Hormones and Cytokines in Relation with Overweight and Hyperglycemia in PCO Patients

Shalan HB Al-Mayoofee [1], Nabiha Missaoui [2], Sihem Hmissa[2], Ali Esmail Al-Snafi [3]

Abstract

Polycystic ovarian syndrome (PCO) is one of the most common endocrine disorders of females. It is frequently accompanying by insulin resistance, hyperandrogenima and hyperlipidemia, that lead to severe consequence in the patients. In the current research, we aimed to evaluate hormones (FSH, LH, total and free testosterone, prolactin, anti-Mullerian, progesterone, estradiol) and cytokines (serum levels of TNF-α, IL-1b, IL-6, IL-8 and IL-10) in relation with overweight and hyperglycemia in PCO patients. Blood samples were collected from one hundred married infertile PCO women (23.30±4.659 years) visiting Al-Shatra and Bint AL-Hoda hospitals in Thi-Qar governorate- Iraq, who diagnosed according to Rotterdam criteria. Blood samples were also collected from 50 healthy, age matched, non-pregnant with regular menstrual cycle, to serve as control. The patients of PCO were classified into four groups according to BMI and fasting serum glucose level, (normal weight nondiabetic patients [BMI: 22.03±4.82 kg/m^2 and serum glucose: 99.45±10.62 mg/dl], normal weight diabetic patients (BMI: 22.58±4.74 kg/m^2 and serum glucose: 128.93±16.34 mg/dl), overweight non-diabetic patients (BMI: 30.27±5.22 kg/m^2 and serum glucose: 100.45±12.54 mg/dl) and overweight diabetic patients (BMI: 34±6.28 kg/m^2 and serum glucose: 130.88±18.22 mg/dl) compared with healthy control (BMI: 22.84±4.63kg/m^2 and serum glucose: 96.14±10.23 mg/dl).

Keywords:

Hormones, Cytokine, BMI, Glucose, PCO, Polycystic Ovary.

[1]Ibn El Jazzar Faculty of Medicine, University of Sousse, Tunisia, [2]Research Laboratory: Oncogenesis and Tumor progression (LR21ES03), Faculty of Medicine of Sousse, University of Sousse, Tunisia, [3]Department of Pharmacology, Faculty of Medicine, Thi-Qar University, Thi-Qar, Iraq.

Address for correspondence:
E-mail:
shalinhashesh@gmail.com

Introduction

Polycystic ovarian syndrome (PCO) is one of the most common endocrine disorders of females. The incidence is highly differ ranging from 2.2% to as high as 26% [1]. Several causes contribute to this variation, firstly, diagnosing the condition is logistically challenging due to the need for blood or ultrasound scans and secondly, there is substantial variation in the interpretation of symptoms, which has led to a lack of consensus about medical guidelines for the disorder.

The Rotterdam guidelines cover a wider range of prevalence than the national institute of health's 1990 standards [2]. The etiology of PCOS was uncertain, it is manifested by two dominant mechanisms namely hyperandrogenism and insulin resistance (IR), both may lead to clinical phenotypes and ovarian morphological alterations in ultrasound investigation. Inflammatory processes, accompaniment these metabolic conditions, are characterized by increased proinflammatory cytokines and oxidative markers which in turn linked to insulin resistance [3].

Many studies revealed an elevated biomarker of inflammation in PCOS, even when body mass index is controlled, which confirmed the role of inflammation in the pathogenesis of PCOS[4]. The etiology of PCOS was not completely explained. Impaired hypothalamic- pituitary- ovary axis was among the etiological factors. Many genetic variants in form of single nucleotide polymorphisms were identify as disease susceptibility factors. In recent years, an increasing number of research have focused on the role of chronic low-grade.

inflammation in PCOS. Some results have concluded that PCOS-associated inflammation depends on visceral adipose tissue. This is an important point, considering that, glucose intake associated with insulin resistance can trigger an inflammatory response that increase oxidative stress and nuclear factor-κB activation. Oxidative stress has been shown to play an important role in chronic low-grade inflammation and can be significantly augmented in PCOS by increasing the proinflammatory cytokines expression [5].

