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Original Research Article

Dysregulation of IL-6 and Iron Biomarkers as Potential Diagnostic Markers for Polycystic Ovary Syndrome

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Abstract: Polycystic Ovary Syndrome is one of the most common endocrinopathies in women of reproductive age group. A Case-control study is carried out in Kirkuk city from 10th of November 2022 to 10th of March 2023 for determination the relationship of ferritin, serum iron and interleukin-6 among PCOS women. The number of PCOS women under study were 60 women whose ages were between 15-45 years old. These patients admitted to obstetrics and gynecology unit at Gynecological and Pediatric Hospital in Kirkuk city. 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, height, and were asked to complete a general questionnaire. Body Mass Index (BMI) was calculated by using the formula: weight (Kg)/height (meters^2). Blood samples collected from each women for measurement of interleukin-6 by Enzyme linked immunosorbent assay (ELISA), and serum ferritin, by immunofluorescence and serum iron by manual biochemistry procedures. The average IL-6 level in PCOS women was (279.8±77.8 pg/ml), whereas the control group had a level of(92.5±11.54 pg/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 of IL-6 in PCOS women with BMI>25 (kg/m2) was (293.1± 81.8pg/ml) which is significantly higher than in PCOS women with normal BMI (231.9±18.9 pg/ml). The study showed that the serum iron and ferritin were elevated significantly in obese PCOS women (151.72±44.85 g/dl and 247.4±147.4 ng/ml) respectively as compared with non-obese PCOS women (140.61±35.08 g/dl and 228.7±38.2 ng/ml). High levels of iron, ferritin and IL-6 were observed in PCOS women as compared with healthy women specialty obese women with PCOS which highlight the importance of inflammation in PCOS and suggest that IL-6 levels could potentially serve as a biomarker for the condition. Additionally, exploring the relationship between IL-6 and other factors, such as hormonal imbalances and metabolic abnormalities, can provide a more comprehensive understanding of the pathophysiology of PCOS.

Keywords: IL-6; Ferritin; Iron: PCOS; Obesity.

INTRODUCTION

The polycystic ovary syndrome (PCOS) is a heterogeneous female endocrine disorder which impact 5-8% of women of reproductive age [1] with a prevalence rate of nearly 5–10% among women of reproductive age [2]. It is characterized by chronic anovulation, hyperandrogenism, and multiple small subcapsular cystic follicles in the ovary on ultrasonography [3]. Polycystic ovarian syndrome 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]. The etiology and pathogenesis of PCOS have remained unknown and it has various phenotypes including 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]. Iron is a strong pro-oxidant, and high levels of it in the body is 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 [7, 8]. The serum IL-6 levels promote in chronic stress and thereby elevate the serum hepcidin levels. During chronic stress, brain cells need more iron for better performance and, iron accumulates in the brain cells. Studies on the changes of serum hepcidin levels in patients with PCOS have shown that serum hepcidin levels in these patients were lower than in non-PCOS patients [9]. Increased hematopoiesis and decreased hepcidin gene expression have been reported to absorb more iron and may cause reduced serum hepcidin level in patients with PCOS in response to iron overload in these patients [10]. This study aims to assess the relationship of ferritin, serum iron and interleukin-6 among PCOS women.

PATIENTS AND METHODS

A Case-control study is carried out in Kirkuk city from 10th of November 2022 to 10th of March 2023.

The number of PCOS women under study were 60 women, their ages were between 15-45 years old. These patients admitted to obstetrics and gynecology unit at Gynecological and Pediatric Hospital in Kirkuk city.

PCOS was diagnosed based on the presence of two of following Rotterdam criteria:

- Oligo and/or anovulation, clinical and/or
- Biochemical signs of hyperandrogenism, and
- Polycystic ovaries in ultrasound, meaning presence of 12 or more follicles measuring 2-9 mm in diameter in each ovary and/or ovarian volume more than 10 cm^3 [2].

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, height, and were asked to complete a general questionnaire. Body Mass Index (BMI) was calculated by using the formula: weight (Kg)/height (meters^2).

Five ml of blood sample was taken by vein puncture from each subject enrolled in this study (women were in 2-5 day of menstrual cycle). Blood samples were added to gel tubes, after blood clotting, centrifuged at 3000 rpm for 15 minute then clot removed and remain re-centrifuged at 3000 for 10 minute and the obtained serum were aspirated using mechanical micropipette and transferred into clean test tubes which labelled and stored at -20ºC until analysis of interleukin-6, serum ferritin and serum iron.

