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Estimating the levels of Vitamin D, Interleukin-17A, Interleukin-22, and Interleukin-34 in Women with Polycystic Ovary Syndrome.

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Abstract

Polycystic ovarian syndrome (PCOS) is the most prevalent endocrine disorder affecting women during their reproductive period. The study aims to estimate the concentrations of interleukins (IL-17A, IL-34, and IL-22) and vitamin D levels, besides some hormonal indices in blood serum, and to explain their roles in PCOS pathogenicity in women. This study included ninety samples categorized into two groups: sixty samples from patients with PCOS and thirty samples from a healthy control group. Participants' ages ranged from 19 to 39 years. The PCOS diagnosis is made by a specialist doctor. Blood samples were collected from each woman, both patients and healthy controls, during the menstrual cycle (days 3 and 4) to measure hormonal profile levels of estradiol, testosterone, Anti-Müllerian Hormone (AMH) and vitamin D using a mini VIDAS device. The ELISA technique was used to determine the concentrations of serum IL-17A, IL-34, and IL-22. The results indicated a significant elevation ($P < 0.05$) in testosterone and Anti-Müllerian Hormone (AMH) levels in patients with PCOS compared to the healthy control group. In contrast, estradiol had a significant decrease in PCOS patients. The vitamin D levels in patients with PCOS were lower than those in the healthy control group, without statistical significance. IL-17A and IL-34 exhibited no significant differences between women with PCOS and the healthy group. IL-22 concentrations were reduced in the patients compared to the healthy control group. Furthermore, it is noteworthy that a weak negative correlation was observed between IL-17A and testosterone ($r = -0.25$), as well as between IL-34 and other variables, including estradiol ($r = 0.27$) and testosterone ($r = 0.28$). In contrast, a weak positive correlation was identified between IL-22 and testosterone ($r = 0.26$). It can be concluded that there are no statistically significant changes in the levels of vitamin D, IL-17A, or IL-34 between patients and healthy controls. However, there was a notable decrease in the levels of IL-22 in patients with PCOS. A drop in IL-22, likely caused by hormonal changes and immune dysregulation, such as chronic low-grade inflammation, may help prevent and treat PCOS.

Keywords: Vitamin D, IL-7A, IL-22, IL-34, PCOS

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تقديرمستويات فيتامين د، انترلوكين 17A ، 22 و34 بين النساء المصابات بمتلازمة تكيس المبايض

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الخلاصة

متلازمة تكيس المبايض (PCOS) هي أكثر اضطرابات الغدد الصماء شيوعًا التي تصيب النساء خلال فترة الإنجاب. تهدف الدراسة إلى تقدير تركيزات الإنترلوكينات (IL−17A , IL−34 و IL−22) ومستويات فيتامين د، بالإضافة إلى بعض المؤشرات الهرمونية في مصل الدم، وشرح دورها في مسببات متلازمة تكيس المبايض لدى النساء. شملت هذه الدراسة تسعين عينة مصنفة إلى مجموعتين: ستون عينة من مريضات مصابات بمتلازمة تكيس المبايض وثلاثون عينة من مجموعة ضابطة سليمة. تراوحت أعمار المشاركات بين 19 و39 عامًا. تم تشخيص متلازمة تكيس المبايض من قبل طبيب مختص. جمعنا عينات دم من كل امرأة، من المريضات وضوابط سليمة، خلال الدورة الشهرية (اليومين الثالث والرابع) لقياس مستويات الهرمونات)الإستراديول، التستوستيرون، AMH، وفيتامين د (باستخدام جهاز VIDAS صغير. حددت تقنية ELISA تركيزات مصل IL−17A و IL−34 و .IL−22 أشارت نتائج البحث إلى ارتفاع كبير (P <0.05) في مستويات هرمون التستوستيرون وهرمون مضاد مولر (AMH) لدى مرضى متلازمة تكيس المبايض مقارنةً بالمجموعة الضابطة الصحية. بينما انخفض هرمون الاستراديول بشكل كبير لدى مرضى متلازمة تكيس المبايض. كانت مستويات فيتامين د لدى مرضى متلازمة تكيس المبايض أقل من تلك الموجودة في المجموعة الضابطة الصحية، دون دلالة إحصائية. لم يُظهر IL−17A و IL−34 أي فروق كبيرة بين النساء المصابات بمتلازمة تكيس المبايض والمجموعة السليمة. انخفضت تركيزات IL−22 لدى المرضى مقارنةً بمجموعة الضبط السليمة. علاوةً على ذلك، تجدر الإشارة إلى وجود ارتباط سلبي ضعيف بين IL−17A والتستوستيرون (r = −0.25)، وكذلك بين IL−34 ومتغيرات أخرى، بما في ذلك الإستراديول (r = 0.27) والتستوستيرون (r = 0.28). في المقابل، وُجد ارتباط إيجابي ضعيف بين IL−22 والتستوستيرون (r = 0.26). يمكن الاستنتاج أنه لا توجد تغيرات ذات دلالة إحصائية في مستويات فيتامين د، IL−17Aو IL−34 بين المرضى والأصحاء. ومع ذلك، قد يساعد انخفاض مستوى IL−22، والذي من المحتمل أن يكون ناجمًا عن تغيرات هرمونية واضطرابات مناعية، مثل الالتهاب المزمن منخفض الدرجة، في الوقاية من متلازمة تكيس المبايض وعلاجها.

