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Research Article

TNF-alpha and IL-10 Levels in Iraqi PCOS and Non-PCOS Patients Undergoing ICSI: An Immunological Perspective

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Abstract

Background: Essential cytokines like tumor necrosis factor-alpha (TNF-α) and interleukin-10 (IL-10) play a critical role in immune regulation, particularly in conditions like polycystic ovary syndrome (PCOS). ***Objective***: To compare TNF-α and IL-10 levels in patients with and without PCOS undergoing intracytoplasmic sperm injection (ICSI) and their effects on ICSI. outcome ***Methods***: This study involved a cohort of 125 patients undergoing ICSI, divided into two groups: those identified with PCOS according to Rotterdam criteria (n=65) and those without PCOS (n=60). The ELIZA method was used to measure the levels of TNF-α and IL-10 in the blood in order to see if they were related to PCOS and to look into a possible connection between these cytokines and the outcome of the ICSI. ***Results***: Significant differences were found in the serum levels of TNF-α and IL-10 between the two groups, suggesting a distinct immunological profile in PCOS patients undergoing fertility treatment. There is no significant correlation between these cytokines and the total number of retrieved oocytes, oocyte maturity, fertilization rate, or pregnancy rate. ***Conclusions***: The study revealed notable differences in serum TNF-α and IL-10 levels between PCOS and non-PCOS groups, suggesting a unique immunological profile in PCOS patients undergoing fertility treatments. Both cytokines did not significantly correlate with the total number of retrieved oocytes, oocyte maturity, fertilization rate, or pregnancy rate. Other factors might be more influential in determining crucial fertility outcomes.

Keywords: Cytokines, ICSI outcome, PCOS, IL-10, Non-PCOS, TNF-α.

مستويات TNF-α و IL-10 لدى العراقيات المصابات وغير المصابات بمتلازمة تكيس المبايض في العراقيات اللواتي يخضعن للحقن المجهري: منظور مناعي

الخلاصة

الخلفية: متلازمة تكيس المبايض هي حالة الغدد الصماء المعقدة التي تؤثر على الخصوبة. تلعب السيتوكينات الأساسية مثل عامل نخر الورم ألفا والإنترلوكين-10 دورًا حاسمًا في تنظيم المناعة، خاصة في حالات مثل متلازمة تكيس المبايض. **الهدف**: تقييم ومقارنة مستويات عامل نخر الورم ألفا والإنترلوكين-10 في المريضات المصابات وغير المصابات بمتلازمة تكيس المبايض ويخضعن للحقن المجهري وتأثيراتها على نتائجه. **الطرق**: شملت هذه الدراسة مجموعة مكونة من 125 مريضة يخضعن للحقن المجهري، مقسمين إلى مجموعتين: مجموعة الذين تم تشخيص إصابتهم بمتلازمة تكيس المبايض وفقاً لمعايير روتردام (العدد = 65) واللواتي لا يعانين من متلازمة تكيس المبايض (العدد = 60). تم قياس مستويات مصل عامل نخر الورم ألفا والإنترلوكين-10 وتحليلها لتقييم ارتباطها بحالة متلازمة تكيس المبايض علاوة على ذلك، دراسة العلاقة المحتملة بين هذه السيتوكينات ونتائج الحقن المجهري. **النتائج**: تم العثور على اختلافات كبيرة في مستويات عامل نخر الورم ألفا والإنترلوكين-10 في مصل الدم بين مجموعات متلازمة تكيس المبايض والمجموعات غير المصابة بمتلازمة تكيس المبايض، مما يشير إلى وجود صورة مناعية متميزة لدى مرضى متلازمة تكيس المبايض الذين يخضعون لعلاج الخصوبة. لا يوجد ارتباط كبير بين هذه السيتوكينات والعدد الإجمالي للبويضات المسترجعة، ونضج البويضات، ومعدل الإخصاب، ومعدل الحمل. **الاستنتاج**: توجد اختلافات ملحوظة في مستويات في المصل بين مجموعات متلازمة تكيس المبايض والمجموعات غير المصابة بمتلازمة تكيس المبايض مما يشير إلى ملف مناعي فريد لدى مرضى متلازمة تكيس المبايض الذين يخضعون لعلاجات الخصوبة. ومع ذلك، لم ترتبط هذه السيتوكينات بشكل كبير بالعدد الإجمالي للبويضات المستردة، أو نضج البويضات، أو معدل الإخصاب، أو معدل الحمل. ويشير هذا إلى أن العوامل الأخرى قد تكون أكثر تأثيرا في تحديد نتائج الخصوبة الحاسمة.

