

## Elevated levels of Ferritin and hs-CRP in type 2 diabetes

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### Abstract

**Objectives:** To measure the levels of high-sensitivity C-reactive protein and ferritin in blood and to assess their association with inflammation in people with Type 2 diabetes.

**Method:** The case-control study was conducted between November 2012 to November 2013 at the Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, and comprised randomly selected patients and healthy controls. Fasting blood samples were analysed for blood glucose, insulin, high-sensitivity C-reactive protein and iron status. SPSS 19 was used for statistical analysis.

**Results:** Of the 210 subjects initially selected, 99(47%) were excluded owing to anaemia. The study population, as such, comprised 111(53%) with an overall mean age of  $38.6 \pm 1.56$  years and mean fasting blood sugar of  $110.78 \pm 3.795$  mg/dl. Group 1 had 44(39.6%) healthy controls while Group 2 had 67(60.3%) diabetic patients. Elevated levels of mean serum ferritin ( $233.11 \pm 43.84$  ng/ml), insulin ( $29.94 \pm 2.19$ ), homeostasis model of insulin resistance ( $10.23 \pm 0.89$ ) and high-sensitivity C-reactive protein ( $5.29 \pm 0.80$  mg/L) with low serum iron levels ( $1.07 \pm 0.115$  ?g/dl) were found in Group 2. There was positive correlation of homeostasis model of insulin resistance with fasting blood sugar ( $r=0.596$ ;  $p<0.001$ ), serum ferritin ( $r=0.306$ ;  $p<0.008$ ), insulin ( $r=0.866$ ;  $p<0.001$ ), and total iron-binding capacity ( $r=0.302$ ;  $p<0.009$ ). There was negative correlation with serum iron ( $r=-0.280$ ;  $p<0.016$ ) and transferrin saturation ( $r=-0.316$ ;  $p<0.006$ ).

**Conclusion:** Elevated ferritin levels without evident iron overload may affect glucose homeostasis, leading to insulin resistance in conjunction with inflammatory changes as seen by elevated C-reactive protein levels.

**Keywords:** Ferritin, hs-CRP, T2DM, Insulin resistance. (JPMA 64: 1389; 2014)

### Introduction

Type 2 Diabetes Mellitus (T2DM) is a major globally emerging lifestyle disorder. Asian countries add to more than 60% of the world's diabetic population.<sup>1</sup> T2DM is a complex disorder involving interaction of both genetic and environmental factors. Individuals with T2DM show both insulin resistance (IR) and beta cell defects. Post-binding defects in insulin action are generally primarily responsible for IR in T2DM.<sup>2</sup>

The relationship between T2DM and iron metabolism has gained interest in both research and clinical practice. Scientific evidences have predicted influences of elevated serum ferritin levels on IR and T2DM either because of increased body iron stores or influenced by several inflammatory diseases.<sup>3,4</sup> Thus it is postulated that circulating levels of ferritin, also an acute phase reactant, are not truly reflective of body iron stores but may reveal other processes such as systemic inflammation.<sup>5,6</sup>

The process of inflammation induces hepatic synthesis of various acute phase proteins such as high-sensitivity C-reactive protein (hs-CRP) and serum ferritin, which are believed to play a role in IR at the cellular level.<sup>7,8</sup> Keeping this model under consideration, the current study aimed at associating increased iron status and hs-CRP levels with diabetes and assessing the correlation of ferritin with IR.

### Patients and Methods

The case-control study was conducted at the Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, between November 2012 to November 2013, and comprised randomly selected patients and healthy controls. In order to achieve minimum 80% power with a 13.14% estimated prevalence of disease in project area and a two-sided 5% level of significance, the minimum sample size required, according to Power and Sample Size (PASS) version 11, was 42 for each group.

After recruiting the subjects randomly from the general population, exclusions were made on the basis of complete blood count (CBC) showing iron deficiency anaemia. The remaining subjects were divided into two groups. Group I had healthy controls and Group 2 had T2DM patients.

