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Abstract.

**Background:** Polycystic Ovary Syndrome (PCOS) is one of the most common endocrinopathies in women of reproductive age group.

**Aim:** to determine the relationship of Anti-Mullerian hormone (AMH) with hepcidin, ferritin, serum iron and interleukin-6 among PCOS women.

**Methods:** A total of 60 PCOS women enrolled in the study, whose ages ranged were between 15-45 years old versus control group (30 healthy volunteer females with regular menstrual cycles aged between 15 to 45 years). All PCOS patients and healthy control underwent full physical examination and anthropometric measurements. Blood samples were collected from each woman for measurement of AMH, and hepcidin. The study showed that the Lowest mean of hepcidin was observed among PCOS women (13.27±1.46 ng/ml) as compared to the control group of non-PCOS women (98.76±2.88 ng/ml). The mean SD of AMH in PCOS women was (7.63±3.66 ng/ml), which was significantly higher than the control group with a mean SD of (2.09±1.11 ng/ml). Based on the study findings, women with PCOS had significantly higher average serum iron levels compared to the control group (223.5±57.3 and 129.144.9 g/dl), serum ferritin levels were significantly elevated in women with PCOS (279.9±44.9 and 189.5 57.3 ng/ml). The mean level of hepcidin was (14.77±1.31 ng/ml) in overweight PCOS women, which was elevated significantly than in PCOS women with normal BMI (12.18±1.58 /ml). The study found a negative correlation of serum hepcidin with each iron, ferritin and AMH among PCOS women.

**Key words:** PCOS, anti-mullerian hormone, hepcidin, ferritin, iron, interleukin-6.

**Introduction.**

Polycystic Ovary Syndrome (PCOS) is a common female gynaecological endocrinopathy disorder that affects women between the ages of 18 to 45 years. It is characterized by a range of signs and symptoms that include androgen excess, ovulatory dysfunction, and dysruptions to the hypothalamic–pituitary–ovarian (HPO) axis function [1-3]. PCOS is diagnosed by the appearance of at least two of the following criteria: increased androgenic hormones, irregular or absent ovulation, and enlarged ovaries comprising over 12 follicles [4].

It has lifelong implications with increased risk for obese and insulin-resistant, obese, and insulin-sensitive, normal-weight and insulin-resistant, and non-insulin-resistant [5]. Women with PCOS may present typical metabolic abnormalities such as insulin resistance (IR) and visceral obesity at a young age. Long-term exposure to these abnormalities throughout fertile life may exacerbate the adverse effects and expose these women to higher risks of metabolic syndrome (MetS), cardiovascular diseases (CVDs) and type II diabetes mellitus (T2DM) [6].

The cause of high production of anti-Mullerian hormone (AMH) in antral follicles of PCOS is currently unknown but there is evidence to support a role played by androgens. Indeed, a positive correlation between serum androgen and AMH levels has been reported and the production of androgens could be an intrinsic defect of thecal cells in PCOS [7]. Some investigators have suggested that increased AMH levels result from the stimulatory effect of androgens in early follicular growth, and others have concluded that AMH can be utilized as a diagnostic marker for ovarian hyperandrogenism [8].

Ferritin is an intracellular storage protein that is essential for the regulation of iron homeostasis. Concentrations of serum ferritin are being used as a biomarker to estimate the levels of body iron stores [9]. Iron is a strong pro-oxidant and high levels of it in the body are associated with an increased level of oxidative stress, which elevates the risk of T2DM and CVD. Whereas mildly elevated body iron stores are associated with impaired glucose tolerance [10].

Hepcidin regulates the body's iron levels, which is important in host defence. The serum hepcidin levels increase during inflammation and infection which is independent of iron levels. The IL-6 plays a major role in this issue [11,12]. The study aimed to determine the relationship between the AMH with hepcidin among PCOS women.

