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Relation of Hyperprolactinemia with Inflammation and Biochemical Characteristics in Women with Polycystic Ovary Syndromes (PCOS)

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Abstract

Since high prolactin levels worsen Polycystic Ovary Syndrome (PCOS) symptoms, they have been the focus recently. The current study aims to shed light on hyperprolactinemia and the role of inflammation and biochemical disturbances in increasing the severity of PCOS. The study involved 90 participants: 68 women with PCOS, aged between 20-45, and 22 apparently healthy women with age match to patients. Blood samples were collected for biochemical analysis, utilizing ElectroChemiLuminescence, ELISA, and NycoCardTM techniques for precise measurement of prolactin, testosterone, estrogen, TSH, FSH, LH, anti-FSH, and CRP. Depending on the prolactin level, samples were categorized into 3 groups: hyperprolactinemia-PCOS (above 23.30 ng/ml), normal prolactin PCOS, and normal prolactin without PCOS. PCOS recorded a significant increase in prolactin level compared with control at p-value (<0.0001). Also, PCOS with hyperprolactinemia displayed notably higher levels of estrogen and CRP compared to both the normal prolactin PCOS and control at p-value (<0.0001). Additionally, PCOS with hyperprolactinemia exhibited notably higher levels of TSH compared to the control at p-value (<0.0001). Also, a significant difference was found in the level of testosterone, LH, LH/FSH ratio, and anti-FSH in PCOS compared to control at p-value (<0.0001). Based on the current study, which showed a statistically significant relationship between hyperprolactinemia and high levels of inflammation as an immune reaction accompanied by hormonal disorders and high levels of anti-FSH, it can be concluded that all the factors mentioned in this study should be included in PCOS treatment strategies to restore hormonal and immune balance, which may be a cause of the condition.

Keywords: PCOS, hyperprolactinemia, inflammation, hormonal imbalance, ELISA.

علاقة فرط بروﻻكتين الدم بالالتهاب والخصائص الكيميائية الحيوية في النساء المصابات بمتلازمة تكيس المبيض المتعدد

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الخلاصة

نظرًا لأن ارتفاع مستويات البرولاكتين يؤدي إلى تفاقم أعراض متلازمة تكيس المبايض (PCOS)، فقد تم التركيز مؤخرًا على هذه المشكلة. يهدف البحث الحالي إلى تسليط الضوء على فرط برولاكتين الدم ودوره في الالتهاب والاضطرابات البيوكيميائية في زيادة حدة متلازمة تكيس المبايض. شملت الدراسة 90 مشاركًا: 68 امرأة مصابة بمتلازمة تكيس المبايض، تتراوح أعمارهن بين 20–45 عامًا، بالإضافة إلى 22 امرأة سليمة ظاهريًا ومتوافقة في العمر مع المرضى. تم جمع عينات الدم لتحليلها بيوكيميائيًا، باستخدام تقنيات ElectroChemiLuminescence و ELISA و NycoCardTM للقياس الدقيق لمستويات البرولاكتين، التستوستيرون، الإستروجين، TSH، FSH، LH، Anti–FSH، و CRP. بناءً على مستوى البرولاكتين، تم تصنيف العينات إلى ثلاث مجموعات: متلازمة تكيس المبايض مع فرط البرولاكتين (أعلى من 23.30 نانوغرام/مل)، متلازمة تكيس المبايض مع برولاكتين طبيعي، وبرولاكتين طبيعي بدون متلازمة تكيس المبايض. سجلت متلازمة تكيس المبايض زيادة كبيرة في مستوى البرولاكتين مقارنة بالمجموعة الضابطة عند قيمة (<0.0001). كما أظهرت مجموعة متلازمة تكيس المبايض مع فرط البرولاكتين مستويات أعلى بشكل ملحوظ من الإستروجين و CRP مقارنة بكل من مجموعة متلازمة تكيس المبايض مع برولاكتين طبيعي والمجموعة الضابطة عند قيمة (<0.0001) .بالإضافة إلى ذلك، أظهرت مجموعة متلازمة تكيس المبايض مع فرط البرولاكتين مستويات أعلى بشكل ملحوظ من TSH مقارنة بالمجموعة الضابطة عند قيمة (<0.0001). كما تم العثور على فرق كبير في مستوى التستوستيرون، LH، نسبة LH/FSH، ومضاد–FSH في متلازمة تكيس المبايض مقارنة بالمجموعة الضابطة عند قيمة (<0.0001). بناءً على الدراسة الحالية التي أظهرت علاقة ذات دلالة إحصائية بين فرط البرولاكتين وارتفاع مستويات الالتهاب كاستجابة مناعية مصحوبة باضطرابات هرمونية ومستويات مرتفعة من Anti–FSH، يمكننا أن نستنتج أن جميع العوامل المذكورة في هذه الدراسة يجب أن تُدرج في استراتيجيات علاج متلازمة تكيس المبايض لاستعادة التوازن الهرموني والمناعي، والذي قد يكون سببًا لهذه الحالة.