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INTRODUCTION

PCOS is a prevalent endocrine disorder, affecting approximately 10% of women of reproductive age [1]. It is characterized by a spectrum of symptoms, including menstrual irregularities, hyperandrogenism, and polycystic ovaries. Beyond these, PCOS is increasingly being recognized for its systemic consequences, mainly metabolic and immunological dysfunctions. Women with PCOS often have many challenges, and one of the main ones is infertility [2]. They usually require assisted reproductive technologies (ART), such as intracytoplasmic sperm injection (ICSI), for conception. The interplay between immune dysregulation and PCOS is a rapidly developing area of interest. Chronic low-grade inflammation, a hallmark of PCOS, is hypothesized to contribute significantly to its pathophysiology. High levels of pro-inflammatory cytokines create this inflammatory environment, which not only makes the endocrine and metabolic problems worse but may also affect the results of fertility treatments [3]. Elevated levels of TNF-α have been associated with insulin resistance, which is predominant in PCOS. It is also implicated in ovarian dysfunction, affecting folliculogenesis and steroidogenesis [3]. TNF-α is a potent cytokine secreted by TH1 (T helper 1) cells with a wide range of effects on various cell types. Accumulating evidence supports its crucial role in early pregnancy, influencing steroid hormone production, embryonic and follicle development, uterine cyclicity, and placental structure [4]. However, it's also well established that excessive TNF-α can be detrimental to pregnancy. TNF-α speeds up the death of trophoblast cells in the placenta, stops the development of fetuses in mice, and limits the growth of human trophoblast cells in the lab [5]. When administered to pregnant mice, TNF-α can lead to abortions, while anti-TNF-α antibodies have been shown to lower resorption rates in a murine model of spontaneous, immunologically mediated miscarriages [6]. On the other hand, IL-10 is an anti-inflammatory cytokine that TH2 (T helper 2) cells produce and is well known for its function in reducing immune responses and inflammation [7]. IL-10 helps maintain immune homeostasis, and its levels are crucial in balancing the inflammatory state in various conditions, including PCOS. Some studies have reported a decrease in a subset of IL-10-producing B cells in patients with recurrent pregnancy loss (RPL) [8]. However, it's worth noting that the involvement of these cells in the pathogenesis of recurrent implantation failure (RIF) remains unclear. Given that common immunological changes have been investigated in both RPL and RIF pathogenesis, it's been suggested that IL-10 might be reduced in RIF patients. This idea comes from the thought that IL-10 might help stop the production of autoantibodies and that the balance between Th1 and Th2 cytokines is important for multiple pregnancies [9]. This study was designed to evaluate the serum levels of TNF-α and IL-10 in PCOS and non-PCOS patients undergoing ICSI, providing an immunological perspective on this condition. By comparing these cytokine levels between the two groups, the study seeks to unravel the immunological details that might influence fertility treatment outcomes and the success rates of ART in women with PCOS.

