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# COMPARISON OF IRON STORES IN HEALTHY INDIVIDUALS AND PATIENTS WITH ISCHAEMIC HEART DISEASE

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**ABSTRACT**

*Excess body iron has been linked to atherosclerosis owing to its pro-oxidative properties. However, inconsistent results have emerged from the epidemiological studies linking iron status and the risk of cardiovascular diseases (CVD). Objective of the present study is to compare iron stores of healthy individuals and patients with ischaemic heart disease (IHD). A total of 137 subjects were included in the study, 90 patients of IHD and 47 healthy subjects with no history of IHD as controls. We compared body iron stores of patients and controls. Serum ferritin, serum transferrin receptor (sTfR) and sTfR/ferritin ratio were used as measures of body iron stores. Our results revealed that mean serum ferritin concentration of cases was significantly higher than controls. Moreover, mean sTfR and sTfR/ferritin ratio of controls was significantly higher than the patients. We conclude from our results that IHD patients have higher iron stores than healthy subjects suggesting a possible association between high iron stores and the risk of IHD.*

***Key Words:*** *Acute myocardial infarction, Serum ferritin. Serum transferrin receptor. Serum transferrin receptor/ferritin ratio.*

## INTRODUCTION

Excess body iron has been linked to atherosclerosis owing to its pro-oxidative properties.[1] As the oxidative modification of low density lipoproteins is assumed to be an initial event in atherogenesis, in vivo oxidative processes are likely to play a role in disease etiology. However, knowledge of the molecular mechanism of iron-related CVD is still limited.[2] The iron hypothesis was first proposed by Sullivan in 1981. He suggested that the development of IHD might be related to acquisition of body iron stores.[3] Since then a number of studies have been conducted to assess the validity of iron hypothesis. These include epidemiological, animal, pathological, molecular and proteomic studies.[4]

However, inconsistent results have emerged from the epidemiological studies linking iron status and the risk of CVD and there are both supportive and non-supportive studies.[5] A number of researchers have provided supportive evidence for iron hypothesis. On the other hand, some studies report that there is no direct association between iron status and the risk of CVD. The discrepancy might have emerged from the employment of different parameters for measuring iron status, differences in sample size and selection biase.[6] The objective of the present study is to compare iron stores of healthy individuals and patients with IHD.

## MATERIALS AND METHODS

### Study Design:

Analytical cross-sectional study.

***Setting:*** The study was conducted at University of Health Sciences (UHS) in collaboration with Punjab Institute of Cardiology (PIC), Lahore.

***Study Population and Sample Size:*** The study included 90 patients of IHD (40-60 years, both sexes) as patients and 47 healthy subjects (40-60 years, both sexes) with no history of IHD as controls.

### Sample Selection:

#### *Inclusion Criteria*

Patients diagnosed cases of acute myocardial infarction (AMI) (within first 48 hours of AMI) admitted to different wards of PIC were selected as patients. Diagnosis of AMI was based on the typical history, suggestive ECG changes and serum cardiac biomarkers.

***Controls:*** Forty seven healthy subjects with no history of IHD were selected as controls.

#### *Exclusion Criteria*

Subjects suffering from acute and chronic inflammatory conditions, malignancies and liver diseases were excluded.

#### *Data Collection Procedure*

Selected subjects were informed about the study and consent was obtained on the consent forms. Relevant history was recorded in proformas. Venous

blood samples were collected after overnight fast (i.e., 12-14 hours fast) between 8:00 am to 9:00 am. As biphasic circadian rhythm has been reported for serum ferritin, specimens were drawn at nearly the same time of the morning for each subject. Blood samples were transferred to labeled vacutainers and placed in an ice box. Samples were transported within an hour of collection to biochemistry laboratory, UHS, Lahore for further processing. Blood was allowed to clot and serum was separated by centrifugation. Approximately 500-700 µL of serum was transferred to each of three labelled aliquots (one for each variable i.e., serum ferritin, sTfR and an extra one). Aliquots for serum ferritin assay were stored at -20°C. Aliquots for sTfR assay were stored at -80°C. The extra aliquots were also stored at -80°C. Storage temperature was checked regularly twice a day i.e., at 9:00 am and 3:00 pm.

Human Ferritin Enzyme Immunoassay Test Kit (BIOCHECK, INC.) was used for the quantitative determination of human ferritin concentration in the serum. Human sTfR ELISA (BioVendor) was used for the quantitative measurement of human sTfR in serum. We calculated sTfR/ferritin ratio from sTfR and ferritin concentrations.

***Statistical Methods:*** The data was entered and analyzed using SPSS (16.0). Independent sample t test was applied to observe group mean differences. A *p*-value of <0.05 was considered as statistically significant.