1. Introduction

In women who suffer from Polycystic Ovary Syndrome (PCOS), an excess of androgens is evident both clinically and biochemically. Multiple endocrine, reproductive, and metabolic concerns have been linked to PCOS, and these risks lower the quality of life for affected women throughout their lives [1], [2]. Basic indicators such as luteinizing hormone (LH), follicle-stimulating hormone (FSH), and androgen level are essential for PCOS diagnosis [3]. PCOS progresses due to an increase in androgen levels, which in turn triggers an increase in LH levels. Two conditions strongly linked to PCOS are hyperinsulinemia and type 2 diabetes mellitus [4]. There is strong evidence that PCOS ovulation abnormalities are associated with increased levels of anti-Mullerian hormone (AMH) and LH [5]. A gonadotropin-releasing hormone (GnRH) stimulation test could cause LH oversupply. Additionally, GnRH stimulation to measure the LH:FSH ratio can help diagnose PCOS [6]. A medical disorder known as hyperprolactinemia is defined as an unusually high prolactin level in the blood that might produce several clinical consequences [7].

The prevalence of hyperprolactinemia in PCOS women is very variable in the literature, ranging from 3% to 67% [8]. Another prevalent endocrine condition in reproductive-aged women that can cause anovulation is hyperprolactinemia, which is similar to PCOS. Female blood donors, with an average age of 30 years, had a prevalence of hyperprolactinemia of about 4% in a population-based cohort study [9], [10]. It is commonly asserted that hyperprolactinemia and PCOS share the same pathophysiological basis. An idea put out that elevated prolactin and LH levels (common in PCOS) are brought about by a reduction in

dopaminergic tone, which in turn reduces the pulsatility of GnRH [8], [11]. Rising levels of prolactin in the blood can prevent the anterior pituitary gland from secreting FSH and the hypothalamus from secreting GnRH [12]. In women, hyperprolactinemia disturbs the ovarian cycle's management of menstruation and ovulation, therefore producing aberrant ovarian function and low estrogen levels [13]. On the other hand, raised prolactin levels in men affect testosterone generation, which causes symptoms including lower libido and less energy [14]. Furthermore, elevated prolactin levels in the bloodstream may be associated with the symptoms of PCOS. However, researchers have yet to find conclusive evidence to support this theory [15]. The medical community has recently been closely looking at and researching the cause-and-effect links between PCOS and hyperprolactinemia, shedding light on the possible links and processes behind the frequent coexistence and interaction between these two diseases [16]. PCOS is often associated with a state of chronic low-grade inflammation [17], [18].

Hyperprolactinemia can contribute to inflammation through several mechanisms; prolactin is not only a hormone associated with lactation but also acts as an immunomodulatory hormone. It can stimulate the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β) [19]. Elevated prolactin levels can lead to an overactive immune response, increasing the levels of these cytokines in the body, which contributes to a state of chronic low-grade inflammation [20]. Women with PCOS often experience insulin resistance, a condition that promotes inflammation. Hyperprolactinemia can exacerbate insulin resistance by interfering with insulin signaling pathways [21], [22]. When prolactin levels are high, they can change how adipose tissue works. This can cause more adipokines, such as leptin and resistin, to be made, which are linked to inflammation [23]. Many women with PCOS struggle with obesity, and hyperprolactinemia can worsen weight gain. Excess adipose tissue, particularly visceral fat, is a source of inflammatory cytokines, contributing further to the chronic inflammation seen in PCOS [24]. Hyperprolactinemia can mess up the Hypothalamic-Pituitary-Ovarian (HPO) Axis Dysregulation axis, which makes GnRH, LH, and FSH less likely to be released. This hormonal disruption can worsen the already imbalanced hormone levels in PCOS, further promoting inflammatory processes [25]. Elevated prolactin can also lead to an increase in adrenal androgens, which are already elevated in PCOS. High androgen levels contribute to the inflammatory milieu, particularly in the ovaries, exacerbating PCOS symptoms [26], [27]. Hyperprolactinemia can make autoimmune diseases worse or even cause them to happen by boosting the immune system, causing inflammation, and may encourage the development of autoimmune disorders [28]. Prolactin has a direct effect on immune cells such as T lymphocytes, B lymphocytes, and macrophages. It promotes the survival and activation of these cells, leading to an enhanced immune response. Elevated prolactin levels can increase the production of autoantibodies, which can attack self-antigens, and then autoimmune disorders settle [29]. The available data on serum prolactin levels in women with PCOS is contradictory. Recent studies have not supported a clear association between hyperprolactinemia, PCOS, and inflammation. Therefore, the current study aims to shed light on hyperprolactinemia and the role of inflammation and biochemical disturbances in increasing the severity of PCOS.

1. Materials and Methods

Subject collection

The study recruited a total of 90 participants between March 2023 and February 2024. Al-Kadhimiya Teaching Hospital or collaborating private clinics and laboratories recruited sixty-eight women diagnosed with PCOS, while 22 women served as healthy controls.

