ORIGINAL ARTICLE

INSULIN RESISTANCE AND SERUM PARAMETERS OF IRON STATUS IN TYPE 2 DIABETICS

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Background: Type 2 diabetes mellitus (T2DM) is a predominant public health concern worldwide, accounting for 90% of the cases of diabetes globally. Pathogenesis of T2DM involves insulin resistance, defective insulin secretion and increased glucose production by the liver. Subclinical haemochromatosis has been considered as one of the probable causes of insulin resistance and diabetes mellitus. The aim of this study was to determine and correlate insulin resistance and serum parameters of iron status (serum ferritin and transferrin saturation) in type 2 diabetics. Methods: It was a correlational study. This study was conducted on sixty male patients with type 2 diabetes mellitus. Fasting blood sample was taken from each subject and analysed for glucose, haemoglobin, insulin, iron, Total Iron Binding Capacity (TIBC) and ferritin. Insulin resistance was determined by HOMA-IR index. Transferrin saturation was calculated from serum iron and TIBC. Data was analysed using SPSS-17. Results: There was significant positive correlation between insulin resistance and transferrin saturation, but there was no significant correlation of insulin resistance with blood haemoglobin, serum iron and serum ferritin in type 2 diabetics. Conclusion: Correlation between insulin resistance and transferrin saturation reveals that iron has negative impact on insulin sensitivity in type 2 diabetics.

Keywords: Insulin resistance, serum ferritin, type 2 diabetics

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism, resulting from defects in insulin action, insulin secretion or both.1 The pathogenesis of type 2 diabetes mellitus (T2DM) is complex and involves the interaction of genetic and environmental factors. Individuals with T2DM show both insulin resistance and beta cell defects. Insulin resistance means that there is decreased ability of target organs like liver, adipose tissues and skeletal muscles to respond to normal circulating concentration of insulin. Post-binding defects in insulin action are primarily responsible for insulin resistance in T2DM.2,3

The relationship between T2DM and iron metabolism has gained interest in both research and clinical practice. Scientific evidence has disclosed unsuspected influences between body iron stores, insulin resistance and T2DM.4,5 Iron influences glucose metabolism even in the absence of significant iron overload. Moderately elevated iron stores below the levels commonly found in genetic haemochromatosis are associated with prevalence of insulin resistance and metabolic syndrome.6,7 Elevated iron stores reflected as elevated plasma ferritin levels, may induce baseline abnormalities that ultimately result in diabetes, or raised ferritin levels may be one of the several metabolic abnormalities related to insulin resistance and T2DM, or both of these abnormalities result from a third independent cause.8

This study was carried out to determine the correlation between insulin resistance and serum iron status (Serum ferritin and transferrin saturation) in type 2 diabetics.

SUBJECTS AND METHODS

It was a correlational study. Study population included sixty male type 2 diabetics. They were selected by convenience sampling from registered cases of type 2 diabetes, in Diabetic Management Centre, Services Institute of Medical Sciences, Lahore.

The selection criteria was patients having haemoglobin level of more than or equal to 13 g/dl. Subjects with history or evidence of conditions leading to iron overload or iron loss like gastrointestinal blood loss, iron supplements, history of blood transfusion/donation in the last one year and haemochromatosis were excluded. Subjects having evidence of any of inflammatory and infectious state that can affect ferritin levels like chronic liver disease, chronic renal disease, chronic and acute infections, malignancies and autoimmune diseases were also excluded.