Materials and methods

Blood samples (after 12 hrs fasting) were collected by venipuncture from hundred PCO married infertile women (23.30±4.66 years) visiting Al-Shatra and Bint Al-Hoda hospitals in Thi-Qar province - Iraq, they were diagnosed by gynecologists according to Rotterdam criteria, from September 2021 to the November 2022. The patients with suspicion of Cushing syndrome, congenital adrenal hyperplasia, androgen-secreting tumor, thyroid dysfunction, hyperprolactinemia, women used antidiabetic, contraceptives, hormonal, and hypolipidemic drugs were excluded. Blood samples were also taken from 50 healthy, age matched (23.84±4.80 years), with regular menstrual cycle, non-pregnant, to serve as control. The research was confirmed by an ethical committee of health directorate of Thi-Qar governorate Iraq. Furthermore, written consents were signed by all participants. FSH, LH, total and free testosterone, prolactin, anti-Mullerian, SHBG, progesterone, estradiol and serum levels of TNF-α, IL-1b, IL-6, IL-8, IL-10 were assayed by using Enzyme-linked immunoassay (BIO TEK 800 analysis instrument USA), Glucose and lipid profile were determined by using spectrophotometer (Apel analysis instrument, Japan), according to the operational manual of the company.

Statistical analysis

The significancy between PCO and healthy control groups for each parameter was determined by using student t-test (Spss version 26). P-value = 0.05 or lower, is considered as significant.

Results

According to BMI and fasting serum glucose level, in comparison with the group of healthy control, the patients of PCO were classified into four groups: normal weight nondiabetic patients [BMI: 22.03±4.82 kg/m² NS and serum glucose: 99.45±10.62 mg/dl (NS)], normal weight diabetic patients [(BMI: 22.58±4.74 kg/m² NS and serum glucose: 128.93±16.34 mg/dl (P<0.01)], overweight non-diabetic patients [BMI: 30.27±5.22 kg/m² (P<0.001) and serum glucose: 100.45±12.54 mg/dl (NS)] and overweight diabetic patients (BMI: 34±6.28 kg/m² (P<0.001) and serum glucose: 130.88±18.22 mg/dl (P<0.001)] compared with healthy control (BMI: 22.84±4.63kg/m² and serum glucose: 96.14±10.23 mg/dl).

Seum LH level was significantly elevated in all PCO subgroups; normal weight nondiabetic (35.06±16 mIU/ml, P<0.01), normal weight diabetic (35.01±17 mIU/ml, P<0.01), overweight non-diabetic (36.08±19 mIU/ml, P<0.01) and overweight diabetic (36.12±20 mIU/ml, P<0.01) compared with healthy control (20.96±14 mIU/ml), and there were no significant variations among PCO subgroups.

In comparison with the serum level of FSH in healthy control (6.6±3.3 mIU/ml), the FSH level was significantly elevated in normal weight diabetic PCO (9.3±5.0 mIU/ml, P<0.05), overweight non-diabetic PCO (9.4±3.0 mIU/ml, P<0.05) and overweight diabetic PCO patients (9.5±4.1 mIU/ml, P<0.05), with no significant variation among PCO subgroups. While, normal weight nondiabetic PCO patients showed no significant changes in the serum level of FSH compared with control group. LH/FSH ratio was not significantly changed in all PCO subgroups in comparison with control.

The serum level of progesterone was significantly decreased in all PCO subgroups; normal weight nondiabetic (10.0±2.0 ng/ml), normal weight diabetic (9.4±1.8 ng/ml), overweight non-diabetic (11.0±3.2 ng/ml) and overweight diabetic (8.3±2.1 ng/ml) (P<0.01 for all) compared with healthy control (21.3 ±5.2ng/ml).

Serum prolactin level was also elevated in all PCO subgroups; normal weight nondiabetic (19.6±9.3 ng/ml), normal weight diabetic (19.2±6.5 ng/ml), overweight non-diabetic (18.3±2.0 ng/ml) and overweight diabetic (19.5±6.0 ng/ml) (P<0.01 for all) compared with healthy control (12.9±4.1 ng/ml).The serum level of total testosterone was significantly increased in normal weight nondiabetic (1.1±0.3 ng/ml, P<0.05), normal weight diabetic (1.4±0.6 ng/ml, P<0.01), overweight non-diabetic (1.3±0.5 ng/ml, P<0.01) and overweight diabetic PCO patients (1.5±0.7 ng/ml, P<0.01) (P<0.01 for all) compared with healthy control (0.8 ±0.7 ng/ml).

