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Study The Role of Interleukin *17A Gene* Polymorphism and Serum Level in Women with Polycystic Ovarian Syndrome

A Thesis

Submitted to the Council of the College of Medicine/University of Karbala for the Fulfillment of the requirements for the Degree of Master of Science in Medical Microbiology

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بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

"نَرْفَعُ دَرَجَاتٍ مَنْ نَشَاءُ ۗ وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ"

صَدَقَ اللهُ العَلِيُّ العَظيم

سورة يوسف آية (٧٦)

Supervisor Certification

We certify that this thesis entitled (Study The Role of Interleukin *17A Gene* Polymorphism and Serum Level in Women with Polycystic Ovarian Syndrome) was prepared under my supervision at the College of Medicine/University of Karbala as a partial Fulfillment of the requirements for the degree of Master of Science in Medical Microbiology.

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DEDICATION

TO THE GREAT GOD, WITHOUT HIS BLESSING, I WOULD NOT BE ABLE TO DO THIS

TO THE PROPHET MOHAMMED AND HIS PROGENY (PEACE BE UPON THEM)

TO IMAM AL-HUSSEIN AL-SHAHEED AND HIS BROTHER AL-ABBAS BIN ALI (PEACE BE UPON THEM)

TO MY PRECIOUS GRANDMOTHER, WHO SUPPORTED ME UNTIL SHE WENT FROM LIFE

MY DEAR FATHER AND MOTHER

MY DEAR AND THE MOST POWERFUL GIRLS IN THE WORLD, MY SISTERS

MY DEAR HUSBAND, MY PURE LOVE

HANEEN KAREEM KHALAF

2023

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Summary

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women of reproductive age, characterized by menstrual abnormalities and clinical or biochemical features of hyperandrogenism. PCOS is associated with multiple comorbidities, including obesity, insulin resistance and type 2 diabetes, mood disorders, Obstructive sleep apnoea (OSA) and cardiovascular disease (CVD). The current study has aimed at determining the influences of genetic variants of interleukin-17A (rs2275913) polymorphism on Polycystic ovary syndrome (PCOS) in addition to ELISA detection of serum IL-17A level.

This study is a case-control study, and the subjects of this study are divided into two groups control (n=50) and patients (n=50) enrolled in Najaf province , AL-Sader Teaching Medical City and AL-Zahraa Teaching Hospital during the period from November 2021 to April 2022.

The samples of blood were collected from each participant, noting that the sera were used to determine serum IL-17A level for all samples by ELISA technique. In contrast, whole blood was used to determine white blood cell and lymphocyte count and DNA extraction. Using Sysmex XN-350, five differential automated hematology analyzers are used to detect white blood cells and lymphocyte count. Moreover, detect IL-17A (rs2275913) gene polymorphism by Real-Time Polymerase Chain Reaction (RT-PCR) technique. MINI VIDAS detects the levels of luteinizing hormone, follicle-stimulating hormone, thyroid stimulating hormone and prolactin hormone.

This study has showed a significant association (P=0.005) between IL-17A serum level and PCOS patients compared with healthy control. The mean of IL-17A in patients was (11.46), and the mean of healthy control was (9.53). Regarding WCC and lymphocyte counts, there was a nonsignificant association between PCOS patients and healthy control with (P=0.230) for WCC and (P=0.231) for lymphocytes. About the genetic polymorphism of the IL-17A gene (rs2275913), the current study demonstrated that the AG genotype was found to be at a higher frequency in patients compared with control (50.0% vs. 42.0%, respectively), the GG genotype was found to be at a higher frequency in control compared with patients (46.0% vs. 34.0%, respectively) and the AA genotype was elevated in patients compared with control (16.0% vs. 12.0%, respectively). In the

genotypes, there was no significant association between the studied groups (*P*=0.421). Also, in the alleles frequency, there were no significant differences between the studied groups (*P*=0.307). In addition, this study found a nonsignificant association of IL-17A serum level with genotypes and alleles frequency of IL-17A gene (rs2275913) polymorphism in PCOS patients.

