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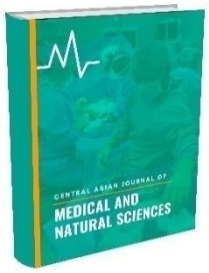

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Evaluation of Some Immunological Aspects of Women with Polycystic Ovary Syndrome in Wasit Governorate

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Key words: polycystic ovary syndrome; Interleukin-15; Interleukin IL6.

Abstract: The aim of the study the Study immune linkage for the occurrence of cysts and Analyzing the results statistically to determine the extent of the immunological causes of the occurrence of polycysticovaries.Polycystic ovary syndrome (PCOS) is one of the commonest endocrine disorders affecting many females in reproductive period which start from menarche till the menopause and commonly caused infertility around the world, and is characterized by irregular menstrual cycle, Hyperandrogensim and polycystic ovary; it can be considered a conditions involving reproductive, metabolic and cardiovascular components leading to lifelong health implication. Its prevalence among infertile women is between (15% to 20%). There is evidence that PCOS is a pro inflammatory disorder, characterized by the presence of low grade chronic inflammation that correlated with obesity or insulin resistance (IR). The present study aims to determine the role of immunological and physiological response in the pathogenesis of PCOS. The current study included 100 females diagnosed with PCOS, there were recruited from Kamal Al-kut hospital in Wasit during November 2022 until January 2023 , the diagnosis of polycystic ovary syndrome was based on the Rotterdam 2003 criteria. The control group consists of 50 fertile women who have regular menstrual cycle with no sign of hyperandrogenism and subjected to ultrasound examination and have normal hormonal levels. The age was identical in both groups and it was between (20-40) years.

The present study was carried out in two parts: immunological study. ELISA technique has been used to determine the serum level of Interlukine-15and IL6.

The results of immunological study demonstrated that IL-15 andIL6 serum level were significantly increased ($p<0.005$)in pcos patients in comparison to control group. The mean level of that interleukin

in patients and control group were (33.991±.57) pg/mL and (36.041±.03) and IL6 (85.944±.29), (84.24±6.39) pg/mL respectively.

Conclusions: In patients with pcos, The plasma level of IL-15, IL-6 is a major independent inflammatory predictor. Also, a ncrease in this hormone levels is an important indicator in predicting polycystic ovary syndrome.

Introduction

Polycystic ovarian syndrome (PCOS) is one of the most common endocrinopathies diseases that affect early reproductive age in females, the first description of PCOS in 1935 by Stein and Leventhal. The diagnose is achieved by three different criteria that were used in this field and was developed by the National Institutes of Health (NIH) in 1990, Rotterdam Criteria (ROT) in 2003, Androgen Excess and PCOS Association (AE-PCOS) criteria in 2006 (Chaudhary et al;2021)

The prevalence of PCOS is depended on the method of diagnosis, Word Health Organizations (WHO) estimate the prevalence of PCOS worldwide is 2% to 26% [Chaudhary et al;2021; Deswal etal;2020), with a high prevalence in obese patients at approximate 73%, the type of obesity is android, with a higher waist to hip ratio and fat in the front abdominal wall (Ghatnatti, etal;2022)

The main pathophysiological characteristic of PCOS is androgen excess with a prevalence of 60–80% (Thomas etal;2021), the biochemical hyperandrogenism includes an increase in total testosterone, free testosterone, dehydroepiandrosterone (DHEA), the dehydroepiandrosterone sulfate (DHEA-S) and androstenedione (Thomas etal;2021).

A dysfunctional interaction of behavioral, environmental, and genetic factors causes PCOS. Enlargement of ovaries, as well as secreting higher levels of androgens than normal theca cells are the most common clinical presentations of PCOS. Increased androgenic secretion results from increased enzyme activity in the steroid production pathway (Conway etal2015).