1. Introduction

Polycystic ovarian syndrome (PCOS) is the most prevalent endocrine disorder affecting women during their reproductive period. It is distinguished by an imbalance of hormones, at least one polycystic ovary accompanied by excessive secretion of androgens, and ovulatory dysfunction [1]. PCOS affects almost 10% of reproductive-age women worldwide, resulting in considerable health and economic challenges. It is a heterogeneous condition that may lead to infertility, irregular menstrual cycles, hirsutism, and acne, among other symptoms. The clinical diagnosis depends mainly on one or more of the three principal symptoms, namely, hyperandrogenism, oligo- or anovulation, and polycystic ovarian morphology [2]. A potential etiological factor of PCOS is vitamin D deficiency (VDD). In fact, the prevalence of VDD in PCOS women is evident, mainly in those with insulin resistance (IR) or obesity. Additionally, a relationship was found between vitamin D levels and the parameters of IR and metabolic syndrome. Subsequently, vitamin D supplementation may thus help with PCOS management[3].VDD (<10 ng/ml) was present in 80% of non-pregnant women and 69% of

pregnant women[4]. Although inflammation is accepted as a contributing factor to many chronic illnesses, it is still unclear how much vitamin D affects it, and in fact, VDD or insufficiency may result from chronic inflammation rather than the other way around [5]. The focus on inflammatory factors and their association with PCOS has risen due to a correlation between the prevalence of PCOS and chronic inflammation [6]. A number of markers, including Tumor Necrosis Factor-alpha (TNF-α), C-reactive protein (CRP), and other interleukins are signs of low-grade chronic inflammation [7].

In the majority of PCOS patients, elevated serum levels of IL-17 have been implicated in the etiology of PCOS through the induction of an inflammatory response [8]. IL-17, also known as IL-17A, is a T-cell derived inflammatory cytokine that Th17 cells, T cells, and neutrophils primarily produce. IL-17 promotes the production of other proinflammatory cytokines and chemokines that mediate immune responses [9]. It was observed that IL-17A, a proinflammatory cytokine, may be linked to infertility in PCOS patients [10]. IL-34 is a newly investigated proinflammatory cytokine that plays a significant role in various disease types. Women with PCOS exhibited elevated levels of IL-34, IL-1β, IL-6, and TNF-α, compared to healthy controls. A positive correlation was observed between the concentration of serum IL-34 and the concentrations of IL-1β, TNF-α, and IL-6. Furthermore, serum levels of IL-34 showed a positive correlation with triglycerides, LDL-C, and HOMA-IR [11]. IL-22 is synthesized by both innate and adaptive immune cells and specifically targets epithelial cells. IL-22 has recently been shown to have a number of metabolic benefits, including improving the protection of the gut mucosal barrier, insulin sensitivity, endocrine functions, chronic inflammation, lowering endotoxemia, and regulating the metabolism of fats in the liver and adipose tissues [12]. The study aimed to determine the concentrations of certain parameters, IL-17A, IL-34, and IL-22, as well as vitamin D levels, and explain their roles in PCOS pathogenicity in women

