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Serum level evaluation of CRP, IL- 22 and IL-35 in women with polycystic ovary syndrome

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SUMMARY

AUTHORS' CONTRIBUTION: (A) Study Design · **(B)** Data Collection. **(C)** Statistical Analysis · **(D)** Data Interpretation · **(E)** Manuscript Preparation · **(F)** Literature Search · **(G)** Funds Collection

Background: Polycystic Ovarian Syndrome (PCOS) is the most well-known endocrine condition among women, with 18- 27% prevalence. PCOS is a pro-inflammatory state, certain cytokines are correlated with women who have PCOS.

Objective: The study aims to estimate the impact of some inflammatory mediators like CRP and interleukin 22 (IL-22) and interleukin 35 (IL-35) on PCOS.

Methods: Eighty women, ranging in age from nineteen to forty, were part of the study; thirty of these women had polycystic ovarian syndrome, and thirty of these women had delayed childbearing. The other twenty women were healthy. We analyzed the CRP, IL-35, and IL-22 levels, after separating the serum by the ELISA method.

Results: The results showed a decrease in CRP, IL-22 and IL-35 in patients with PCOS and in delayed childbirth women. Conclusion: In PCOS, we notice an imbalance in inflammatory indicators such as CRP, IL-22 and IL-35, and in women who have delayed childbearing.

Keywords: PCOS; CRP; IL-22; IL-35; Cytokines; ELISA

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INTRODUCTION

Polycystic Ovarian Syndrome (PCOS) is the most well-known endocrine condition among women of this generation with a prevalence 18- 27% [1]. Nevertheless, this complicated and diverse endocrinopathy's aetiology is still a mystery. In addition to insulin resistance and irregular menstrual cycles, polycystic ovary syndrome is characterised by hyperandrogenism. The risk of complications such as infertility, insulin resistance, and type 2 diabetes is higher in women who have Polycystic Ovary Syndrome (PCOS) [2].

A pro-inflammatory state is PCOS. This condition of persistent low-grade inflammation may be associated with the onset of metabolic problems and ovarian dysfunction. On top of that, research has linked PCOS to certain cytokines in females [3].

A variety of immune cells release cytokines, which are signaling protein molecules that influence the activity of other cells. Because leukocytes, oocytes, and follicular cells release these molecules in the ovary, cytokine levels are altered in women with polycystic ovarian syndrome. Next, we will look at the paracrine and autocrine pathways by which cytokines regulate ovarian function. Inflammatory indicators and polycystic ovary syndrome (PCOS) has been the subject of few investigations, leaving much to speculation. Understanding polycystic ovary syndrome (PCOS) may so benefit from investigating the role of cytokines in this endocrine condition [4].

One acute-phase protein that the liver produces in response to inflammation-promoting chemicals such as TNF-α and IL-6 (cytokines) is CRP, or C-reactive protein. Inflammation prognosis is most strongly predicted by it. In response to tissue injury or inflammation, the body secretes C-Reactive Protein (CRP) into the circulation [5].

Though mostly secreted by subsets of T cells and group 3 Innate Lymphoid Cells (ILC3s), the cytokine Interleukin-22 (IL-22) is generated by both adaptive and innate immune cells and targets epithelial cells [6]. For the purpose of maintaining mucosal homeostasis and immunity, it serves as a recursive connection. Improving insulin sensitivity, preserving the gut mucosal barrier and endocrine functioning, decreasing endotoxemia and chronic inflammation, and regulating lipid metabolism in liver and adipose tissues are just a few of the metabolic benefits that IL-22 has been shown. Prior research indicated that insulin-like growth factor 22 (IL-22) levels dropped in PCOS patients' blood and follicular fluid, and that IL-22 treatment might alleviate insulin resistance, normalize ovarian function, and end the irregular menstrual cycle [7].