Those excluded were older than 65 years and/or with

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clinically significant concurrent conditions (iron deficiency anaemia, cardiovascular disease, acute infectious disease or chronic inflammatory or debilitating disease), smoking and alcoholism and medication (multivitamins and iron supplements) usage. The research protocol was approved by the institutional ethics committee and all clinical investigations were conducted according to the Declaration of Helsinki. After obtaining written informed consent from all subjects, 10ml of blood was collected from each participant. Biomarkers were analysed in the serum samples. Commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits were used to determine serum ferritin by BioCheck (Foster city, CA, USA, cat #. BC-1025), hs-CRP by Dia Source Immunoassay SA (Belgium, Ref# KAPDB4360) and serum insulin by Dia Source Immunoassay SA (Belgium, cat# KAP1251). Additionally, all subjects had their fasting blood sugar (FBS) estimated by Glucose-Oxidase-Phenol-Aminophenazone (GOD-PAP) method (Merck, France). The IR level was calculated using the homeostasis model assessment of insulin resistance (HOMA-IR) index [fasting insulin (units per milliliter) x fasting glucose milligram/deciliter]/405].<sup>9</sup> Serum iron and total iron-binding capacity (TIBC) was determined by enzymatic colorimetric method by BioMerieux (S.A. France). Transferrin saturation% was calculated by 100 x serum iron / TIBC.<sup>10</sup>

Data was analysed using SPSS 19. Mann Whitney U test was applied for comparison between groups. Mean  $\pm$  standard deviation (SD) were calculated for quantitative variables. Spearman correlation was applied to correlate variables and  $p < 0.05$  was considered significant.

## Results

Of the 210 subjects initially selected, 99(47%) were

**Table-1:** Anthropometric, serum glycaemic and serum iron parameters.

| Parameters                       | Controls n= 44      | Diabetics n= 67      | P value |
|----------------------------------|---------------------|----------------------|---------|
| Age                              | 29.93 $\pm$ 1.81    | 47.27 $\pm$ 1.322**  | < 0.001 |
| Weight                           | 62.52 $\pm$ 1.87    | 76.30 $\pm$ 2.74     | <0.001  |
| Fasting blood sugar (mg/dl)      | 84.54 $\pm$ 2.96    | 137.02 $\pm$ 4.63**  | < 0.001 |
| Serum Insulin(IU/ml)             | 22.59 $\pm$ 1.68    | 29.94 $\pm$ 2.19*    | 0.037   |
| Insulin resistance (HOMA-IR)     | 4.61 $\pm$ 0.46     | 10.23 $\pm$ 0.89**   | <0.001  |
| Serum iron (?g/dl)               | 2.44 $\pm$ 0.26     | 1.07 $\pm$ 0.155**   | <0.001  |
| Serum TIBC (?g/dl)               | 1.91 $\pm$ 0.21     | 2.98 $\pm$ 0.14**    | <0.001  |
| Serum transferrin saturation (%) | 306.63 $\pm$ 142.57 | 65.51 $\pm$ 18.14**  | <0.001  |
| Serum ferritin (ng/ml)           | 132.45 $\pm$ 38.08  | 233.11 $\pm$ 43.84** | <0.001  |
| hs-CRP (mg/L)                    | 2.49 $\pm$ 0.66     | 5.29 $\pm$ 0.80*     | 0.002   |

HOMA-IR: Homeostasis model of insulin resistance. TIBC: Total iron-binding capacity.

hs-CRP: High-sensitivity C-reactive protein.

Values are expressed as Mean  $\pm$  SD. Comparison between groups was tested by Mann - Whitney U test. \*Statistically significant as compared to non-diabetic, where  $p$  value < 0.05.

\*\*Statistically significant as compared to non-diabetic, where  $p$  value < 0.01.