Many PCOS women have abdominal obesity which it lead to adipose tissue dysfunction, characterized by hypertrophic adipocytes, lipolysis impairments and insulin action. The secretion and expression of adipokines involved in insulin resistance such as adiponectin hormone and dysfunction in the adipose tissue that plays a key role in the metabolic abnormalities of PCOS patients(Villa and Pratley 2011) Many PCOS women showed an increased risk for nonalcoholic fatty liver disease (NAFLD) which is one of the most serious hepatic multiplication of metabolic abnormalities with a wide spectrum ranged from hepatic steatosis, inflammation, fibrosis to hepatocellular carcinoma. (Vassilatou, et al,2011,; Baranova et al.2011) Obesity, androgen excess, dyslipidemia and insulin resistance are the prime factors related to NAFLD in PCOS patents. (Hasan et al; 2017) The immune ascendancy cytokines in follicular fluid lymphocytes may be the immunological feature of PCOS ovary, Many evidence shows that immune dysregulation and chronic inflammation may be involved in the etiology of PCOS but the underlying mechanisms still an unclear. (Qin et al;2016) The decreasing of dendritic cells percentage and cytokines in follicular fluid of PCOS women indicate a confusion in the immunological microenvironment of the follicles in the ovary, that may be involved in the folliculogenesis dysfunction. (Zhang et al;2017)

Material and Method

A case control study collected 150 sample from the Al-Batool Hospital, Infertility Center from (1/11/2022) to (30/1/2023). This study involved 150 women, age range 20-40 year, those women comprised two groups; first group consisted of 100 diagnosed women with PCOS the patient's group

who were diagnosed by consultant gynecologist based on Rotterdam criteria (Rotterdam, 2004), and second group consisted of 50 healthy women (as controls).

Sampling (Blood samples):

Fasting venous blood samples were collected from each case during 2nd – 5th day of the menstrual cycle (early follicular phase) for those of normal cycle. about 5 ml of blood samples were obtained from veins of patients having polycystic ovarian syndrome and healthy control subjects.

about 3 ml of blood sample was placed into a gel tube and kept at room temperature for 15 minutes, following coagulation, the serum was separated by a centrifuge 4000 R per M for 10 min used to test.

Measureme1 Measurement of Human IL-15 (Interleukin 15) ELISA Kit

A. Principle

This ELISA kit uses the Sandwich-ELISA principle. The micro-ELISA plate provided in this kit has been pre-coated with an antibody specific to Human IL-15. Samples (or Standards) are added to the micro-ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human IL-15 and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human IL-15, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Human IL-15. You can calculate the concentration of Human IL-15 in the samples by comparing the OD of the samples to the standard curve.

Measurement of Human IL-6 (Interleukin 6) ELISA Kit

A. Principle

Test principle This ELISA kit uses the Sandwich-ELISA principle. The micro-ELISA plate provided in this kit has been pre-coated with an antibody specific to Human IL-

6. Samples (or Standards) are added to the micro-ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human IL-6 and Avidin, Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human IL-6, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 ± 2 nm. The OD value is proportional to the concentration of Human IL-6. You can calculate the concentration of Human IL-6 in the samples by comparing the OD of the samples to the standard curve.

Statistical Analysis

The data were analyzed statistically using SPSS vet.25, and the averages were compared using Chi-square under a probability level of 0.05.

Results and Discussion

Serum levels of IL-15, IL6 in patients and controls.

Table (3-2) illustrates the serum levels of IL-6, and IL-15 inPCOS patients and controls group.

Samples	IL-6	IL-15
Patients	85.94 ± 4.29	33.99 ± 1.57
Control	84.24 ± 6.39	36.04 ± 1.03
Sig	0.01	0.02

Table (3-2) : Serum levels of IL-15, IL6 in patients and controls.

The results for the three parameters IL-15 , and IL-6 for PCOS patients were (33.99 ± 1.57pg/mL AND 85.94 ± 4.29pg/mL) respectively; while the result for the two parameters IL-15 and IL-6 for controls group were (36.04 ± 1.03pg/Ml AND 84.24 ± 6.39pg/mL) respectively, all results were with no significant differences as compared to controls group. the levels of IL-6 were significantly higher in women with PCOS compared to BMI-matched controls. Higher IL-6 levels are related with the IR and total testosterone levels observed in women with PCOS compared with controls. Interestingly, the levels of IL-6 were high in both lean and obese women with PCOS. Significant heterogeneity was observed across the studies,

Although this study found no relationship between IL-6 levels and the BMI of PCOS patients, the data analysis pointed to a direct relationship between IR, androgen, and elevated IL-6 levels. The findings of the present study indicate that high IL-6 levels are not an intrinsic characteristic of PCOS. Therefore, IL-6 should not be used as a biomarker for the diagnosis of PCO.

