**Evaluation of iron profile in type 2 diabetes mellitus patients of tertiary care center of central India**

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**Abstract**

**Introduction:** Iron profile is one of the most important indexes for good health. Altered iron profile is a risk factor for many diseases. Excessive iron accumulation in body causes damages of many cellular structures and leads to oxidative damage and causes many complications. So, Iron profile must be studied in many diseases like diabetes mellitus.

**Materials and Methods:** 100 subjects were taken in this study, out of which 50 were type 2 diabetes and 50 were normal healthy controls. BMI was calculated by dividing body weight (Kg) to square of height (meters). The waist / hip circumference ratio was calculated by dividing the waist circumference (cm) to hip circumference (cm). Sphygmomanometer was used for blood pressure measurement. The serum iron, TIBC, UIBC, plasma glucose and HbA1c were measured by semiautoanalyzer. The serum ferritin was estimated by chemiluminescence method. The % transferrin saturation was calculated by formula serum iron (µg/dl) x 100 / TIBC (µg/dl).

**Result:** The anthropometric parameters like weight, BMI, waist circumference, hip circumference, waist hip circumference ratio, systolic and diastolic blood pressure was statistically extremely significantly (p<0.001) increased in type 2 diabetes mellitus subjects as compared to healthy control subjects. The plasma fasting glucose, HbA1c, serum iron, % saturation and serum ferritin were statistically extremely significantly (p<0.001) increased and UIBC was statistically extremely significantly (p<0.001) decreased while TIBC and hemoglobin were statistically significantly (p<0.05) decreased in type 2 diabetes mellitus subjects as compared to healthy control subjects.

**Conclusion:** Elevated iron and ferritin is a risk factor for diabetes and produce many complications. Proper management must be taken to remove the excess iron that can be very harmful to the body.

**Keywords:** T2DM, Iron, Radical, Ferritin, Insulin resistance, Oxidative stress.

**Introduction**

Diabetes mellitus is a metabolic disorder of various causes which leads to hyperglycemia due to alteration in metabolism with derangement of carbohydrate, fat, and protein metabolism.¹ This disease is mainly caused by insulin deficiency, impaired insulin action or both.²⁻³ The prevalence of diabetes in South East Asia including India was 82 million in 2017 and will expect to increase up to 51 million in 2045 as per international diabetes federation.³ Diabetic patients have various microvascular (like neuropathy, nephropathy, and retinopathy), macrovascular (like atherosclerosis) and miscellaneous (like diabetic cardiomyopathy) complications.⁵ The various complications are produced by reactive oxygen species leads to oxidative damage which is generated by free radicals like free iron.⁶ Iron is one of the most important micronutrients for good health⁷ and disturbed iron metabolism leads to lipid-protein oxidation and also damages RBC membrane.⁸ Various research studies found that the reactive free iron or iron overloads was responsible for diabetes.⁹⁻¹¹ The exact mechanism by which the altered iron metabolism leads to disease like diabetes is remains not understood.

**Aim and Objectives**

The main aim of this study was to evaluation iron profile in type 2 diabetes mellitus (T2DM) patients of tertiary care center of central India as compared to healthy age and gender matched controls. The objectives for this study were as followed:

1. Comparative study of anthropometric parameters of T2DM patients and control subjects.
2. Comparative study of biochemical parameters of T2DM patients and control subjects.

**Materials and Methods**

**Study Design:** This case-control study was carried out in the department of biochemistry, Gajara Raja medical college, Gwalior (Madhya Pradesh) India. The study was approved by Institutional Ethical Committee, G.R. Medical College, Gwalior.

**Study Population:** This study was conducted on 100 subjects in which 50 were diagnosed cases of T2DM patients and 50 were age and gender matched healthy control subjects.

**Study Record:** Clinical histories of patients were recorded in study proforma. Questionnaires and consent was taken from each patient before the study was started.

**Inclusion Criteria:** Diagnosed T2DM patients having fasting blood glucose ≥ 126mg/dl or HbA1c ≥ 6.5%.¹²

**Exclusion Criteria:** Patients taking drugs which disturbed iron metabolism, patients undergo previous blood transfusion, patients having haemoglobinopathies, genetic mutations which causes iron overload and pregnant women were excluded from the study.
Anthropometric Parameters Measurements: BMI was calculated by dividing body weight (Kg) which was calculated by using digital scale nearest to 0.1 kg to square of height (meters) which was measured by using commercial stadiometer to the nearest 0.1cm. The waist/hip circumference ratio was calculated by dividing the waist circumference (cm) measured by using measuring tape midway between lower border of rib cage, and the iliac crest to hip circumference (cm) measured by using measuring tape around the point with the maximum circumference over the buttocks. The blood pressure was measured by average of two reading of sphygmomanometer.