## RESULTS

Significant difference was observed between serum ferritin concentration of cases and controls (313.27 ± 255.640 vs. 84.79 ± 139.171 ng/ml, $p < 0.001$) showing that cases have a mean serum ferritin concentration higher than controls (Fig. 1). Moreover, we utilized sex-specific cut-off criteria for defining high iron stores.[12,6] According to the above mentioned cut-off value, among 63 male cases, 32 (51%) had high iron stores and one (1%) had borderline high iron stores (i.e., 300 µg/L). Four male cases had very high serum ferritin concentration i.e., 1000 µg/L. Thirty (48%) male cases had serum ferritin <300 µg/L. Among the 18 male controls, only 02 (11%) had high iron store and none had very high iron store. Sixteen (89%) male controls had serum ferritin <300 µg/L. According to the above mentioned criteria for females, among the 27 female cases, 11 (41%) had high iron stores. None of the females had very high serum ferritin i.e., 1000 µg/L. Sixteen (59%) females had serum ferritin <200 µg/L. among the 29 female controls, only 01 (3%) had high iron stores and none had very high iron stores. Twenty eight (97%) females had serum ferritin < 200 µg/L. Significant difference was also observed between sTfR concentration of cases and controls (2252.22 ± 2823.077 vs. 3956.38 ± 4066.759 ng/ml, $p = 0.005$) showing that controls have a mean sTfR concentration higher than cases (Fig. 2). Similarly, significant difference was observed between sTfR/ferritin ratio of cases and controls (13.599 ± 18.707 vs. 389.006 ± 624.401, $p < 0.001$) showing that controls have a mean sTfR/ferritin ratio higher than cases (Fig. 3).

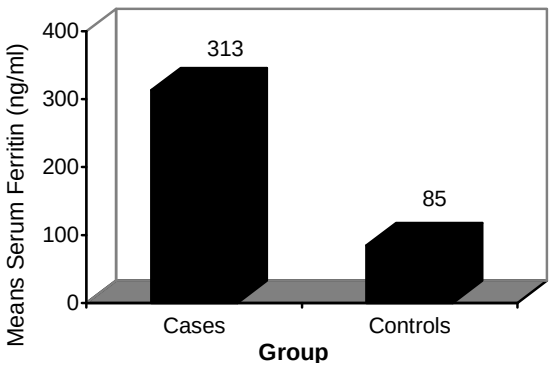


**Fig. 1:** *Differences in serum ferritin of cases and controls.*

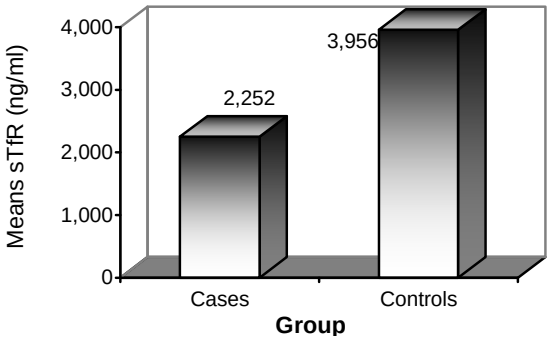


**Fig. 2:** *Differences in sTfR of cases and controls.*

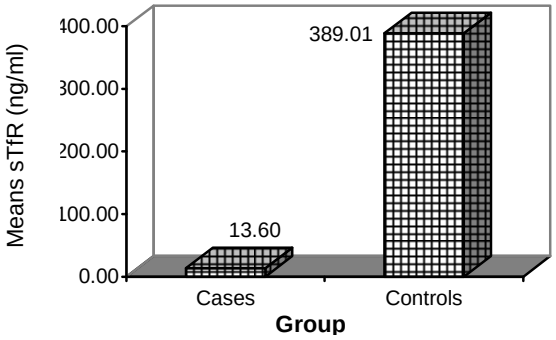


**Fig. 3:** *Differences in sTfR/ferritin ratio of cases and controls.*

## DISCUSSION

Our results revealed that the patients hade a mean serum ferritin concentration higher than controls. These findings suggest that relative iron depletion offers protection against IHD and support Sullivan's hypothesis that the development of IHD might be related to acquisition of body iron stores.[7] Our results are in agreement with the findings of Salonen et al. and Klipstein-Grobusch et al. who have reported higher risk of MI in subjects with higher serum ferritin.[8,9] Moreover, Kiechi et al. have reported that iron-deficient men and women constitute a low-risk group whereas subjects with prominent iron stores face a high-risk burden of carotid atherosclerosis.[10]

Our results reveal lower mean sTfR concentration in patients than controls indicating relatively higher iron stores in this group of study subjects. These findings provide additional support to the iron hypothesis. As the role of sTfR in the detection of iron overload has to the yet established, we have also compared the sTfR/ ferritin ratio of cases and controls.[11] Our results showed that controls have a mean sTfR/ferritin ratio higher than the patients. These findings are generally in agreement with the ones reported by Tuomainen et al. They evaluated the association between body iron stores and the risk of AMI in men and reported that the mean sTfR/ferritin ratio was lower among the patients than controls suggesting the role of high body iron stores in the development of first MI in men.[10] Although our mean sTfR/ferritin ratio for cases (13.599) is very close to the value reported by Tuomainen et al. among their cases (15.1) but the mean sTfR/ferritin ratio for controls (389.006) in our study population is much higher than the value reported by Tuomainen et al. among their controls (21.3). This difference might have emerged from the increased prevalence of dietary deficiencies and intestinal parasitism contributing to depletion of iron stores in subjects from the developing countries.[12]

We **conclude** that IHD patients have higher iron stores, as indicated by higher serum ferritin and lower sTfR and sTfR/ ferritin ratio, than healthy subjects. These findings suggest a possible association between high iron stores and the risk of IHD. Although our results suggest an association between high iron stores and IHD, however, to establish a causal relationship we recommend that cohort and intervention studies must be carried out in future.

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