Iron and Ferritin as novel screening markers of Acute Myocardial Infarction

S. Aishwarya¹, M.Vasanthan^{1*}, V.M.Vinodhini¹, Renuka. P¹ & V.E. Dhandapani²

¹ Department of Biochemistry, SRM Medical College Hospital & Research Centre, Faculty of Medicine and Health Sciences, SRM Institute of Science and Technology, SRM Nagar, Kattankulathur, 603203, Kanchipuram, Chennai, TN, India.

² Department of Cardiology, SRM Medical College Hospital & Research Centre, Faculty of Medicine and Health Sciences, SRM Institute of Science and Technology, SRM Nagar, Kattankulathur, 603203, Kanchipuram, Chennai, TN, India.

***Corresponding author:** Dr.M.Vasanthan, Department of Biochemistry, SRM Medical College Hospital & Research Centre, Faculty of Medicine and Health Sciences, SRM Institute of Science and Technology, SRM Nagar, Kattankulathur, 603203, Kanchipuram, Chennai, TN, India.

Email: vasanthm1@srmist.edu.in

ORCID LINK: <https://orcid.org/0000-0002-4879-0860>

Abstract

Introduction: Cardiovascular disease is a public health problem globally. In India, the prevalence of Acute Myocardial Infarction (AMI) was 6.43%. Several causes lead to AMI, among which excess serum Iron and Ferritin is responsible for an increase in the generation of free radicals, thus accelerate atherogenesis through oxidation of Low-density lipoprotein – cholesterol (LDL-C). Assess serum Iron and Ferritin status and compare them with the cardiac marker (CK-MB) among patients with AMI.

Methods: The study was performed in a tertiary care centre in TamilNadu, India. This case-control study was conducted on 25 patients diagnosed with AMI and 25 age and sex-matched healthy participants as control group. Iron, Ferritin, CK-MB and lipid profile parameters were analyzed in serum samples of the participants.

Result: The mean levels of serum Iron, Ferritin, CK-MB and lipid profile parameters were increased significantly among the cases when compared to the control group. CK-MB had a strong positive correlation with serum Iron and Ferritin. Serum Iron and Ferritin also had a positive correlation with total cholesterol and LDL-C, and a negative correlation with High-density lipoprotein – cholesterol. The odds of developing AMI in those with serum Iron >51.5µg/dl and Ferritin >137ng/dl were 36 and 61.7 times at higher risk respectively.

Conclusion: Higher levels of serum Iron and Ferritin seem to be stronger risk factors for AMI, and shall be considered as early screening cardiac markers for AMI.

Keywords: Acute Myocardial Infarction, Iron, Ferritin.

1. Introduction

In 2014, coronary heart disease (CHD), a worldwide community health problem contributed to 10% of the global disease load and 30 % of the global mortality rate [1]. The incidence of CHD in 2000- 2010 was 300.6/100,000 persons/year for men and 47.9/100,000 persons/ year for women worldwide, of that 17.5 million death from CHD is reported [2]. In the year 2009, the prevalence of Acute Myocardial Infarction (AMI) in India was reported as 64.37 / 1000 people in men between the age group of 29-69 years, which were slightly lesser in women. The mortality rate of AMI in South India was 13.2%, of which the survival probability was less among males [3].

Myocardial Infarction (MI) is the result of myocardial necrosis due to significant and sustained ischemia, according to the World Health Organization (WHO). Therefore, obstruction of blood flow as the result of plaque formation or due to other obstructing mechanisms results in AMI. Plaque formation is always a magnitude of atherosclerosis [4]. Several causes lead to AMI in the adult population, which include age, sex, smoking, lack of exercise, hyperlipidemia, Diabetes Mellitus (DM), obesity, high blood pressure (Hypertension), alcoholism, high levels of Homocysteine and serum Folate [5]. In the majority of cases, AMI was found to have a direct link with hyperlipidemia. The most predominant form of hyperlipidemia was found to have increased LDL cholesterol and low HDL cholesterol levels [6]. Hyperlipidemia is a condition that results in the deposition of lipids in the sub endothelium of the arteries. The lesions at the earlier stages will be consisting of subendothelial accumulations of macrophages engorged with cholesterol, called 'foam cells', as they are the precursors of 'fibrous cap'. This Plaque formation undergoes ulceration and calcification which results in hemorrhage from small arteries that develop into a lesion. This lesion advances and blocks the blood flow, resulting in MI or stroke [7].