Serum level of free testosterone also significantly increased in normal weight nondiabetic (2.0±0.8 pg/ml), normal weight diabetic (1.8±1.7 pg/ml), overweight non-diabetic (2.2±1.8 pg/ml) and overweight diabetic PCO patients (2.0±1.3 pg/ml) (P<0.05 for all) compared with healthy control (1.3±1.3 pg/ml).

SHBG was significantly elevated in normal weight nondiabetic (24.0±11.0 nmol/l, P<0.01), normal weight diabetic (36.0±4.0 nmol/l, P<0.001), overweight non-diabetic (22.0±1.8 nmol/l, P<0.01) and overweight diabetic (25.0±14.1 nmol/l, P<0.01) compared with healthy control (17.0±8.7 nmol/l). AMH was increased (P<0.01) and E2 was highly elevated (P<0.001) in all PCO subgroups compared with control. Serum estradiol was significantly increased in all subgroups of PCO patients (137.0±11.0 to 192.0±10.2 ng/ml, P<0.001 for all)

compared with control (118.0±19.1 ng/ml). TNF α was significantly declined in all PCO subgroup16.0±5.1 to 21.0±10.0 ng/ml (P<0.01) compared with control group (25.2±4.1 ng/ml). IL-6 was significantly elevated in normal weight nondiabetic (27.0±19.0 ng/ml, P<0.001), normal weight diabetic (22±4.5 ng/ml, P<0.01), overweight non-diabetic (21±7.9 ng/ml, P<0.01) and overweight diabetic (19±5.6 ng/ml, P<0.01) compared with healthy control (13.1±2.5ng/ml). IL-8 showed only significant decline in the overweight diabetic subgroup compared with healthy control, while, IL-10 showed no changes in the normal weight nondiabetic, normal weight diabetic, overweight non-diabetic and overweight diabetic subgroups compared with healthy control. IL-1 β was significantly elevated in normal weight nondiabetic (39.0±13.0 ng/ml, P<0.01), normal weight diabetic (53.0±25.0 ng/ml, P<0.001), overweight non-diabetic (38±8.5 ng/ml, P<0.01) and overweight diabetic (41±8.7 ng/ml, P<0.01) compared with healthy control (17.3±7.8 ng/ml).

Table 1: Serum hormones and cytokines levels in relation with BMI and hyperglycemia in PCO patients.

