THE RELATIONSHIP BETWEEN PCOS AND THE LEVEL OF (TNF-A) AND (IL-1B) IN IRAQI FEMALE PATIENTS

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Aims: The study aimed to examine immunological bases of (PCOS) by estimate serum level of (TNF-α) and (IL-1β) pro-inflammatory cytokines in Iraqi PCOS women.

Methods and Material: In vitro quantitative determination of (TNF-α) and (IL-1β) pro-inflammatory cytokines in serum of PCOS patients by using ELISA technique.

Statistical analysis used: Data were analyzed using (SPSS) version 26. Independent sample t-test was used to study difference in mean between any two groups. One way Anova test used to study difference in mean between more than two groups. Chi-square test was used to study association between any two categorical variables. In order to detect the cutoff value that predict a positive finding, (ROC) curve analysis was used.

Results: IL-1β level was highly significant higher in women patients with PCOS in comparison with apparently healthy control (P < 0.001). Mean TNF–α level was highly significant higher in women patients with polycystic ovary syndrome in comparison with apparently healthy control (P < 0.001).

Conclusions: Current study revealed that PCOS is a pro-inflammatory disease, characterized by high presence of chronic low- grade inflammation, levels of IL-1β and TNF–α were highly significant higher in women patients with PCOS in comparison with healthy controls. According to ROC curve results serum level of IL-1β can use as a valuable diagnostic marker in early diagnosis of PCOS.

KEY MESSAGES: The present study demonstrated highly significant association between the the level of IL-1β and TNF–α and PCOS

INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the commonest endocrine disorder of female at reproductive age and it's prevalence in general population as 20%–33%.[1] PCOS is characterized by irregular menstrual periods, infertility, acne, obesity, amenorrhea, oligomenorrhea, hirsutism, high serum testosterone levels, raised LH, and low FSH levels.[2,3,4] Obesity is an independent risk factor for PCOS, and it plays a significant role in the development and manifestations of the clinical, biochemical, and metabolic characteristics of the disease.[5] The majority of PCOS patients are overweight or obese. Obesity is characterized as having a BMI of 30 kg/m2 or greater, according to World Health Organization criteria [6]. immune cell dysregulation, including both innate and adaptive immune cells, is said to be a cause of cross-talk in PCOS.[7] PCOS has been linked in several studies to low-grade chronic inflammation, which involves an imbalance in the production of pro-inflammatory factors.[8] Hyperandrogenemia leads to monocyte infiltration in the ovary and an increase the release of pro-inflammatory cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), causing long-term ovarian tissue inflammation, affecting the maturation of new follicles in the ovary, and leading to cystic follicle development.[9] In women with PCOS, these pro-inflammatory cytokines affect ovarian function and the processes of ovulation, fertilization, and implantation.[10] TNF-α has a function in PCOS patients with obesity, insulin resistance, hyperandrogenism, in addition to interfering with immune and inflammatory responses, differentiation,

proliferation, and cell death.[11] Due to its claimed involvement in inflammatory-related processes in the ovaries, IL-1B has been the subject of several studies looking at its function in PCOS patients.[12]

Aim of this study: Estimate the serum level of (TNF-α) and (IL-1β) pro-inflammatory cytokines in PCOS women and compare their levels between PCOS women with apparently healthy control group

SUBJECTS AND METHODS:
1. Patients and apparently healthy control

The current study includes a case control study for a group of (84) participants. (54) females suffering from polycystic ovary syndrome (PCOS), and (30) match ages of the apparently healthy females serve as control. Patients were collected from Women's and Children General Hospital and out clinic patients in Al-Qadisiyah Governorate, age range from (15-41) years, during the period from October 2022 to the end of February 2023. The clinical assessment of patients with PCOS was evaluated by physician according to sonography and laboratory assessment; hormones levels FSH, LH were measured to all participated in this study in the follicular phase (2-5) days of cycle. Age, BMI were taken for each patient and control. Each patient and control have filled out the questionnaire.