**Patients and Methods.**

A Case-control study is carried out in Kirkuk City from the 10th of November 2022 to the 10th of March 2023. The number of PCOS women under study was 60 women whose ages were between 15-45 years old. These patients were admitted to the obstetrics and gynaecology unit at Gynecological and Pediatric Hospital in Kirkuk City. PCOS was diagnosed based on the presence of two of the following Rotterdam criteria:

- Oligo and/or anovulation, clinical and/or Biochemical signs of hyperandrogenism, and Polycystic ovaries in ultrasound, meaning the presence of 12 or more follicles measuring 2-9 mm in diameter in each ovary and/or ovarian volume of more than 10 cm³.

In addition, the control group consisted of 30 healthy volunteer females with regular menstrual cycles aged between 15 to 45 years. All PCOS patients and healthy control underwent full physical examination and anthropometric measurements including weight, and height, and were asked to complete a general questionnaire. Body Mass Index (BMI) was calculated by using the formula: weight (kg)/height (meters²)

Patients with metabolic or endocrinology disorders including thyroid disorder, diabetes, hypertensive, and hyperprolactinemia, and excluded from the study by specific laboratory tests. Subjects with medication like ovulation induction agents, antiandrogens, antidiabetic, antiobesity, hormonal drugs and current or previous use were also excluded. Approval
permission was presented to the director of Kirkuk Health Directorate / Gynecological and Pediatric Hospital in Kirkuk City. Five ml of blood sample was taken by vein puncture from each subject enrolled in this study (women were in 2-5 days of menstrual cycle). Blood samples were added to gell tubes, after blood clotting, centrifuged at 3000 rpm for 15 minutes then the clot was removed and remained re-centrifuged at 3000 for 10 minute and the obtained serum were aspirated using a mechanical micropipette and transferred into clean test tubes which labelled and stored at -20°C for measurement of AMH, hepcidin by Enzyme-linked immunosorbent assay (ELISA), and serum ferritin, by immunofluorescence and serum iron by manual biochemistry kits.

Results.

The study showed that Women with PCOS were characterized by increased BMI (Body Mass Index) and were associated with various health conditions such as hirsutism, acne, menstrual cycle disturbance, and alopecia. In total, 60 patients were diagnosed with PCOS. Patients with Alopecia included 17 patients (28.33%) and 30 patients (71.67%) (Table 1).

Table 2 displays the hepcidin levels in two studied groups. In the patient group consisting of PCOS women, the mean ± SD hepcidin level was 13.27 ± 1.46 ng/ml. In contrast, the control group of non-PCOS women exhibited a mean ± SD hepcidin level of 98.76 ± 2.88 ng/ml. Notably, the hepcidin levels were significantly lower in PCOS women compared to non-PCOS women, as indicated by a p-value of 0.0001.

According to Table 3, the study revealed that the mean ± SD of AMH in PCOS women was 7.63 ± 3.66 ng/ml, which was significantly higher than the control group with a mean ± SD of 2.09 ± 1.11 ng/ml. The difference between the two groups was statistically significant, as indicated by a p-value of 0.0001.

Based on the study findings, women with PCOS had significantly higher average serum iron levels compared to the control group (223.5±57.3 and 129.1±44.9 g/dl, respectively). Additionally, the study demonstrated that serum ferritin levels were significantly elevated in women with PCOS (279.9±44.9 ng/ml) compared to the control group (189.5±57.3 ng/ml) (Table 4).

Table 5 shows that the mean level of hepcidin was (14.77±1.31 ng/ml) in overweight PCOS women, which was elevated significantly than in PCOS women with normal BMI (12.18±1.58 /ml) (P=0.006).

The study showed that the Mean±SD of AMH in PCOS women with BMI>25 (kg/m2) was (8.27±3.03 ng/ml) was significantly higher than in PCOS women with normal BMI (7.01±2.91 ng/ml) at P. value 0.001 (Table 6).

In addition, the study found a negative correlation of serum hepcidin with each iron, ferritin, and AMH among PCOS women. However, the study did not find any significant correlations between AMH and ferritin, or between serum iron and AMH (Table 7).

Discussion.