Variables	N=26 Overweight diabetic	N=50 Overweight non-diabetic	N=8 Normal weight diabetic	N=16 Normal weight nondiabetic	N=50 Control
BMI (kg/m^2)	22.84±4.63[a]	22.03±4.82[a]	22.58±4.74[a]	30.27±5.22[b]	34±6.28[b]
Glucose mg/dl	96.14±10.23[a]	99.45±10.62[a]	128.93±16.34[b]	100.45±12.54[a]	130.88±18.22[b]
LH (mIU/ml)	20.9±14.0[a]	35.1±16.2[b]	35.1±17.4[b]	36.0±19.1[b]	36.1±20.3[b]
FSH (mIU/ml)	6.6±3.3[a]	7.5±2.6a[b]	9.3±5.0[b]	9.4±3.0[b]	9.5±4.1[b]
LH/FSH ratio	4.08±3.39[a]	5.22±3.2[a]	4.63±3.2[a]	5.33±3.4[a]	4.64±2.1[a]
PROG (ng/ml)	21.3 ±5.2[a]	10.0±2.0[b]	9.4±1.8[b]	11.0±3.2[b]	8.3±2.1[b]
PRO (ng/ml)	12.9±4.1[a]	19.6±9.3[b]	19.2±6.5[b]	18.3±2.0[b]	19.5±6.0[b]
Total Testo (ng/ml)	0.8 ±0.7[a]	1.1±0.3[b]	1.4±0.6[c]	1.3±0.5[c]	1.5±0.7[c]
Free Testo (pg/ml)	1.3±1.3[a]	2.0±0.8[b]	1.8±1.7[b]	2.2±1.8[b]	2.0±1.3[b]
SHBG (nmol/l)	17.0±8.7[a]	24.0±11.0[b]	36.0±4.0[c]	22.0±1.8[b]	25.0±14.1[b]
AMH (ng/ml)	1.4±0.8[a]	2.4±1.2[b]	2.4±0.9[b]	2.4±1.6[b]	2.4±0.2[b]
E2 (ng/ml)	118.0±19.1[a]	192.0±10.2[c]	137.0±11.0[c]	177.0±17.5[c]	174.0±13.4[c]
TNF α (ng/ml)	25.2±4.1[a]	18.0±10.0[b]	19.0±7.5[b]	21.0±10.0[b]	16.0±5.1[b]
IL-6(ng/ml)	13.1±2.5[a]	27.0±19.0[c]	22±4.5[b]	21±7.9[b]	19±5.6[b]
IL-8 (ng/ml)	17.5±3.2[a]	14.0±11.0[a]	17.0±9.4[a]	15.0±12.0[a]	12.0±5.0.9[b]
IL-10 (ng/ml)	22.36±17.4[a]	22.0±18.0[a]	18.7±11.0[a]	20±17[a]	24.0±19.0[a]
IL-1 β (ng/ml)	17.3±7.8[a]	39.0±13.0[b]	53.0±25.0[c]	38±8.5[b]	41±8.7[b]

Similar letter horizontally means not significant

Discussion

This study assessed the incidence, pattern and predictors of serum hormones and cytokines levels in relation with overweight and hyperglycemia in PCO patients in comparison with the group of healthy control. According to BMI and fasting serum glucose level, the patients of PCO were classified into four groups: normal weight non diabetic patients, normal weight diabetic patients, overweight non-diabetic patients and overweight diabetic patients (Table 1). Our study revealed that women with PCOS had higher BMI than control women, these results were in agreement with many previous studies [6, 7]. The obesity was existed in 30-70% of women with PCOS [8, 9].

In the current research, we recorded significant increase in the levels of LH, prolactin, testosterone, E2, AMH, with significant decline in progesterone and SHBG, in all subgroups of PCOS compared with healthy control. Elevated LH has been described in women with PCO and with other hyper-androgenism disorders, like androgen-secreting ovarian tumors [10]. Androgens affected the releasing of gonadotropin by aromatization to estrogens [11]. Although androgen in the normal level didn't alter directly, the gonadotropin release in PCOS women or in healthy women [12]. Obesity is one of the

signs of the PCOS, but, not all PCOS patients were obese and not all obese females showed PCOS [13]. As in the current study, previous the researches revealed that the androgens were significantly increased in women with PCOS [14], associate with many consequences including acne, alopcia and hirsutism, and its physiological effects predisposed in long term to infertility [15]. Excess androgen also increased insulin resistance, weight, and elevate blood pressure, which contributes to cardiovascular disease [16]. The results were also consistent with that recorded by Piouka et al. [17], who found that AMH was elevated in PCO women, and its higher levels reflected the PCOS severity [17, 18].

On the other hand, it has been noted that the responsiveness of endometrium to progesterone was decreased PCOS women [19, 20], and the expression of progesterone expression was higher in PCOS patients who have anovulation in comparison to those who still ovulate [21]. In addition, expression of progesterone in epithelial cells was higher than that in stromal cells in PCOS women [22], suggested that the lower binding of progesterone in stromal cells may resulted in the promotion of E2-induced epithelial cell proliferation in PCOS [23]. In the current research, we recorded significantly elevation in the levels of inflammatory cytokines IL6 and IL1β and decline of the level of TNFα

with no significantly changes in the level of IL8 and IL10 in all subgroups of PCOS compared with healthy control. The increased levels and expressions of IL-6 and IL-1β in the ovary was correlated with overweight, insulin resistance and hyperglycemia which occurred in most cases of PCO [24, 25].