Inclusion and exclusions criteria

The inclusion criteria included an age range of 20–45 years and confirmation of PCOS diagnosis by a consulting physician based on the Rotterdam criteria (2003) outlined in the Rotterdam ESHRE/ASRM Consensus of 2004 [30], with age-matched healthy women enrolled as controls. The physician made the diagnosis they were accustomed to based on the Rotterdam criteria: (1) clinical and/or biochemical hyperandrogenism. The modified Ferriman-Gallwey (MFG) score assesses hirsutism by examining specific body areas such as the upper lip, face, jaw and neck, upper and lower back, upper arm, thigh, chest, upper and lower abdomen, and perineum. A score of 8 or higher out of 36 is considered significant. Furthermore, elevated serum testosterone levels are observed, with levels surpassing 0.8 ng/mL. (2) Oligo/Anovulation: irregular menstrual cycles (oligomenorrhea) ranging from 35-45 days or amenorrhea (absence of menstruation for >3 months). (3) Polycystic Ovary Morphology: A qualified ultrasound specialist physician conducts a transvaginal ultrasound examination on cycle days 3–4 to assess ovarian morphology [30]. Disease history in the family, duration of disease, and menstrual cycle were obtained according to a questionnaire provided by patients. Exclusions criteria Patients with thyroid, cardiovascular, autoimmune, diabetes mellitus, hypertension, chronic renal failure, and malignant disorders were excluded. Also excluded were participants who had taken any additional drugs within six months after the sample collection, including lipid-lowering, ovulation-stimulating, corticosteroids, antidiabetic, and antihypertensive drugs.

The Waist-to-hip Ratio measurement

The Waist-to-Hip Ratio (WHR) assesses the body's fat distribution, specifically around the waist and hip regions. It is a straightforward yet informative indicator of fat distribution. The calculation entails dividing waist circumference by hip circumference, with the hips representing the widest part of the buttocks. The formula is expressed as WHR = waist circumference/hip circumference. In females, a desirable waist-to-hip ratio is considered to be 0.8. It is noteworthy that women with PCOS exhibited an elevated waist-to-hip ratio, with a value of 0.87, indicating the presence of PCOS [31].

Blood collection

Four ml of peripheral blood was withdrawn from each participant and collected by disposable syringe; the 4 ml were set in a gel tube for the ELISA and biochemical study and centrifuged to separate serum using several Eppendorf tubes to prevent repeated freeze-thaw cycles.

Biochemical and hormonal tests

A fully automated analyzer, the cobas® e 411 analyzer, uses patented ElectroChemiLuminescence (ECL) technology for immunoassay analysis to measure all hormones, including testosterone, prolactin, estrogen, TSH, FSH, and LH. The NycoCardTM CRP (C-reactive protein) Test is a quantitative assessment of the best immunity to C-reactive protein (CRP) in blood or serum. In the case of prolactin level, after checking, if the level of prolactin was above 23.30 ng/ml, which exceeded the normal range, consider it hyperprolactinemia [32]. Thus, patients were categorized into 3 groups: hyperprolactin with PCOS (G1), normal prolactin with PCOS (G2), and normal prolactin without PCOS as the control group (G3).

Enzyme-linked immunosorbent assay (ELISA):

This assay uses the "Double Antibody Sandwich" method from ELISA Kits available from MyBioSource, USA. The process entails measuring the levels of Autoantibody-FSH Anti-FSH in (Catalog No.: MBS7612282) protein in the samples. This is accomplished by

comparing the optical density (O.D.) of the samples to a computed and established reference curve. To guarantee precision and uniformity, all standards, samples, and reagents were carefully prepared according to the test preparation instructions included in the kit brochure.

LH/FSH ratio measurement

Most (but not all) female PCOS patients exhibit this phenomenon when they have an abnormal FSH to LH ratio. The blood levels of FSH and LH are usually 1:1. Both FSH and LH are, in most cases, in the range of about 4–8 in young fertile women. In women with PCOS, the LH to FSH ratio is often higher, for example, 2:1 or even 3:1. In healthy women, the ratio between LH and FSH typically falls between 1 and 2. Women with PCOS experience the ratio for LH/FSH, potentially reaching as high as 2 or 3 [6].

Statistical analysis

The results of the collected data were analyzed using the GraphPad Prism (10) program using an independent T-test and one-way ANOVA as appropriate. The data were presented as Mean ± S.E., while the significant differences were considered at $p \leq 0.001$.

2. Result

Table 1 presents the mean serum concentrations of prolactin in the PCOS and control groups. The study found that the mean concentration ± standard error (S.E.) (ng/mL) of prolactin was significantly higher in women with PCOS (24.03±1.41) compared to women without PCOS (14.46±0.84), with a P-value of <0.0001.

Table 1: Serum level of prolactin in PCOS and control.