The finding that patients with PCOS have reduced serum hepcidin concentrations compared to healthy controls, as observed by Tawfeq et al. [1], suggests a potential link between PCOS and altered iron metabolism. Several other studies also found that patients with PCOS had serum hepcidin concentrations reduced than healthy controls [2,3]. Moreover, Sarhat et al. [4], in a recent study indicated a notable reduction in the serum levels of hepcidin PCOS group compared to the

Table 1. Clinical data of patients' group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
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<td></td>
</tr>
<tr>
<td>&lt;30 year</td>
<td>34</td>
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</tr>
<tr>
<td>≥30 year</td>
<td>26</td>
<td>43.33</td>
</tr>
<tr>
<td>Total</td>
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<td>100</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>17</td>
<td>28.33</td>
</tr>
<tr>
<td>25-29.9</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>≥30</td>
<td>13</td>
<td>21.67</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>Hirsutism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>7</td>
<td>11.67</td>
</tr>
<tr>
<td>Present</td>
<td>53</td>
<td>88.33</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>Acne</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>9</td>
<td>15.00</td>
</tr>
<tr>
<td>Present</td>
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<td>85.00</td>
</tr>
<tr>
<td>Total</td>
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</tr>
<tr>
<td>Menstrual cycle disturbance</td>
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</tr>
<tr>
<td>Regular</td>
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</tr>
<tr>
<td>Irregular</td>
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<tr>
<td>Total</td>
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<td>100</td>
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<td>Family history of PCOS</td>
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</tr>
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<td>10.00</td>
</tr>
<tr>
<td>Yes</td>
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<td>90.00</td>
</tr>
<tr>
<td>Total</td>
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<td>100</td>
</tr>
<tr>
<td>Alopecia</td>
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<td></td>
</tr>
<tr>
<td>Yes</td>
<td>17</td>
<td>28.33</td>
</tr>
<tr>
<td>No</td>
<td>30</td>
<td>71.67</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2. Mean levels of hepcidin in PCOS women and the control group.

<table>
<thead>
<tr>
<th>Hepcidin (ng/ml)</th>
<th>Studied groups</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients group</td>
<td>Control group</td>
</tr>
<tr>
<td>No.</td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>13.27±1.46</td>
<td>98.76±2.88</td>
</tr>
<tr>
<td>Minimum</td>
<td>10.77</td>
<td>23.55</td>
</tr>
<tr>
<td>Maximum</td>
<td>17.27</td>
<td>78.77</td>
</tr>
<tr>
<td>p value=0.0001</td>
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</tr>
</tbody>
</table>

Table 3. The level of AMH in blood serum.

<table>
<thead>
<tr>
<th>AMH (ng/ml)</th>
<th>Studied groups</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients group</td>
<td>Control group</td>
</tr>
<tr>
<td>No.</td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>7.63±3.66</td>
<td>2.09±1.11</td>
</tr>
<tr>
<td>Minimum</td>
<td>1.32</td>
<td>1.03</td>
</tr>
<tr>
<td>Maximum</td>
<td>13.56</td>
<td>2.98</td>
</tr>
<tr>
<td>P-value=0.0001</td>
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</table>