This study demonstrated that the IL-17A gene (rs2275913) polymorphism was non significant associatied with PCOS. therefore, might have a protective role in PCOS patients. In addition, the IL-17A may be used as a predictive marker for diagnosing PCOS.

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List of Abbreviations

Code	Words
bp	**Base Pair**
BP	**Blood Pressure**
DBP	**Diastolic Blood Pressure**
DM	**Diabetes Mellitus**
DNA	**Deoxyribonucleic Acid**
CD4 T cell	**Clusters for Differentiation 4 T Cell**
CD8 T cell	**Clusters for Differentiation 8 T Cell**
EDTA	**Ethylene Diamine Tetra Acetic Acid**
HRP	**Horse Radish Peroxidase**
IL	**Interleukin**
SBP	**Systolic Blood Pressure**
SNP	**Single Nucleotide Polymorphisms**
Th17	**T Helper 17**
WBC	**White Blood Cell**
WHO	**World Health Organization**
µl	**Microliter**

Chapter One

Introduction

and

Literature Review

1.1. Introduction

Polycystic ovarian syndrome (PCOS) is a complex, enigmatic, and public disease. It is the most common endocrinopathy faced by reproductive-aged women and affects up to 1 in 5 women (Teede, Deeks and Moran, 2010)

The reported prevalence of PCOS in women of reproductive age varies from 6% to 20%, depending on which diagnostic criteria are used to define the syndrome (Azziz *et al.,* 2001; March *et al.,* 2010). During the reproductive years, PCOS is associated with crucial reproductive morbidity, including infertility, abnormal bleeding, increased pregnancy loss, and pregnancy complications (Carmina and Lobo, 1999).

Polycystic ovarian syndrome is often associated with psychological impairments, including depression and other mood disorders and metabolic derangements, chiefly insulin resistance and compensatory hyperinsulinemia, which is a significant factor responsible for altered androgen production and metabolism. Most women with PCOS are also overweight or obese, further enhancing androgen secretion while impairing metabolism and reproductive functions and possibly favoring the development of the PCOS phenotype. The definition of PCOS has led to an impressive increase in scientific interest in this disorder, which should be further directed to improve individualized clinical approaches and therapeutic strategies (Pasquali *et al.,* 2011).

Women with PCOS are more likely to develop many metabolic and reproductive health complications, including dyslipidemia, abnormal glucose level, insulin resistance, miscarriage, gestational diabetes, and others (Escobar-Morreale, 2018).

Diagnosis frequently is delayed (Gibson-Helm *et al.,* 2017), and physicians often are poorly informed about PCOS (Lin *et al.,* 2018). PCOS is characterized by enhanced luteinizing hormone (LH) relative to follicle-stimulating hormone release, increased LH-dependent ovarian testosterone (T) production, frequent adrenal androgen excess, profound insulin resistance, dysglycemia, and obesity (Diamanti-Kandarakis and Dunaif, 2012; Dunaif, 2016).

The underlying cause of PCOS is a defect in the ovarian cells, most likely the theca cells, which leads to excessive androgen synthesis and the condition's clinical and biochemical signs and symptoms (Bednarska and Siejka, 2017). Extensive research

suggests that the etiology of PCOS involves an interaction between environmental factors and gene variants. However, it has been suggested that genetic factors contribute less than 10% to disease susceptibility (Parker *et al.*, 2022).

Among women with PCOS, inflammation is associated with insulin resistance and hyperandrogenism (Shorakae *et al.*, 2015). Interleukin-17A (*gene IL-17A*) is a pro-inflammatory cytokine that is mainly secreted by T-helper 17 cells and whose expression depends on transforming growth factor-β (TGF-β) and IL-6. IL-17A triggers the expression of many pro-inflammatory mediators, including chemokines, metalloproteinases, nitric oxide synthase-2, tumor necrosis factor-α (TNF-α), IL-6 and IL-1, thereby recruiting monocytes and neutrophils and triggering an inflammatory response (Özçaka *et al.*, 2013). TNF-α is produced by immune cells, granulosa–luteal cells, and ovarian macrophages (Spaczynski, Arici and Duleba, 1999) and is involved not only in immune and inflammation reactions and cell differentiation, proliferation and death but also in the obesity associated with PCOS (MacEwan, 2002).

