Iron Indices are higher in Women with Polycystic Ovary Syndrome than Healthy Control.

Dr. Hussein Kadhem Al-Hakeem (PhD)*
Dr. Mohammed F. A. M. El-Hadi (M.B.Ch.B., M.Sc) **
Dr. Raheem Mahdy Raheem (M.B.Ch.B, FICMS/Hematology) ***

(*)- Department of Chemistry, College of Science, Al-Kufa University, Iraq.
(**)- Department of Biochemistry, College of Medicine, University of Karbala, Iraq.
(***)- Correspondence to: Dr. Hussein Kadhem Abdul Hussein, College of Science, Al-Kufa University, Iraq. (Mobile: 07801035399) E-mail: headm2000@yahoo.com

Abstract
Background: The prevalence of the metabolic syndrome in Poly Cystic Ovary Syndrome (PCOS) is approximately 43-47%, a rate 2-fold higher than that for women in the general population. The pathogenic link between the metabolic syndrome and PCOS is most likely insulin resistance that present in obese and non-obese women. Increased body iron stores are associated with insulin resistance and type 2 diabetes. The aim of the present study is to evaluate the iron status parameters in PCOS patients as a possible cause of the associated features of PCOS.

Patients and methods: 48 patients with PCOS diagnosed according to the Rotterdam revised consensus meeting in 2003 with matched control of 37 apparently healthy women were examined. Blood were aspirated from individuals in the morning and serum iron, total iron binding capacity TIBC, serum ferritin, estimated total iron body
stores (ETIBS), Transferrin, and Transferrin saturation percentage (TSP) were determined.

**Result:** There is a significant difference ($p<0.05$) in all iron indices of PCOS patients in comparing with healthy control group. All parameters are increased in PCOS patients except TIBC which decrease in these patients in comparing with healthy control group. There is no or very small correlation between ETIBS and each serum index in PCOS patients while there is a small positive correlation in ETIBS in comparing with both serum iron and TIBC.

**Conclusion:** In addition to the laboratory tests that should be measured in PCOS women including lipid profile and fasting serum glucose \(^{(51)}\), this study suggests estimation of serum ferritin as a possible important tool in the follow up of routine care.

**Introduction:** There is an increasing evidence that patients with polycystic ovary syndrome (PCOS) have an increased cardiovascular risk compared with age matched controls and it has been estimated that myocardial infarction is seven times more likely in patients with PCOS \(^{(1, 2)}\). Many researches have shown that PCOS is not only a gynaecological condition affecting women of reproductive age but also, a comprehensive syndrome with a variety of associated metabolic disorders (insulin resistance, obesity, hyperinsulinaemia and dyslipidaemia) \(^{(3, 4, 5)}\) began commonly to be described as associated with PCOS. These disorders are also the features of the so-called metabolic syndrome or syndrome X, as defined by either the World Health Organization or the Adult Treatment Panel (ATP III) \(^{(6)}\). There is also some discussion as to whether PCOS itself could be another feature of syndrome X \(^{(7)}\).

The prevalence of the metabolic syndrome in PCOS is approximately 43-47%, a rate 2-fold higher than that for women in the general population women \(^{(8, 9)}\). High body mass index and low serum HDL cholesterol are the most frequently occurring components of the metabolic syndrome in PCOS \(^{(10)}\). The pathogenic link between the metabolic syndrome and PCOS is most likely insulin resistance that present in obese and nonobese women \(^{(11, 12)}\). Obesity, atherogenic dyslipidemia, hypertension, impaired fasting glucose/impaired glucose tolerance, and vascular abnormalities are all common metabolic abnormalities present in PCOS \(^{(8)}\).

Many parameters used as multiple risk factors for cardiovascular diseases in PCOS including atherogenic disturbance of lipid profile (high triglycerides, high LDL, and low HDL which is associated with the presence of insulin resistance) \(^{(13, 14)}\), homocysteine \(^{(15)}\), endothelial dysfunction markers \(^{(16)}\), oxidative stress, and decreased antioxidant capacity may contribute to the increased risk of cardiovascular disease in women with PCOS \(^{(17)}\).

Increased body iron stores are associated with insulin resistance and type 2 diabetes. In conceptual agreement, increased serum ferritin levels are positively associated with the prevalence of the metabolic syndrome in men and adult pre- and postmenopausal women \(^{(18)}\) and with an increased risk of type 2 diabetes in both men and women \(^{(19, 20)}\). Given that insulin resistance and an increased risk of type 2 diabetes are frequent in patients with polycystic ovary syndrome (PCOS) \(^{(3, 21)}\). The aim of the present study is to evaluate the iron status parameters in PCOS patients in comparing with healthy control as a possible cause (by precipitation the iron in certain tissues and subsequent damage to those tissues) of the associated features of POCS.