Parameter	Normal value, U	Control (n=22) (Mean±S.E)	PCOS (n=68) (Mean±S.E)	P value
Prolactin	4.97 - 23.30, ng/mL	14.46±0.84	24.03±1.41	<0.001**

After measuring the prolactin level, if it exceeds the normal range of 23.30 ng/mL, it is considered hyperprolactinemia [32]. Therefore, the samples were categorized into three distinct groups based on their prolactin levels. Group 1, labeled as Hyperprolactin PCOS (G1), comprised 38.89% of the participants, indicating a significant portion with elevated prolactin levels and PCOS. Group 2 (G2) consisted of 36.67% of the subjects, characterized by individuals with PCOS but normal prolactin levels. The remaining 24.44% fell into Group 3 (G3), serving as the control group, presumably with neither elevated prolactin levels nor PCOS, as shown in Figure 1. Accordingly, the percentage of women with PCOS who have hyperprolactinemia was 51%.

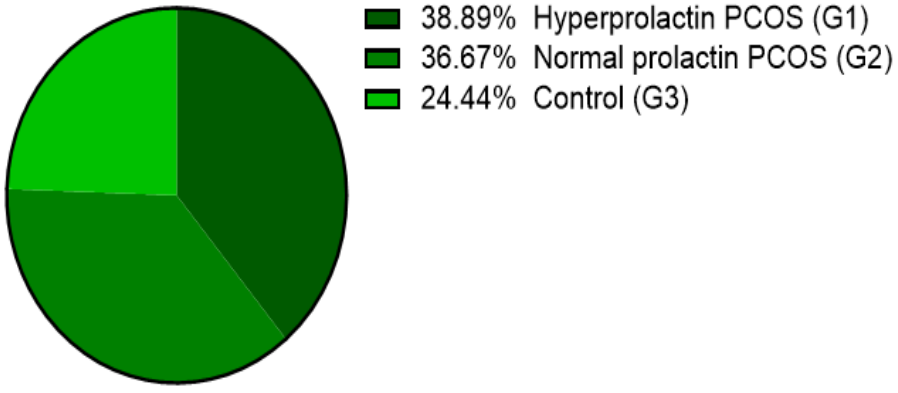

Figure 1: Distribution of Prolactin Levels in PCOS Patients.

Table 2 and Figure 2 summarize the comparison of demographic and biochemical characteristics between three groups: hyperprolactinemia PCOS, normal prolactin PCOS, and a control group. The levels of testosterone in G1 and G2 were (0.36± 0.02, 0.38± 0.04) respectively, with a significant difference compared to G3 at p-value 0.0004 as compared to the normal value (0.08–0.48) ng/mL, as shown in Figure 2 (a). Based on prolactin levels, a significant increase was found in G1 (33.23±2.052) compared to G2 & G3 (15.13±1.030 & 14.46±0.8387) respectively, at p-value <0.0001 with a normal value of 4.97 - 23.30 ng/mL, as shown in Figure 2 (b). Also, estrogen level increased significantly in G1 (50.99±4.18) compared to G2 and G3 (28.16±0.41 & 28.48 ± 3.14) at p-value <0.0001 with a normal value of 12.5 - 166 pg/mL, as shown in Figure 2 (c). The TSH level showed a significant increase in G1 (2.22±0.18) as compared to G3 (1.6±0.12) at p-value 0.0194 with a normal value of 0.27 - 4.2 ulU/mL, as shown in Figure 2 (d). In FSH with a normal value of 3.5–12.5 mlU/mL, a non-significant difference was found among the groups at a p-value of 0.3142, as shown in Figure 2 (e). In contrast, in LH, LH/FSH ratio, and anti-FSH level, it was recorded that there was a significant increase in G1 and G2 compared to G3 at p-values (<0.0001, <0.0001, and <0.0001), respectively, as shown in Figure 2 (f, g, and h). Finally, the CRP level was significantly higher in G1 (10.65±1.107) compared to G2 and G3 (5.9±0.84 and 1.3±0.18), with a p-value <0.0001 and a normal range of 0–5 mg/mL, as depicted in Figure 2 (i).

Table 2: Comparison of Demographic and Biochemical Characteristics between Groups

Quantitative variables (Mean ± SE)	Normal value, U	Group 1 (Hyperprolactinemia PCOS) (n= 35)	Group2 (Normal prolactin PCOS) (n= 33)	Group3 (Control) (n= 22)	P value
Testosterone	0.08 - 0.48, ng/mL	0.36± 0.02	0.38±0.04	0.19±0.02	**0.0004**
Prolactin	4.97 - 23.30, ng/mL	33.23±2.052	15.13±1.030	14.46±0.8387	**<0.0001**
Estrogen	12.5 – 166, (pg/mL)	50.99±4.18	28.16±0.41	28.48±3.14	**<0.0001**
TSH	0.27 - 4.2, ulU/mL	2.22±0.18	1.76±0.14	1.6±0.12	**0.0194**
FSH	3.5-12.5, mlU/mL	6.6±0.72	10.9±3.57	6.5±0.36	0.3142
LH	2.40- 12.60, mlU/mL	13.01±0.76	16.36±1.86	7.44±0.48	**0.0001**
LH/FSH ratio	0-2	2.22±0.17	2.14±0.14	1.18±0.07	**<0.0001**
Anti-FSH	- (ng/mL)	15.27±0.81	15.90±0.81	6.79±0.18	**<0.0001**
CRP	0-5 (mg/L)	10.65±1.107	5.9±0.84	1.3±0.18	**<0.0001**