METHODS

Study design and Patients selection

A prospective comparative study, conducted at the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies consultation clinic, Al-Nahrain University, Baghdad, Iraq. The study spanned from December 2021 to April 2023. All participants, aged between 18 and 39 years, underwent the ICSI program. The calculation of the sample size was based on the formula:

$N = p\ (1\text{-}p)\ z2/me2$

where p represents the prevalence rate of infertility in females, set at 12% based on findings from a previous study [10]. The Z-value was 1.96 for a 95% confidence interval, and the margin of error (me) was 0.05. This calculation resulted in a sample size of 162. However, out of these 162 female patients, 31 declined to participate, and 6 were excluded due to their use of corticosteroids, which could impact immune function and potentially alter the cytokine profiles central to the study. In total, 125 women were involved in the study: 65 women with PCOS and 60 women without PCOS. The PCOS diagnosis was based on the Rotterdam criteria, requiring the presence of at least two of the following: oligo- or anovulation, clinical or biochemical signs of hyperandrogenism, and polycystic ovaries on ultrasound. The non-PCOS group consisted of women with normal menstrual cycles and no clinical signs of hyperandrogenism or polycystic ovaries. All participants provided informed consent, and the Institutional Review Board approved the study.

Inclusion Criteria

The selection of participants was based on the following criteria: PCOS and non PCOS women undergoing ICSI treatment using the antagonist protocol. Women aged between 18 and 39 years.

Exclusion Criteria

Females undergoing pituitary downregulation with GnRH agonists, patients with poor ovarian reserve, known cases of endometriosis, autoimmune diseases or endocrine disorders such as diabetes mellitus, hyperthyroidism, congenital adrenal hyperplasia, and hyperprolactinemia were excluded. These diseases cause widespread inflammation and alter cytokine profiles, which could interfere with the study's assessment of TNF-α and IL-10 levels. Additionally, females with a history of ovarian surgery, radiation or chemotherapy exposure, as well as female participants whose male partners had severe oligo-asthenoteratozoospermia or azoospermia with surgically obtained frozen sperm, were also not included.

Assessment of the participants

All the enrolled women in this study underwent a detailed assessment of their medical history, physical examination and gynecological evaluation. Additionally, hormonal profiling included the measurement of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), anti-Müllerian hormone (AMH), thyroid-stimulating hormone (TSH), and prolactin. The Body Mass Index (BMI) was calculated using the established formula: BMI = weight in kilograms / height in meters squared. Transvaginal ultrasound was used to check the ovarian reserve, mostly by counting the number of small follicles (2–6 mm in diameter) in both ovaries. It was also used to check the endometrial thickness (ET) and see if there were any problems with the endometrium. These assessments were conducted on the second day of the menstrual cycle. The study also included a follow-up phase focusing on the assessment of oocytes, fertilization outcomes, the grading of embryos, and the rate of pregnancy accomplishment.

Sample collection and measurements

Approximately 6 ml of blood was collected from each participant via venipuncture. Following collection, the blood was allowed to clot at room temperature and was then centrifuged at 3000 rpm for 15 minutes to separate the serum. The serum was immediately aliquoted and stored at -20°C until analysis. We used the enzyme-linked immunosorbent assay (ELISA) kits from YL Biont Shanghai to measure the levels of TNF-α and IL-10 in the blood, following the manufacturer's instructions. A microplate reader was used to read the ELISA plates at 450 nm, and standard curves made with known amounts of serum TNF-α and IL-10 were used to figure out the cytokine concentrations.