**Materials and Methods:**
Patients: After ethics committee approval, 47 women with PCOS recruited (mean age, 28.5; SD, 6.5 years. Patients with PCOS were recruited from the gynecological clinics in Najaf, Iraq. PCOS was defined according to the Rotterdam revised consensus meeting in 2003, it was proposed that oligomenorrhoea, clinical or biochemical hyperandrogenaemia and the presence of polycystic ovaries should serve as the diagnostic criteria for PCOS \(^{(22)}\). Control subjects were 38 healthy control women with a normal menstrual cycle and with no clinical or biochemical features of hyperandrogenism.

Measurements: Blood were aspirated from individuals in the morning and collected in plain tube for serum separation by centrifugation in order to estimate the iron status parameters. Serum levels of iron were estimated using colorimetric method, total Iron Binding Capacity (TIBC) were estimated colorimetrically by the following procedure: An excess of iron is added to the serum iron to saturate the transferrin. The unbound iron is precipitated with basic magnesium carbonate. After centrifugation the iron in the supernatant was determined. The Ferritin Quantitative Test is based on a solid phase enzyme-Linked immunosorbent assay (ELISA). The assay system utilizes one rabbit anti-ferritin antibody for solid phase (microtitre wells) immobilization and a mouse monoclonal anti-ferritin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. Estimated total iron body stores (ETIBS) were estimated using the following formula \(^{(23, 24)}\):

Estimated Total Iron Body Stores in µmol = (serum ferritin in µg/L) * 143

Transferrin saturation percentage (TSP) was calculated by dividing serum iron concentration by TIBC \(^{(25)}\). The percentage of transferrin saturation was calculated from the serum iron and transferrin concentrations, using the formula: serum iron (µmol/L)/transferrin (g/L) x 3.98. The formula is based on the maximal binding of 2 mol Fe\(^{3+}\)/mol of transferrin and a molecular weight of 79,570gm/mol for transferrin \(^{(26)}\). From this formula

\[
\text{Transferrin (g/L)} = \frac{\text{S.Iron(µmol/L)}}{\text{Transferrin saturation%} \times 3.98}
\]

In order to exclude the patients with chronic inflammation (which affects serum ferittin level), semiquantitative estimation of C - reactive protein was used in these women to assess the potential confounding effect of chronic inflammation on the observed increase in ferritin levels \(^{(27)}\). This study excluded any patient with a positive C-reactive protein (more than 6mg/L).

Biostatistical analysis:- The results were expressed as (mean±standard deviation ). Pooled t-test was used for the comparison of significant difference between the healthy and control groups in the measured parameters. Correlation coefficient (r) was also used for searching about any correlation between the parameters.

Results:

The results of iron indices expressed as mean ± standard deviation are presented in Table (1). There is a significant difference (p<0.05) in all iron indices of PCOS patients in comparing with healthy control group. All parameters are increased in PCOS patients except TIBC which decrease in these patients in comparing with healthy control group.

| Table (1): Iron parameters in PCOS patients and control group expressed as mean ± standard deviation. |
Iron Index | PCOS patients M±SD | Control M±SD | Significance (p<0.05)
---|---|---|---
S.Ferritin (ug/L) | 137.3±31.3 | 71.9±26.4 | Significant
ETIBS (mmol) | 19.6±4.5 | 10.3±3.8 | Significant
S.Iron (umol/L) | 28.5±8.6 | 19.1±4.4 | Significant
Transferrin Conc. (g/L) | 0.117±0.025 | 0.138±0.025 | Significant
S.TIBC (umol/L) | 46.5±9.8 | 55.1±10.1 | Significant
TSP (%) | 86.6±29.8 | 33.5±8.6 | Significant

In order to study the correlation between ETIBS, which distributed in all body tissues, and iron indices in serum, the correlation coefficient (r) were calculated for ETIBS and each serum index. From the results in Table (2), it is obvious that there is no or very small correlation between ETIBS and each serum index in PCOS patients while there is a small positive correlation in ETIBS in comparing with both serum iron and TIBC.

Table (2): Correlation coefficients (r-values) of the comparisons between ETIBS and other iron indices.