A multi-marker approach is available that uses several biomarkers, with each representing a different pathway. The approach includes the presently available markers of myocardial damage such as Creatine Kinase (CK), CK-MB isoform, Heart-type fatty acid-binding protein, cardiac Troponin I and T, and Myoglobin [8]. This in turn, is followed by the inflammatory markers such as C-Reactive protein (CRP), D-dimer, Renin, Plasminogen activator inhibitor type I (PAI 1), N-terminal pro-atrial and B-type natriuretic peptide, Fibrinogen, Aldosterone, Urinary Albumin Creatine Ratio and Homocysteine [7]. In addition to these risk factors and biomarkers, it seems that there are yet many other risk factors causing MI [9].

National Health and Nutrition Examination Survey III (NHANES III) reported a significant positive relationship with increase Iron loading and risk of AMI [10]. In our body, Iron is stored as a protein complex, Ferritin. Increased serum Ferritin is a highly capable risk factor for CHD. The intracellular Ferritin concentration is a measure of total Iron store of the body [11]. Hence, an elevated level of serum Iron and Ferritin leads to an elevated risk of AMI. Excessive Iron load mainly in myocardial tissue is a probable risk factor of AMI [6].

Besides, there are several discrete pathways and molecular mechanisms involved in atherogenesis as a result of increased Iron load that leads to AMI. The role of Iron in the pathogenesis of CHD like AMI is involved by catalyzing various free-radical in various oxidative reactions. Iron in the reduced form (Fe^{2+}) contributes to the following reactions

known as the Haber–Weiss and Fenton reaction that catalyzes the free radicals such as hydrogen peroxide (H_2O_2) and superoxide (O_2^-) that result in the generation of hydroxyl radicals ($\text{OH}^\cdot$) [12].

From Heme and Ferritin, Iron is released by Reactive Oxygen Species (ROS) during oxidative stress. This leads to the complex development of atherosclerosis, by increasing the low-density lipoprotein (LDL) peroxidation and thereby its absorption by macrophages that leads to the development of foam cells, leading to AMI [11]. The Fenton and Haber-Weiss reactions are also related to the elucidation of Iron's role in cardiac reperfusion injury. Following ischemic injury, there is reperfusion of cardiac myocytes which realizes O_2^- and H_2O_2 . These ROS generate the highly toxic $\text{OH}^\cdot$, in the presence of intracellular iron, thus supporting lipid peroxidation, and release of hydrolytic enzymes with increase intracellular lysozyme fragility complicating pre-existing atherosclerosis, leading to AMI [11]. Another evidence is that Iron oxidatively damages the atherosclerotic plaque by promoting endothelial activation, enhancing adhesion molecule expression. Circulating Iron oxidize LDL-C thus enhances LDL retention in sub-endothelium with macrophage progression to form foam cells leading to sudden AMI [14]. On the other hand, Iron deficiency is also found to have a strong association with adverse outcomes in acute coronary syndrome (ACS) [15]. There is also evidence that a comparatively low level of Iron may be a risk factor for coronary events [16]. Hence, this study is intended to find the Iron and Ferritin status and compare them with a cardiac marker (CK-MB) in AMI patients.

2. Materials and Methods

The case-control study was conducted from December 2020 to March 2021 on 50 participants in the age group 25-55 years. The participants were divided into two groups out of which, 25 Acute Myocardial Infarction patients admitted in CICU (Cardiac Intensive Care Unit) were included in the case group and 25 included the control group with age and sex-matched healthy participants who attended the MHC (Master Health Checkup).

Inclusion Criteria:

Clinically diagnosed Patients with AMI, both male and female within the age group of 25-55 years for the case and healthy patients of the same age as control.

