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Research Article

CORRELATION OF IRON AND MYELOPEROXIDASE LEVELS IN ISCHEMIC HEART DISEASE

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ABSTRACT

Objective: The objective of the study was to estimate, compare, and correlate serum iron and myeloperoxidase (MPO) levels in acute ischemic heart disease (IHD) cases.

Methods: Subjects for the study were divided into two groups of 35 acute IHD cases and 35 age and sex matched controls. Serum iron and MPO levels were assayed, and results compared.

Statistical analysis: The comparison between two groups was done using the Student's t-test. Correlation between iron and MPO was established using Pearson's correlation test.

Results: High serum MPO levels and low iron levels were observed in acute IHD cases in comparison to controls. There was no significant correlation between serum iron and MPO in acute IHD cases.

Conclusion: High iron levels have a beneficial role, whereas high MPO levels have an inflammatory role in the development of IHD. Determination of serum iron and MPO levels in high-risk subjects may help in taking proper measures to prevent acute IHD.

Keywords: Myeloperoxidase, Iron, Ischemic heart disease, Atherosclerosis.

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INTRODUCTION

Ischemic heart disease (IHD) is a condition that occurs when there is an imbalance between myocardial oxygen supply and demand. It is the leading cause of cardiovascular death worldwide, and the majority of deaths occur in countries like India [1,2]. Iron and myeloperoxidase (MPO) play an important role in the development of cardiovascular disorder, especially IHD, by promoting atherogenesis. The most common cause of myocardial ischemia is atherosclerosis of coronary arteries [1].

Iron plays an important role in physiologic functions because of its ability to serve both as an electron donor and acceptor. Being present as heme and non-heme iron in proteins, it is essential to many synthetic and enzymatic processes such as electron transfer and oxygen utilization [3,4]. Iron promotes free radical generation through the Fenton and/or Haber-Weiss reactions and increases plasma low-density lipoprotein (LDL) oxidation. Oxidized LDL promotes the accumulation of lipids in macrophages and foam cells and finally progresses to atherosclerosis and cardiovascular disease. Thus, iron acts as an independent risk factor for myocardial infarction [4].

MPO is a hemoprotein released by activated neutrophils and is characterized by pro-oxidative and pro-inflammatory properties that play an important role in thrombosis, plaque instability, and vasoconstriction [5]. Published literature suggests MPO as a cardiac biomarker [6]. MPO generates free radicals, induces inflammation, decreases nitric oxide (NO) levels, and is involved in all stages of atherogenesis from endothelial dysfunction to plaque rupture. This leads to acute coronary syndromes [5,7,8].

High iron levels increase the activity of MPO, and high levels of MPO reduce NO bioavailability and favor atheroma formation [4,7]. As MPO is an iron containing protein, its level can be increased with an increase in iron level. Similarly, iron depletion decreases the activity of harmful iron dependent enzymes and protects against IHD [4]. This study was conducted to estimate, compare, and correlate serum iron and MPO levels in acute IHD cases.

METHODS

The present case control study was conducted in the Department of Biochemistry, in collaboration with the Department of Cardiology, K.S. Hegde Charitable Hospital, Mangaluru, from October 2015 to March 2016. After obtaining ethical clearance from the Institutional Ethical Committee, 35 acute IHD cases and 35 healthy age and sex matched controls were recruited for the study. Acute IHD cases were recruited from the cardiology ward and were selected based on the clinical history and clinical examination. The diagnosis of IHD was based on the findings in ECG or echocardiogram or elevation of cardiac markers. Control subjects were selected from the general population.

Acute IHD cases had hemoglobin > 12 g/dL and were within the age group of 30–60 years. Patients with a history of diabetes and hypertension (HTN) who were on regular treatment were also included in this study group. Control group subjects did not have any risk factors or addictions. Patients with a history of chronic kidney disease, chronic liver disease, blood donation in the past 3 months, on iron or antioxidant therapy, infections, polycythemia, malignancy, anemia, and pregnant women were excluded from the study.

After obtaining informed consent, 5 ml of blood samples were collected in plain red-topped Vacutainer tubes containing clot activator. Blood samples were centrifuged, and sera were separated. Serum iron was estimated immediately in the autoanalyzer by ferrozine method [9]. Sera were stored at −20°C till analysis for the estimation of MPO.

Serum MPO was estimated by Matheson *et al.* method [10] using a spectrophotometer.