PCOS Patients were diagnosed according to the 2003 Rotterdam criteria. For the diagnosis, two of the following three criteria were required: (1) Hyperandrogenism.(Hirsutism, Acne, Alopecia). (2) Oligo ovulation and /or non-ovulation; Oligomenorrhea or amenorrhea (3) Ultrasound polycystic ovary morphology. Exclusion criteria included all patients over the age of 45 who were suffering from menopause and menstrual irregularities. Patients suffering from congenital adrenal hyperplasia or adrenal tumors . Diabetic patients, hyperprolactinemia patients, and thyroid dysfunction patients, Body mass index (BMI) for both of patients and healthy control group was measured as following:

(weight (kg))/(Height² (m)).

2. Collection of Blood

Using 5ml disposable syringes, vein punctures were used to collect blood samples from all subjects. 5ml of blood was taken from each participant woman in this study.

Each patient had a 2ml sample of their withdrawn blood put into an EDTA tube for genetic analysis. The remaining 3ml were put in a gel tube, and then serum was taken out following a 10-min centrifugation at 4000 rpm. To avoid multiple freezing and thawing cycles, serum samples were aliquoted into two Eppendorf tubes and then kept frozen until the time of analysis.

3. Immune Assay

levels of cytokines in patient and apparently healthy control serum samples including (TNF-α) and (IL-1β) , were measured using the commercial ELISA kits and done according to company instruction (BT-LAB).

4. Statistical Analysis

The statistical program for social sciences (SPSS) version 26 and Microsoft Office Excel 2010 were employed to collect, summarize, analyze, and display data. Numeric data were presented as mean, standard deviation after performance of Kolmogorov- Smirnov normality test and making decision about normally and non-normally distributed variables. One way Anova test used to study difference in mean between more than two groups. Chi-square test was used to study association between any two categorical variables. In order to detect the cutoff value that predict a positive finding, receiver operator characteristic (ROC) curve analysis was used.

Ethical approval: The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 2115 (including the number and the date in 30/10/2022) to get this approval.

RESULT

The current study includes a case control study for a group of (84) participants. (54) females suffering from (PCOS), and (30) match ages of the apparently healthy females serve as control. Patients were collected from Women's and Children General Hospital and out clinic patients in Al-Qadisiyah Governorate whom age range from (15-41) years, during the period from October 2022 to the end of February 2023. The basic subject characteristics are shown in Table 1. There was no significant difference in mean age, or age group distribution between cases and controls. there was significant difference between patients and control subjects in mean weight (P = 0.001), and there was significant difference between women patients with PCOS and control subjects in mean BMI (P = 0.001).

Table (1): Demographic characteristics of women patients with polycystic ovary syndrome and healthy control subjects

Characteristic	Patients with PCOS n = 54	Healthy control n = 30	P
Age (years)			
Mean ±SD	28.17 ± 7.27	26.91 ± 7.02	0.415 † NS
Range	15– 41	16 – 41	
< 20, n (%)	6 (11.1%)	6 (20.0%)	0.199 ¥ NS
20-29, n (%)	21 (38.9%)	16 (53.3%)	
30-39, n (%)	25 (46.3%)	7 (23.3%)	

≥ 40, *n* (%)	2 (3.7%)	1 (3.3%)	
Weight (Kg)			
Mean ±SD	77.17 ± 6.96	61.68 ± 7.27	0.001
Range	43 − 110	45 − 79	† S
Body mass index(BMI) (kg/m²)			
Mean ±SD	30.52± 4.72	23.17 ± 1.44	0.001
Range	18.67 − 41.62	18.73 − 24.84	† S

n: number of cases; **SD**: standard deviation; †: independent samples t-test; ¥: Chi-square test; **S**: significant at *P* < 0.05: NS: not significant at P > 0.05