Aim of the Study

The aim of this study was planned to estimate the association between IL-17A gene polymorphism and serum level in a patient with polycystic ovarian syndrome in a group of Iraqi people; the following objectives achieved this:

1. Determination of some demographic data in patients and control.

2. Estimation of IL-17A gene polymorphism by RT-PCR technique.

3. Determination of IL-17A serum level by ELISA test.

4. Evaluation of some laboratory tests in patients and control.

1.2. Literature Review

1.2.1. Definition of Polycystic Ovarian Syndrome

Polycystic Ovarian Syndrome (PCOS) is an endocrine disorder commonly found in women during their reproductive age (Abinaya *et al.,* 2019). Clinical or biochemical hyperandrogenism is the most commonly presented symptom, along with ovulation problems, menstrual irregularities, and multiple cysts in their ovaries. It has been reported that 5 – 20% of women of reproductive age have PCOS. Nearly 70% of the women are undiagnosed (Boyle and Teede, 2012).

The current clinical Definition recognizes PCOS as a broad spectrum of presentations consisting of three key features: oligomenorrhea, hyperandrogenism and polycystic ovaries, with the proviso of secondary causes excluded. Hyperandrogenism and ovarian dysfunction are the salient features of PCOS. In addition, metabolic abnormalities such as insulin resistance and hyperinsulinemia, abdominal obesity, hypertension and dyslipidemia, type 2 diabetes mellitus (T2DM), cardiovascular disease and endometrial hyperplasia are commonly associated with the disease (Dunaif *et al.,* 1989; Legro *et al.,* 1999; Korhonen *et al.,* 2001; Kim and Choi, 2013). Hence, PCOS is a life-long condition that manifests from puberty and has severe adverse reproductive and metabolic implications (Dunaif and Fauser, 2013).

1.2.2. Epiemiology of PCOS

Understanding the global prevalence and phenotype of PCOS is essential, considering that geographic factors and ethnic/ racial variations can shape the clinical presentation of the syndrome. The first studies to determine prevalence in a medically unselected (unbiased) population were initiated by Azziz and colleagues. They reported PCOS prevalences ranging from 4% to 6.6% using the National Institutes of Health (NIH) 1990 criteria among unselected reproductive-age women residing in the southeastern United States. These studies detected no statistically significant differences between black and white women. (Knochenhauer *et al.,* 1998; Azziz *et al.,* 2004)

Worldwide prevalence of PCOS ranges from 4% to 21%, depending on the diagnostic criteria used (Ma *et al.,* 2010; Boyle *et al.,* 2012).

The prevalence of PCOS among different geographic regions ranges from 5% to 10% according to NIH 1990 criteria; from 10% to 15% according to the Androgen Excess

& PCOS Society AE-PCOS 2006 criteria, and from 6% to 21% when the ESHRE/ASRM 2003 criteria [European Society of Human Reproduction and Embryology (ESHRE)/The American Society for Reproductive Medicine (ASRM)] were applied. More significant estimates of PCOS prevalence with the Rotterdam 2003 and AE-PCOS 2006 criteria are primarily attributed to their more expansive Definition and inclusion of additional phenotypes, compared with NIH 1990 diagnostic criteria (Sirmans *et al.*, 2014).

Variations in the reported prevalence within the exact Definition across countries can partly be explained by ethnic differences, the variety of approaches used to define study population(s), and the application of varying methods to evaluate key PCOS features. The results of epidemiologic studies of PCOS largely depend on how the study population and the PCOS phenotypes were defined. For example, in some studies, a population-based model was used to identify the study population, whereby subjects were randomly selected from a particular geographic area (Mehrabian *et al.,* 2011; Tehrani *et al.,* 2011; Li *et al.,* 2013). These studies overall are highly representative of the reference population and are considered standard for the evaluations of authentic relationships among variables of interest, even if those were not prespecified in the original study hypothesis (Kumarapeli *et al.,* 2008; Tehrani *et al.,* 2011; Li *et al.,* 2013; Rashidi *et al.,* 2014).