Presence of the mutant allele of *IL6* was found to influence the clinical characteristics (i.e., BMI, hyperandrogenism, and impaired glucose tolerance) of affected women Most authors agree that hyperandrogenism, anovulation, and impaired glucose tolerance are key symptoms of this disorder H.AZcar et al (2001),R.SLegro,et al (1999), Jfemandez.Real etal (2001). A remarkably high percentage, ranging from 40% to 50% of women with PCOS, also exhibit signs of obesity R.SLgro,et al (1999),SSieg ,et al (2002). The investigated *IL6* polmorphism has been shown to be associated with lipid abnormalities (i.e., increased serum triglyceride levels, decreased serum high-density lipoprotein-2 cholesterol levels, and elevated fasting and post-glucose-load free fatty acid levels) J.Mfernandes-Rerl (2000) and with impaired insulin sensitivity JFrtanadez –REAL ,et al (2001) Whether the wild-type genotype or the presence of the mutant allele of *IL6* predisposes women to a pathological phenotype has been a subject of debate J.Cpikup et al (2021),JHnmGen et al (2003),AkubasZEk,(2003) To the best of our knowledge, we provide the first data concerning *IL6* in a relatively large series of women with PCOS.

The results indicate that IL-15 is involved in the pathogenesis of PCOS potentially by affecting survival, the inflammation state and steroidogenesis.

Relationship between Interleukin 6 and pcos

(IL-6), a pleiotropic cytokine, plays an important role in the endocrine system, especially as related to ovarian maturation and the processes of fertilization and implantation. IL-6 has also been shown to modulate ovarian development and function (Tumu VR etal,;2013). Therefore, IL-6 may be a key mediator of low-grade chronic inflammation in PCOS. Moreover, markers of chronic subclinical inflammation, such as IL-6, have been shown to be independent risk predictors for the development of type 2 diabetes (Mohli M etal ;2004). Several studies have now documented an increase in IL-6 levels in PCOS patients (Lin YS etal .2011;Escobar- Morreale HF .2003) .Both Mohlig et al. and Gonzalez et al. have pointed out that elevated IL-6 levels may have links with insulin resistance and hyperandrogenism in PCOS(Mohlig M etal .2004 ;Gonzalez F etal .2012) .Taken together, these studies support the hypothesis that PCOS increases the risk of diabetes by activating chronic inflammation (Mohlig M etal,;2004).

More than half of women with PCOS have insulin resistance and hyperinsulinemia. These insulin abnormalities might play a significant role in the pathogenesis of PCOS, not only by influencing the reproductive abnormalities of PCOS, but also by amplifying metabolic defects (Diamanti-kandarakisE. 2006; Dunaif A.2008),Hyperinsulinemia may contribute to a hyperandrogenic state by increasing androgen production of theca cells and influencing hepatic production of sex hormone binding globulin, resulting in higher concentrations of free androgens(Sirmans M and pate KA ;2013).

Hyperinsulinemia is more common in affected women with reproductive morbidities such as gestational diabetes mellitus (GDM) and pre-eclampsia, which are also associated with insulin resistance (Dunaif A; 2008). Therefore, in PCOS patients, these findings have led to the development of an important therapeutic strategy based on insulin-sensitizing drugs, such as metformin.

Metformin has insulin-lowering effects by improving insulin sensitivity and, in turn, can decrease circulating androgen levels. In addition, it also plays a critical role in the treatment of PCOS because women with PCOS are at an increased risk of insulin resistance(Escobar-Morreale HF etal ;2012).Indeed, metformin improves insulin- mediated glucose disposal in women with PCOS(Dunaif A,; 2008)Thus, metformin has become one of the key drugs in the treatment of PCOS. Considering the relationship between IL-6 levels and insulin resistance, metformin has the potential to affect serum IL-6 levels in PCOS patients

This research on IL-6 is shedding further light on the pathogenesis of PCOS and the long-term cardiovascular disease risk associated with PCOS (Ibanez L et al; 2004) .Therefore, strategies ameliorating inflammation may be useful for the management of PCOS and associated conditions(Ojeda-ojeda M etal ;2013).