Interleukin-35 (IL-35) a heterodimeric cytokine, is the

newest identified member of interlokin-12 cytokine family, this family mediate diverse immunological functions [8,9]. IL-35 is produced by monocytes, smooth muscle cells, Endothelial Cells (ECs), dendritic cells, and regulatory (T and B) cells, IL-35 plays a powerful role in inflammatory suppression and immunomodulatory functions [10]. IL-35 is anti-inflammatory cytokine (immune-suppressive), it decreases autoimmune disease and mediate many immune pathways [11]. Our research area in this study is to find the relationship between interleukins and their effect on PCOS.

METHODS

The current study conducted during the period from November 2022 to March 2023, involved eighty women at the age of 19-42 years. These cases were selected from The Kamal Al Samarrai Hospital /Baghdad, Iraq. The participant women are divided into three groups, first group 30 women with polycystic ovary syndrome (PCOS), Second group 30 women with delayed childbirth, third group 20 healthy women as control with regular menstrual cycle and normal level of hormones test. Women with PCOS have been checked medically by ultrasound waves to confirm that they have PCOS by radiologist. The study was approved by the ethics committee of Baghdad University, carried out at the Department of Biotechnology, College of Science, Baghdad.

Collecting blood sample: About 5 ml of venous blood was withdrawn by a medical syringe from participant women (patients and controls). The withdrawn blood sample put in gel tubes after a while, they were placed in a centrifuge at 3000 rpm for 10 minutes to separate the serum to be frozen in multiple Eppendorf tubes to avoid multiple thawing.

Laboratory methods: The serum level of CRP, IL-22, and IL-35 was determined by using Enzyme Linked Immunosorbent Assay (ELISA) by follow the instructions of manufacturer for the kits (biont, China). Body mass index calculated by this equation (BMI = weight (kg)/ length square (m^2)).

Statistical analysis

Mean ± Standard Division (SD), ANOVA Table (Duncan test), Pearson's correlation was calculating for parametric data. Median, 25%-75% percentile, Kruskal-Wallis, Sperman's correlation was calculating for non-parametric data by using Statistical program: IBM SPSS V27.0.

RESULTS

The Median (25% - 75% percentile) age of the PCOS group was 26.0 (22.0-29.25) years and 30.0 (25.75-36.0) years in women with delayed childbirth compared with 27.0 (21.75-33.25) years in the control group as shown in **Tab. 1.** The mean ± SE body mass index (BMI) in the PCOS group, 30.28 ± 0.82 (kg/m^2) was significantly higher ($p<0.05$) than that in women with Delayed childbirth 26.94 ± 1.31(kg/m^2) and control group 26.19 ± 1.08 (kg/m^2) as shown in **Tab. 1.**

CRP serum level

The result showed decrease in C-reactive protein CRP but not significant in PCOS compared with control, women with delayed childbirth it showed the lowest result between the study groups as showed in **Tab. 2.**

IL-22 serum level

The result of IL-22 showed the lowest level in delayed childbirth women, and few in PCOS patient women but not significant ($p>0.05$), in compared with control group as explained in **Tab. 3.**

IL-35 serum level

The result of IL-35 showed significant decrease ($p<0.05$) in PCOS women in compared with healthy women, the results of delayed childbirth women as will be showed in **Tab. 4.**

Tab. 1. Demographic characteristics of study groups.

Parameters	PCOS	Delayed childbirth	Controls
Age (Years) Median (25% - 75% percentile)	26 (22.0-29.25)	30 (25.75-36.0)	27 (21.75-33.25)
BMI (kg/m^2) Mean ± SE	30.28 ± 0.82	26.94 ± 1.31	26.19 ± 1.08

Tab. 2. CRP serum level in studied groups.

Parameter	Mean ± SE		
	PCOS	Delayed childbirth	Control
CRP (mg/dl)	0.40 ± 0.05	0.29 ± 0.05	0.48 ± 0.09

Tab. 3. Interleukin-22 level in groups.

Parameter	Mean ± SE		
	PCOS	Delayed childbirth	Control
IL-22 (pg/ml)	72.61 ± 11.84	58.97 ± 10.75	80.80 ± 22.41

Tab. 4. Interleukin-35 level in groups.