Exclusion Criteria:

1. Patients with clinical conditions that will affect the serum Iron and Ferritin levels like liver disease, kidney disease, malabsorption syndrome, post-surgical patients on recovery, patients with carcinoma disease, smoker, pregnancy, patients with the acute and chronic bleeding condition, hemochromatosis, tuberculosis, chronic inflammatory diseases, those on medications that affect the serum Iron and Ferritin were excluded from the study.
2. Past History of AMI or CHD.
3. Age group <25 & >55 years.

All patients registered in the study were explained about the study. Informed written consent was taken from all the participants. A detail history and relevant anthropometric measurements like height, weight and BMI were recorded. Under aseptic precautions, 2 ml venous blood sample was collected from antecubital vein after overnight fasting of 10-12 hours from all the subjects in a plain vacutainer tube. Analysis of cardiac marker (CK-MB) by UV Kinetic G6P, serum Iron by TPTZ Binding method, Total Cholesterol by CHOD-POD, Triglycerides by enzymatic GPO-PGO, VLDL-C by calculation using Freidwald's formula, HDL-C and LDL-C by Direct Antibody Inhibition by Immunoturbidimetric method using Beckman Coulter Auto analyzer were carried out. Serum Ferritin was estimated by Enhanced CLIA VITROS Eci Immunoanalyzer by Ortho Clinical Diagnostics.

Data were analyzed using Statistical Package of Social Service (SPSS 22.0). Continuous variables were presented as Mean $\pm$ SD for normally distributed data. Categorical variables were expressed as frequencies and percentages. Student's t- test was used to compare the difference between the mean levels of parameters between the two groups. Pearson's correlation and calculation of odds ratio were done using statistical software. For all statistical tests, a p value $< .05$ was taken to indicate a significant difference. Results were graphically represented. Graphical representation was done in MS Excel 2010.

3. Results

A total of 50 subjects were studied in which 25 subjects diagnosed with acute myocardial infarction were in case group and 25 healthy subjects were in control group. The mean ages of controls and cases were 39.4 ± 9.35 years and 45.24 ± 8.36 years respectively age range 25-55 years. Among 50 subjects about 72% control and 76% case were male and 28% control and 24% case were female (Figure I). The mean BMI (Kg/m^2) for control (24.58 ± 3.2) and case (26.6 ± 3.2) were calculated and found there is a significant difference ($p=.025^*$) between mean levels of case and control (Table I).

Table I: Demographic features of the study group

Parameters	Control (n= 25)	Case (n = 25)	p value
Mean age (years, mean $\pm$ SD)	39.48 ± 9.35	45.24 ± 8.36	-
Male %	72%	76%	-
Female %	28%	24	-
BMI (Kg/m^2 , mean $\pm$ SD)	24.58 ± 3.2	26.57 ± 3.23	.029*

The values are statistically significant based on the p value.*p value $< .05$, ** p value $< .01$, *** p value $< .001$, NS-Not Significant.

Figure I: Distribution of Case and control according to Sex

The mean values of CK-MB, Iron, Ferritin, Total Cholesterol, Triglyceride, LDL-C, and VLDL-C were found to be elevated in the AMI patients when compared to the healthy individuals. The HDL-C levels did not vary significantly (Table II).

Table II: Comparison of Mean $\pm$ SD of the Measured Biochemical Parameters between the AMI Patients (Case) and Healthy Subjects (Control)

PARAMETERS	CASE (n =25)	CONTROL (n =25)	p VALUE
CK-MB (U/L)	149.08 $\pm$ 140.49	12.4 $\pm$ 4.48	<.0001***
Iron (μg/dl)	83.16 $\pm$ 34.1	48.64 $\pm$ 10.2	<.0001***
Ferritin (ng/dl)	202.71 $\pm$ 109.53	20.36 $\pm$ 14.15	<.0001***
T. Cholesterol (mg/dl)	221.76 $\pm$ 25.74	177.8 $\pm$ 29	<.0001***
Triglyceride (mg/dl)	162.8 $\pm$ 65.97	111.16 $\pm$ 35.38	.001**
HDL-C (mg/dl)	37.28 $\pm$ 10.89	42.72 $\pm$ 11.58	.03*
LDL-C (mg/dl)	152 $\pm$ 27.2	112.36 $\pm$ 23.90	<.0001***
VLDL-C (mg/dl)	32.48 $\pm$ 13.28	22.72 $\pm$ 7.83	.002**

CK-MB- Creatine Kinase-MB, TC- Total Cholesterol, Tgl- Triglyceride, HDL-C – High density Lipoprotein-Cholesterol, LDL-C – Low density Lipoprotein-Cholesterol, VLDL-C – Very Low density Lipoprotein-Cholesterol

Values are expressed in mean $\pm$ standard deviation.