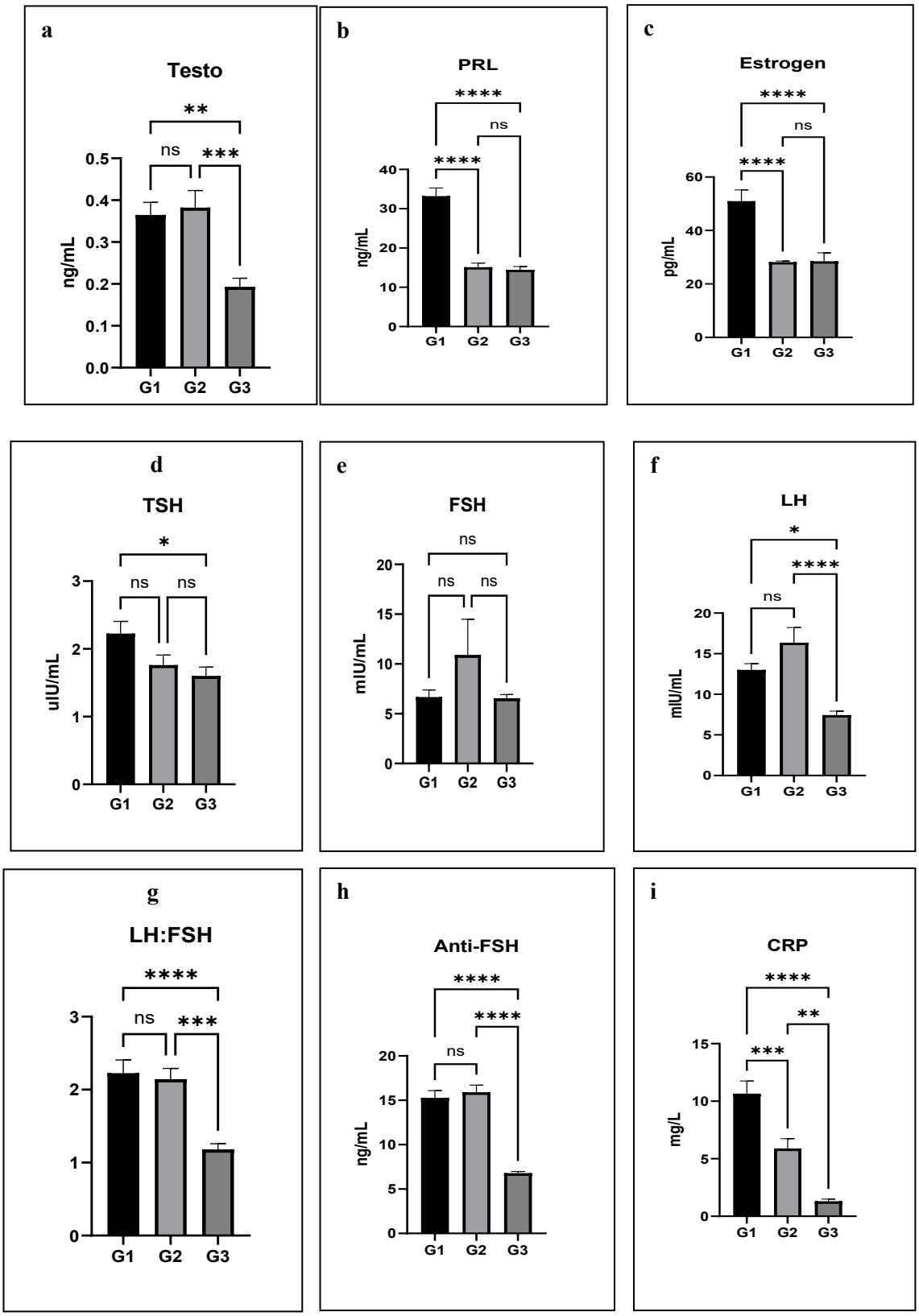

Figure 2: The demographic comparison and biochemical characteristics between groups

The comparison of clinical findings between patients with hyperprolactinemia PCOS is detailed in Table 3 and Figure 3. The study involved a total of 35 patients in Group 1, with various quantitative variables analyzed and compared.

Age was categorized into two groups: those aged 25 years or younger and those older than 25. In Group 1, there were 13 patients aged 25 or younger, with a mean age of (32.05± 2.186) years. For patients older than 25, comprising 22 individuals, the mean age was (33.93±3.028) years, and the statistical analysis revealed no statistical significance at a p-value of 0.6656.