Ovarian stimulation and oocyte and embryo quality assessment

The male partners underwent seminal fluid analysis as per the World Health Organization (WHO) 2010 guidelines. For controlled ovarian stimulation, recombinant FSH (r-FSH) gonadotropins, Gonal-F from Merck, were administered at dosages varying between 75 and 300 IU. Once follicular recruitment began and a sufficient number of follicles reached 14 mm in size, pituitary down-regulation was initiated using a GnRH antagonist (Cetrotide 0.25 mg) once daily. Ovulation was induced using 500 micrograms of recombinant human chorionic gonadotropin (r-hCG; Ovitrelle®). The oocyte collection was conducted 34–36 hours post-ovulation under general anesthesia. This process involved a transvaginal ultrasound-guided follicular puncture utilizing a 17-gauge double-lumen needle connected to a Cook® suction pump. The maturity and quality of oocytes were microscopically assessed, followed by ICSI, where a single sperm was injected into each oocyte using a fresh semen sample prepared by the swim-up technique. Oocyte and embryo quality was evaluated using specific grading systems [11,12], and the fertilization rate was calculated as the number of fertilized oocytes divided by the total number of injected mature oocytes multiplied by 100% [13]. Two to three good-quality embryos were transferred to the uterus using an embryo transfer catheter. Luteal phase support was provided with vaginal progesterone suppositories (Cyclogest 200 µg three times daily) and injectable progesterone (Primolute Depot® 250 µg every third day), starting from the evening of oocyte retrieval until the pregnancy test. The pregnancy test, measuring β-hCG in the serum, was conducted 14 days after embryo transfer, with pregnancy rates calculated based on the proportion of women with positive tests relative to the total number of women who underwent embryo transfer. The treatment protocol for each patient was comprehensively documented to ensure uniformity and accuracy in treatment administration and data collection.

Statistical Analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS), while Microsoft Office Professional Plus 2021 was employed for writing purposes. Descriptive statistics (mean ± standard deviation) were calculated for all variables. Depending on how the data was distributed, an independent *t*-test was used to compare the levels of TNF-α and IL-10 between the PCOS group and the non-PCOS group. The cut-off value, sensitivity, and specificity were determined using the receiver operating characteristic (ROC) curve. Pearson's correlation coefficient (r) was employed to assess the association between continuous variables. A *p*-value less than 0.05 was considered statistically significant.

RESULTS

Table 1 compares the demographic and hormonal profiles of the PCOS and non-PCOS groups. Age and BMI, the duration of infertility, FSH, TSH, prolactin and day two estradiol levels did not significantly differ between the two groups.

Table 1: demographic and Hormonal Profile comparison between PCOS and non PCOS patients

Parameter	Non-PCOS (n=60)	PCOS (n=65)	*p*-value
Age (year)	29.93±5.53	30.17±5.17	0.806
BMI (kg/m²)	28.66±5.9	29.22±5.11	0.571
Infertility Duration (year)	7.02±4.09	7.74±4.21	0.332
FSH (mIU/mL)	5.97±1.84	5.52±1.58	0.076
LH (mIU/mL)	3.94±1.43	5.13±1.88	<0.001
Day2 E2 (pg/mL)	34.91±9.47	36.31±10.42	0.432
Prolactin (mIU/mL)	21.22±2.82	20.75±4.54	0.061
AMH (pg/ml)	1.99±0.42	3.81±1.20	<0.001
TSH (mIU/mL)	2.64±0.82	2.62±0.83	0.910

Values were expressed as mean± SD. BMI: body mass index; PCOS; polycystic ovary syndrome; FSH: follicle stimulating hormone; LH: luteinizing hormone; E2: Estradiol hormone; AMH: anti mullerian hormone; TSH: Thyroid stimulating hormone.

However, LH levels were significantly higher in the PCOS group compared to the non-PCOS group ($p<0.001$), which is consistent with the typical endocrine profile observed in PCOS. AMH levels were also significantly higher in the PCOS group ($p<0.001$). As seen in Table 2, the levels of serum TNF-α and IL-10 showed notable differences

between the two groups. Serum TNF-α levels were significantly higher in the PCOS group than in non-PCOS patients (p<0.001).

Table 2: Comparison of serum TNF-α and IL-10 levels between the PCOS and non-PCOS groups

Parameters	Non-PCOS (n=60)	PCOS (n=65)	*p-value*
S. TNF-α (ng/L)	57.46±12.00	75.99±15.99	<0.001
S. IL-10 (ng/L)	85.29±13.43	78.44±21.15	0.034

Values were expressed as mean±SD. PCOS: polycystic ovary syndrome, S.TNF-α: serum tumor necrotic factor-alpha; IL-10: interleukin-10.

