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The role of CTLA-4, epiregulin and PD-1 in women with polycystic ovarian syndrome

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Abstract

Polycystic ovarian syndrome (PCOS) is a hormonal, reproductive, metabolic disorder, affect female at reproductive age. PCOS is associated with hormonal and immunological defect, and chronic low-grade inflammation, that affect some proteins expression, like cytotoxic T Lymphocyte Associate protein 4 (CTLA-4), programmed death-1 (PD-1), epiregulin (EREG) in association with T-cell activation. These immunological parameters may indicate autoimmune diseases and PCOS. CTLA-4 and PD-1 are immune checkpoints that regulate the immunity during the chronic inflammation, by suppressing T-cells activation, natural killer cell, B-cell production. Epiregulin, a member of the epidermal growth factor (EGF) family, is released in response to luteinizing hormone(LH) released from granulosa cells in ovaries. There is an indirect link between PCOS and epiregulin in chronic low-grade inflammation. The purpose of this study was to indicate the role of CTLA-4, epiregulin, and PD-1 in women with polycystic ovarian syndrome, also the estimation of some related hormones level in PCOS, like prolactin, testosterone anti-Mullerian hormone (AMH). This study included 65 patients with PCOS and 56 normal controls (healthy women). Their average ages were from 20 to 45 years. The study subjects were from the private women's infertility clinics at the Medical City Hospital, Baghdad. The study lasted from November 2024 to January 2025. Metformin, vitamin D, and oral contraceptives were prescribed by consultant to patients. The blood samples were collected and CTLA-4, PD-1, EREG were measured by enzyme-linked immunosorbent assay (ELISA) technique. The results showed a significant decrease in CTLA-4 in patients (46.04 ±4.51) than in controls (70.46 ±9.41), ($p \leq 0.01$). Also, there was no difference in PD-1 level in patients (250.37 ±23.37), and controls (247.81 ±29.80), ($p > 0.05$). EREG showed a significant increase in patients (1099.12 ±138.51), compared to controls (835.02 ±48.62), ($p \leq 0.05$). In addition, prolactin hormones recorded a significant increase in patients (154.86 ±27.22) compared to controls (13.91 ±1.50), ($p \leq 0.05$). While for the testosterone hormone there was a significant decrease in patients (16.36 ±2.58), as compared to controls (28.73 ±1.75), ($p \leq 0.05$). The AMH showed no difference between patients (5.79 ±0.40), and controls (4.18 ±0.44), ($p > 0.05$). In conclusion, based on the current findings, the decrease in CTLA-4, and the increase in EREG in the patient's group was associated with low-grade inflammation like PCOS.

Keywords: PCOS, AMH, CTLA-4, epiregulin (EREG), and PD-1.