The values are statistically significant based on the p value. * p value < .05, ** p value < .01, *** p value <.001, NS-Not Significant.

Pearson correlation analysis with CK-MB showed a strong positive correlation with serum Iron and serum Ferritin (Table III).

Table III: Pearson correlation analysis of CK-MB with iron and ferritin in subjects with AMI

Parameters	CK-MB	p value
Iron	.829 ^a	<.0001***
Ferritin	.821 ^a	<.0001***

The values are statistically significant based on the p value.* p value < .05, ** p value < .01, *** p value <.001, NS-Not Significant.

^aPositive Correlation

Pearson correlation analysis with serum Iron and Ferritin showed a strong positive correlation with total cholesterol and LDL-C and a negative correlation with HDL-C (Table IV).

Table IV: Pearson correlation analysis between iron and ferritin with lipid profile parameters in subjects with AMI

Parameters	Iron	p value	Ferritin	p value
Total Cholesterol	.719 ^a	<.0001	.735	<.0001***
LDL-C	.853 ^a	<.0001	.840	<.0001***
HDL-C	-.667 ^b	<.0001	-.716	<.0001***

The values are statistically significant based on the p value.* p value < .05, ** p value < .01, *** p value <.001, NS-Not Significant.

^aPositive Correlation

^bNegative Correlation

Receiver operating characteristic (ROC) curve was created to evaluate the risk of AMI by measuring serum Iron. Serum Iron cut-off value of ≥ 51.5 $\mu\text{g/dl}$ is shown to be predictive of AMI with 96% sensitivity and 40% specificity (Figure II).

Figure II: ROC curve computed in SPSS software 25 for optimal cut-off level of serum iron with AMI. The cut-off determined by the area near the highest sensitivity and specificity.

The area under curve (AUC) was moderately high (AUC = .888, 95% CI = 0.802-0.974, p value< .0001) indicative of high sensitivity and specificity to predict AMI with serum Iron.

On calculating the odds ratio to predict the risk of AMI, high serum Iron >51.5µg/dl (P = 0.001, OR = 36, 95% CI = 4.17- 310.45), high serum Ferritin >137ng/dl (P = 0.0002, OR = 61.71, 95% CI = 6.96 - 547.36), high serum Total Cholesterol >200 mg/dl (P = <0.0001, OR = 21, 95% CI = 4.92 – 89.56), high Triglyceride >150mg/dl (P 0.01, OR = 4.84; 95%, CI = 1.28 – 18.25) and high LDL-C >130 mg/dl (P =0.0001, OR = 16; 95%, CI = 4.001 – 63.97) were found to be independently associated with AMI (Table V).

Table V: Association between parameters in different group of participants.

Variables	Odds ratio	95% CI interval	p Value
Iron	36	4.17-310.45	0.001**
Ferritin	61.71	6.96 - 547.36	.0002**
Total cholesterol	21	4.92 - 89.56	<.0001***
Triglyceride	4.84	1.28 - 18.25	.01*
HDL-C	0.47	0.04 - 5.65	.55
LDL-C	16	4.001 - 63.97	<.0001***
VLDL-C	4.57	0.47 - 44.17	0.18

The values are statistically significant based on the p value. * p value < .05, ** p value < .01, *** p value <.001, NS-Not Significant.

4. Discussion

Cardiovascular disease is a worldwide community health problem. Several causes lead to the increased risk of AMI [1]. From various epidemiological studies, a positive relationship between body Iron stores and CHD have been found [11]. Later, an indication of relation with increased serum Ferritin concentration and the risk of AMI was evident from numerous findings, which is in concordance to the present findings [17]. Some studies, in contrast, show an insignificant relationship between Ferritin and AMI [16].

