EVALUATION OF SERUM FERRITIN IN TYPE 2 DIABETES MELLITUS

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ABSTRACT

BACKGROUND

Ferritin, one of the key proteins regulating iron homeostasis, is a widely available clinical biomarker to evaluate iron status and especially important for detecting iron deficiency. Recent studies indicate that increased body iron stores have been associated with the development of glucose intolerance and type 2 diabetes mellitus. There is a bi-directional association between diabetes mellitus and iron metabolism. Systemic iron overload contributes to abnormal glucose homeostasis by: (i) insulin deficiency as a result of oxidative stress on the pancreatic beta cells leading to cell death and decreased secretion of insulin (ii) insulin resistance caused directly by iron overload. This study was carried out to investigate the association between iron status, as assessed by circulating serum ferritin and type 2 diabetes mellitus as well as to assess the relationship between serum ferritin and glycated hemoglobin in the study group. The present comparative cross sectional study comprised of 80 apparently healthy controls and 80 type 2 diabetic patients who attended the outpatient departments of Medical College, Kozhikode. Levels of fasting blood glucose, serum ferritin, glycated hemoglobin and hemoglobin were estimated. Significantly elevated ferritin was found in type 2 diabetic patients (Mean 143.34±59.23) when compared to non-diabetic control (Mean 53.87±27.33) of the same age group. Elevated ferritin in diabetic patient was positively correlated with HbA1c and FBS. So Ferritin may be used as one of the important biomarkers in predicting diabetes mellitus.

KEYWORDS

Diabetes Mellitus, Ferritin, Glycated Haemoglobin, Insulin.


INTRODUCTION

Diabetes mellitus is one of the most prevalent endocrine disorders in the world. Diabetes is a significant and growing threat to global health worldwide, diabetes probably affects 250 million people. This number was eight fold less in 1985 (30 million) and the world prevalence is expected to reach 380 million by 2025. Iron is the most abundant transitional metal in the body. The crucial role of iron in the pathophysiology of disease is derived from the easiness with which iron is reversibly oxidized and reduced. This property, while essential for its metabolic functions, makes iron potentially hazardous because of its ability to participate in the generation of powerful oxidant species such as hydroxyl radical. The catalytic iron converts poorly reactive free radicals like hydrogen peroxide (H2O2) into highly reactive ones such as hydroxyl radical and superoxide anion that can initiate and propagate the cascades leading to oxidative damage. Generation of the hydroxyl radical by iron occurs via the following reactions.

\[
\begin{align*}
\text{Fe}^{2+} + \text{H}_2\text{O}_2 & \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}^- \quad \text{(Fenton reaction).} \\
\text{Fe}^{2+} + \text{O}_2 & \rightarrow \text{Fe}^{2+} + \text{O}_2^- \\
\text{H}_2\text{O}_2 + \text{O}_2^- & \rightarrow \text{OH}^- + \text{OH}^- + \text{O}_2 \quad \text{(Haber-Weiss reaction).}
\end{align*}
\]

Elevated iron stores could enhance oxidation of lipids, especially of free fatty acids; through accelerated production of free radicals. The complex process of advanced glycation end product formation produces reactive oxygen species (ROS) by metal catalyzed reactions. Advanced glycation end products themselves bind transition metals, potentiating their toxic effects, including insulin resistance. ROS interfere with insulin signaling at various levels, impairing insulin uptake through a direct effect on insulin receptor functions and inhibiting the translocation by GLUT4 in the plasma membrane. Iron through Fenton’s reaction participates in the formation of highly toxic free radicals such as hydroxide and the superoxide anion that are capable of inducing lipid peroxidation. Hydroperoxides react with transition metals to form stable aldehydes, such as malondialdehyde (MDA). ROS can stimulate vascular smooth muscle cell growth and proto-oncogene expression. In patients with type 2 diabetes mellitus, higher levels of MDA, a marker of lipid peroxidation is found. Oxidative stress induces both insulin resistance by decreasing internalization of insulin and increased ferritin synthesis.

Raised Serum ferritin may possibly be related to the occurrence of long term complications of diabetes, both micro vascular and macro vascular. The explosive increase of Diabetic population worldwide is a major public health concern both in developing and developed countries. Hence this study was carried out to examine the relationship between serum ferritin and type 2 diabetes mellitus.