RESULTS

The characteristics of the study group are shown in Table 1. In each group out of 35, 29 were male and 6 were female. Out of 35 acute IHD cases, 26 had ST-segment elevated myocardial infarction (STEMI), 5 had non-ST-segment elevated myocardial infarction (NSTEMI), and 4 had unstable angina (UA). Among acute IHD cases, one subject had diabetes mellitus (DM), 12 had hypertension (HTN), and 5 had both DM and HTN. Numerically, among acute IHD cases, seven subjects were alcoholics, 11 were smokers, and 3 had a history of both alcoholism and smoking. A comparison between the above subgroups was not done as the number of subjects in each subgroup was less in number. A comparison of mean values of iron and MPO between acute IHD cases and controls is shown in Table 2. Correlation of serum iron with MPO levels in acute IHD cases (r=0.304) is shown in Fig. 1.

DISCUSSION

IHD contributes to 12.2% of total deaths worldwide and is expected to be one of the four major causes of death by 2030 [11]. The major cause of IHD is atherosclerosis [1]. The most common clinical presentations of IHD are STEMI, NSTEMI, and UA [12]. STEMI results from complete and prolonged occlusion of a coronary blood vessel and is defined based on ECG criteria. NSTEMI is defined by an elevation of cardiac biomarkers in the absence of ST elevation. The syndrome is termed UA in the absence of elevated cardiac enzymes. History, physical examination, ECG, biochemical markers, and echocardiography remain important tools to make an appropriate diagnosis [13].

Table 1: Characteristics of the study groups

Parameter	Acute IHD cases	Controls
Age (years) (mean±SD)	54.09±6.73	53.11±5.24
BMI (kg/m^2) (mean±SD)	22.92±2.21	23.38±1.54
Hb (%) (mean±SD)	13.90±1.34	13.68±0.79

BMI: Body mass index, Hb: Hemoglobin, SD: Standard deviation. IHD: Ischemic heart disease

Table 2: Serum iron and MPO levels in acute IHD cases and controls

Parameter	Acute IHD cases	Controls	p-value
Iron (µg/dL) (mean±SD)	78.43±30.4	94.17±18.01	<0.05
MPO (µmoles/L) (mean±SD)	393.59±135.29	75.16±21.03	<0.01

MPO: Myeloperoxidase, SD: Standard deviation. IHD: Ischemic heart disease

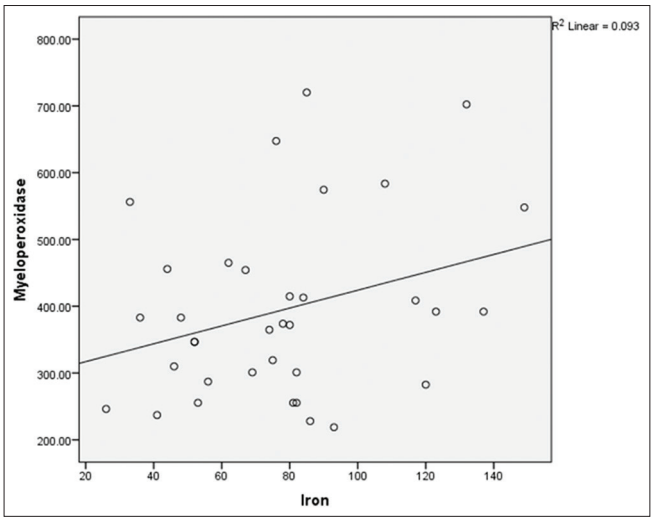

Fig. 1: Correlation of serum iron and myeloperoxidase levels in acute ischemic heart disease cases

MPO generates a number of reactive species, including hypochlorous acid, chloramines, tyrosyl radicals, and nitrogen dioxide, that oxidize the protein, lipid, and antioxidant constituents of LDL. Many of the primary oxidation products are unstable and serve as reactive intermediates that promote further modification of LDL particles and their aggregation. Modified LDL can contribute to atherogenesis by promoting cholesterol deposition and the transformation of macrophages into foam cells [14,15]. Furthermore, MPO converts cardioprotective lipoprotein into a dysfunctional form. Dysfunctional HDL particles lack atheroprotective properties and promote pro-inflammatory effects [5].