In the present study, the patients in both the study groups were in the age group of 46-55 years (52%). The mean age in the cases group was 45.24 ± 8.36 years as compared to 39.48 ± 9.35 years among the control, in which majority of the patients in both the groups were males (18 control and 19 cases) compared with females (7 control and 6 cases). Mudiappa Herakall et al study found the mean age was 48.3 ± 14.24 case and 47.6 ± 8.15 among the control group and the of male female ratio was 71/29 [17]. There was a significant difference in BMI of the patients (.029*) between groups as per the Student's t-test (p value <0.05). This

was similar to the study of M.P. Holay et al found a significant difference in BMI of patients in the controls and cases group [11].

In the present study, Total Cholesterol, Triglycerides, LDL-C, and VLDL-C values were significantly higher and HDL-C values were significantly lower in the case group as compared to the control group as per the Student's t-test (p value <0.05). A study by Jitender Sharma et al showed raised serum Total Cholesterol, LDL-C, and Triglycerides levels among AMI patients than the controls. Serum HDL-C levels were found to be decreased among the AMI patients than the controls [18].

Several types of research have delivered a strong proposal that oxidized LDL had a major activity in the development of atherosclerosis and CVD. Oxidation of LDL increases by the catalytic role of Iron in reaction those results in the generation of highly reactive OH^- . O_2^- anions generated during oxidative stress are capable of mobilizing Iron from Ferritin [19].

According to a study conducted by Moacir Fernandes de Godoy et al, severe obstructive CHD was reported among women with serum Ferritin levels greater than 80 ng/mL than those with lesser serum Ferritin levels. On the other hand, serum Ferritin did not play any role in the prediction of the degree of obstruction in men [16]. However, a study by Birger Wolff et al reported a stronger association of serum Ferritin levels in carotid atherosclerosis in men [20].

In this study, a significant increase in body Iron store was found in AMI patients compared with healthy controls, and serum Ferritin concentration was also significantly increased in AMI patients compared to the healthy controls. Iron and Ferritin status were compared with a cardiac marker (CK-MB) in AMI patients, as CK-MB is considered as a reliable marker for myocardial infarction. As CK-MB is an early marker of AMI that reaches a peak in 16-30 hours in MI [21]. CK-MB values were significantly elevated in AMI patients and in the present study a strong positive correlation of CK-MB with serum Iron and Ferritin ($r = .829$ and $r = .821$ respectively) was found.

A cross-sectional study by Morad Rostami et al reported a significant increase in the levels of serum Iron and Ferritin in patients with AMI when compared to controls [22]. The study by Vijaya BM et al concluded that, higher concentration of Ferritin is a potential risk factor for AMI [23]. The study by Chanchal L et al, also suggested increased serum Ferritin level is associated with AMI in the Manipuri male population and estimating of serum Ferritin may be used in confirming AMI [24].

A study by Silvia WD et al, noted that serum Ferritin was significantly direct associated with serum Cholesterol ($r=0.439$, $p<0.01$) and serum LDL-C ($r=0.381$, $p < 0.01$). Serum Ferritin had a significant inverse correlation with serum HDL-C ($r=-0.210$, $p < 0.05$) [25]. In the present study, similar finding was observed, that a strong positive correlation between serum Iron with serum Total Cholesterol ($r = .719$) and serum LDL-C ($r = .853$) and serum Ferritin is strongly negative correlated with HDL-C ($r = -.667$) and also a strong positive correlation was observed between serum Ferritin with serum Total Cholesterol ($r = .735$) and serum LDL-C ($r = .840$) and serum Ferritin is strong negatively correlated with HDL-C ($r = -.716$).