In addition, the results of studies measuring the prevalence of PCOS also suffer from the fact that the assessment of the PCOS phenotype is a complex multistep process, which requires multiple clinical and laboratory evaluations, pelvic ultrasound, and possibly several visits for some subjects. Thus, these studies may suffer from under-reporting of PCOS because these patients require more intensive study and follow-up than unaffected individuals. Furthermore, detecting PCOS in a study population requires more effort than diagnosing unaffected subjects. Population-based studies are the most difficult to complete and suffer from significant incomplete data (March *et al.,* 2010; Tehrani *et al.,* 2011).

Another standard model used to determine the prevalence of PCOS is the institution-based study, wherein subjects are undergoing a physical and medical assessment for nonmedical reasons, for example, a pre-employment or yearly employment assessment (Knochenhauer *et al.,* 1998; Azziz *et al.,* 2004; Yildiz *et al.,* 2012). In this approach, the study cohort is less likely to represent the general population

than population-based cohorts because individuals undergoing a pre-employment or employment assessment may be of higher socioeconomic and educational status than the general population. However, this approach usually makes obtaining a complete phenotype assessment in most subjects (Azziz *et al.,* 2004; Yildiz *et al.,* 2012).

1.2.3. Etiology of Polycystic Ovary Syndrome

The etiological factors associated with PCOS are not yet so clear and still under debate. Different factors could contribute to the etiopathology of PCOS, such as genetic, biochemical, environmental and immunological. Many genes are crucial contributors to PCOS; however, none of these factors could be implicated as the leading cause (Abbott, Dumesic and Franks, 2006).

1.2.3.1. Genetics

The genetic and environmental factor is responsible for the etiology of PCOS. An unhealthy lifestyle, diet or infectious mediators increase the risk of PCOS (Goodarzi *et al.,* 2011). Due to insulin resistance and its elevated level, the ovaries' function disturbs that rises androgen level, leading to anovulation (Diamanti-Kandarakis, Kandarakis and Legro, 2006). Apart from environmental factors, genetic factors are responsible for the etiology of PCOS. Its cause involves candidate genes and SNPs. According to databases, PCOS etiology involves 241 gene variations (Joseph *et al.,* 2016). Polymorphism or any nucleotide change causes a defect in the transcriptional activity of a gene that leads to PCOS (Strachan and Read, 1999).

Moreover, associated comorbidities, such as hyperandrogenemia, hyperinsulinemia and disturbed insulin secretion, cluster within these families of patients with PCOS (Legro *et al.,* 1998; ColillaCox and Ehrmann, 2001; Legro *et al.,* 2002; Yildiz *et al.,* 2006). PCOS appears more common amongst monozygotic twin pairs sisters than dizygotic twins, indicating a high degree of heritability in patients with PCOS (Vink *et al.,* 2006).

Previous studies have verified that PCOS shares similar properties with many chronic inflammatory disorders, and classical inflammatory mediators such as C-reactive protein (CRP), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) have also been found to be elevated in patients with PCOS (Deligeoroglou *et al.,* 2012; Ojeda-Ojeda *et*

al., 2013; Bhatnager *et al.,* 2019). Moreover, pro-inflammatory cytokines and their over-activated immune responses have also been associated with higher androgen levels. They can impact ovarian function and jeopardize the processes of ovulation in PCOS. Therefore, if a polymorphism can impact gene expression or the protein structure of cytokines, it is highly likely that it might alter inflammation status and influence predisposition to PCOS (González, 2012; Boots and Jungheim, 2015).

1.2.3.2. Environmental Factors

Environmental factors associated with PCOS can be classified as prenatal (fetal developmental programming) or postnatal (diet, Obesity, sedentary lifestyle, environmental toxins and prescription drugs) (Diamanti-Kandarakis, Kandarakis and Legro, 2006). Evidence suggests that environmental stimuli mimic hormonal actions and activate pre-existing, predisposing factors that trigger the endocrine activity characteristic of PCOS (Escobar-Morreale, Luque-Ramírez and San Millán, 2005).