After subject selection, written informed consent was taken from the subjects. Demographic information was taken, history and physical examination was completed. Waist circumference (Cm) was measured in the horizontal plane midway between the costal margin and the iliac crest at the end of normal expiration. Hip circumference was taken at the widest point of gluteal region. Waist to hip ratio was calculated by dividing waist circumference by hip circumference. Height (m) and weight (Kg) were measured in subjects wearing usual clothes, without shoes. Body Mass Index (BMI) was calculated as weight divided by the square of height in meters. Blood pressure was measured with a
mercury sphygmomanometer on the right arm with the subjects in sitting position after a five minute period of rest. After 8–10 hours of overnight fast, 10 ml of venous blood was drawn by aseptic techniques. Blood haemoglobin was estimated on non clotted blood using spectrophotometer by cyanmethaemoglobin method with haemoglobin kit10 (Diamate Biotechnologies, Bardwell, Milton Keynes MK 13 8BE UK). Serum glucose was determined after enzymatic oxidation in the presence of glucose oxidase by enzymatic colorimetric test for Glucose11 (Human Gesellschaft for Biomedica and Diagnostica D-65205 Wiesbaden-Germany). Serum iron and serum UIBC (Unbound iron binding capacity) were measured by colorimetric method using spectrophotometer with the kit (AMP Diagnostics, Graz, Austria).12 Serum Total iron binding capacity (TIBC) was determined by adding serum iron and serum UIBC.12 Transferrin saturation was calculated by: (Serum iron ×100/serum TIBC).13 Insulin was measured in human serum quantitatively by immunoenzymometric assay with an automated EIA analyser CODA, Bio-Rad laboratories, Hercules, CA, USA with the kit (Monobind Inc. Lake Forest, CA 92630, USA).14 Serum ferritin was determined quantitatively by Enzyme-immunoassay for ferritin with the kit (Globe Diagnostics Milan Italy) using CODA automated EIA analyser.15 Insulin resistance was calculated from fasting serum glucose (mmol/l) and fasting serum insulin (µIU/ml) by Homeostatic model assessment for insulin resistance (HOMA-IR) using following formula.16 HOMA-IR= Fasting serum glucose ×Fasting serum insulin/22.5. 

The data was entered and analysed using SPSS-17.0. Mean±SEM were given for normally distributed quantitative variables and median with Interquartile range (IQR) were given for non-normally distributed quantitative variables. Spearman Rho correlation was applied to correlate non-normally distributed quantitative variables, and p<0.05 was considered as statistically significant.

RESULTS
The results of this study are summarised in Tables-1, 2, and Figure-1. Mean±SEM age of the subjects was 44.05±0.82 years, BMI was 28.70±0.560 Kg/m², waist circumference was 99.33±1.59 Cm, waist to hip ratio was 0.99±0.21, systolic BP was 127±1.7 mmHg mercury and diastolic BP was 84.0±0.97 mmHg.

Mean±SEM serum glucose was 8.66±0.20 mmol/l. Median serum insulin was 18.50 (9.7–31) µIU/ml and median insulin resistance was 7.53 (3.2–11.5). Median serum iron was 96.6 (77–118) µg/dl, serum TIBC was 267.0 (220–299) µg/dl, serum transferrin saturation was 35.0 (30–50)%, serum ferritin was 72.34 (37–115) ng/ml and blood haemoglobin was 14.22±0.12 g/dl, (Table-1). Significant positive correlation was observed between insulin resistance and transferrin saturation (ρ=0.346, p=0.008), but there was no significant correlation between insulin resistance and serum ferritin (ρ=0.119, p=0.366) blood haemoglobin (ρ=0.054, p=0.682) and serum iron (ρ=0.144, p=0.28), (Table-2, Figure-1).

Table 1: Anthropometric, serum glycaemic and serum iron parameters in type 2 diabetics

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>44.05±0.82</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>28.70±0.56</td>
</tr>
<tr>
<td>Waist circumference (Cm)</td>
<td>99.3±1.59</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.99±0.21</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>127±1.7</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>84.0±0.97</td>
</tr>
<tr>
<td>Serum glucose (mmol/l)</td>
<td>8.66±0.20</td>
</tr>
<tr>
<td>Serum insulin* (µIU/ml)</td>
<td>18.50 (9.7–31)</td>
</tr>
<tr>
<td>Insulin resistance*</td>
<td>7.53 (3.2–11.5)</td>
</tr>
<tr>
<td>Serum iron* (µg/dl)</td>
<td>96.6 (77–118)</td>
</tr>
<tr>
<td>Serum TIBC* (µg/dl)</td>
<td>267 (220–299)</td>
</tr>
<tr>
<td>Serum ferritin saturation* (%)</td>
<td>35.0 (30–50)</td>
</tr>
<tr>
<td>Serum ferritin* (ng/ml)</td>
<td>72.34 (37–115)</td>
</tr>
<tr>
<td>Blood haemoglobin (g/dl)</td>
<td>14.22±0.12</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SEM or *Median (Range)