In this study, the optimal cut-off values obtained by the analysis for serum Iron is 51.5 $\mu\text{g/dl}$ (AUC = .888, 95% CI = 0.802-0.974, p value $< .0001$). On calculating the odds ratio to predict the risk of AMI, high serum Iron $>51.5\mu\text{g/dl}$ ($P = 0.001$, OR = 36, 95% CI = 4.17- 310.45), high serum Ferritin $>137\text{ng/dl}$ ($P = 0.0002$, OR = 61.71, 95% CI = 6.96 - 547.36), high

serum Total Cholesterol >200 mg/dl ($P = <0.0001$, OR = 21, 95% CI = 4.92 – 89.56), high Triglyceride >150mg/dl ($P = 0.01$, OR = 4.84; 95%, CI = 1.28 – 18.25) and high LDL-C >130 mg/dl ($P = 0.0001$, OR = 16; 95%, CI = 4.001 – 63.97) were found to be independently associated with AMI. This is similar to the study done by Holay MP et al, which shows high serum Ferritin, Total Cholesterol, Triglyceride, HDL-C, LDL-C and VLDL-C were reported to be significantly associated with AMI [11]. In the present study, HDL-C and VLDL-C were not associated with AMI.

Indian population with high body Iron stores is at greater risk of AMI [18]. Chelating of free Iron with Deferoxamine can reduce arrhythmia and improved myocardial function after AMI and the amount of Iron available in tissue can determine tissue susceptibility to ischemic. Iron reduction by Iron-chelating therapy or restrictions on the intake of Iron-rich foods could reduce atherosclerotic lesion size and increased plaque stability [22]. Therefore, the assessment of Iron and Ferritin status along with dyslipidemia can accelerate AMI.

The limitation of the study is the relatively small sample size and it was conducted in specific group of population. In addition, other risk factors like diet and physical inactivity could have been considered.

5. Conclusion

Elevated levels of serum Ferritin and Iron are seen in Acute Myocardial Infarction. Based on the “Iron hypothesis”, Iron is assumed to be injurious to the cardiovascular system in promoting atherosclerosis. Hence, higher levels of serum Iron and Ferritin seem to be strong risk factors for Acute Myocardial Infarction, which can be considered as early screening cardiac markers for Acute Myocardial Infarction. Therefore, screening for iron and ferritin levels among the high-risk subjects shall be beneficial in early prediction, diagnosis and management of Acute Myocardial Infarction.

6. Ethical approval

The institutional ethical committee approval {Ethics clearance number: 1872(A)/IEC/2019} was obtained before starting the study.

7. Acknowledgement

The authors sincerely thank the management of SRM Medical College Hospital and Research Centre, SRM IST for the support.

8. Conflict of interest

Declared none by the authors.

9. References

1. World Health Organization (WHO). *Global status report on non-communicable diseases*. Geneva Switzerland: World health organization; 2014.
2. Arriola L, Martinez- Camblor.P, Larranaga.N et al. Alcohol intake and the risk of coronary heart disease in the Spanish EPIC cohort study. *Heart*: 2010; 96:124–130. doi:10.1136/hrt.2009.173419.