WHR was another parameter examined, with a cutoff of 0.8 separating the groups. Among 35 patients in Group 1, 4 individuals had a WHR of 0.8 or lower, with a mean of 26.82 ± 0.9377. In contrast, 31 patients had a WHR greater than 0.8, with a mean of 34.06 ± 2.275, and the statistical analysis revealed no statistically significant at a p-value of 0.2680

The presence or absence of a disease history was also investigated. Seven patients had a history of the disease, with a mean of 45.43 ± 7.427. In comparison, 28 patients had no previous disease history, with a mean of 30.18 ± 1.357, and this difference was statistically significant at a p-value of 0.0018.

Regarding the duration of the disease, patients were divided based on whether they had been diagnosed for less than or more than one year. Seventeen patients had a disease duration of less than one year, with a mean of 33.41 ± 3.142, in comparison to 18 patients who had a disease duration exceeding one year, with a mean of 33.06 ± 2.756 and the analysis showed a non-significant p-value 0.9334. Menstrual cycle regularity was also considered, with two patients having a regular cycle and a mean age of 26.23 ± 2.475. oppositely, 33 patients had an irregular cycle, with a mean of 33.66 ± 2.153 and a p-value of 0.4085. This comparison reveals no significant difference regarding menstrual cycle regularity in this patient group.

Table 3: Comparison of Hyperprolactinemia PCOS Clinical Findings of Patients

Quantitative variables (Mean ± SE)		Number	Group 1 (Hyperprolactinemia PCOS) (N= 35)	P value
Age	**≤25 years**	13	32.05±2.186	0.6656
	>25 years	22	33.93±3.028	
WHR	**≤0.8**	4	26.82± 0.9377	0.2680
	>0.8	31	34.06±2.275	
Disease history	**Yes**	7	45.43 ± 7.427	**0.0018**
	No	28	30.18 ± 1.357	
Duration of Disease	**≤ 1Yrs**	17	33.41 ± 3.142	0.9334
	>1Yrs	18	33.06 ± 2.756	
Menstrual cycle	**Regular**	2	26.23± 2.475	0.4085
	Irregular	33	33.66±2.153	

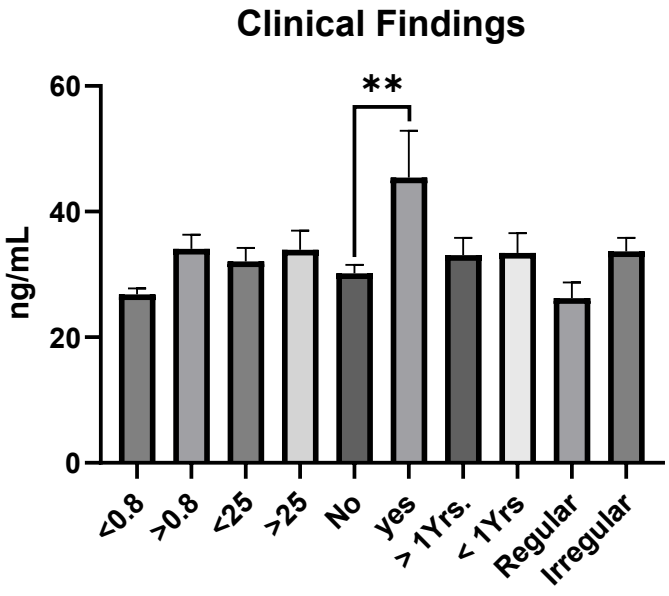

Figure 3: Comparison of Hyperprolactinemia PCOS Clinical Findings of Patients

3. Discussion

The serum prolactin levels in PCOS women are inconsistent. Recent studies have not consistently shown PCOS-related hyperprolactinemia. A significant number of PCOS patients with elevated prolactin levels had other concurrent factors responsible for the hyperprolactinemia when using a comprehensive diagnostic approach, which includes prolactin chromatography, pituitary magnetic resonance imaging (MRI), and ruling out hypothyroidism, pregnancy, and prolactin-raising drugs. These include prolactinomas, hyperprolactinemic medications, and macroprolactin. Hyperprolactinemia was detected in less than 5% of PCOS women. Mechanisms linking prolactin levels and PCOS require further study [16]. The marked rise in prolactin levels, which is one of the most important hormonal changes, supports the crucial role of hyperprolactinemia among the groups that may be one of the factors of the ovulatory dysfunction and menstrual irregularities that are characteristic of PCOS [15]. Women diagnosed with PCOS display a wide spectrum of prolactin levels, which can be greater, equal to, or lower than those observed in women without PCOS [8]. The study results showed an increase in prolactin levels in women with PCOS compared to those without the PCOS, a finding that contradicts previous studies such as [33], [34]. These studies suggested that elevated blood prolactin levels were not associated with PCOS and should not be considered a characteristic of PCOS.