Figure 1: Scatter plot showing significant correlation (p=0.008) between insulin resistance and serum transferrin saturation in type 2 diabetics

DISCUSSION
The aim of this study was to determine the correlation between insulin resistance and iron status (expressed as
serum ferritin and transferrin saturation) in type 2 diabetics. There was no significant correlation between insulin resistance and serum ferritin, however significant positive correlation was observed between insulin resistance and serum transferrin saturation. Regarding anthropometric parameters, type 2 diabetics fulfilled the WHO clinical criteria for metabolic syndrome. Their mean BMI was in the range of overweight category, their waist to hip ratio was more than 0.9, although their mean blood pressure was less than 130/90 but they were on anti-hypertensive drugs and their fasting blood glucose was also above the limit of 6.1 mmol/L.\(^1\) (130 mg/dl).

Increased serum ferritin has also been recognized as a feature of the insulin resistance syndrome and type 2 diabetes mellitus.\(^8\) Hepatic iron deposition has been detected in up to one third of patients with non-alcoholic fatty liver disease (NAFLD), and is considered a manifestation of the metabolic syndrome.\(^9\) The relationship between iron levels and type 2 diabetes is bidirectional. Iron stores influence insulin actions in type 2 diabetes and insulin affects several iron metabolic pathways. Insulin stimulates ferritin synthesis and facilitates iron uptake by enterocytes and fat cells. Insulin causes redistribution of transferrin receptors from an intracellular membrane compartment to the cell surface suggesting that regulation of iron uptake by insulin occurs in parallel with its effects on glucose transport.\(^10,11\) Reciprocally, iron influences insulin actions. Iron interferes with insulin inhibition of glucose production by the liver. The initial abnormality commonly observed in iron overload is liver insulin resistance. Hepatic extraction and metabolism of insulin is reduced leading to peripheral hyperinsulinemia. Iron also affects the skeletal muscles, the main regulator of insulin actions.\(^12,13\)

In the present study, we did not find any significant correlation between insulin resistance and serum ferritin. This finding differs from the results of the previous studies conducted in China and Korea. According to these studies patients having type 2 diabetes mellitus were in higher quartile of ferritin and the association between insulin resistance and serum ferritin remained significant even after adjustments for dietary factors, body mass index and inflammatory markers.\(^14,15\) One of the reasons for lack of any association between insulin resistance and serum ferritin in our study may be the small sample size and secondly most of the patients were on drugs like pioglitazone and metformin. These drugs not only affect insulin resistance but they also influence the absorption as well as metabolism of iron at the level of hepatocytes.\(^16,17\) In this study, serum transferrin saturation was also taken as an index of body iron status and significant positive correlation was observed between insulin resistance and serum transferrin saturation in type 2 diabetics. This result is in concordance with a previous study conducted in Greece depicting strong correlation between insulin resistance and transferrin saturation in non diabetic offspring of type 2 diabetics.\(^18,19\)

**CONCLUSION & RECOMMENDATIONS**

Correlation between insulin resistance and serum transferrin saturation in the present study reveals that stored iron has negative impact on insulin sensitivity in type 2 diabetics. In Pakistan, anaemia is very prevalent and many measures are taken for treatment of anaemia which can influence the co-existing diabetic state. Modestly elevated iron levels, below levels diagnostic of haemochromatosis whether cause or the result of insulin resistance must be checked, as this potentially free iron is worsening subclinical hepatic steatosis and thus insulin resistance in type 2 diabetics. We recommend that more studies need to be performed regarding the role of ferritin in pre-diabetic stage as well as in type 2 diabetics.

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