Table 4. Comparison between studied groups regarding serum iron and ferritin levels.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Studied groups</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient group</td>
<td>Control group</td>
</tr>
<tr>
<td>S. iron (g/dl)</td>
<td>223.5±57.3</td>
<td>129.1±44.9</td>
</tr>
<tr>
<td>S. Ferritin (ng/ml)</td>
<td>279.9±44.9</td>
<td>189.5±57.3</td>
</tr>
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</table>
PCOS, may play a role. Androgens and insulin have been hypothesized that the dysregulation of androgens and levels in PCOS are not yet fully understood. However, it has possible in PCOS [2,3]. Secondly, altered hepcidin levels in PCOS women compared to the healthy control group and found that these biochemical indicators may be a reliable diagnostic measure for PCOS cases. Ferritin, the cellular storage protein for iron, serves as a biomarker for estimating iron status, inflammatory signals, and erythropoietic demands [1]. The lower serum hepcidin concentrations observed in patients with PCOS could have several implications. Firstly, reduced hepcidin levels may lead to increased iron absorption, potentially resulting in higher iron levels in the body. This could contribute to iron overload or excess iron accumulation in tissues, although further research is needed to explore this possibility in PCOS [2,3]. Secondly, altered hepcidin levels in PCOS may be associated with disrupted iron utilization. Iron is essential for various physiological processes, including red blood cell production and cellular metabolism. Altered hepcidin levels could impact iron availability for these processes, potentially leading to anaemia or impaired cellular function [4]. The underlying mechanisms contributing to reduced hepcidin levels in PCOS are not yet fully understood. However, it has been hypothesized that the dysregulation of androgens and insulin signalling, which are both characteristic features of PCOS, may play a role. Androgens and insulin have been shown to influence hepcidin expression and iron metabolism in experimental studies [8]. The study's findings support the hypothesis that AMH levels are significantly higher in women with PCOS compared to the control group. In agreement with these findings, Yetim et al. [9] findings proved a high level of AMH was recorded among PCOS women and suggested that AMH can be used as a biomarker for the diagnosis of PCOS. In a recent study, Tunc et al. [10] found that AMH level was significantly higher in the PCOS women than in the control group. Alfatlawi (2017) [11] also AMH showed a significant statistical increase between PCOS patients and the control group P<0.05. The exact mechanisms underlying this relationship are not fully understood. However, it is believed that the excess production of androgens (male hormones) in conditions like PCOS can lead to disrupted follicular development, resulting in anovulation. This disruption may contribute to higher levels of AMH [12]. Also, another study reports similar results that PCOS had higher levels of AMH than control [13]. Muharam et al. [14] suggested that the women with PCOS have high levels of AMH in comparison with the control group AMH level was markedly increased in the PCOS group and these results agree with our results. In PCOS, there is a disruption in this follicular development process. Multiple follicles start to grow but do not fully mature or ovulate. These immature follicles accumulate in the ovaries, leading to an increased number of small, undeveloped follicles. It is in these small antral follicles that AMH is primarily produced. AMH is secreted by the granulosa cells surrounding the follicles and acts as a suppressor of follicle-stimulating hormone (FSH) secretion from the pituitary gland [15]. The higher number of small follicles in PCOS contributes to elevated AMH levels in the bloodstream. Therefore, the increased production of AMH in PCOS is closely linked to the excessive growth of preantral and small antral follicles, which are characteristic of the condition. Elevated AMH levels are often used as a diagnostic marker for PCOS and can provide insights into the ovarian reserve and follicular activity in affected individuals [16]. Measuring serum AMH levels has become a useful tool in diagnosing PCOS and assessing ovarian reserve. The elevated AMH levels in PCOS indicate increased follicular activity and the presence of a larger pool of developing follicles in the ovaries. It's important to note that while elevated AMH levels are commonly associated with PCOS, they are not exclusive to this condition. Other factors, such as age and certain ovarian conditions, can also contribute to elevated AMH levels. Therefore, clinical judgment and consideration of other diagnostic criteria are necessary when evaluating an individual for PCOS [17].

According to the study, the average Serum iron and ferritin level in women with PCOS was significantly higher than that of the control group. Mathew et al. [18], also indicated that the average serum iron level in women with PCOS was significantly higher than that of the control group and serum ferritin was elevated significantly in PCOS women than those in the control group and found that these biochemical indicators may be a reliable diagnostic measure for PCOS cases. Ferritin, the cellular storage protein for iron, serves as a biomarker for estimating the levels of iron stored in the body [19]. Several factors potentially contribute to the elevation of serum ferritin levels