Nevertheless, it is now necessary to monitor serum prolactin levels in individuals diagnosed with PCOS. A study by [35] suggested that infertile PCOS individuals may be at increased metabolic risk due to low serum prolactin. However, the result of this study agreed with previous studies, such as [36]. It was suggested for the detection of the reasons for hyperprolactinemia, particularly macroprolactinemia, suggesting that PCOS patients have elevated prolactin levels. A meta-analysis study conducted by [15] revealed that patients diagnosed with Rotterdam-defined PCOS had significantly higher prolactin levels compared to those without the condition. One may use a slightly elevated prolactin level to diagnose PCOS. A study by [37] indicates that PCOS women aged ≤35 years had a 1.5-fold greater upper reference limit of serum PRL compared to controls. PCOS may cause prolactin elevation by decreasing central dopaminergic tone, which raises prolactin and LH levels. The

relationship between prolactin and PCOS remains uncertain despite the proposal of numerous theories. Studies indicate that prolactin release is influenced by environmental, psychological, and physical factors [38]. Previous studies suggest that hyperprolactinemia may trigger PCOS symptoms; a decrease in central dopaminergic tone may cause prolactin elevation in PCOS, leading to an increase in both PRL and LH levels [37], [39].

According to the findings of this study, 51% of PCOS patients have hyperprolactinemia. The prevalence of hyperprolactinemia in PCOS women reported in the literature is not homogeneous; it ranges between less than 5% and more than 65% due to several factors that influence the diagnosis of both PCOS and hyperprolactinemia [40].

This study revealed that the CRP level increased in PCOS compared to the control group, irrespective of the prolactin level. This suggests that PCOS is linked to systemic inflammation, which includes the elevation of CRP and pro-inflammatory cytokines [41], [42]. It was also found that there was a significant rise in CRP in PCOS with hyperprolactinemia compared to PCOS with normal prolactin levels. These findings may support the idea that prolactin is an immunomodulating substance that may affect different types of inflammation in PCOS. The study conducted by [28] revealed that women with hyperprolactinemia had elevated levels of certain immunological markers, such as CRP, and suggested that prolactin may exert immunomodulating effects in vivo, potentially impacting various inflammatory disorders. Other studies showed no correlation between high levels of prolactin and CRP in PCOS [43]. Prolactin is known to have immunomodulatory effects, meaning it can influence the immune response. Depending on the context, prolactin can have both pro-inflammatory and anti-inflammatory actions [28]. The most common features of PCOS are insulin resistance [4]. The combination of insulin resistance and hyperprolactinemia can lead to higher levels of circulating insulin (hyperinsulinemia), which in turn can increase the production of inflammatory markers and exacerbate the inflammatory state in PCOS [21]. Therefore, this study suggested that the pathways leading to hyperprolactinemia and inflammation in PCOS may overlap. For instance, insulin resistance, which is common in PCOS, can contribute to both elevated prolactin levels and inflammation. Additionally, obesity, a frequent comorbidity in PCOS, is associated with both hyperprolactinemia and increased inflammatory markers.
Furthermore, the study discovered higher estrogen levels in PCOS with hyperprolactinemia compared to PCOS with normal prolactin levels, even though these levels are within normal limits. The main ovarian hormone, estrogen, interferes with PRL secretion through different mechanisms: regulation of prolactin gene expression, downregulation of dopamine receptor expression, and stimulation of lactotroph proliferation. Therefore, estrogen is considered a prolactin-releasing factor [44]. Several previous studies suggested that PCOS causes hyperprolactinemia because it induces a relative increase in estrogen levels [8], [45]. This study also agreed with a previous study that found there is an increase in estrogen in PCOS with hyperprolactinemia [36]. Indeed, various experimental studies have shown an increase in prolactin secretion under estrogen action [46], [47]. Nevertheless, other considerations challenge this postulation. First, several studies have demonstrated that combined oral contraceptives (including estrogens) do not cause prolactinoma size to increase [48]. So, this study suggested high estrogen levels in the context of hyperprolactinemia PCOS can exacerbate many of the syndrome's symptoms, including menstrual irregularities, metabolic disturbances, and fertility issues.

Another statistical finding in this study was the revealed increase in TSH level in hyperprolactinemia PCOS compared to the control. This study disagreed with the previous study, which found there is no significant difference in TSH level between

hyperprolactinemia PCOS and control [33], [36]. Elevated TSH and prolactin levels can worsen menstrual irregularities in PCOS, leading to more pronounced oligomenorrhea (infrequent menstruation) or amenorrhea (absence of menstruation) [49].