PCOS, as a chronic low-grade inflammatory condition, can stimulate the immune response and elevated inflammatory agents such as hsCRP and IL-6 [26]. Cytokines including IL-6 enhanced the secretion of hsCRP. Increased levels of hsCRP in PCOS have been reported in many studies[27, 28]. Production of CRP in the liver primarily is under the control of IL-6 stimulation. Both IL-6 and CRP markers of systemic inflammation are associated with insulin resistance, hyperglycemia and type 2 diabetes [29, 30]. Adiopcity plays a role in the metabolic alterations through the cytokines and adipokines production [31]. The pro-inflammatory cytokine can stimulate the *in vitro* proliferation of androgen producing theca cells [32]. Therefore, the local inflammatory response may stimulate androgen produced by ovary in PCOS.

Although several pro-inflammatory markers were elevated in PCOS, but the majority of the studies found that the level of TNFα either unchanged or declined [33, 34]. No significant variations were recorded in IL-10 between PCOS and healthy control. Although IL-10 in the adipose tissue is regulated to limit the systemic pro-inflammatory response noted in obesity, but the comparison between overweight PCOS women with overweight controls revealed no significant variations in the IL-10 levels, which in agreement with our study [35].

The results of this study are also in agreement with those recorded by Wu *et al*. [36], who noted that serum IL-8, IL-10 levels were not different between PCOS and healthy control. Interactions between PCOS, hyperinsulinemia, hyper-androgenism, abdominal obesity and inflammation, may be key to explain the phenotypes presented by women with PCOS and suggesting possible interventions to treat PCOS and related complications.

Conclusion

According to our results, inflammatory cytokines IL6, IL1β are an important part of the pathophysiology of PCO, and may serve as beneficial indicator in the diagnosis and treatment. Furthermore, the results also confirmed that IL6, IL1β were positively correlated with serum levels of (total and free testosterone, FSH, LH, prolactin, anti-Mullerian). While TNFα was inversely correlated with these parameters.

Abbreviations
AMH: anti-Mullerian hormone, E2: estradiol, FSH: follicle stimulating hormone, IL: Interleukin, IR: insulin resistance, LH: luteinizing hormone, SHBG: sex hormone binding globulin, TNFa: tumor necrosis factor-α.

References

1. S. V. Kirthika, J. Paul, S. Sudhakar, and P. S. Selvam, "Polycystic ovarian syndrome-interventions for the emerging public health challenge: A scoping review," *Drug Invention Today,* vol. 12, no. 3, pp. 1-4, 2019.

2. L. Rasquin and J. Mayrin, "Polycystic Ovarian Disease (Stein-Leventhal Syndrome). Authors Source StatPearls [Internet]. Treasure Island (FL)," ed: StatPearls Publishing, 2017.

3. M. Maiorino, G. Bellastella, D. Giugliano, and K. Esposito, "From inflammation to sexual dysfunctions: a journey through diabetes, obesity, and metabolic syndrome," *Journal of Endocrinological Investigation,* vol. 41, no. 11, pp. 1249-1258, 2018.

4. N. Boulman *et al.*, "Increased C-reactive protein levels in the polycystic ovary syndrome: a marker of cardiovascular disease," *The Journal of Clinical Endocrinology & Metabolism,* vol. 89, no. 5, pp. 2160-2165, 2004.

5. J. Dalmau Torres, E. Gargallo Ibort, J. Tamayo Fajardo, and A. Nuviala Nuviala, "CONVERGENT VALIDITY OF NPS AND ASSESSMENT OF LOYALTY MODELS IN SPORTS SERVICES," *Revista Internacional de Medicina y Ciencias de la Actividad Física y del Deporte,* vol. 22, no. 85, 2022.

6. H. Lee, J.-Y. Oh, Y.-A. Sung, H. Chung, and W. Y. Cho, "The prevalence and risk factors for glucose intolerance in young Korean women with polycystic ovary syndrome," *Endocrine,* vol. 36, pp. 326-332, 2009.

7. R. S. Legro, A. R. Kunselman, W. C. Dodson, and A. Dunaif, "Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women," *The journal of clinical endocrinology & metabolism,* vol. 84, no. 1, pp. 165-169, 1999.