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Introduction

Polycystic ovarian syndrome (PCOS), is one of the most increasingly endocrine disorder in women at reproductive age, causing a several symptoms such as a high androgen level, anovulatory sub-fertility, acne, hirsutism, insulin resistance, which leads to type 2 diabetes mellitus and obesity (where its related with the elevation of leptin hormone), also causing a cardiovascular disease. The chronic low-grade inflammation is also accompanying PCOS.[1,2] Insulin resistance and hyperandrogenemia are the key roles of PCOS development. However, some hormonal, immunological, genetic, and environmental factors affect PCOS.[3] The luteinizing hormone (LH) became in high level in PCOS, which directly induced the epiregulin (EREG). Where EREG is a part of the ovary's direct response to the LH hormone. EREG is a member of the epidermal growth factor family (EGF), released by granulosa cells in response to the luteinizing hormone (LH) surge that occurs mid-cycle. Together with amphiregulin and betacellulin, it will interact with the EGF receptor (EGFR) on granulosa and cumulus cells, activating intracellular signaling pathways-particularly extracellular signal-regulated kinases 1/2 (ERK1/2), that are essential for cumulus expansion, oocyte maturation, and follicle rupture.[4] The involvement of EREG in ovarian follicle development was hypothesized to have a potential role in PCOS pathophysiology, since epiregulin was implicated in inflammatory responses, also PCOS associated with chronic low-grade inflammation. Also on immunological level, the cytotoxic T-lymphocyte associated protein 4 (CTLA-4) that also called CD152, and programmed cell death protein 1 (PD-1), are co-expressed on effector T-cells and play a role in the balance of immune response. The CTLA-4 and PD-1 are both co-inhibitory receptors on T-cells (which is a type of immune cell that matures in thymus and plays a role in specific immunity in an organ), help suppress excessive immune response and inflammation. Dysfunction of these receptors may remove critical immune checkpoints, promoting a chronic inflammatory state characteristic of PCOS. In patients with PCOS the metabolic disorder was associated with the expression of PD-1, and CTLA-4.[5] The CTLA-4 gene consists of 4 exons encoded by chromosome one and two.[6] Moreover, some hormones associated with PCOS beside LH and follicle-stimulating hormone (FSH), like prolactin (PRL), Anti-Mullerian Hormone (AMH), and Testosterone. Prolactin is produced from lactotrophic cells by anterior pituitary gland, play a role in regulation of menstrual cycle and ovulation, stimulate the mammary glands to produce milk after childbirth, and suppress the reproductive hormones when elevated. The high and low level of PRL causes negative metabolic disturbance such as obesity, and disturbance of lipid profile, also reduced libido, where PRL associated with reproductive system regulation and immunity.[7,8] However, AMH is an indicator for PCOS, fertility disorder, and resistance to ovulation stimulation. A glycoprotein hormone, released from granulosa cells in the ovary's small antral follicles, is widely recognized as valuable marker for quantifying ovarian reserve, and AMH causes a decrease in Follicle stimulating hormone (FSH).[9,10] Also the testosterone hormone (where its androgens), increased in level in women with PCOS, especially in patients who had insulin resistance, its level associated with the sexual function in women.[11] The Metformin drug can improve obesity, insulin resistance, and type 2 diabetes, this made the testosterone decreased in level in women with PCOS, that means there is a positive correlation between testosterone hormone and insulin resistance.[12]

Subjects and Methods

The study was performed in Baghdad, the samples were randomly collected from Medical City of Baghdad, hospital, and the private women's infertility clinics. A total of 65 women with PCOS, and 56 apparently healthy women as controls were studied. The range of women's age was between 19 to 50 years. The patients were taking the following medications, Metformin, vitamin D, also an oral contraceptive.

The hormones Prolactin, Testosterone, and AMH were measured in serum of PCOS at early follicular phase and control women using an AFIAS-6 apparatus. Immunological parameters were determined by the enzyme-linked immunosorbent assay (ELISA) technique using commercial kits, the CTLA-4 kit (Cat.No. E0277Hu), PD-1 (Cat.No. E4711Hu), and EREG (Cat.No. E0399Hu).

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS), 2019 program was used to assess the differences between groups. The t-test was used to compare between means. The chi-square test was used to compare between percentages in this study. A p value <0.05 was considered significant.

Results

The current study reported that the patients with PCOS in age range <30 years old were 45 (66.67%) significantly higher compared to patients with ages >30 years old 21 (33.33%), (p≤0.05). However, the controls with age range <30 ware 29 (71.43%), not significantly different compared to controls with age range >30, 13 (28.57%), (p>0.05), as shown in Table 1.

Table 1. Distribution of study sample according to Age.

Factor	Patients No. (%)	Control No.(%)	*p*-value
Age groups (years)			
<30 yr	45 (66.67%)	29 (71.43%)	0.0398
≥30 yr	21 (33.33%)	13 (28.57%)	-
Mean ±SE	28.80 ±0.78	25.64 ±1.91	NS

p > 0.05 is not significant (NS).

Also, this study reported a significant decrease in CTLA-4, in patients with PCOS (46.04 ±4.51) compared to controls (70.46 ±9.41) (p≤0.01). While PD-1 had reported a non-significant difference between patients with PCOS where it was (250.37 ±23.37) and controls (247.81 ±29.80) (p>0.05). The patients with PCOS showed a significant increase of EREG (1099.12 ±138.51), compared to controls (835.02 ±48.62), where the level of EREG in control also considered high, (p≤0.05) as shown in Table 2.

Table 2. Comparison of cytotoxic T-lymphocyte associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1) and epiregulin (EREG) between patients and control groups.

Group	CTLA-4 Means ±SE	PD-1 Means ±SE	EREG Means ±SE
Patients	46.04 ±4.51	250.37 ±23.37	1099.12 ±138.51
Control	70.46 ±9.41	247.81 ±29.80	835.02 ±48.62
**p*-value	0.0066	NS	0.0349

*:T-test p > 0.05 is not significant (NS).