Sample Collection and Preparation: 5 ml of fasting blood samples were withdrawn from the antecubital vein. Blood samples were collected in plain vacutainers, fluoride vacutainers and EDTA vacutainers. The plain vacutainers was incubated at 37°C for 30 minutes then clot was removed from plain vacutainers and remaining sample was taken in centrifuged test tube. Samples were centrifuged at 3000 rpm for 10 to 20 minutes. Supernatant collected in clean and dry test tube for analysis of biochemical test.

Analysis of Sample: The serum iron, total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC) and plasma glucose was estimated semi automated chemistry analyzer (Auto lab 200) using ferrozine method. The Glycated hemoglobin (HbA1c) was measured by boronate affinity chromatography using Nyco card reader. The serum ferritin was estimated by chemiluminescence method. The percentage transferrin saturation was calculated by formula serum iron (μg/dl) x 100/ TIBC (μg/dl).13

Statistical Analysis
The data was documented in Microsoft Excel 2010 and formatting was done in the same. The data was analyzed in SPSS-24. Z test was used to compare the anthropometric and biochemical parameters of T2DM and control cases. The Graph was plotted with the help of graph pad prism 7.

Results
The anthropometric parameters like weight, BMI, waist circumference, hip circumference, waist hip circumference ratio, systolic and diastolic blood pressure was statistically extremely significantly (p<0.001) increased in T2DM subjects as compared to healthy control subjects which is shown in table 1. The graphical presentation of anthropometric parameters of T2DM patients and control subjects was shown in Fig. 1. The plasma fasting glucose, HbA1C, serum iron, % saturation and serum ferritin were statistically extremely significantly (p<0.001) increased and UIBC was statistically extremely significantly (p<0.001) decreased while TIBC and hemoglobin were statistically significantly (p<0.05) decreased in T2DM subjects as compared to healthy control subjects which is shown in table 2. The graphical presentation of glycemic parameters and iron profile parameters of T2DM patients and control subjects was shown in Fig. 2.

![Graphical presentation of anthropometric parameters of T2DM patients and control subjects](image-url)
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**Fig. 2:** Graphical presentation of Glycemic parameters and iron profile parameters of T2D patients and control subjects

**Table 1:** Showing the comparative changes of anthropometric parameters and iron profile parameters of T2DM patients and control subjects

<table>
<thead>
<tr>
<th>Groups</th>
<th>Weight (Kg)</th>
<th>Height (meters)</th>
<th>BMI (Kg/m²)</th>
<th>Waist (cm)</th>
<th>Hip (cm)</th>
<th>W/H Ratio</th>
<th>Systolic BP (mmHg)</th>
<th>Diastolic BP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control subjects (n=50)</td>
<td>Min 49</td>
<td>1.45</td>
<td>20.17</td>
<td>70.10</td>
<td>87.75</td>
<td>0.69</td>
<td>134</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Max 62</td>
<td>1.65</td>
<td>24.67</td>
<td>79.50</td>
<td>105.80</td>
<td>0.89</td>
<td>149</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>Mean 54.8</td>
<td>1.53</td>
<td>23.39</td>
<td>75.92</td>
<td>95.78</td>
<td>0.79</td>
<td>142.58</td>
<td>79.86</td>
</tr>
<tr>
<td><strong>± SD</strong></td>
<td>3.37</td>
<td>0.05</td>
<td>1.05</td>
<td>2.29</td>
<td>4.06</td>
<td>0.04</td>
<td>3.75</td>
<td>2.78</td>
</tr>
<tr>
<td>Diabetic subjects (n=50)</td>
<td>Min 55</td>
<td>1.46</td>
<td>21.34</td>
<td>76.66</td>
<td>91.10</td>
<td>0.75</td>
<td>112</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>Max 74</td>
<td>1.65</td>
<td>32.37</td>
<td>89.49</td>
<td>105.00</td>
<td>0.94</td>
<td>127</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>Mean 63.92</td>
<td>1.54</td>
<td>27.22</td>
<td>82.30</td>
<td>99.62</td>
<td>0.83</td>
<td>120.58</td>
<td>90.86</td>
</tr>
<tr>
<td><strong>± SD</strong></td>
<td>5.00***</td>
<td>0.05NS</td>
<td>2.96***</td>
<td>3.82***</td>
<td>3.40***</td>
<td>0.04***</td>
<td>3.75***</td>
<td>2.58***</td>
</tr>
</tbody>
</table>

* Statistically significant at 0.05 (p<0.05) **Statistically highly significant at 0.01 (p<0.01)
*** Statistically extremely Significant at 0.001 (p<0.001) NSNon significant

**Table 2:** Showing the comparative changes of glycemic parameters and iron profile parameters of T2DM patients and control subjects

