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RESEARCH ARTICLE

Correlation between serum free iron, glycated hemoglobin and insulin resistance in uncontrolled type-2 diabetic patients

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ABSTRACT

Background: The link between iron and diabetes was first recognized in pathologic conditions—hereditary hemochromatosis and thalassemia—but high levels of dietary iron also impart diabetes risk. Free iron may contribute to the pathogenesis and progress of this disease and its complication. Iron causes hyperinsulinemia by decreasing the insulin uptake and metabolism by hepatocytes. Elevated iron stores are commonly found in insulin resistance. Iron in its free form is known to induce oxidation of biomolecules by producing harmful hydroxyl radicals. **Aims and Objectives:** In this study, we aimed to estimate and compare the serum levels of free iron in diabetes and healthy individuals. **Methods and Materials:** This study included 253 subjects in 2 groups. Group-I comprised 207 subjects with diabetes mellitus and Group-II comprised 46 healthy subjects. Blood sugar, free iron, and glycated hemoglobin were analyzed in blood samples using standard kits. The results of all the parameters were expressed as mean ± standard deviation. Student *t*-test was done to assess the statistical significance between 2 groups. The association between the parameters was studied by Pearson correlation. **Result:** In this study, we found a significant increase in serum free iron in Group-I ($P < 0.01$), when compared with Group-II. A significant correlation between the serum free iron and glycated hemoglobin ($r = 0.59$; $P < 0.001$) and fasting blood sugar ($r = 0.43$; $P < 0.001$). **Conclusion:** The elevated serum free iron in uncontrolled diabetes may contribute to oxidative stress which may be associated with complications of diabetes.

KEY WORDS: Free Iron; Glycated Hemoglobin; Hyperinsulinemia; Diabetes Mellitus

INTRODUCTION

Iron is a very important transition metal for the cells in the body and its abnormal homeostasis is associated with the pathogenesis of various chronic diseases, including diabetes.[1,2] Iron plays a key role in vital biochemical activities.[3] Iron is potentially hazardous because of its ability to participate in the generation of powerful oxidant species such as hydroxyl radical.[4,5] Dietary iron is a critical determinant of body's iron status since once absorbed it is not actively excreted.[6,7] However, the ability of iron to cycle between its two stable oxidation states is also potentially to generate reactive oxygen or nitrogen species (ROSs or RNSs) such as hydroxyl radical via Fenton's and Haber-Weiss reactions.[3,6,8]

To control and balance the production of ROSs and RNSs, the cell builds up a set of antioxidants and detoxifying enzymes such as superoxide dismutase, catalyze, and glutathione peroxidase that can scavenge excessive ROSs or RNSs beyond the antioxidant capacity of the organism called oxidative stress is encountered in many pathological conditions such as diabetes and diabetic complications.[3,6,9] Iron can be released from ferritin by the action of reducing agents that convert Fe^{3+} into Fe^{2+}.[10,11] Apoferritin, the protein fraction of ferritin, that holds oxidized iron molecules. When

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the concentrations of antioxidants are low, the reducing potential and anaerobiosis progressively increase, facilitating a rapid release of iron from ferritin.

The overall result of oxidative reactions is an increase in the availability of free iron from the ferritin.[10,12] Iron influences insulin action interferes with insulin inhibition of glucose production by the liver.[10,13-15] Hepatic extraction and metabolism of insulin are reduced with increasing iron stores, leading to peripheral hyperinsulinemia. In fact, the initial and most common abnormality seen in iron overload conditions is liver insulin resistance.[10,15]

The exact mechanism of iron-induced diabetes is mediated by 3 key mechanisms, "insulin deficiency, insulin resistance, and hepatic dysfunction.[4,16] The mechanisms for insulin resistance include to possibility of iron overload causing resistance directly or through hepatic dysfunction. Patients with unexplained hepatic iron overload mostly found to be insulin resistant, which suggests a common etiologic link among hepatic iron, hepatic dysfunction, and insulin resistance.[17]

This study was aimed to determine serum free iron in person with diabetes and healthy individuals.