Frequency distribution of women patients with PCOS according to obesity is shown in Figure 1. There was significant difference between patients and controls regarding grades of obesity (P = 0.001). The proportion of patients with overweight (BMI ≥ 25 kg/m2) was 31 (57.4%), the proportion of patients with obese (BMI ≥ 30 kg/m2) was 22 (40.7%), whereas the proportion of patients with normal weight (BMI = 18.5 - 24.9 kg/m2) was only 1 (1.9 %).
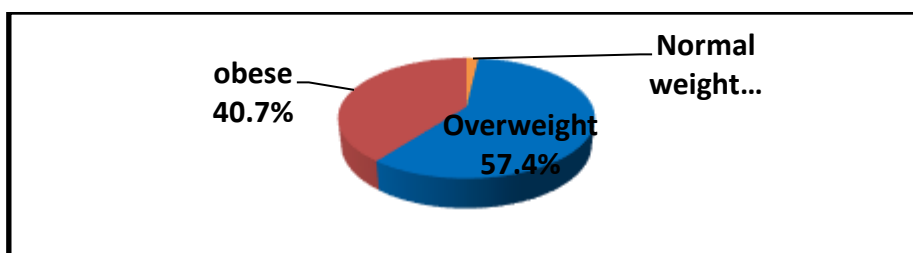

Figure 1: Pie chart showing the frequency distribution of women patients with polycystic ovary syndrome according to obesity

The levels of pro-inflammatory cytokines are shown in Table 2. There was highly significant difference in levels of (IL-1β) and (TNF-α) between PCOS patients and controls. The IL-1β mean level was highly significant higher in women patients with PCOS in comparison with healthy control (P < 0.001). The mean TNF–α level was highly significant higher in women patients with PCOS in comparison with healthy control (P < 0.001).

Table 2: Cytokines levels in cases and controls

	Cases −control comparison		*P*
	Patients with PCOS ***n* = 54**	**Healthy control** ***n* = 30**	
IL-IB (pg/ml)			
Mean± SD	**3779.09 ± 739.90**	**2346.64 ± 1136.19**	**< 0.001** **†** **HS**
Range	2364.52 − 6560.42	23.16- 3471.0	
TNF-α (ng/ml)			
Mean± SD	**527.45 ± 128.92**	**308.78 ± 158.99**	**< 0.001** **†** **HS**
Range	391.81 − 980.15	10.39- 492.63	

n: number of cases; **SD**: standard deviation; †: independent samples t-test; HS: Highly significant at P ≤ 0.001.

Table 3 shows the effect of BMI on cytokines levels in patients with PCOS. The mean IL-1β levels was higher in patients with obesity in comparison with other groups, but the difference was non-significant (P= 0.941) and the mean TNF-α levels was higher in patients with obesity in comparison with other groups, but the difference was non-significant (P= 0.172). There is no significant difference in the level of pro-inflammatory cytokines between obese and nonobese PCOS women.

Table 3: Effect of BMI on cytokines levels in polycystic ovary syndrome women

	BMI comparison			
	Normal Weight *n=1* **(BMI = 18.5 - 24.9 kg/m²)**	*Overweight* *n=31* **(BMI ≥ 25 kg/m²)**	*Obesity* *n=22* *(BMI ≥ 30 kg/m2)*	*P value*
IL-1β				

Mean± SD	3748.76	3793.6 ± 763.13	3980.57 ± 736.03	0.941 † NS
Range	…..	2574.17– 6560.42	2364.52– 5617.0	
TNF-α				
Mean± SD	426.35	491.9 ± 67.75	547.83 ± 154.49	0.172 † NS
Range	…..	394.98 – 630.81	391.8 – 980.15	

n: number of cases; **SD**: standard deviation; †: one way ANOVA; NS: not significant at P > 0.05.

The receiver operator characteristic (ROC) curve analysis was carried out To evaluate the IL-1β cutoff value as well as to predict the polycystic ovary syndrome as diagnostic tests and the results are shown in table (4), and figure (2).The results indicate that an IL-1β cutoff value of >3134.69-fold demonstrates high sensitivity (90.7%) and specificity (86.7%) in diagnosing PCOS. These results suggest that IL-1β may serve as a potential diagnostic marker for PCOS.