Conversely, serum IL-10 levels, an anti-inflammatory cytokine, were significantly higher in the non-PCOS group compared to the PCOS group, with a p value of 0.034. Figures 1 and 2 illustrate how the Receiver Operating Characteristic (ROC) analysis conducted in this study assesses the diagnostic capabilities of serum tumor necrosis factor-alpha (TNF-α) and Interleukin-10 (IL-10) levels in distinguishing between patients with PCOS and non-PCOS.

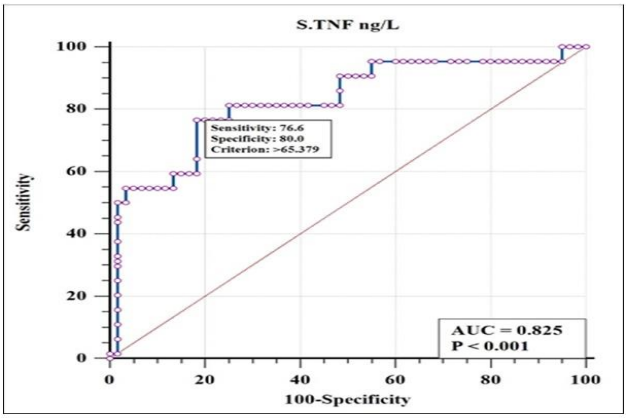

Figure 1: Receiver Operating Characteristic (ROC) Analysis of Serum TNF-α in Differentiating PCOS from Non-PCOS.

This analysis is crucial in determining the effectiveness of these cytokines as biomarkers in the context of PCOS. Twenty-two women were excluded from the pregnancy rate calculations due to the onset of ovarian hyperstimulation syndrome (OHSS).

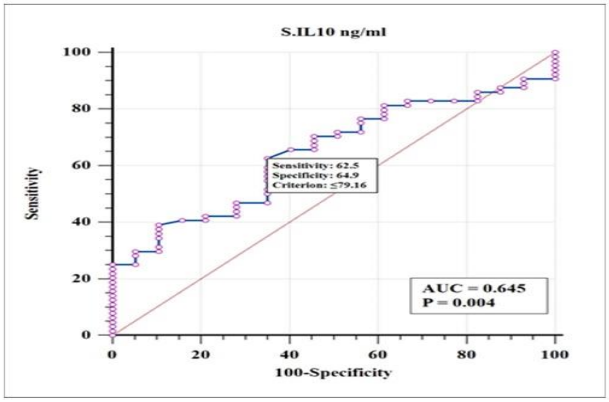

Figure 2: Receiver Operating Characteristic (ROC) Analysis of Serum IL-10 in Differentiating PCOS from Non-PCOS.

This condition necessitated the freezing of all embryos and the subsequent cancellation of fresh embryo transfers. Table 3 showed the pregnancy rate of the patients involved in this study.

Table 3: Pregnancy rate of patients involved in the study (n=103)

Pregnancy	n(%)
Positive	36(34.95)
Negative	67(65.048)
Total	103(100)

Table 4 demonstrates generally weak associations between cytokine levels and the parameters. It was observed that both S. TNF-α and S. IL-10 exhibited a non-significant correlation with the number of oocytes retrieved, the mature (MII) oocyte count, FR%, GI embryo, and pregnancy rate.

Table 4: Correlation between the serum inflammatory cytokines with ICSI outcome in PCOS and non PCOS patient (n=125)

Parameters	S. TNF-α		S. IL-10	
	r	*p*	*r*	*p*
Total Oocyte	-0.03	0.232	0.05	0.228
MII oocyte	-0.10	0.270	0.01	0.919
FR%	-0.06	0.257	0.2	0.09
No. of Grade I embryo	0.12	0.184	0.10	0.295
Pregnancy rate	0.050	0.572	0.038	0.603

PCOS: polycystic ovary syndrome, S.TNF-α: serum tumor necrotic factor, IL-10: interleukin-10, MII: metaphase 2, FR: fertilization rate, GI: grade I, *r*: Pearson's correlation.