METHODS AND MATERIALS

Selection of Participants

After the Institutional Ethical Committee clearance, 253 subjects were selected following written and informed consent in both English and Vernacular Language. Study subjects were categorized into 2 groups.

1. Diabetes mellitus (DM) group consists of 207 subjects. The exclusion criteria are type-1 DM, hypothyroidism, hypertension, hyperthyroidism, cancer, and pancreatitis. Inclusion criteria are type-2 DM above 30 years of age.
2. Healthy group was consist 46 subjects. Exclusion criteria are smokers, alcoholics, pregnant, and lactating women. The inclusion criteria are above 30 years of healthy persons without any disease.

Sample Type

Serum, plasma and whole blood.

Sample Collection

The blood sample from 253 individuals was collected in fasting state using vacutainers with (sodium fluoride and EDTA) without anticoagulants in a sterile gel tube. These tubes were centrifuged at 3500 rpm for 10 minutes and serum was separated.

Blood Sample Analysis

Fasting blood sugar (FBS) by Hexokinase method and free iron by direct iron assay using chromophore ferene® were analyzed in blood sample using standard kits in Dimension RxL autoanalyzer, USA and HbA1c by high-performance liquid chromatography method was analyzed in blood sample using Bio-Rad autoanalyzer, USA. Glucose present in plasma in expressed as mg/dl. Iron present in serum is expressed as µg%. HbA1c present in whole blood is expressed as g%.

Statistical Analysis

The results of all the parameters were expressed as mean ± standard deviation. Student t-test was done to assess the statistical significance between 2 groups. The association between the parameters was done by correlation coefficient.

RESULT

Our this study was performed on 207 type-2 diabetes and 46 healthy subjects. A direct relationship was found between serum free iron and type-2 DM. We found a significant increase in serum free iron in Group-I 98.33 ± 51.27 ($P < 0.01$), when compared with Group-II 78.07 ± 33.13 (Table 1).

In this study, we found a significant positive correlation between the serum free iron and FBS ($r = 0.43$; $P < 0.001$) (Figure 1) and HbA1c ($r = 0.59$; $P < 0.001$) (Figure 2), respectively.

DISCUSSION

In this study, we have observed a significant increase in serum free iron in Group-I cases, compared to Group-II cases who were in good glycemic control.[18] Hyperglycemia observed in Group-I cause increased glycation of protein and release the iron in its free state. Several studies have shown that serum free iron is significantly increased in type-2 DM.[19,20] This observation is contradicted by the studies of El-Nabarawy et al.[1] Glycemic control reflects the interaction between hyperinsulinemia with iron. This is further supported by evidence suggesting that hyperglycemia precedes the elevation of iron in diabetes.[21,22]

Table 1: Demographical data and biochemical parameters in the study groups

Paraemters	Group-I (*n*=207)	Group-II (*n*=46)
Age (in years)	55.54±10.32	43.35±8.83
Sex (F: M)	78:129	29:17
FBS (mg %)	156.78±48.10***	91.72±8.82
HbA1c (g %)	8.43±2.12***	-
Serum free iron (µg %)	98.33±41.27**	78.07±13.03