Table 4: Sensitivity and specificity of IL-1β level (> 3134.69-fold) in polycystic ovary syndrome

IL-1β level	Patients with PCOS *n* = 54	Healthy control *n* = 30
> 3134.69	49 (%) True positive	4 (%) False negative
< 3134.69	5 (%) False positive	26 (%)True negative
Sensitivity %	90.7 %	
Specificity %	86.7%	
PPV %	92.5%	
NPV %	83.9%	
AUC (95% CI)	0.925 (0.874- 0.976)	

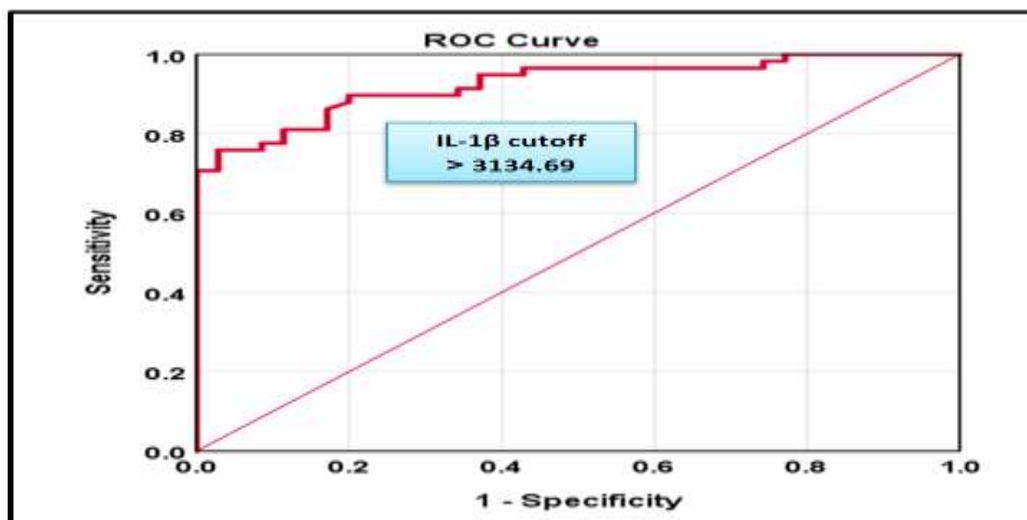CI: Confidence interval, AUC: Area under curve.

Figure 2: Receiver operator characteristic curve analysis of IL-1β for the calculation of possible diagnostic cutoff value.

The receiver operator characteristic (ROC) curve analysis was carried out To evaluate the TNF–α cutoff value as well as to predict the PCOS as diagnostic tests and the results are shown in table (5), and figure (3). .The results indicate that an TNF-α cutoff value of >417.89-fold exhibits moderate sensitivity (88.9%) and specificity (80.0%) in diagnosing PCOS. These results imply that TNF-α may have potential as a diagnostic marker for PCOS.

Table 5: Sensitivity and specificity of TNF-α level (> 417.89-fold) in polycystic ovary syndrome

TNF-α level	Patients with PCOS *n* = 54	Healthy control *n* = 30
> 417.89	48 (%) True positive	6 (%) False negative
< 417.89	6 (%) False positive	24 (%) True negative
Sensitivity %	88.9 %	
Specificity %	80.0%	
PPV %	88.9%	
NPV %	80.0%	
AUC (95% CI)	0.898 (0.835- 0.960)	

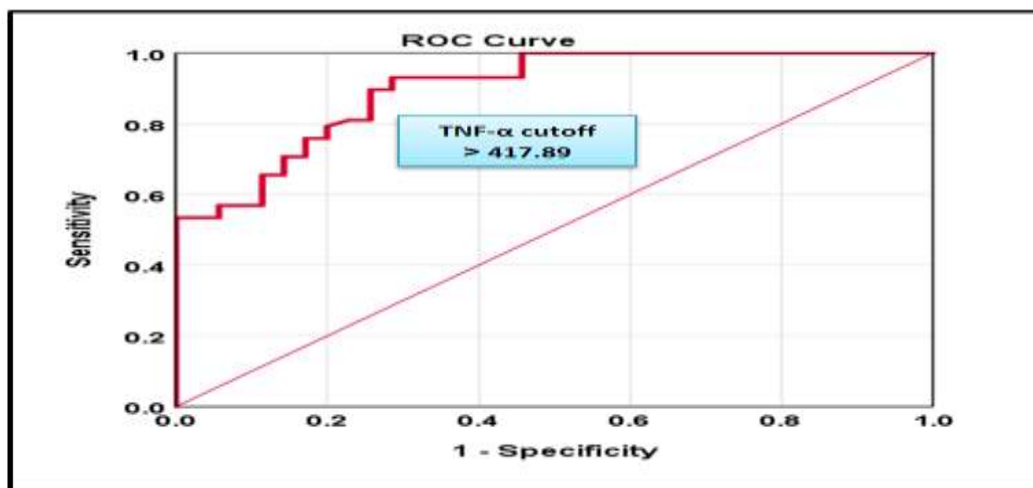CI: Confidence interval, AUC: Area under curve.