<table>
<thead>
<tr>
<th>Table 5. Distribution of hepcidin levels according to BMI in patients' group.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hepcidin (ng/ml)</strong></td>
</tr>
<tr>
<td><strong>BMI≤25 (kg/m²)</strong></td>
</tr>
<tr>
<td><strong>No.</strong></td>
</tr>
<tr>
<td><strong>Mean</strong></td>
</tr>
<tr>
<td><strong>P-value= 0.006</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 6. Distribution of AMH levels according to BMI in the PCOS group.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AMH (ng/ml)</strong></td>
</tr>
<tr>
<td><strong>BMI ≤ 25 (kg/m²)</strong></td>
</tr>
<tr>
<td><strong>No.</strong></td>
</tr>
<tr>
<td><strong>Mean</strong></td>
</tr>
<tr>
<td><strong>p value= 0.001</strong></td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Table 7. Correlation between hepcidin and different parameters of PCOS group.</th>
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</thead>
<tbody>
<tr>
<td><strong>Sample 1</strong></td>
</tr>
<tr>
<td>Hepcidin</td>
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<tr>
<td>Hepcidin</td>
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<tr>
<td>Hepcidin</td>
</tr>
</tbody>
</table>
in women with PCOS, including the iron-sparing effect caused by the prolonged menstrual cycle and hyperinsulinism [20]. Meanwhile, higher insulin may facilitate intestinal absorption and deposition of iron in tissue, with IR leading to higher levels of ferritin [21]. Several studies indicated a relationship between PCOS and iron levels in the body, as increased insulin in PCOS women excesses iron storage in the body and raise ferritin levels because the insulin stimulates the intestines to absorb iron, and this indicates an indirect relationship between hepcidin levels and PCOS [22,23].

The increase in serum ferritin levels observed in PCOS patients suggests a potential dysregulation in iron metabolism and storage. Elevated ferritin levels could indicate higher iron stores, which may be associated with underlying hormonal imbalances, insulin resistance, or other factors involved in PCOS pathogenesis [24]. In line with the current findings, previous studies have also reported that the mean level of hepcidin was significantly elevated in overweight PCOS women compared to PCOS women with a normal BMI [25]. Another study reported that the mean level of hepcidin was significantly elevated in overweight PCOS women and this elevation in hepcidin levels suggests a potential association between BMI and hepcidin regulation in PCOS [26]. The specific mechanisms underlying this relationship are not yet fully understood. However, adipose tissue-derived factors and chronic inflammation associated with overweight, or obesity may influence hepcidin production and iron metabolism [27]. Another study indicated that excess adipose tissue, especially in the visceral region, can secrete pro-inflammatory cytokines that may affect hepcidin synthesis and chronic inflammation has been linked to alterations in iron homeostasis, potentially leading to changes in hepcidin levels [4].

Furthermore, factors such as insulin resistance and hormonal imbalances, commonly observed in PCOS and often associated with overweight or obesity, may also contribute to the dysregulation of hepcidin levels. The current findings align with the existing body of literature, suggesting that overweight PCOS women exhibit higher levels of hepcidin compared to those with a normal BMI [28]. The exact mechanisms underlying the relationship between BMI and AMH levels in PCOS are not fully understood. However, it was hypothesized that factors associated with excess adiposity, such as chronic inflammation, insulin resistance, and hormonal imbalances, may influence AMH production [29]. Obesity is defined as abnormal or excessive fat accumulation that presents a risk to human health and has been linked to alterations in reproductive hormones and ovarian dysfunction. Adipose tissue, particularly visceral fat, can secrete various bioactive substances, including hormones and inflammatory cytokines, which may influence AMH levels [30]. This dysregulation of iron metabolism may contribute to the higher iron stores often observed in PCOS [31]. While the study by Hossein Rashidi et al. [7], didn't show negative relation of serum hepcidin with iron, or ferritin, in PCOS women, which contrasts Variations in study design, methodology, and participant characteristics may contribute to the differences in findings. It is important to note that the relationship between hepcidin and serum iron levels in PCOS is still an area of active research, and there may be variations in findings among different studies. Factors such as sample characteristics, methodologies, and variations in PCOS phenotypes may contribute to the inconsistencies. These parameter measurements could be applied to the disease status of the patients even during obstetric emergencies [32], however, the limitation is that the endogenous cellular milieu is affected by cell quasi-equilibrium environment at cellular surrounding environments[33,34].

Conclusion.

Low levels of hepcidin accompanied by high levels of iron, ferritin and AMH were observed in PCOS women as compared with healthy women. More research is needed to elucidate the underlying mechanisms and establish the clinical significance of reduction hepcidin concentrations in PCOS women.

REFERENCES