3. Dr. Abhishek Sharma, Dr. MN Bhat. *Evaluation of mortality related to acute myocardial infarction in a tertiary care center in south India: A descriptive study. International Journal of Medical and Health Research. Volume 4; Issue 12; December 2018; Page No. 48-52.*
4. Shanthi Mendis, Kristian Thygesen, Kari Kuulasmaa et al. *World Health Organization definition of myocardial infarction: 2008–09 revision. International Journal of Epidemiology 2011; 40:139–146; doi:10.1093/ije/dyq165.*
5. Alberto Ascherio and David J. Hunter. *Iron and Myocardial Infarction. Lippincott Williams & Wilkins. Tue, 27 Aug 2019; Vol. 5, No. 2.*
6. Naresh Kumar, Suresh Kumar, Anil Kumar et al. *Lipid Profile of Patients with Acute Myocardial Infarction (AMI). Cureus 2019 March 18. Doi: 10.7759/cureus.4265.*
7. Aldons J. Lasis. *Atherosclerosis. NIH Public Access, 2000 September 14; 407(6801): 233–241. doi:10.1038/35025203*
8. Ulrich Forstermann, Ning Xia, Huige Li. *Roles of Vascular Oxidative Stress and Nitric Oxide in the Pathogenesis of Atherosclerosis. Circulation Research; 26, 2016; DOI: 10.1161 / CIRCRESAHA. 116.309326.*
9. Trushna Shah, Geetanjali Purohit, R M Shah et al. *Prevalence of Coronary Heart Disease in different socioeconomic status: Is dyslipidemia a future threat? International Journal of Biomedical and Advance Research 2015; 6(02): 120-123*
10. Shipra, Bharat Kumar Gupta, Ranjan Solank et al. *Relationship of Lipid Profile and Serum Ferritin levels with Acute Myocardial Infarction. Journal of Clinical and Diagnostic Research. 2014 Aug, Vol-8(8): CC10-CC13.*
11. M.P. Holay, A.A. Choudhary, S.D. Suryawanshi. *Serum ferritin—a novel risk factor in acute myocardial infarction. Indian Heart Journal 6402 (2012) 173–177.*
12. J P Kehrer. *The Haber–Weiss Reaction and Mechanisms of Toxicity. Toxicology 2000 Aug 14;149(1):43-50. doi: 10.1016/s0300-483x (00)00231-6.*
13. Gautam Ramakrishna, Thom W Rooke and Leslie T Cooper. *Iron and peripheral arterial disease: revisiting the iron hypothesis in a different light. Vascular Medicine 2003; 8: 2003–2010.*
14. Francesca Vinchi, Martina U. Muckenthaler, Milene C. Da Silva et al. *Atherogenesis and iron: from epidemiology to cellular level. Frontiers in pharmacology; published: 05 May 2014 doi: 10.3389/fphar.2014.00094.*
15. Tanja Zeller, Christoph Waldeyer, Francisco Ojeda et al. *Adverse Outcome Prediction of Iron Deficiency in Patients with Acute Coronary Syndrome. Biomolecules 2018, 8, 60; doi:10.3390/biom8030060.*
16. Moacir Fernandes de Godoy; Isabela Thomaz Takakura; Rafael Dinardi Machado et al. *Serum ferritin and obstructive coronary artery disease: angiographic correlation. Arq. Bras. Cardiol, Apr. 2007. vol.88 no.4.*
17. Mudiappa Herakall, MS Biradar. *A Study of Serum Ferritin in Acute Myocardial Infarction. Annals of International Medical and Dental Research, June 2018, Vol (4), Issue (5).*
18. Jitender Sharma, Bhawna Mahajan1, Roopali Rajput et al. *Analysis of Iron and Lipid Profiles in Indian Male Acute Myocardial Infarction Patients. Scholars Academic Journal of Biosciences, 2017; 5(5):359-362.*

19. Vijaya Lokary, M Vijaya Bhaskar, M Madhulatha et al. *Iron Status in ST- and Non-ST-elevated Myocardial Infarction. International Journal of Scientific Study, October 2016, Vol 4, Issue 7.*
20. Birger Wolff, MD, Henry Völzke, MD, Jan Lüdemann, PhD et al. *Association Between High Serum Ferritin Levels and Carotid Atherosclerosis in the Study of Health in Pomerania (SHIP). Stroke 1 February 2004, Volume 35, Issue 2, Pages 453-457.*
21. C. Daniel Cabaniss. *Creatine Kinase. Butterworth Publishers. 1990, Bookshelf ID: NBK352.*
22. Morad Rostami, Mohammad Aberomand, Alireza Khirollah et al. *Evaluation of Serum Iron and Ferritin Levels in Myocardial Infarction Patients. Journal of Bioinformatics and Intelligent Control Vol. 2, 79–82, 2013.*
23. Vijaya Bhaskar M, Srilekha S, Saranya M et al. *Assessment of ferritin and its association with C - reactive protein and malondialdehyde in acute myocardial infarction. International Journal of Research in Medical Sciences, 2015 Dec;3(12):3581-3585.*
24. Chanchal L, Shaini L, Sachin Deba Th et al. *Serum Ferritin Level in Male with Established Coronary Artery Disease. IOSR Journal of Dental and Medical Sciences, Dec. 2014, e-ISSN: 2279-0853, p-ISSN: 2279-0861. Volume 13, Issue 12 Ver. I, PP 58-62.*
25. Silvia WD, Biswas S, Uthappa S et al. *Ferritin, a Potent Threat for Acute Myocardial Infarction? JAPI. 2003;51;947-950.*