RESULTS

The study showed that Women with PCOS were characterized with increased in BMI (Body Mass Index) and was 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 1: Clinical data of patients group

Parameters		**No.**	**%**
Age	<30 year	34	56.67
	≥30 year	26	43.33
	Total	60	100
BMI (kg/m^2)	≤25	17	28.33
	25-29.9	30	50
	≥30	13	21.67
	Total	60	100
Hirsutism	Absent	7	11.67
	Present	53	88.33
	Total	60	100
Acne	Absent	9	15.00
	Present	51	85.00
	Total	60	100
Menstrual cycle disturbance	Regular	7	11.67

	Irregular	53	88.33
	Total	60	100
Family history of PCOS	No	6	10.00
	Yes	54	90.00
	Total	60	100
Alopecia	Yes	17	28.33
	No	30	71.67
	Total	60	100

According to the study, the average of IL-6 level in women with PCOS was significantly higher than that of the control group. Specifically, the Mean of IL-6 in PCOS women was 279.8±77.8 pg/ml, whereas the control group had a level of 92.5±11.54 pg/ml. This difference was found to be statistically significant with at P-value of 0.0001, Table 2

Table 2: The level of IL-6 in the studied groups

IL-6 (pg/ml)	**Studied groups**	
	Patients group	**Control group**
No.	60	30
Mean±SD	279.8±77.8	92.5±11.54
Minimum	199.3	67.4
Maximum	465.1	113.6

P-value: 0.0001

Based on the study findings, women with PCOS had significantly higher level of 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 (189.5.6±57.3 ng/ml). These results are presented in Table 3.

Table 3: Comparison between studied groups regarding serum iron and ferritin levels

Parameters Mean±SD	**Studied groups**		**P-value**
	Patients group	**Control group**	
S. iron (g/dl)	223.5±57.3	129.1±44.9	0.001
S. Ferritin (ng/ml)	279.9±44.9	189.5.6±57.3	0.001

The study showed that the Mean±SD of IL-6 in PCOS women (patients group) with BMI>25 (kg/m^2) was (293.1±81.8 pg/ml) was significantly higher than in PCOS women with normal BMI (231.9±18.9 pg/ml) at P. value 0.0011, Table 4.

Table 4: Distribution of IL-6 levels according to BMI in PCOS group

IL-6 (pg/ml)	**(patients group)**	
	BMI≤25 (kg/m^2)	BMI>25 (kg/m^2)
No.	17	43
Mean	231.9±18.9	293.1±81.8

P-value: 0.011

The study showed that the serum iron and ferritin were elevated significantly obese PCOS women (151.72±44.85 g/dl and 247.4±147.4 ng/ml) respectively as compared with non-obese PCOS women (140.61±35.08 g/dl and 228.7±38.2 ng/ml). The differences in both serum iron and ferritin levels between these two groups were statistically significant, with a p-value less than 0.05, Table 5.

Table 5: Mean levels of serum iron and ferritin in obese and normal weight PCOS women

Parameter (mean±SD)	**PCOS women**		**P-value**
S. iron (g/dl)	BMI≤25 (kg/m^2)	140.61±35.08	0.046
	BMI>25 (kg/m^2)	151.72±44.85	
S. Ferritin (ng/ml)	BMI≤25 (kg/m^2)	228.7±38.2	0.036
	BMI>25 (kg/m^2)	247.4±147.4	