Figure 3: Receiver operator characteristic curve analysis of TNF-α for the calculation of possible diagnostic cutoff value.

DISCUSSION

PCOS is recognized as one of the most common a heterogeneous, complicated genetic, endocrine-metabolic condition in reproductive-aged women. Is a major cause of ovulatory and menstrual irregularity, subfertility and infertility, clinically apparent hyperandrogenism, and metabolic dysfunction [13]. PCOS's actual etiology is uncertain. However, it is believed to be a complex disorder with a genetic component [14]. According to studies, PCOS is characterized by chronic low-level inflammation, and this chronic condition may be a contributing factor to the long-term consequences of PCOS.[15] Individuals with PCOS have been reported to have higher levels of classical inflammatory mediators such as tumor necrosis factor-α (TNF-α).[16]

Immune system is affected by estrogen/progesterone ratio.[17] Patients with PCOS present low progesterone level as a result of oligoovulation or anovulation; therefore, the immune system could be overstimulated by excess estrogen leading to production of autoantibodies in these patients.[18] The increase in IL-1β and TNF-α in PCOS patients is thought to be due to anovulation.[19] During PCOS, immune cells and immune regulatory molecules play substantial roles in maintaining metabolic balance and regulating immunological responses. Patients with PCOS have low progesterone levels due to oligo/anovulation. Low progesterone levels in PCOS, as a result, overstimulate the immune system, triggering it to secrete more estrogen. [9]

The mean age of patients was (28.17 ± 7.27) which is in agreement with Al-Musawy *et al.* [1](27.7 ± 5.8 years) and higher than in studies of both Alteia *et al.* and Agacayak *et al.* [20,21] (25.8 ± 4.4 years) (mean 26.2 ± 4.0 years). Majority of PCOS patients were in the age between (30-39 years old). While more than one third of the patients (38.9%) were in the age group between (20-29 years old), The majority of patients might not be aware they have PCOS until they are trying to get pregnant, despite the fact that it can manifest at any age following puberty and can lead to infertility. The similarities between these studies regarding the same age group administration because PCOS appeared at menarche and the females became symptomatic later but most women with polycystic ovarian syndrome are diagnosed when their age between 20 and 30 years.[22].

The mean weight of patients was (77.17 ± 6.96). The present study showed that women with PCOS in our country have a higher body weight than control group . overweight and obesity are a prevalent finding in PCOS and exacerbates several of its metabolic and reproductive characteristics. It is difficult to understand the connection between PCOS and obesity, although it almost certainly includes a combination of hereditary and environmental factors. [23]

The mean BMI of women patients with PCOS was (30.52± 4.72) This result is in agreement with several studies [24,25,26,27], who showed that women with PCOS had a higher BMI when Compared to non-PCOS subjects and prevalence of overweight and obesity in women with PCOS is much higher than in their matching control.

Mean levels of serum IL-1β were 3779.09 ± 739.90 pg/ml and 2346.64 ± 1136.19 pg/ml, in women patients with polycystic ovary syndrome and healthy control subject respectively. The mean IL-1β level was highly significant higher in women patients with polycystic ovary syndrome in comparison with healthy control (P < 0.001). This result is in agreement with studies done by Zangeneh et al.[15] Başer *et al.* and Taha *et al.*[28,29] They found that IL-1 β level were significantly higher in patients with PCOS. Kolbus *et al.*[30] contradicted these results and suggested there was no significant association between presence of the IL-1B within PCOS women and controls. The reasons for persistent low-grade inflammation in PCOS are adipocyte hypertrophy, which promotes stromal vascular compression, resulting in adipose tissue hypoperfusion and, consequently, hypoxia.IL-1 is also involved in the development of IR and DM 2.[31]