MATERIALS AND METHODS

The comparative cross sectional study comprised of 80 apparently healthy controls and 80 type 2 diabetic patients who attended the outpatient departments of Medical College, Kozhikode.
Study was conducted in 160 subjects of either sex with prior informed consent. Detailed history was taken (Age, sex, duration of illness, history of hypertension). Blood pressure was recorded. Body mass index (BMI) was calculated as weight in kilogram/(Height in m)^2.

The following Blood Parameters were done

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
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<tbody>
<tr>
<td>Fasting blood glucose</td>
<td>By glucose oxidase method</td>
</tr>
<tr>
<td>Glycated Haemoglobin</td>
<td>By turbidimetric inhibition immunoassay</td>
</tr>
<tr>
<td>Serum ferritin</td>
<td>By electro chemiluminescence immunoassay</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>By cyanmethaemoglobin method</td>
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RESULTS

A cross-sectional study was conducted among 160 subjects, with females comprising 78.5% and males 22.5% of the age group 30-65 years, the mean age being 54.30±6.58 years and was grouped into two. The first group included 80 diabetic patients of both sexes, the second group included 80 gender matched apparently healthy individuals. The statistical package for the Social Sciences (SPSS, Version: 16.0) software for windows were used for the analysis of the data. Microsoft Word and Excel have been used to generate tables and graphs. Results were analyzed statistically for significance by Independent ‘t’ test and chi square test. And Pearson correlation ‘r’ test (Correlation coefficient test) was done to assess the relation of ferritin with FBS and HbA1c. At p-value <0.05, results were considered significant.

In group one, n=80, 26% were males and 74% were females with the mean age being 54.59±6.25 years. The BMI of this group was 26.88±6.04 kg/m^2. The mean FBS in this group were 189.66±77.81 mg/dl. The mean HbA1c level in this group was 9.11±1.52% and we categorized group1 into two depending on the HbA1c.

Controlled DM - < 7%
Uncontrolled DM - >7%

Majority (95%) of diabetics had uncontrolled DM.

The mean ferritin level in this group was 143.34±59.23 ng/ml. The normal ferritin level is different in males and females.

Males: 30-400 µg/L (ng/ml)  
Females: 15-150 µg/L (ng/ml)  

So we categorized male diabetics into two groups depending on the ferritin level.

Low normal: 30-100 ng/ml.  
High normal: 100-400 ng/ml.  

Category of female diabetics depending on the ferritin level

Low normal: 15-100 ng/ml.  
High normal: 100-150 ng/ml.  
High: >150 ng/ml.  

Majority of male diabetics (80.95%) had high normal ferritin level while in females, majority of female diabetics (40.68%) had high ferritin level, 32.2% had high normal ferritin level and 27.22% had low normal ferritin level.

In group two (n=80), 20% were males and 80% were females with the mean age being 54.02±6.94 years. The BMI of this group was 24.84±3.26 kg/m^2. The mean FBS in this group were 83.78±9.73 mg/dl. The mean HbA1c level in this group was 5.39±0.54% and the mean ferritin level in this group was 63.87±5.45 ng/ml.  

There wasn’t any significant difference between the two groups for age and gender. The mean level of haemoglobin in diabetics was not different from those of normal controls statistically. The serum ferritin level in the diabetic group was more than that of the controls and was found to be statistically significant (p=0.00).

Pearson correlation between serum ferritin and FBS in the study group was positively correlated (r=+0.698, p=0.00). Pearson correlation between serum ferritin and HbA1c in the study group was also positively correlated (r=+ 0.363, p=0.00).

GROUP COMPARISON OF VARIOUS PARAMETERS

CORRELATION OF FERRITIN WITH FBS

Tested using pearson correlation bivariate analysis  
(r value=0.498). There was a significant positive correlation between ferritin and FBS.