**Table-2:** Correlation of insulin resistance with iron parameters, FBS and CRP (n= 111).

| Correlations of insulin resistance with: | Spearman's Correlation Coefficient | P value |
|------------------------------------------|------------------------------------|---------|
| Serum transferrin saturation             | -0.316                             | 0.006*  |
| Serum ferritin                           | 0.306                              | 0.008*  |
| Serum iron                               | -0.280                             | 0.016*  |
| Serum total iron binding Capacity        | 0.302                              | 0.009** |
| Fasting blood Glucose                    | 0.596                              | 0.000** |
| hs-CRP                                   | 0.089                              | 0.450   |

FBS: Fasting blood sugar. hs-CRP: High-sensitivity C-reactive protein.

\*Statistically significant as compared to non-diabetic, where  $p$  value < 0.05.

\*\*Statistically significant as compared to non-diabetic, where  $p$  value < 0.01.

excluded owing to anaemia. The study population, as such, comprised 111(53%) with an overall mean age of 38.6 $\pm$ 1.56 years (range: 15-65 years) and mean fasting blood sugar of 110.78 $\pm$ 3.795mg/dl. Group 1 had 44(39.6%) healthy controls while Group 2 had 67(60.3%) diabetic patients.

The mean levels of blood glucose ( $p < 0.001$ ), insulin ( $p = 0.037$ ), IR ( $p < 0.001$ ), ferritin ( $p < 0.001$ ), and hs-CRP ( $p < 0.05$ ) were significantly higher in T2DM compared to the controls. However, serum iron were considerably lower in Group 2 compared to Group 1 ( $p < 0.001$ ) (Table-1).

There was significant positive correlation with ferritin ( $r = 0.306$ ) and TIBC ( $r = 0.302$ ) in T2DM. A negative but significant correlation was found between IR and serum iron ( $r = -0.28$ ) and serum transferrin saturation ( $r = -0.316$ ) in Group 2 (Table-2).

## Discussion

According to the contemporary understanding, IR has been conventionally regarded as the key stage of T2DM pathogenesis. The well-established IR markers are glucose, insulin, and C-peptide concentrations.<sup>11</sup> Sub-clinical inflammation with activated cytokines is also a key feature of this disease. Along with cytokine production, inflammation is accompanied by the production of acute-phase proteins, primarily CRP.<sup>12</sup> It is postulated that CRP induces serine phosphorylation in the insulin-receptor domain, which impairs the ability of the latter to activate phosphatidylinositol 3-kinase and leads to IR development.<sup>13</sup> However, in our study we did not find any significant correlation between hs-CRP and IR despite having high levels in T2DM.

Our results showed significant correlation between elevated serum ferritin concentrations and IR. This finding is consistent with previous studies conducted on Chinese and Korean populations.<sup>14,15</sup> Elevated levels of ferritin

have been recognised as a feature of T2DM. The relationship between iron levels and T2DM is complex. Insulin stimulates ferritin synthesis and activates the iron upload and contrarily iron influences the insulin inhibition of glucose production from liver.<sup>16,17</sup> On the other hand, ferritin acts as an indicator of pancreatic inflammation and thus referred to as marker for IR.<sup>18</sup>

One study<sup>19</sup> reported a 2.5 times higher ferritin concentration in T2DM compared to healthy people, although the rate of transferrin receptors showed no significant difference, suggesting a link with increased serum ferritin, and negative iron levels may be due to inflammatory problem rather than iron overload. Our results support the same hypothesis. Another study<sup>20</sup> reported that serum ferritin concentration can possibly be an indicator of systemic fat content and degree of IR. Perhaps because of selection bias, in our study group, the possible association was not solely linked to diabetes.

The results are consolidated by correlation of IR with low iron levels, low transferrin saturation, high TIBC and high ferritin levels, revealing that high levels of ferritin are not due to iron overload but due to inflammatory changes which subsequently cause IR leading to T2DM irrespective of gender. Influence of iron deficiency does not change the observation, as ferritin levels are clearly non-parallel with the iron levels.

The results of our study did not consider gender as a discriminator as inflammation in T2DM patients of any gender will show the same results but still we consider it a limitation of the study.

## Conclusion

Elevated ferritin levels without evident iron overload may affect glucose homeostasis. This may lead to the development of IR in conjunction with inflammatory changes as seen by elevated CRP levels.

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