**P<0.01 ***P<0.001, FBS: Fasting blood sugar

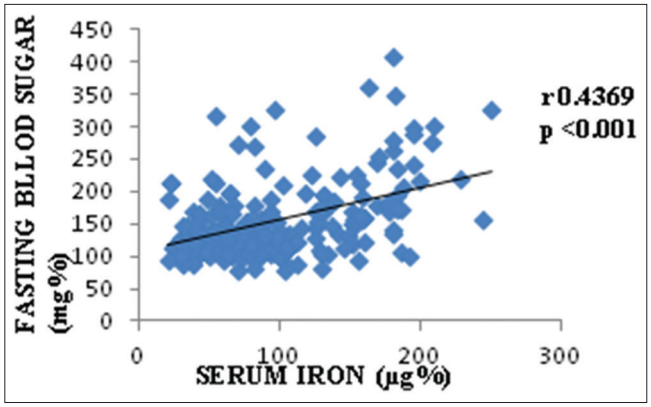

Figure 1: Correlation between serum free iron and fasting blood sugar in Group-I

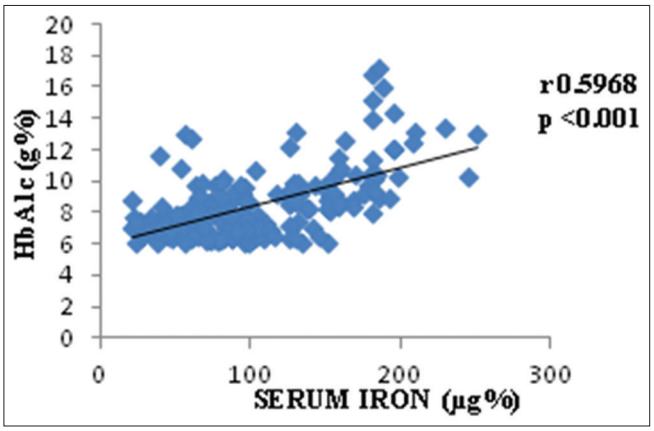

Figure 2: Correlation between serum free iron and HbA1c in Group-I

In this study, we found a positive correlation between FBS and free iron as well as free iron and HbA1c in Group-I. Increased glycation of proteins as a result of poor glycemic control triggers the increased release of free iron from glycated proteins like hemoglobin which generates a vicious cycle of hyperglycemia, glycation of hemoglobin, and increase in levels of free iron.[17] Evidence that systemic iron overload could contribute to abnormal glucose metabolism was first derived from the observation that the frequency of diabetes is increased in classic hereditary hemochromatosis. However, with the discovery of novel genetic disorders of iron metabolism, it is obvious that iron overload, irrespective of the cause or the gene involved, results in an increased incidence of type 2 diabetes. The role of iron in the pathogenesis of diabetes is suggested by (1) an increased incidence of type 2 diabetes in diverse causes of iron overload and (2) reversal or improvement in diabetes (glycemic control) with a reduction in iron load achieved using either phlebotomy or iron chelation therapy. The relationship between free iron and type-2 diabetes is bidirectional.[23] Insulin influences the iron uptake and storage by increasing the cell surface transferring receptors,[24] reciprocally iron influences the insulin activity by interfering with glucose uptake and utilization.[25] This increased presence of free iron pool will enhance oxidant generation leading damage to biomolecules.[8] Free iron is known to induce oxidation of biomolecules through Fenton's and Haber–Weiss reaction by producing harmful hydroxyl radicals.[8,23] Free radical formation may play a role in the pathogenesis of diabetes by disrupting insulin action and total body glucose disposal. The iron act as a powerful pro-oxidant and the oxidative stress are increased in glycose intolerant states.[26-28] The presence of poor glycemic control, hyperglycemia, iron overload, and protein glycation will all lead to oxidative stress causing early appearance of microvascular complications (retinopathy and nephropathy).[8,23] Among cases of incident diabetes, the mean concentration of serum ferritin was significantly higher compared with control subjects, and the mean ratio of transferrin receptors to ferritin was significantly lower. This relationship with markers of body iron stores persisted after correction for various other risk factors for diabetes, including markers of obesity and inflammation.[29]

The common presence (59-92% of patients) of non-transferrin-bound iron, a form of iron most susceptible to redox activity, in excess amounts in type 2 diabetes with a strong gradient for severity,[30] and the preliminary evidence that reduction in body iron stores with bloodletting in type 2 diabetes results in improvement in glycemic control and insulin resistance,[31] suggests a pathogenic role of iron in type 2 diabetes. The limitations of this study we have not assessed the markers of oxidative stress and insulin resistance.

CONCLUSION

The significantly increased serum free iron and glycated hemoglobin in DM reveals the fact that serum free iron contributes indirectly to oxidative stress in DM. In summary, there is suggestive evidence that iron plays a pathogenic role in diabetes and its complications such as microangiopathy and atherosclerosis. Reliable and sensitive methods need to be developed to precisely measure the free/catalytic iron that participates in oxidative injury. Iron chelation therapy may present a novel way to interrupt the cycle of catalytic iron-induced oxidative stress and tissue injury and consequent release of catalytic iron in diabetes and to prevent diabetes-related complications.

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