In the present study, serum MPO levels were significantly increased in new IHD cases when compared to controls indicating its role in inflammation. Results of the present study are also in accordance with Hameed *et al.* and Ndrepepa *et al.* who reported that serum MPO levels were high in acute coronary syndromes when compared to controls [16,17]. Kaya *et al.* observed higher plasma MPO levels in patients with acute STEMI when compared to controls and also found that there was a higher risk of major adverse cardiovascular events in patients with higher MPO levels among the cases after a follow-up period of 2 years [18].

MPO levels differ significantly with the difference in age and body mass index (BMI) [19,20]. Hence, in the present study, age- and sex-matched controls were taken to remove the false variation created by these factors. Furthermore, there was no significant difference in the mean age and BMI between the two groups. MPO levels were significantly higher in the case of subjects who later developed CAD during 8 years of follow-up according to Meuwese *et al*. and also MPO levels correlated with C-reactive protein (CRP) [8]. In a case–cohort study conducted on middle-aged healthy men and women, serum levels of MPO were higher in cases of coronary heart disease (CHD) after a follow-up time of 10–15 years. There was no significant interaction of MPO with sex or increased weight in CHD, indicating that MPO levels were independently associated with increased risk of CHD [21].

Zhang *et al.* showed that blood and leukocyte MPO activity were higher in patients with CAD than angiographically verified normal controls. Results were independent of the patient's age, sex, HTN, smoking, or diabetes status, LDL concentration, leukocyte count, and Framingham global risk score [22]. In a study done by Roman *et al.* in the emergency department, they observed higher levels of MPO and high sensitivity CRP (hsCRP) in patients with acute coronary syndromes like UA compared to those with stable angina and also MPO was associated with major adverse cardiac events in patients with acute coronary syndromes [23].

Patients with higher MPO levels and a significant coronary artery calcium score were at an increased risk of cardiovascular disease events after a follow-up of 3.8 years in a study done by Wong *et al.* [24].

The risk factors such as diabetes and smoking can increase MPO levels [19]. A study done by Van der Zwan *et al.* observed an association of MPO with blood pressure in patients with diabetes and obesity [25]. Nahon *et al.* observed that increased alcohol consumption leads to reactive oxygen species formation and is associated with high MPO levels [26]. In this study, the effect of a single risk factor could not be studied because most of the study subjects had multiple risk factors. However, it was observed that serum MPO levels were higher in subjects with risk factors such as HTN, smoking, or both.

Increased levels of free iron promote the free radical generation, which, in turn, causes LDL oxidation and lipid accumulation and finally leads to atherosclerosis [4]. The results of the present study are contradictory to

the above theory. The serum iron levels in both the groups were within the normal range, but mean serum iron levels in the acute IHD were low in comparison to controls. Kervinen *et al.* found an association between low serum iron levels and CHD [27]. Liao *et al.* also observed that there was an inverse relationship between serum iron and MI risks in women [28]. Ekblom *et al.* found that there exists a low risk for MI with iron levels in the normal upper range [29]. In a 6-month follow-up study done by Huang *et al.* in STEMI patients who underwent angioplasty, low serum iron levels observed at the time of STEMI correlated with an increase in thrombolysis in myocardial infarction risk score after 6 months and also with inflammatory markers [30]. Contrary to the above studies, Marniemi *et al.* found that there was no association between iron levels and CHD [31].

In the present study, there was no significant correlation between serum MPO and iron in acute IHD cases. These results agree with a study done by Hameed *et al.* who found no correlation between MPO and iron in IHD patients [32].

Correlations between serum iron and MPO would probably have been better understood if the study was performed on a larger population. Ferritin analysis, along with serum iron, could have been a better parameter for understanding the correlations. There is no universally validated method for the estimation of MPO. Further standardization is required. Serial estimation of serum iron and MPO levels during the acute phase would have given a better understanding of the role of these parameters in acute IHD subjects.

CONCLUSION

High iron levels have a beneficial role, whereas high MPO levels have an inflammatory role in the development of IHD. Determination of serum iron and MPO levels in high-risk subjects may help in taking proper measures to prevent acute IHD.

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AUTHORS' CONTRIBUTIONS

Thejaswini Muppala – Data acquisition, literature search, and manuscript preparation. Dasaraju Rajesh – Data analysis, manuscript editing, and review. Priya Patil – Literature search. Ashalatha V Rao – Guiding and writing of the article.

CONFLICTS OF INTEREST

There are no conflicts of interest connected with this article.

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