2. Methodology

2.1. Study design

Prospective comparative research was conducted at the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, AL-Nahrain University, Baghdad, Iraq, from November 2023 to July 2024. The ethical approval was issued by the Ethics Committee of the College of Science, University of Baghdad, Iraq (number CSEC/1123/0080, dated November 9, 2023), and all participants provided written consent. All participants were aged between 19 and 39 years. The study includes 90 Iraqi women divided into two groups: 60 PCOS patients and 30 healthy controls.

2.2. Enzyme-linked fluorescence assay (ELFA)

The mini VIDAS device was used to measure levels of testosterone, estradiol, AMH, and vitamin D using an enzyme-linked fluorescence assay (ELFA) as directed by the manufacturer, bioMérieux, a French company. Each test uses a solid-phase receptacle (SPR) that is internally coated with specific antibodies and a sealed reagent strip that has all the necessary reagents prepared for use. The equipment automatically executes all procedures, including incubation, washing, and detection.

2.3. Enzyme-linked immunosorbent assay (ELISA)

The serum concentrations of IL-17A, IL-34, and IL-22 were measured using the enzyme-linked immunosorbent assay (ELISA) method with a sandwich-ELISA principle, according to the manufacturer's instructions. BT LAB ELISA Kit / China. The standards and samples were added to the well of the pre-coated plate with the antibody. Then, the biotinylated antibodies

were added, followed by streptavidin-HRP. Plates were incubated for 60 minutes at 37°C, then washed five times. Substrate solutions A and B were added, and after 10 minutes of incubation in the dark at 37°C, the stop solution was added. Thereafter, the colour formed was measured in terms of absorbance at 450 nm.

2.4. Inclusion and exclusion criteria

The inclusion criteria for the PCOS group included women aged between 19 and 39 years. The PCOS diagnosis by a specialist doctor was based on the Rotterdam criteria, requiring the presence of at least two of the following: oligo/anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries on ultrasound.

Exclusion criteria included individuals with adrenal disorders, thyroid dysfunction, diabetes mellitus, and hyperprolactinemia. The control group, consisting of fertile women with regular menstrual cycles and no clinical signs of hyperandrogenism or endocrine disorders, was matched in age to the PCOS patients. These women underwent ultrasound examinations to confirm normal ovarian morphology. The blood samples were collected during the follicular phase (days 3 or 4) of the menstrual cycle from each woman in both patients and healthy controls.

2.5. Statistical analysis

All statistical analyses were performed with the Statistical Analysis System (SAS) (2018). Data were analysed by an independent samples t-test in statistical analysis. The data are presented as mean ± SE (standard error). The correlation coefficient -r test was calculated to examine the association among the parameters.

3.Results

The comparison between PCOS patients and the healthy control group showed several statistically significant differences in the measured parameters. The PCOS group had lower serum estradiol levels (37.75 ± 1.56) than the control group (47.06 ± 2.97), which was a significant difference (t (88) = 6.041, p = 0.0029). In contrast, testosterone levels were elevated in patients with PCOS (0.502 ± 0.04) relative to the control group (0.155 ± 0.2) (t (88) = 0.130, p = 0.0001). Additionally, the levels of the AMH hormone were higher in patients (5.96 ± 0.33) than in the control group (2.07 ± 0.16), and this difference was statistically significant (t (88) = 0.959, p = 0.0001), as shown in Table 1.

Table 1: Hormone estradiol, testosterone, and AMH levels in the studied groups

Parameters/ Hormones	Means ±SE		T-test	P-value
	Patients	Control		
Estradiol (pg/ml)	37.75 ±1.56	47.06 ±2.97	6.041 **	0.0029
Testosterone (ng/ml)	0.502 ±0.04	0.155 ±0.2	0.130 **	0.0001
AMH (ng/ml)	5.96 ±0.33	2.07 ±0.16	0.959 **	0.0001
* (P≤0.05), ** (P≤0.01).				