Other biochemical analyses revealed significant changes in testosterone, LH, LH:FSH ratio, and anti-FSH in PCOS compared to controls, regardless of hyperprolactinemia. An increase in testosterone levels is one of the diagnostic criteria used to identify PCOS. Women diagnosed with PCOS may encounter several challenges, including reduced fertility, skin problems like acne, excessive hair growth (hirsutism), obesity, and resistance to insulin [50]. PCOS groups in this study showed higher LH/FSH ratios than the control. This spike fits the accepted pathophysiology of PCOS, in which hyperinsulinemia and hyperandrogenism cause relatively decreased FSH levels and increased LH output, thereby upsetting follicular maturation [6]. Though it is not the only criteria for diagnosis, the LH/FSH ratio is a vital diagnostic sign in PCOS. Compared to the more balanced ratio seen in women without PCOS, women with PCOS commonly have an increased LH/FSH ratio, usually exceeding 2:1 or even 3:1 [51]. Because of the heightened pulsatility of GnRH from the hypothalamus, women with PCOS commonly show hypersecretion of LH. Insulin resistance and hyperinsulinemia cause this hypersecretion, which is common in PCOS. [52]. LH levels are high; FSH levels are either somewhat low or normal. This imbalance stunts ovarian follicle formation, which causes anovulation, one of PCOS's defining traits. Increased LH drives the theca cells in the ovaries to generate more androgens (testosterone and androstenedione), hence aggravating hyperandrogenism. Many of the clinical PCOS symptoms, hirsutism, acne, and alopecia are caused by hyperandrogenism [6], [52].

Many previous studies [53], [54], and [55] found that anti-FSH increased PCOS relative to control. "Anti-FSH antibody" refers to an autoantibody that targets FSH. These antibodies may alter FSH activity, affecting fertility and reproductive health. Anti-FSH antibodies reduce FSH's biological activity. Anovulation and decreased follicular development are the major signs of PCOS [54]. Anti-FSH antibodies neutralize FSH, disrupting reproductive hormone balance. This could worsen PCOS's hormonal imbalance of low FSH and high LH, causing ovulation and polycystic ovaries [55]. Anti-FSH antibodies may indicate autoimmune PCOS. PCOS shares pathophysiology with several autoimmune illnesses, suggesting autoimmune reactions may be involved [53].

Age and WHR are regarded as pivotal factors in the assessment of PCOS and its associated risks. Our findings indicate that these parameters, although variably distributed across the groups, have non-significant differences within the hyperprolactinemia PCOS group based on the age demarcation of 25 years or a WHR cutoff of 0.8 and agreed with the previous study [36]. While age and obesity are critical factors in the pathophysiology of hyperprolactinemia PCOS, previous studies suggest that the biochemical milieu characterizing each PCOS subtype may overshadow their individual impact [56], [57].

The study also revealed that there were non-significant differences in the duration of the disease menstrual cycle within the hyperprolactinemia PCOS group. The assessment of menstrual period regularity did not reveal any significant differences, which could be attributed to the variety of PCOS symptoms and the multiple factors that influence menstrual regularity beyond just hyperprolactinemia. However, patients who had the disease before showed very different symptoms, which could be because they had been exposed to the disease's detrimental effects for a long time, such as insulin resistance and hyperandrogenism [50]. Women with a family history of PCOS may experience symptoms that overlap with hyperprolactinemia, such as irregular menstrual cycles, infertility, and galactorrhea (milk production unrelated to childbirth). This can prompt investigations into both conditions within the same family [58].

There are some limitations that should be noted in the current study. Primarily, it has favored hormonal-level investigations using a case-control design. Second, this study may not have an adequate sample size; further studies with a larger sample size are recommended. Also, it is recommended imaging, preferably MRI, of the pituitary fossa to determine the presence of a prolactin-secreting pituitary tumor or other lesion for hyperprolactinemia diagnosis rather than relying solely on serum level. While PCOS is a recognized condition, a larger patient cohort would strengthen the generalizability of the findings. Furthermore, the underlying causes and optimal treatment strategies for PCOS remain elusive. Future research with larger and more diverse patient populations is warranted to solidify the observed associations between hyperprolactinemia and inflammation in PCOS and to explore their potential as therapeutic targets.

Conclusion

Based on the results of the current study, a statistically significant relationship between hyperprolactinemia and high levels of inflammation (CRP) as an immune reaction accompanied by hormonal disorders (ES, TSH, testosterone, and LH) in addition to high levels of autoantibodies (anti-FSH), it can be concluded that all the factors mentioned in this study should be included in the therapeutic strategies for PCOS patients, in order to restore hormonal and immune balance in these patients, which may be a cause of their cardiovascular diseases and insulin resistance diseases in addition to infertility.

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Ethics approval and consent to participate

The study protocol received approval from the Ethics Committee of the University of Baghdad, the Baghdad Health Directorate, and the Al-Kadhimiya Teaching Hospital, and it was carried out under the principles outlined in the Declaration of Helsinki according to No. 18363 on 23/11/2022. Before taking part in the study, all participants provided written informed consent after receiving comprehensive information about the purpose, nature, and potential risks and benefits of the research. Participants were given the assurance that they had the right to withdraw from the study at any time without facing any negative consequences. The study process strictly maintained the confidentiality of participant information, and data were made anonymous for analysis. Each participant was required to complete a detailed Consent to Participate declaration form before being included in the study. However, it is not applicable in the headings.

Conflict of interest

There are no conflicts of interest.

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