8. R. Azziz, K. S. Woods, R. Reyna, T. J. Key, E. S. Knochenhauer, and B. O. Yildiz, "The prevalence and features of the polycystic ovary syndrome in an unselected population," *The Journal of Clinical Endocrinology & Metabolism,* vol. 89, no. 6, pp. 2745-2749, 2004.

9. J. Vrbikova and V. Hainer, "Obesity and polycystic ovary syndrome," *Obesity facts,* vol. 2, no. 1, pp. 26-35, 2009.

10. A. Pérez, J. Calaf, S. Webb, J. Prat, and A. De Leiva, "Postmenopausal androgen secreting ovarian tumour: pathophysiological implications; a case report," *European Journal of Obstetrics & Gynecology and Reproductive Biology,* vol. 35, no. 1, pp. 97-105, 1990.

11. R. A. Lobo and U. Goebelsmann, "Adult manifestation of congenital adrenal hyperplasia due to incomplete 21-hydroxylase deficiency mimicking polycystic ovarian disease," *American Journal of Obstetrics and Gynecology,* vol. 138, no. 6, pp. 720-726, 1980.

12. P. Serafini, P. D. Silva, R. J. Paulson, K. Elkind-Hirsch, M. Hernandez, and R. A. Lobo, "Acute modulation of the hypothalamic-pituitary axis by intravenous testosterone in normal women," *American journal of obstetrics and gynecology,* vol. 155, no. 6, pp. 1288-1292, 1986.

13. L. qasime Ali, N. M. Luaibi, and B. J. Majeid, "USED INOSITOL

DECREASED Anti-Müllerian Hormone (AMH) IN POLY CYSTIC OVARY SYNDROME WOMEN," *International Journal,* vol. 3, no. 4, pp. 857-869, 2015.

14. A. Huang, K. Brennan, and R. Azziz, "Prevalence of hyperandrogenemia in the polycystic ovary syndrome diagnosed by the National Institutes of Health 1990 criteria," *Fertility and sterility,* vol. 93, no. 6, pp. 1938-1941, 2010.

15. S. West *et al.,* "Irregular menstruation and hyperandrogenaemia in adolescence are associated with polycystic ovary syndrome and infertility in later life: Northern Finland Birth Cohort 1986 study," *Human reproduction,* vol. 29, no. 10, pp. 2339-2351, 2014.

16. L. L. Yanes Cardozo, D. G. Romero, and J. F. Reckelhoff, "Cardiometabolic features of polycystic ovary syndrome: role of androgens," *Physiology,* vol. 32, no. 5, pp. 357-366, 2017.

17. A. Piouka, D. Farmakiotis, I. Katsikis, D. Macut, S. Gerou, and D. Panidis, "Anti-Mullerian hormone levels reflect severity of PCOS but are negatively influenced by obesity: relationship with increased luteinizing hormone levels," *American Journal of Physiology-Endocrinology And Metabolism,* vol. 296, no. 2, pp. E238-E243, 2009.

18. P. Pigny, S. Jonard, Y. Robert, and D. Dewailly, "Serum anti-Mullerian hormone as a surrogate for antral follicle count for definition of the polycystic ovary syndrome," *The Journal of Clinical Endocrinology & Metabolism,* vol. 91, no. 3, pp. 941-945, 2006.

19. R. F. Savaris *et al.,* "Progesterone resistance in PCOS endometrium: a microarray analysis in clomiphene citrate-treated and artificial menstrual cycles," *The Journal of Clinical Endocrinology & Metabolism,* vol. 96, no. 6, pp. 1737-1746, 2011.

20. Z.-Q. Shen, H.-T. Zhu, and J.-F. Lin, "Reverse of progestin-resistant atypical endometrial hyperplasia by metformin and oral contraceptives," *Obstetrics & Gynecology,* vol. 112, no. 2 Part 2, pp. 465-467, 2008.

21. L. Margarit *et al.,* "MUC1 as a discriminator between endometrium from fertile and infertile patients with PCOS and endometriosis," *The Journal of Clinical Endocrinology & Metabolism,* vol. 95, no. 12, pp. 5320-5329, 2010.

