during iron oxidation.Free Radic Biol Med. 2012; 53(2): 375-82.