DISCUSSIONS

The presence of hirsutism in 88.33% of the patients in the given PCOS group , this finding agreed with several studies, they reported that hirsutism rates was around 80% among women with PCOS [1-4]. The underlying cause of hirsutism is commonly attributed to increased androgen production from either the ovaries or adrenal glands. Androgens are male sex hormones, but both men and women produce them, although in different amounts. In conditions like polycystic ovary syndrome (PCOS), there can be an excess production of androgens, leading to hirsutism among other symptoms [5]. Elevated serum levels of androgens, including testosterone, androstenedione, and dehydroepiandrosterone sulfate (DHEAS), are commonly observed in women with polycystic ovary syndrome (PCOS). These elevated androgen levels contribute to the development of various symptoms associated with PCOS [6]. Occasionally masculinization and obesity centered on the lower half of the torso 'apple-shaped" were also noticed in addition to other symptoms [7]. Some studies have explored the potential associations between menstrual irregularities and endocrine or metabolic parameters in PCOS. For example, elevated levels of androgens, such as testosterone, have been found to correlate with menstrual irregularities in some studies. Additionally, insulin resistance, which is common in PCOS, has been implicated in menstrual disturbances and may contribute to the metabolic features of the condition [8-12]. The research findings indicate that IL-6 levels were significantly elevated in patients with PCOS compared to the control group. This finding was in agreement with the finding of Sarhat *et al.,* [1] who revealed that interleukin-6 (IL-6) was significantly higher in Polycystic ovary syndrome patients in comparison to the control group. Furthermore, several previous reports were also found an elevated level of when serum IL-6 levels in Polycystic ovary syndrome patients compared to healthy people [2-4]. In addition to the recent findings, several previous reports have also consistently demonstrated elevated serum IL-6 levels in patients with PCOS compared to healthy individuals. These findings further support the notion of an association between PCOS and increased levels of IL-6 [5, 6]. The elevated IL-6 levels in PCOS patients suggest the presence of chronic low-grade inflammation in this condition. IL-6 is a pro-inflammatory cytokine that is involved in immune responses and plays a role in promoting inflammation. The dysregulation of IL-6 in PCOS may contribute to the development of insulin resistance, disrupted ovarian function, and other symptoms associated with the syndrome [7]. These consistent findings across different studies indicate that elevated IL-6 levels are a common characteristic in PCOS. It suggests that inflammation and immune system dysfunction may be significant factors in the pathophysiology of PCOS [8, 9]. Understanding the elevated IL-6 levels in PCOS can have important clinical implications. It can provide insights into the underlying mechanisms of the syndrome and aid in the development of targeted therapeutic approaches. By addressing inflammation and its associated metabolic disturbances, interventions targeting IL-6 may have the potential to improve the management and outcomes of PCOS patients [10]. The association between IL-6 and PCOS remains inconclusive, as indicated by conflicting results in the literature [11, 12]. A meta-analysis conducted by Escobar-Morreale et al. reported no significant relationship between IL-6 concentration and PCOS. Their findings suggest that IL-6 may not play a prominent role in the pathophysiology of PCOS [13]. These conflicting results highlight the complexity of the relationship between IL-6 and PCOS. It is possible that factors such as sample size, population characteristics, and methodological differences across studies contribute to the discrepancies observed. According to the study, the average of serum iron and ferritin level in women with PCOS was significantly higher than that of the control group Several factors potentially contribute to elevation of serum ferritin levels in women with PCOS, including the iron-sparing effect caused by prolonged menstrual cycle and hyperinsulinism [2]. Meanwhile, higher insulin may facilitate intestinal absorption and deposition of iron in tissue, with IR leading to higher levels of ferritin [3]. Several studies confirmed the significant increase in serological level of ferritin in diagnosed polycystic syndrome patients. The main role of ferritin is to sequester iron, and ferroxidase converts Fe II to Fe III, and iron in cellular systems is locked inside the cells and its concentration decreases within the cytoplasm, and it is considered a basic element for the survival of the organism [14-16]. Other studies also reported a significant increase in serum ferritin levels in diagnosed polycystic ovary syndrome (PCOS) patients [17, 18]. Ferritin plays a crucial role in iron homeostasis by sequestering and storing iron. It functions as a storage protein, helping to regulate iron levels in the body. Additionally, the ferroxidase activity of ferritin converts ferrous iron (Fe II) to ferric iron (Fe III), contributing to iron stabilization and storage within cellular systems [19]. 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 [20]. In line with the current finding, The study of Alissa *et al.,* [18] revealed that IL-6 levels were increased in obese PCOS women compared to non-obese PCOS women. This finding suggests that obesity may contribute to higher levels of IL-6 in individuals with polycystic ovary syndrome (PCOS). Several studies also showed this elevation in obese PCOS women [22, 23]. The proinflammatory cytokine IL-6, in addition to stimulating the liver to produce acute-phase reactants, takes an active part in the inflammation process along with CRP, both of which are related to obesity in PCOS [2]. The study findings suggest that plasma IL-6 levels are significantly increased in obese women with Polycystic Ovary Syndrome (PCOS) compared to their lean counterparts. This observation may indicate a pathophysiological connection between elevated cytokine levels and visceral fat accumulation in women with PCOS [24, 25]. The exact mechanisms underlying this association are not fully understood. However, it is postulated that the increased IL-6 levels in obese PCOS women may be a result of the inhibitory effect of estradiol and estrone on IL-6 secretion in these patients [26]. According to the study findings, the serum iron and ferritin levels were significantly elevated in obese women with Polycystic Ovary Syndrome (PCOS)

compared to non-obese PCOS women. In line with these finding, Several studies have investigated this relationship and have reported interesting findings [27, 28]. Obesity, characterized by excess adipose tissue accumulation, has been linked to disturbances in iron homeostasis. Adipose tissue, particularly visceral fat, is known to secrete adipokines and cytokines that can impact iron regulation. The chronic low-grade inflammation associated with obesity may also contribute to dysregulated iron metabolism in PCOS [29]. In addition to the association between obesity and altered iron metabolism, research has explored the relationship between serum ferritin levels and various diagnostic components of metabolic syndrome in PCOS women. Metabolic syndrome is a cluster of conditions that include abdominal obesity, high blood pressure, high blood sugar, and abnormal lipid levels. It is characterized by increased cardiovascular risk [30]. The exact mechanisms underlying the association between obesity and iron metabolism in PCOS are not fully understood. However, it is hypothesized that adipose tissue-derived factors, chronic inflammation, and hormonal imbalances associated with obesity may influence iron homeostasis [31].

Adipose tissue is active endocrine organ that releases many metabolically active peptide hormones, cytokines, chemokines and adipokines, which can affect iron metabolism. Chronic inflammation, commonly observed in obesity, may disrupt iron regulation and lead to higher serum iron and ferritin levels [32, 33].

CONCLUSIONS

High levels of iron, ferritin and IL-6 were observed in PCOS women as compared with healthy women specialty obese women with PCOS which highlight the importance of inflammation in PCOS and suggest that IL-6 levels could potentially serve as a biomarker for the condition. Additionally, exploring the relationship between IL-6 and other factors, such as hormonal imbalances and metabolic abnormalities, can provide a more comprehensive understanding of the pathophysiology of PCOS.

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