Dietary habits, exercise and cultural, social and economic factors might modify environmental exposure. For that reason, among others, the prevalence of the metabolic conditions associated with PCOS (Obesity, metabolic syndrome and disorders of glucose metabolism) might vary as a function of the type of environmental exposure, especially in racially mixed populations that do not have a predominant genetic background. Thus, although environmental factors cannot be homogeneous in studies of the pathogenesis of PCOS in human subjects, the internal validity of such studies can be increased by including women of the same ethnicity from the same geographic area. It has been suggested that PCOS presents a nongenetic inheritance pattern in populations with a poor lifestyle (high-saturated-fat diet, sedentary lifestyle, alcoholism and smoking). In such populations, changes in the fetal–placental unit, the onset of IUGR (intrauterine growth retardation) and the frequency of SGA newborns (Small for gestational age) may occur. Hyperinsulinaemia and visceral obesity are more likely to develop during childhood and to culminate in a higher prevalence of insulin resistance, systemic arterial hypertension (SAH) and hyperandrogenism in reproductive-age women (Ibáñez *et al.,* 2001).

During pregnancy, women who were born SGA (Small for gestational age) also present a higher risk for placental disorders and the delivery of SGA newborns (Small for gestational age), which suggests a nongenetic inheritance pattern of PCOS. If they

maintained a proper lifestyle throughout their childhood and their reproductive period, these women would not experience placental changes; Thus, the process described earlier would be interrupted, and their children would be born AGA (Appropriate for gestational age) (Fig. 1) (Escobar-MorrealeLuque-Ramírez and San Millán, 2005). Contrarily, diets high in saturated fat and sedentary lifestyles are associated with developing PCOS and its metabolic consequences, mainly Obesity (Fauser *et al.*, 2012).

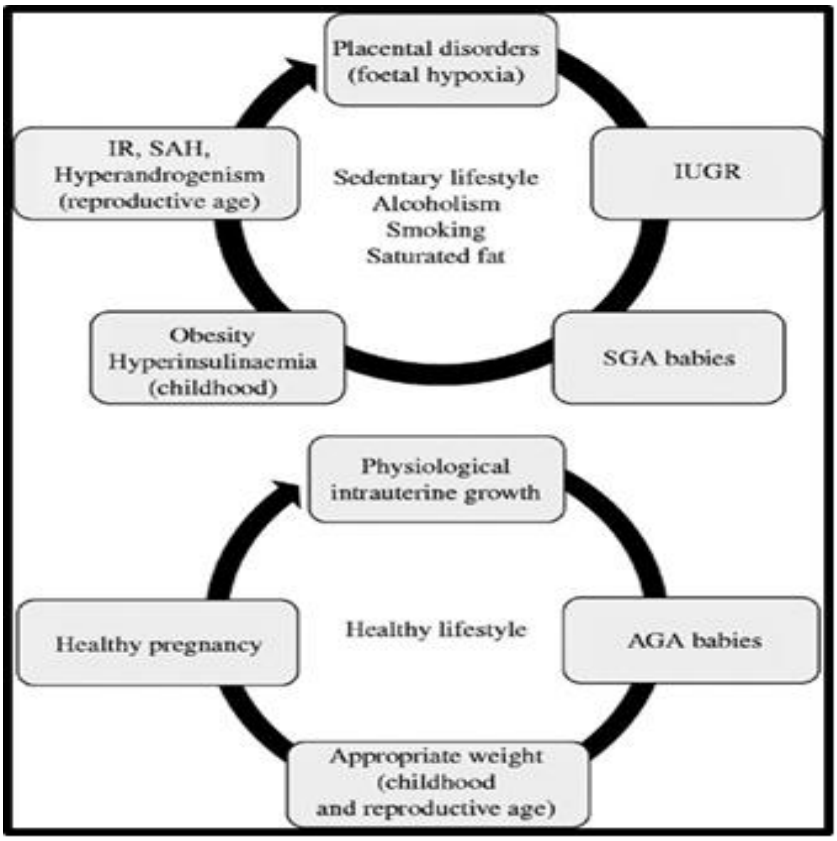


Figure (1-1): Environmental Factors of Polycystic Ovary Syndrome. Adapted from IUGR, Intrauterine Growth Restriction; IR, Insulin Resistance; SAH, Systemic Arterial Hypertension; SGA, Small for Gestational Age; AGA, Appropriate for Gestational Age. (Escobar-Morreale, Luque-Ramírez and San Millán, 2005)