Mean levels of serum TNF-α were 527.45 ± 128.92 ng/ml and 308.78 ± 158.99 ng/ml, in women patients with polycystic ovary syndrome and healthy control subject respectively. The mean TNF–α level was highly significant higher in women patients with polycystic ovary syndrome in comparison with healthy control (P < 0.001). This result is in agreement with studies done by Başer et al.[28], found that TNF-α were statistically significantly higher in the PCOS group. According to this, inflammation is crucial to the pathophysiology of PCOS. In contrary to this results, another study

reported no significant difference in TNF–α levels in PCOS patients as compared to controls.[32] Increased levels of proinflammatory mediators such as TNF- α in women with PCOS, as well as its involvement in immunological over-activation, may have an impact on ovarian function and the ovulation process. [33]

Mean levels of serum IL-1β were (3748.76, 3793.6 ± 763.13 and 3980.57 ± 736.03) in normal weight, overweight patients and obese patients respectively. The mean IL-1β levels was higher in patients with obesity in comparison with other groups, but the difference was non-significant (P= 0.941). This result is in agreement with studies done by Alkhuriji *et al*.[34], observed that IL-1β levels were high in patients with PCOS with obesity. The increase in IL-1β in these patients is thought to be due to anovulation.[19]

Mean levels of serum TNF-α were (426.35, 547.83 ± 154.49 and 491.9 ± 67.75) in normal weight, overweight patients and obese patients respectively. The mean TNF-α levels was higher in patients with obesity in comparison with other groups, but the difference was non-significant (P= 0.172). This result is in agreement with studies done by Hashoosh *et al*.[35] Women with PCOS had greater levels of TNF-α , which had a clear correlation with higher body fat percentage.[36] Obesity increases the pro-inflammatory milieu created by PCOS. When a woman has PCOS, her TNF-α level increases as her BMI rises.[37]

To evaluate the IL-1β cutoff value as well as to predict the polycystic ovary syndrome as diagnostic tests or adjuvant diagnostic tests, receiver operator characteristic (ROC) curve analysis was carried out. The IL-1β cutoff value was >3134.69-fold with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under curve 90.7%, 86.7%, 92.5%, 83.9% and 0.925 (0.874- 0.976).

Our findings indicate that an IL-1β cutoff value of >3134.69-fold demonstrates high sensitivity (90.7%) and specificity (86.7%) in diagnosing PCOS. These results suggest that IL-1β may serve as a potential diagnostic marker for PCOS. The elevated IL-1β levels observed in PCOS patients indicate the presence of chronic low-grade inflammation in the condition. Inflammation is believed to contribute to the development of insulin resistance, hyperandrogenism, and impaired ovarian function in PCOS.[38] The ROC analysis highlights the ability of IL-1β levels to discriminate between PCOS patients and healthy controls, underscoring its potential as a diagnostic tool for PCOS.

To evaluate the TNF-α cutoff value as well as to predict the polycystic ovary syndrome as diagnostic tests or adjuvant diagnostic tests, receiver operator characteristic (ROC) curve analysis was carried out. The TNF-α cutoff value was >417.89-fold with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under curve 88.9%, 80.0%, 88.9%, 80.0% and 0.898 (0.835- 0.960).

Our findings suggest that an TNF-α cutoff value of >417.89-fold exhibits moderate sensitivity (88.9%) and specificity (80.0%) in diagnosing PCOS. These results imply that TNF-α may have potential as a diagnostic marker for PCOS. Several previous studies have investigated the role of TNF-α in PCOS and its diagnostic potential. For example, Thathapudi *et al*.[39] mention TNF-α is a common features with (PCOS), also reported the PCOS had elevated body mass index, and serum TNF-α when compared with controls (p<0.05). The ROC analysis TNF- α levels are highlighted for their ability to distinguish between PCOS patients and healthy controls, emphasizing its use as a PCOS diagnostic tool.

CONCLUSION

There was a significant difference between patients and control subjects in mean weight and BMI , PCOS patients have a higher body weight and BMI when compared to controls. The majority of PCOS patients had BMI falling in the range of overweight and obese. The mean levels of IL-1β and TNF-α were higher in PCOS patients with obesity in comparison with other groups, but the difference was non-significant. According to ROC curve results the serum level of IL-1β can use as a valuable diagnostic marker in the early diagnosis of PCOS.

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