22. S. Quezada, C. Avellaira, M. C. Johnson, F. Gabler, A. Fuentes, and M. Vega, "Evaluation of steroid receptors, coregulators, and molecules associated with uterine receptivity in secretory endometria from untreated women with polycystic ovary syndrome," *Fertility and sterility,* vol. 85, no. 4, pp. 1017-1026, 2006.

23. J. Barbado Solorzano and A. Martínez-Moreno, "SPORTS CENTRE WORKERS'PERCEPTIONS OF LEADERSHIP, ENGAGEMENT AND STRESS," *Revista Internacional de Medicina y Ciencias de la Actividad Física y del Deporte,* vol. 22, no. 85, 2022.

24. J. Adams *et al.,* "Enhanced inflammatory transcriptome in the granulosa cells of women with polycystic ovarian syndrome," *The Journal of Clinical Endocrinology & Metabolism,* vol. 101, no. 9, pp. 3459-3468, 2016.

25. V. Wieser, A. R. Moschen, and H. Tilg, "Inflammation, cytokines and insulin resistance: a clinical perspective," *Archivum immunologiae et therapiae experimentalis,* vol. 61, pp. 119-125, 2013.

26. M. Abedini, E. Ghaedi, A. Hadi, H. Mohammadi, and R. Amani, "Zinc status and polycystic ovarian syndrome: A systematic review and meta-analysis," *Journal of Trace Elements in Medicine and Biology,* vol. 52, pp. 216-221, 2019.

27. D. Glintborg and M. Andersen, "An update on the pathogenesis, inflammation, and metabolism in hirsutism and polycystic ovary syndrome," *Gynecological endocrinology,* vol. 26, no. 4, pp. 281-296, 2010.

28. C. C. Kelly, H. Lyall, J. R. Petrie, G. W. Gould, J. M. Connell, and N. Sattar, "Low grade chronic inflammation in women with polycystic ovarian syndrome," *The Journal of Clinical Endocrinology & Metabolism,* vol. 86, no. 6, pp. 2453-2455, 2001.

29. G. Blake and P. Ridker, "Inflammatory bio-markers and cardiovascular risk prediction," *Journal of internal medicine,* vol. 252, no. 4, pp. 283-294, 2002.

30. A. D. Pradhan, J. E. Manson, N. Rifai, J. E. Buring, and P. M. Ridker, "C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus," *Jama,* vol. 286, no. 3, pp. 327-334, 2001.

31. R. Pasquali, A. Gambineri, and U. Pagotto, "The impact of obesity on reproduction in women with polycystic ovary syndrome," *BJOG: An International Journal of Obstetrics & Gynaecology,* vol. 113, no. 10, pp. 1148-1159, 2006.

32. R. Z. Spaczynski, A. Arici, and A. J. Duleba, "Tumor necrosis factor-α stimulates proliferation of rat ovarian theca-interstitial cells," *Biology of Reproduction,* vol. 61, no. 4, pp. 993-998, 1999.

33. L. J. Moran, M. Noakes, P. M. Clifton, G. A. Wittert, D. P. Belobrajdic, and R. J. Norman, "C-reactive protein before and after weight loss in overweight women with and without polycystic ovary syndrome," *The Journal of Clinical Endocrinology & Metabolism,* vol. 92, no. 8, pp. 2944-2951, 2007.

34. A. C. Souza dos Santos, N. P. Soares, E. C. Costa, J. C. F. de Sá, G. D. Azevedo, and T. M. A. M. Lemos, "The impact of body mass on inflammatory markers and insulin resistance in polycystic ovary syndrome," *Gynecological Endocrinology*, vol. 31, no. 3, pp. 225-228, 2015.

35. Å. Lindholm *et al.,* "No difference in markers of adipose tissue inflammation between overweight women with polycystic ovary syndrome and weight-matched controls," *Human Reproduction,* vol. 26, no. 6, pp. 1478-1485, 2011.

36. R. Wu *et al.,* "Ovarian leukocyte distribution and cytokine/chemokine mRNA expression in follicular fluid cells in women with polycystic ovary syndrome," *Human reproduction,* vol. 22, no. 2, pp. 527-535, 2007.