<table>
<thead>
<tr>
<th>Groups</th>
<th>FBS (mg/dl)</th>
<th>HbA1C (%)</th>
<th>Iron (µg/dl)</th>
<th>TIBC (µg/dl)</th>
<th>UIBC (µg/dl)</th>
<th>% Saturation</th>
<th>Ferritin (ng/ml)</th>
<th>Hemoglobin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control subjects (n=50)</td>
<td>Min 78</td>
<td>4.2</td>
<td>79.4</td>
<td>267</td>
<td>127.2</td>
<td>20.75</td>
<td>92</td>
<td>10.9</td>
</tr>
<tr>
<td></td>
<td>Max 98</td>
<td>4.9</td>
<td>148.4</td>
<td>401.5</td>
<td>303.2</td>
<td>52.48</td>
<td>287</td>
<td>14.9</td>
</tr>
<tr>
<td></td>
<td>Mean 87.18</td>
<td>4.57</td>
<td>111.84</td>
<td>326.18</td>
<td>214.34</td>
<td>35.7</td>
<td>190.94</td>
<td>12.7</td>
</tr>
<tr>
<td><strong>± SD</strong></td>
<td>12.13</td>
<td>0.21</td>
<td>21.45</td>
<td>46.52</td>
<td>65.29</td>
<td>10.91</td>
<td>55.64</td>
<td>1.04</td>
</tr>
<tr>
<td>Diabetic subjects (n=50)</td>
<td>Min 138</td>
<td>7.0</td>
<td>57.80</td>
<td>133.8</td>
<td>41</td>
<td>18.87</td>
<td>268</td>
<td>9.1</td>
</tr>
<tr>
<td></td>
<td>Max 315</td>
<td>13.4</td>
<td>27.40</td>
<td>447.1</td>
<td>305.1</td>
<td>78.31</td>
<td>560</td>
<td>14.5</td>
</tr>
<tr>
<td></td>
<td>Mean 212.1</td>
<td>9.24</td>
<td>134.66</td>
<td>300.16</td>
<td>165.5</td>
<td>42.08</td>
<td>400.24</td>
<td>12.13</td>
</tr>
<tr>
<td><strong>± SD</strong></td>
<td>48.92***</td>
<td>1.65***</td>
<td>42.06***</td>
<td>70.57***</td>
<td>15.39***</td>
<td>64.28***</td>
<td>1.54*</td>
<td></td>
</tr>
<tr>
<td>SE 6.969</td>
<td>0.236</td>
<td>6.67</td>
<td>12.966</td>
<td>13.596</td>
<td>2.567</td>
<td>12.02</td>
<td>0.263</td>
<td></td>
</tr>
</tbody>
</table>

* Statistically significant at 0.05 (p<0.05) **Statistically highly significant at 0.01 (p<0.01)
*** Statistically extremely Significant at 0.001 (p<0.001) NSNon significant

**Discussion**

In this study we found that the body weight, BMI, waist circumference, hip circumference, waist-hip circumference ratio, systolic and diastolic blood pressure were significantly increased in T2DM patients as compared to normal age and gender matched healthy control subjects. Similar results had been found in various research studies in T2DM patients. The FBG, HbA1C, serum iron, % saturation and serum ferritin were significantly increased while TIBC, UIBC and hemoglobin were significantly decreased in T2DM patients as compared to normal age and gender matched healthy control subjects. Various scientific studies also found similar results in T2DM patients. There is a close relationship between iron profile and T2DM because altered glucose metabolism altered the iron profile and vice versa. This bidirectional relationship occurs because of the altered iron profile or free iron induces oxidative stress and produces inflammatory cytokines. Huang J et al., 2015

found that the serum ferritin concentration was negatively associated with insulin sensitivity indicated close association between insulin resistance and total body iron stores. Similar study conducted in Brazil by Monteiro SCM et al. 2016 found the association between prediabetes, insulin resistance and serum ferritin. Obesity is a complication and risk factor for T2DM. There is accumulation of fat in the fat cell, and these fat cells produce chemicals that lead to inflammations. In T2DM patients, hyperglycemia negatively influences many metabolic processes including iron metabolism. Various studies had shown iron overload in type 2 diabetes mellitus. Via Haber–Weiss and Fenton reactions, the free iron radicals initiate oxidation of biomolecules leading to generation of hydroxyl radical (OH*). These radicals damage cellular membrane protein and nucleic acid. These events lead to insulin resistance and finally type 2 diabetes mellitus. The free OH* causes non-enzymatic glycation of protein. The non-enzymatic glycation of proteins followed by a series of reactions and rearrangements resulting in the formation of advanced glycation end products (AGEs). These mechanisms, together with the interaction of the AGEs with their receptors (RAGE), induce ROS production. The Glycation transferrin has decreased ability to bind Fe3+ and thus induces the pool of free iron. The free iron and oxidative stress also promote the synthesis of ferritin.

**Conclusion**

Hence, the evaluation of iron profile can be a useful outcome of the predictable studies on diabetes and related complications. Elevated iron and ferritin is a risk factor for diabetes and produce many complications. Proper management must be taken to remove the excess iron that can be very harmful to the body.

**Conflict of Interest:** None.

**References**


**How to cite this article:** Dhakad GS, Sharma AK, Kanwar G, Singh AK, Sharma S. Evaluation of iron profile in type 2 diabetes mellitus patients of tertiary care center of central India. *Int J Clin Biochem Res* 2019;6(1):15-19.