The result demonstrated that no statistically significant differences were observed between the study groups in vitamin D (16.41 ± 1.38 vs. 19.47 ± 2.53; t (88) = 5.268, p = 0.2514), IL-17A levels (110.14 ± 4.36 vs. 110.95 ± 6.78; t(88) = 15.540, p = 0.9180), and IL-34 levels (16.21 ± 1.34 vs. 19.14 ± 2.10; t(88) = 4.782, p = 0.2271). However, the levels of serum IL-22 were lower in the PCOS group (114.89 ± 5.53) compared to the control group (213.42 ± 45.16), and this difference was statistically significant between the groups (t (88) = 65.005, p = 0.0034), as shown in Table 2.

Table 2: Biochemical analysis and immunological parameters in the studied groups

Parameters/ biochemical	Means ±SE		T-test	P-value
	Patients	Control		
Vitamin D (ng/ml)	16.41 ±1.38	19.47 ±2.53	5.268 NS	0.2514
IL-17A (ng/L)	110.14 ±4.36	110.95 ±6.78	15.540 NS	0.9180
IL-34 (ng/L)	16.21 ±1.34	19.14 ±2.10	4.782 NS	0.2271
IL-22 (ng/L)	114.89 ±5.53	213.42 ±45.16	65.005 **	0.0034
* (P≤0.05), ** (P≤0.01), NS: Non-Significant.				

In Table 3, the results showed no statistically significant correlation between vitamin D and other variables, such as estradiol (r = 0.04), testosterone (r = 0.12), and AMH (r = 0.10). On the other hand, there was a weak negative correlation between IL-17A and testosterone (r = -0.25) and a weak negative correlation between IL-34 and other variables, such as estradiol (r = 0.27) and testosterone (r = 0.28), while there was a weak positive correlation between IL-22 and testosterone (r = 0.26).

Table 3: Correlation coefficient between hormones and biochemical analysis in patients

Parameters	Correlation coefficient-r		
	Estradiol	Testosterone	AMH
Vitamin D	-0.04 NS	-0.12 NS	0.10 NS
IL-17A	-0.07 NS	-0.25 *	0.13 NS
IL-34	-0.27 *	-0.28 *	0.13 NS
IL-22	-0.06 NS	0.26 *	0.02 NS
* (P≤0.05), ** (P≤0.01), NS: Non-Significant.			

4.Discussion

One of the most common endocrine illnesses affecting reproductive-age women, 5-10% is PCOS, and its etiology is complex, and patients exhibit elevated ovarian androgen production, which correlates with diminished menstruation, acne, and hirsutism [13]. The results of estradiol, testosterone, and AMH levels in the present study were in agreement with those of previous investigations [14, 15], which showed that elevations in testosterone and AMH levels, accompanied by reduced estradiol levels, were the primary endocrine changes observed in women with PCOS. Women with PCOS who are VDD or insufficient might greatly improve their hormonal, metabolic, and androgen-related abnormalities with just VD supplements (50.000 IU/week) [16]. A review reported that VDD is correlated with the onset and symptoms of PCOS, including calcium dysregulation and follicular arrest, which may contribute to menstrual irregularities and fertility issues. These metabolic disturbances have also been linked to vitamin D receptor (VDR) polymorphisms, and vitamin D is directly associated with insulin resistance, which is one of the hallmark features of the PCOS phenotype[17]. A study by Pergialiotis and his colleagues found no significant impact of vitamin D on the hormonal profile of women with PCOS, indicating its effect as minimal [18]. The current findings were consistent with those of Figurova *et al.,* [19]. Furthermore, a study by Kharb *et al.,* demonstrated that vitamin D levels in the patient and control groups were not statistically significant [20]. Also, Lejman-Larysz *et al*., obtained similar results [21]. Another study in 2025 shows a significant decrease ($p \leq 0.001$) in the mean vitamin D level in the sera of women with PCOS compared to the control [22].