Parameter	Median (25% - 75% percentile)		
	PCOS	Delayed childbirth	Control
IL-35 (pg/ml)	0.31 (0.24–0.35)	0.35 (0.25–0.36)	0.36 (0.33–0.41)

DISCUSSION

In this study 30 PCOS women were involved, the median age was 26.0 years (range 22.0 - 29.25), which close with Baracat, et al. 25.0 years (range 21.0-29.0) [12]. Lower than results obtained by Monika Sarkar, et al. 35 years [13], and M Jacewicz, et al 35 years [14] and Katarzyna, et al 27.0 years [15]. PCOS appears and is more common in women in the reproductive age, so with age reproductive disorders decrease (like anovulation and infertility) and turn into metabolic problems (such as insulin resistance and type 2 diabetes). These changes in the syndrome occur because of follicle loss and PCOM disappearance [16].

In this current study, PCOS women participants recorded a high level of BMI 30.28 ± 0.82. This result disagreed with Ashraf Ganie, et al. [17] and Ramezani, et al. [18] who they reported slightly difference between patient and control groups. But this result agreed with Alsamarai, et al. [19] who reported same result with PCOS women that have a high level of BMI than control. Obese females are more susceptible to PCOS. Globally 38-88% of females are either obese or overweight. Having a BMI>or = 30 Kg/m^2 is termed as obesity. Hyperandrogenism have close association with PCOS women that influence deposition of fats in adipose tissue and in turn cause obesity in affected women especially in abdominal area [20].

In the present study low level of CRP was encountered in PCOS patient, and lowest in women suffer from delayed childbirth. These results disagreed with Rudnicka, et al. [21] and Dabravolski, et al. [22] and Zhai and Pang [23] who they report in their studies that CRP was elevated in PCOS women than in without PCOS women. But these results agreed with Kim, et al. [24] who showed in their result decrease in CRP level compared to healthy participant. Soares and partners also showed in their results no difference in CRP level between study groups [25].

Studies have shown that women with PCOS when they take drugs to reduce the symptoms of the syndrome and reduce its impact, their CRP level decrease [22,26].

The current study found decrease in IL-22 serum level in PCOS women (72.61 ± 11.84 pg/ml) and lowest in delayed childbirth women (58.97 ± 10.75 pg/ml) compared with control group (80.80 ± 22.41 pg/ml) ($p>0.05$). Our results compatible with the study of Qi, et al. [7] that also have decrease in IL-22 in PCOS women. This results incompatibles with Seren, et al. that indicated no difference in IL-22 level between PCOS and healthy at baseline of study [27]. Patients with polycystic ovary syndrome may have a number of metabolic disorders that impact several metabolic pathways. The lipids, carbs, amino acids, steroid hormones, purines, bile acid production, citric acid cycle and steroid hormone metabolism are particularly disrupted in this condition [28]. So, because IL-22 is produced by intestinal group 3 Innate Lymphoid Cells (ILC3), its affect by these metabolic substances [6]. Qi X, et al. founds the therapeutic effect of IL-22 on PCO syndrome, so they indicate the valid treatment of IL-22 on PCOS patients [8].

In this study the results showed significant decrease level of IL-35 in PCOS patient 0.31 (0.24–0.35) pg/ml, in delayed childbirth 0.35 (0.25-0.36) pg/ml, and in healthy women 0.36 (0.33-0.41) pg/ml ($p<0.05$). Our study compatible with Nehir, et al. [29], in addition to Zhai and Pang [23], where found decrease level of anti-inflammatory cytokines in general among them IL-35, PCOS patients show low levels of anti-inflammatory cytokines. Low level of anti-inflammatory can cause immunological infertility, when they elevated pregnancy can happened and that showed in study on Iraqi PCOS women by Hantoosh, et al. [30].

CONCLUSION

The current findings showed decrease in CRP and IL-22, in addition to IL-35 in PCOS patient and delayed childbirth women. These inflammatory markers have correlation with infertility and symptoms of the syndrome. Research and further clarification is important to know more about this syndrome and impact on the immune aspect.

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