<table>
<thead>
<tr>
<th>Comparisons</th>
<th>PCOS</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETIBS vs. S.Iron</td>
<td>-0.24</td>
<td>0.49</td>
</tr>
<tr>
<td>ETIBS vs. Transferrin</td>
<td>0.12</td>
<td>0.27</td>
</tr>
<tr>
<td>ETIBS vs. TSP</td>
<td>-0.27</td>
<td>0.31</td>
</tr>
<tr>
<td>ETIBS vs. TIBC</td>
<td>0.13</td>
<td>0.53</td>
</tr>
</tbody>
</table>

Discussion:
The increase in iron indices in PCOS patients, especially ETIBS (Table 1) indicating high availability of iron in different tissues. This increase in tissues iron are in agreement with the result of other researches (27, 28). It is hypothesized that genetic factors, the absence of a regular menstrual blood loss, or even hyperinsulinemia resulting from insulin resistance, considering that insulin might stimulate intestinal iron absorption by upregulating the activity of hypoxia-inducible factor-1alpha and down regulating hepcidin expression (29, 30), may have contributed to the increased body iron stores and serum ferritin levels observed in PCOS patients.

Elevated iron stores were positively associated with the prevalence of the metabolic syndrome and with insulin resistance (31, 32, 33, 34). Insulin resistance, hyperinsulinaemia, and obesity are all seen in patients with PCOS (21, 35, 36, 37, 38). The presence of insulin resistance and obesity contribute a high prevalence of the metabolic syndrome in PCOS patients (39, 40, 41).

The increased iron stores might contribute to the insulin resistance and β-cell dysfunction frequently found in PCOS patients, as has been proposed for insulin resistance, the metabolic syndrome, and type 2 diabetes (18, 19, 42, 43). The results of Luque-Ramírez et al (2007) (28) suggested that insulin resistance and hyperinsulinism,
and not the reduced menstrual losses secondary to from oligo- or amenorrhea, are responsible of the increased ferritin levels and body iron stores found in overweight and obese women with PCOS.

Yet several of these factors might collaborate in the increased body stores observed in overweight and obese PCOS patients. As proposed for type 2 diabetes (31), the insulin resistance intrinsic to PCOS, exacerbated by obesity and perhaps dietary influences, may facilitate iron absorption and deposition in tissues, a mechanism possibly amplified by the reduced menstrual losses of PCOS patients. Iron deposition in certain tissues increases insulin resistance, closing the vicious circle of iron overload and predisposing these women to disorders of glucose tolerance and other components of the metabolic syndrome. The contrary is also noticed when reduce ETIBS, for instance by blood donation (44), improves insulin sensitivity (45).

Because the periodic blood loss resulting from regular menstruation protects premenopausal women against excessive iron accumulation, oligomenorrhea and amenorrhea might contribute to the increase in ferritin observed in overweight and obese PCOS patients. When PCOS patients and control subjects studied as a whole, ferritin levels were increased in women with amenorrhea compared with women with regular menstrual cycles, whereas women with oligomenorrhea presented with intermediate values (27). However, the increase in serum ferritin levels in PCOS may be a secondary, not a pathogenic, event in PCOS. The absence of regular menstrual blood loss in PCOS patients might contribute to iron overload, as serum ferritin levels were increased in our amenorrheic patients compared with regularly menstruating women. Oxidative stress increases ferritin synthesis, partly to avoid further oxidative damage, given that ferritin neutralizes the highly toxic unbound iron (31), and oxidative stress may be increased in PCOS women (17, 46). Also, the hyperinsulinemia resulting from insulin resistance may contribute to increased body iron stores and serum ferritin levels because insulin may stimulate intestinal iron absorption. (47). Additionally, it's well known that in PCOS there is hyperandrogenemia which affects on erythropoiesis processes (48). This phenomenon correlated hyperandrogenemia in PCOS with one of the important iron function.

Therefore, and considering that insulin might stimulate intestinal iron absorption by upregulating activity of hypoxia-inducible factor-1alpha and down regulating hepcidin expression (29, 47), the amelioration of insulin resistance and hyperinsulinemia by metformin may explain the reduction in serum ferritin levels and iron stores found in the PCOS patients treated with this insulin sensitizer, especially when, to our best knowledge, no direct interaction of metformin with the intestinal absorption of iron has been described to date (27).

It is considered that increased iron stores contribute to insulin resistance and hyperinsulinemia by reducing hepatic insulin extraction and metabolism (49) and by decreasing glucose uptake in muscle (50).

The results in Table (2) indicating lack, or very slight correlation, between ETIBS and any other iron indices in serum. This fact revealed that the iron that precipitate in any tissue do not extracted and circulated easily. While there is accumulation of iron in certain tissues, the circulating iron is low and does not reflect the iron status in other tissues except ferritin which is the precursors of the ETIBS (23, 24).
Conclusion:
In addition to the laboratory tests that should be measured in PCOS women including lipid profile and fasting serum glucose (51), this study suggests estimation of serum ferritin as a possible important tool in the follow up of routine care.

References:


32. Megan Jehn, MHS¹, Jeanne M. Clark, MD¹,² and Eliseo Guallar, Serum Ferritin and Risk of the Metabolic Syndrome in U.S. Adults Diabetes Care 27:2422-2428, 2004.