In the study, the mean levels of IL-22 were significantly ($p < 0.01$) decreased in PCOS patients compared with healthy subjects. There are conflicting facts on the impacts of IL-22 in PCOS. A research by Aksun and his colleagues did not reveal a significant difference in the level of IL-22 between PCOS patients and healthy individuals [23]. Otherwise, Zhou *et al.,* found that women with PCOS had lower IL-22 levels [24]. These results agree with the current study. Besides, two independent groups observed reduced IL-22 secretion in both PCOS patients and PCOS-like murine models. This result could potentially guide the use of IL-22 therapy in both experimental studies and clinical trials related to PCOS [25]. One study has reported that IL-22 may improve PCOS by enhancing insulin resistance and systemic inflammation [26]. According to an earlier study, giving IL-22 to several PCOS animals reduced insulin resistance, thereby enhancing ovarian function [27]. While exhibiting research, Vasyukova *et al.,* reported that IL-22 levels were elevated in women with PCOS, whether they were thin or overweight, in comparison to the control group [28]. Serum IL-22 levels showed a strong negative relationship with parts of Metabolic Syndrome(MetS), inflammation markers, and reproductive characteristics of PCOS. This underscores the important role of IL-22 in diagnosing metabolic dysfunction associated with PCOS, which may facilitate the prevention and treatment of PCOS and its related metabolic disorders [29].

The levels of IL-17A and IL-34 in the blood of women with PCOS and healthy controls were not statistically significant. While the study by Hadi Khorsheed *et al.,* found that IL-17A levels were elevated in PCOS patients compared with healthy controls [30]. Also, Zhou *et al.,* obtained similar results [24]. Elevated IL-17A levels in PCOS women may indicate an inflammatory response within the ovaries. This cytokine is a vital component of the body's immune response, and overproduction of IL-17 may exacerbate inflammatory responses, contributing to tissue damage [31]. Inflammatory responses in PCOS, particularly elevated IL-17A, are associated with complications, such as insulin resistance and metabolic disorders[32]. Cai *et al.,* study revealed significant findings regarding IL-34. IL-34 levels were significantly higher in PCOS women compared to normal women. These high levels were associated with other cytokines (IL-6, TNF-α, and IL-1β), HOMA-IR, triglycerides, and LDL-C [11]. A crucial aspect of PCOS is the implication of the immune system in its pathophysiology. Hyperandrogenism affects different types of immune cells in several ways, but hyperandrogenism affects different types of immune cells in several ways, but commonly, extreme androgen levels stimulate some types of immune cells, like neutrophils, while others, such as dendritic cells, are suppressed by hyperandrogenism. These immune cells subsequently generate a diverse array of cytokines that influence several characteristics of PCOS [33]. Prior research has also demonstrated that hyperandrogenism in PCOS affects immune cells through several pathways, with a primary function in PCOS being the generation of cytokines such as IL-22, IL-6, and TNF-α. These cytokines have negative impacts on many physiological systems and participate in many of the clinical symptoms of PCOS, including obesity, insulin resistance, infertility, and hypertension [34]. The majority of women with PCOS exhibited clinical hyperandrogenism, which included alopecia and hirsutism [35].

5.Conclusion

The current study found a significant increase in both levels of testosterone and AMH, whereas estradiol had a significant decrease in PCOS patients. The levels of vitamin D, IL-17A, and IL-34 did not exhibit statistically significant differences between patients and healthy controls. Individuals with PCOS simultaneously showed a significant decrease in the serological levels of IL-22. A decrease in IL-22 may indicate an improvement in the condition and reduced inflammation associated with PCOS. Therefore, it has the potential to serve as a

therapeutic target in the future. Further studies are necessary to confirm these findings and elucidate the specific role of IL-22 in PCOS. However, the study encountered an important limitation, namely, the sample size of PCOS. Although PCOS has been a well-known disease for a long time, its causes and treatment are still unknown.

6. Disclosure and conflict of interest

There is no conflict of interest

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