1.2.3.3. Insulin Resistance (IR)

A link between PCOS and IR was first highlighted in 1980, whereby obese women with PCOS were shown to have an increased insulin response to an oral glucose tolerance test compared with obese controls (Jeanes and Reeves, 2017). Prevalence rates of IR have

been reported between 44% and 85% (Diamanti-Kandarakis and Dunaif, 2012; Stepto *et al.,* 2013). This variability is partly due to differences in PCOS phenotype and ethnicity (Daan *et al.,* 2014; Bil *et al.,* 2016).

Insulin resistance and compensatory hyperinsulinemia are fundamental to exacerbating PCOS symptoms and metabolic complications (Moran *et al.,* 2010). Several studies have shown that most women with PCOS, both lean and overweight, have a form of IR that is intrinsic to the syndrome (Stepto *et al.,* 2013). Studies have shown that Obesity and PCOS have a separate and synergistic relationship with IR (Dunaif and Abraham, 2001). Insulin sensitivity and secretion are defects in lean women with PCOS (Dunaif *et al.,* 1989). The pathogenesis of IR in PCOS is complex and incompletely understood, though it is agreed that there is a post-binding defect in insulin receptor signaling (Diamanti-Kandarakis and Dunaif, 2012). There is an increasing body of evidence to suggest a genetic susceptibility to PCOS and its associated IR, as well as evidence to suggest that PCOS may also have fetal origins due to androgen exposure at critical periods of growth or due to intra-uterine growth restrictions (Obermayer-Pietsch *et al.,* 2015).

Chronic inflammation and its role in IR in PCOS continue to be investigated (Victor *et al.,* 2016). The increase in multiple markers of inflammation such as leucocyte count, C-reactive protein, IL-18, protein-1, and monocyte chemo-attractant, in addition to increased oxidative stress and endothelial dysfunction, is evidence that PCOS is commonly coupled with low-level systemic inflammation (Escobar-Morreale, Luque-Ramírez and González, 2011). However, limited studies assess the presence of chronic inflammation independent of adiposity in women with PCOS. Circulating oxidative stress markers are abnormal in women with PCOS independent of weight excess and IR (Murri *et al.,* 2013). The close association between oxidative stress and inflammation makes it difficult to determine their contribution to PCOS, and the mechanism of oxidative stress-induced IR remains unclear (Zuo, Zhu and Xu, 2016). Oxidative stress and inflammation markers positively correlate with androgen levels in PCOS patients; thus, they seem to contribute to hyperandrogenaemia. However, detailed interactions still need to be identified, and as of yet, few investigations have been done (González *et al.,* 2005).

Insulin acts directly on the ovary by stimulating the thecal cells to increase androgen production and activate the cytochrome P450c17α (Conway, Avey and

Rumsby, 1994; Nestler *et al.,* 1998). Hyperinsulinaemia further exacerbates the pathogenesis of PCOS by inhibiting the production of insulin-like growth factor-1 (IGF-1) binding protein in the liver, leading to elevated circulating levels of IGF-1, which in turn stimulates ovarian thecal cell androgen production (Hopkinson *et al.,* 1998). Hyperinsulinaemia reduces the hepatic production of sex hormone-binding globulin, thus increasing free testosterone levels (Cassar *et al.,* 2016). In combination, hyperinsulinemia and hyperandrogenaemia can disrupt follicle growth, accompanied by menstrual irregularity, anovulatory sub-fertility and accumulation of immature follicles (Diamanti-Kandarakis and Dunaif, 2012).

Women with PCOS commonly have postprandial dysglycaemia (Legro *et al.,* 1999), which reflects peripheral, primarily skeletal muscle IR. In lean women with PCOS, hyperinsulinemia is often evident postprandially but not in the fasted state; IR and compensatory hyperinsulinemia have also been proposed as a cause of reactive hypoglycemia in women with PCOS (Morales *et al.,* 1996).