The prolactin hormone levels recorded a significant increase (154.86 ±27.22) in patients with PCOS compared to controls (13.91 ±1.50) (p≤0.05). However, the testosterone hormone showed a significant decrease in patients as it was 16.36 ±2.58, compared to controls (28.73 ±1.75) (p≤0.05). As such the levels of AMH hormone in this study reported a significant difference between the patients with PCOS (5.79 ±0.40) compared to controls as it was (3.18 ±0.44) (p<0.05) as shown in Table 3.

Table 3. Comparison of hormones between patients and control groups.

Group	Prolactin (ng/ml) Means ±SE	Testosterone (ng/ml) Means ±SE	AMH (ng/ml) Means ±SE
Patients	154.86 ±27.22	16.36 ±2.58	5.79 ±0.40
Control	13.91 ±1.50	28.73 ±1.75	3.18 ±0.44
**p*-value	0.0035	0.0328	0.048

*:T-test $p < 0.05$ is significant.

Discussion

PCOS is an endocrinological, hormonal, multisystem, metabolic and reproductive disorder, affects women at the reproductive age. The diagnosis of PCOS is by clinical feature hyperandrogenism, ovary dysfunction leading to infertility, and by ultrasound of multi-cysts in ovaries. The diagnosis confirms by using the AMH.[13] A previous study reported that the levels of PRL hormone in women with PCOS could be high due to the decrease in dopaminergic tone that related with a high secretion of LH, but the real causing still unclear. Generally, the high PRL levels suppress ovulation and lead to PCOS development.[14] This is consistent with the present study where the level of PRL in patients was higher than in the controls. However, the testosterone level was low in patient compared to controls. This may be related to Metformin drug that used to treat insulin resistant in PCOS, as reported in a previous study.[15] Metformin drugs improve insulin sensitivity in women with PCOS, leading to reduction in hyperinsulinemia-driven androgen production, particularly (testosterone).[16] As shown in a previous research the high levels of AMH hormone related with PCOS, where it is a best indicator for PCOS and ovarian reserve,[17] In the current study results the AMH level showed a significant increase in patients with PCOS compared to controls, However, the difference was not high, and this may be related to the treatment or the patient and the control in the same age group. As such, a former study showed that genetic variation caused a decrease in CTLA-4 gene expression, this led to the thinking that PCOS related with low-grade inflammation beside other factors as shown in Figure 1.

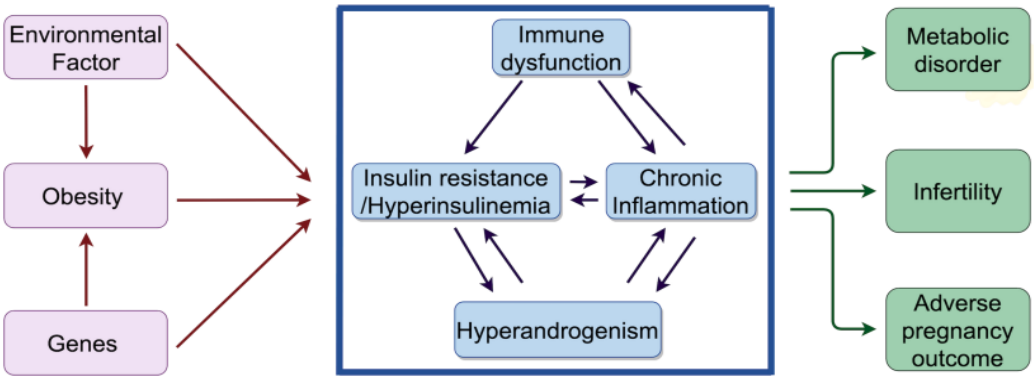

Figure 1. The relationship of polycystic ovarian syndrome (PCOS) with some factors.

Only active T-cell express CTLA-4, and PD-1 to suppress the immune response,[18] therefore, the CTLA-4, is considered as an immune check point receptors expressed on activated T-cell, and regulatory T-cells by competing with cluster of differentiation 80 (CD80) and CD86, as in Figure 2.