DISCUSSION

The exploration of inflammatory markers, such as TNF-α and IL-10, provides a window into the complex interplay between the immune system and reproductive health. These cytokines, essential components of the body's immune response, play crucial roles in modulating inflammation, a factor increasingly recognized for its impact on various aspects of reproductive physiology [14]. The goal of this study is to understand our results in relation to other research, highlighting how immune system factors might affect fertility outcomes and treatment for women undergoing ICSI. Significantly, this study found elevated levels of TNF-α in the serum of women with PCOS. These findings are in agreement with many studies that revealed significantly higher TNF levels in PCOS patients [15,16]; however, other studies conducted by Escobar-Morreale *et al*. did not identify a significant difference in TNF-α levels between PCOS women and non-PCOS groups [17]. The presence of elevated TNF-α levels in women with PCOS emphasizes a substantial inflammatory environment of the syndrome, potentially exacerbating insulin resistance and metabolic syndromes, increasing androgen production, and contributing to PCOS symptoms like menstrual irregularities and fertility issues [18]. Additionally, elevated TNF-levels in PCOS raise concerns about the possibility of increased cardiovascular risks in affected individuals. Chronic inflammation is a well-established risk

factor for cardiovascular diseases, and its presence in PCOS emphasizes the need for a comprehensive approach to address the inflammatory component of the syndrome [18,19]. Contrary to this, the findings of the study showed lower IL-10 levels in the PCOS group, supporting the hypothesis of an imbalanced immune regulation in PCOS. This is in line with the findings of Pedro-Regidor *et al*., who reported reduced anti-inflammatory cytokines in PCOS, implying a tilt towards a pro-inflammatory state [20]. However, a study by Velez DR et al. indicated no significant difference in IL-10 levels between PCOS and non-PCOS women, suggesting the possibility of variability in immune responses based on ethnicity or environmental factors [21]. The lower levels of IL-10 in the PCOS group indicate a potential disruption in the anti-inflammatory pathways. This could contribute to the chronic inflammatory state seen in PCOS and might influence the effectiveness of treatments like ICSI. No significant correlation was found between TNF-α and IL-10 levels and oocyte number, maturation, and fertilization rate. The findings align with research suggesting that the roles of TNF-α and IL-10 in fertility are more intricate and perhaps less influential on specific aspects like oocyte quality [22–24]. Studies support this perspective, indicating that other factors might play a more pivotal role in determining reproductive success. These factors could include genetic polymorphisms affecting cytokine expression and response, environmental and lifestyle influences on overall reproductive health, and metabolic conditions that alter hormonal and follicular environments [25–27]. In contrast, some studies revealed a significant link between these cytokines and oocyte maturation [28–4]. In the same manner, no significant link was demonstrated between TNF-α and IL-10 and the G1 embryo and pregnancy rate. Parallel results were revealed in studies that exhibited a negative correlation between TNF-α and IL-10 levels and embryo quality and pregnancy rate [29–31], while others showed a significant correlation [32].

Limitations of the study

The fact that this study only used a single center and had a small sample size limited its scope. Additionally, the evaluation of oocyte and embryo quality was based on indirect methods, primarily microscopic assessments reliant on visual inspection using an inverted microscope. Consequently, this approach did not allow for precise estimation of the genetic, biochemical, and molecular characteristics of the oocytes and embryos.

Conclusion

This study identified notably higher TNF-α and lower IL-10 levels in individuals with PCOS compared to those without, highlighting a distinct immunological profile in PCOS patients. However, the investigation did not find a significant correlation between TNF-α and IL-10 levels and the outcomes of ICSI. These findings suggest that, while TNF-α and IL-10 are significant markers in the context of PCOS, their direct impact on the success of ICSI treatments might be limited.

Conflict of interests

No conflict of interests was declared by the authors.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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