CORRELATION OF FERRITIN WITH HBA1c

Tested using Pearson correlation bivariate analysis  
(r value=0.363). There was a significant positive correlation between ferritin and HbA1c.
DISCUSSION
Two important observations were found in this study. First observation was that, significantly elevated ferritin was found in type 2 diabetic patients (Mean 143.3±59.23 ng/ml, p=0.00) when compared to non-diabetic control (Mean 63.87±27.33 ng/ml) of the same age group. Second observation was that elevated ferritin in diabetic patient was positively correlated with Hba1c (r=0.363, p=0.00) and FBS(r=0.498, p= 0.00).

In some epidemiological studies serum ferritin was the second strongest determinant of blood glucose(After BMI). In regression models, ferritin was the third strongest determinant of blood glucose (After BMI, age). The probable correlation between ferritin and diabetes mellitus (DM) was considered first in 1993 by Kay etal.

Jukka T Salonen et al tested the hypothesis that the accumulation of iron in the body predicts the development of non-insulin dependent diabetes. They followed 1038 randomly selected men from eastern Finland aged 42-60 for four years; there was a high incidence of non-insulin dependent diabetes in eastern Finland. Fifty three men who were found to have diabetes at baseline(Fasting blood glucose concentration ≥6.0 mmol/L or who were already receiving treatment for diabetes) were excluded. They estimated iron stores by determining the ratio of the concentrations of transferrin receptors to ferritin in frozen serum samples drawn during baseline examinations. Serum samples from 41 men who developed diabetes during follow up and from 82 controls were analysed in a logistic regression model, men with high stores of iron(Those in the lowest quarter of the ratio of transferrin receptors to ferritin, that is <9.4 µg/µg) were 2.4 times more likely(95% confidence interval[CI]) of odds ratio 1.03 to 5.5, P=0.04) to develop diabetes than men with lower stores of iron. In a step up model in which baseline concentrations of serum triglycerides and glycosylated proteins were adjusted for as continuous variables, the odds ratio for developing diabetes was 2.5(1.1 to 6.0, P=0.04).

This was the first study to show an association between stores of iron and the incidence of diabetes. Their data supported the theory that increased iron stores, even in the range not considered to be associated with haemochromatosis, contribute to the development of non-insulin dependent diabetes.

In EPIC-Norfolk prospective study, baseline serum ferritin was higher among incident case participants (360) than in control participants (758) in both men (Geometric means: 96.6[95% CI 84.7–110.2] vs 67.8 [95% CI 62.6–73.5]ng/ml in case and control participants, respectively) and women(45.9 [95%CI 38.1–55.3] vs 34.8 [95% CI 31.6–38.4] ng/ml).They found that elevated ferritin was predictive of diabetes independently of a comprehensive range of risk factors and confounders, where previously only a limited number of confounders were accounted for. Specifically, they found that elevated ferritin was associated with diabetes independently of known risk factors for diabetes(Age, BMI, sex, family history, physical inactivity and smoking), as well as dietary factors and alcohol intake measured by food diary.

In the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam case–cohort study, higher body iron stores(reflected by elevated ferritin concentration and a lower ratio of sTfR to ferritin) were associated with increased risk of type 2 diabetes. These associations remained significant after adjustment for a wide range of known risk factors and markers of different pathways of the development of diabetes. Of the individual markers, adjustment for alanine amino transferase (ALT) and gamma glutamyl transferase (GGT) had the strongest impact on the association observed between ferritin and diabetes risk. In contrast, only a marginal impact on the inverse association between sTfR-to-ferritin ratio and type 2 diabetes was observed in models adjusting for biomarkers of inflammation, hepatic fat accumulation, insulin resistance and dyslipidaemia. Levels of sTfR were not significantly associated with risk of type 2 diabetes.

It can be concluded that high ferritin level is an indication that iron plays an important role in the etiology of type 2 DM.

CONCLUSION
Serum ferritin level in type 2 diabetics is significantly elevated than non-diabetics. So among the large number of environmental and genetic factors contributing to the development of diabetes, excess body iron appears to be a potential new risk factor. Serum ferritin was much higher in uncontrolled diabetes mellitus.Ferritin may be used as one of the important biomarkers in predicting diabetes mellitus.

Iron exerts a detrimental effect on β-cell function that may be reversible with removal of excess iron, either through phlebotomy or possibly iron chelation.

REFERENCES