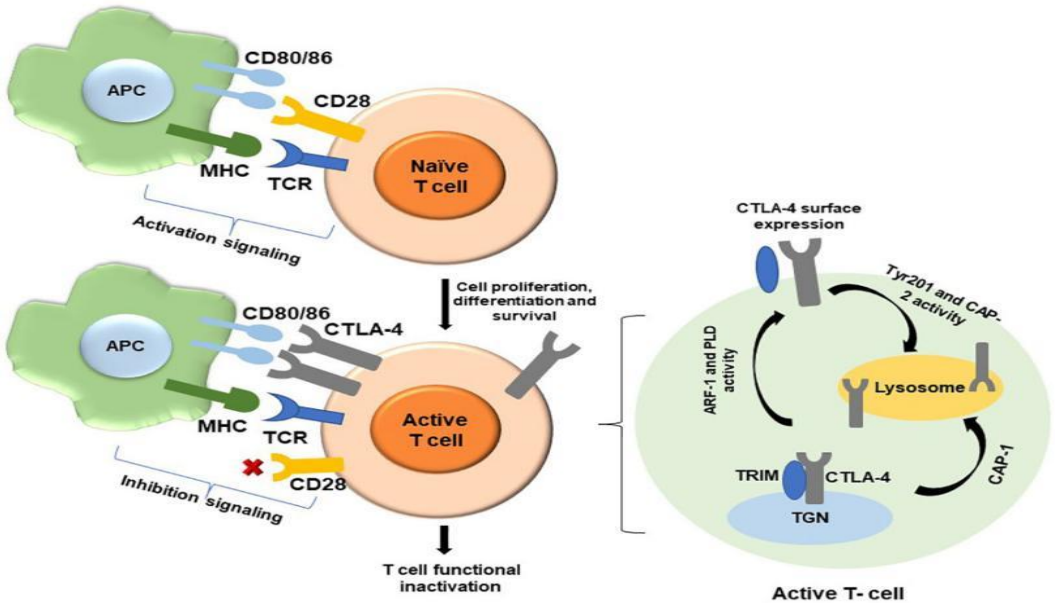

Figure 2. The mechanism of T-cell activation and cytotoxic T-lymphocyte associated protein 4 (CTLA-4) when exerts an inhibitory function on T-cell.

There was a negative correlation between body mass index (BMI) and CTLA-4, a chronic inflammation decreased CTLA-4 expression, that increased the immune dysregulation in obese women.[19,20] Also, a previous study showed that patients with deficient CTLA-4 had lower B-cell death and B-cell receptor (BCR) proliferation. In addition, the deficiency of CTlA-4 revealed a reduce number of natural killer cells, affecting its function, like cytotoxicity and inflammatory cytokine generation.[21] As for this study, there was a significant decrease in CTLA-4. PD-1 plays a negative regulatory role in inflammation; it suppresses the inflammatory response. Where it is a member of B7 binding CD28 co-stimulatory molecule family (a second signal for T- cell activation, to express CTLA-4). In addition, PD-1 stimulates T-cell apoptosis and secrets interleukin 10 (IL-10) to suppress the immunity, it's function is T-cell checkpoint.[22,23] The PD-1 in previous study showed a decrease in levels of patients with PCOS.[24] This study revealed a non-significant difference in PD-1 among patients and controls. In addition, EREG as mentioned in previous research is one of EGF family, that plays a role in oocytes maturation, and cumulus expansion (it is the cells that surround the ova in ovaries). Any disruption in these paracrine and autocrine loops especially in low-grade inflammation as in PCOS causes aberrant follicular growth and anovulation. So, the level of EREG is elevated in response to LH surge.[25] There was no correlation between EREG and testosterone, FSH, LH, estradiol, progesterone. While AMH hormone may have a correlation with EREG, due to their related function, where AMH is an indicator of ovarian reserve, and the EREG plays a role in oocytes maturation to release the ovum. However, this was not proved in research yet.[26] The current study concluded an elevation in EREG in patients and reduction in CTLA-4 associated with elevated prolactin and AMH among patients and controls, as a previous study concluded that EREG levels are significantly elevated in PCOS women.[27]

Author Contributions

SFA designed the research, performed the statistical analysis and revised the manuscript. SKI collected the data and wrote the manuscript. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved by the Ethic committee, of the College of Science, University of Baghdad (Ref.: CSEC/1124/0100, November, 2024).

Informed consent

A written informed consent was obtained from each patient and control before participated in this study.

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