Modern chronic drug treatment for PCOS patients is typically based on the administration of oral contraceptives, antiandrogens, and/or insulin sensitizers(Luque –Ramire ZM and Escobar – Morreale HF; 2010)As a biguanide that improves insulin sensitivity, metformin has been extensively evaluated in PCOS (Sirmans SM and pate KA ;2013)and has been shown to play a critical role in improving low-grade inflammation in PCOS(Diamanti –kandara kis E etal ;2006) . found there was a significant decrease in IL-6 levels after metformin treatment in PCOS women(Lin YS. 2011,; Luque-Ramirez et al .2010). confirmed that metformin may have beneficial effects on the inflammatory background associated with PCOS .(Ciaraldi et al; 2013). indicated that treatment-related reductions in IL-6 levels were significantly correlated with drops in fasting insulin levels .However(Mohlig et al. and Jakubowska et al;2018). failed to find any changes of plasma IL-6 levels with metformin therapy in PCOS patients(Jakubowskad et al ;2008)

Obesity is frequently present in women with PCOS. Continuous release of inflammatory mediators such as IL-6 perpetuates the inflammatory condition associated with obesity in PCOS, possibly contributing to insulin r esistance and other long-term cardiometabolic risk factors,(Ojeda-ojeda M et al ;2013).

Relationship between Interleukin 15 and pcos

IL-15 is an anabolic cytokine that is produced in skeletal muscle and In fact, skeletal muscle levels of IL-15 are among the highest of any tissue (LiH et al ;2021), and IL-15 is one of the most abundant cytokines in skeletal muscle (Duan Y et al; 2017).directly affects muscle anabolism in animal and in vitro models. IL-15 is an immunostimulatory cytokine *trans*-presented with the IL-15 receptor α-chain to the shared IL-2/IL-15Rβ and common γ-chains displayed on the surface of T cells and NK cells.

IL-15, a cytokine with biological functions on cells of lymphoid lineage, mediates its activities through the β and γ chains, IL-15 also binds to endothelial cells with high affinity that IL-15 is a stimulator of angiogenesis in vivo.

Interleukin-15 (IL-15) induces proliferation and promotes cell survival of human T and B lymphocytes, natural killer cells, and neutrophils.

PCOS patients have permanently elevated serum and ovarian levels of inflammatory markers interleukin-2 (IL-15), IL-6 compared with normal controls(Duan NMP et al.2016,; LiH Y. 2021) The association between inflammatory cytokines and ovarian dysfunction implies that inflammation might be reckoned as the most potent risk factor of PCOS Abraham (Guanadass S et al;2021). Further investigating the role of inflammatory mediators in the commencement and development of PCOS could be critical for better understanding the pathophysiology of the disease and developing a potential therapeutic target. IL-15 is secreted by many cell types, including both immune and nonimmune cells such as T-lymphocytes, macrophages, neutrophils and skeletal muscle cells (Giri JG et al,;1995)). IL-15 has attracted considerable attention for its beneficial effects, including improving lipid and glucose metabolism, suppressing white adipose tissue inflammation, enhancing mitochondrial function, and attenuating endoplasmic reticulum stress (YeJ et al;2015). In contrast with beneficial effects of IL-15, IL-15 was also reported to participate in chronic inflammation of adipose tissue leading to obesity-associated metabolic syndrome, Furthermore, serum IL-15 concentrations were higher in overweight subjects, suggesting that adipose tissue depots might be a source of IL-15 (DOzioE etal ;2014). IL-15 was increased in follicular fluid (FF) from women with endometriosis, suggesting that IL-15 may have impaired oocyte quality leading to lower fertilization rates (Falconer H etal ;2009). IL-15 concentration in FF of follicles with immature oocytes were significantly higher than those with mature oocytes, suggesting that IL- 15 should be investigated as a possible

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