1.2.3.4. Hyperandrogenism

Hyperandrogenism is the defining feature of women with PCOS. It is caused by the disruption of normal ovarian or adrenal function resulting in the production of excess androgens. The first impact of androgen excess in PCOS is impaired folliculogenesis. Increased androgens in the early gonadotropin-independent stage stimulate the formation of primordial follicles and increase the number of small antral follicles. Usually, the gonadotropin-releasing hormone is secreted in a pulsatile manner by the hypothalamus that stimulates the pituitary gland to release gonadotrophins, such as LH and FSH (Nisenblat and Norman, 2009).

Luteinizing hormone acts primarily on the ovarian theca cells carrying LH receptors and induces the production of androgens. Concomitantly, FSH acts on the ovarian granulosa cells and converts the androgens formed in theca cells into estrogens, principally estradiol, which is responsible for developing follicles. However, in women with PCOS, it has been hypothesized that dysregulation in the neuroendocrine system leads to an imbalance in the hypothalamic-pituitary-ovarian axis, leading to the overproduction of gonadotrophins. An increased hypothalamic GnRH favors the production of the β-subunit of LH over the β-subunit of FSH, favoring LH over FSH,

hence resulting in the classical hormonal hallmark of elevated LH/FSH ratio in PCOS. Owing to the increased LH stimulation, numerous follicles in the theca cells of ovaries get arrested mostly in the preantral and antral stages, causing hyperplasia of theca cells and subsequent accumulation of follicular fluid forming cyst-like structures along the periphery of the ovary, giving it a string of pearls-like appearance (Ashraf *et al.*, 2019).

An increased number of follicles and increased expression of key enzymes involved in the androgen synthesis thus produce an excessive amount of androgens, as shown in (Fig.1-2).

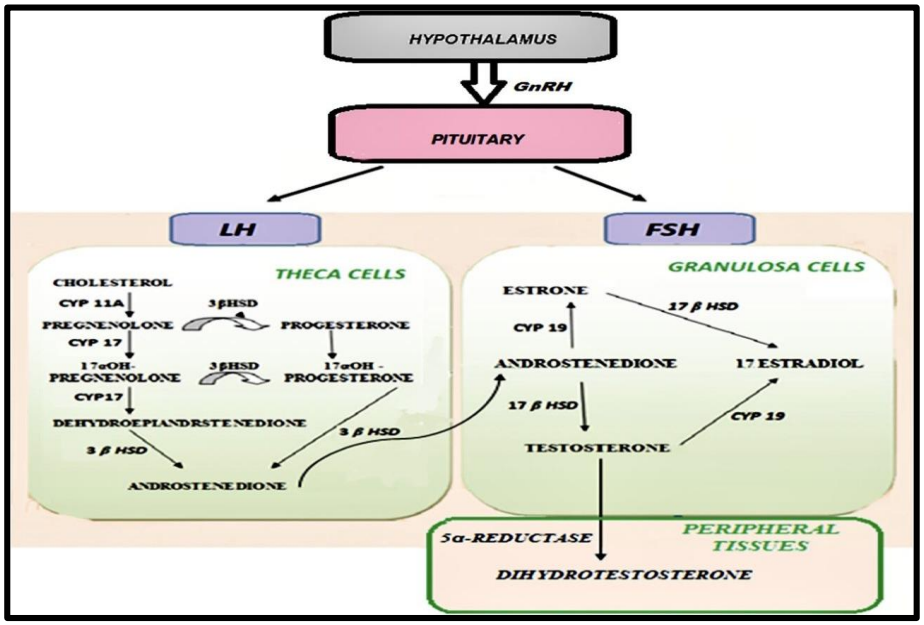


Figure (1-2): Hypothalamic-Pituitary-Ovarian Axis and Steroidogenesis (Ashraf *et al.*, 2019)

Furthermore, the hyperandrogenic state in PCOS also seems to be linked with the action of insulin. The increased insulin secretion possibly mimics the tropic action of luteinizing hormone on ovarian theca cells (Wu *et al.*, 2014), which further causes an increase in androgens; this is further validated by the fact that the improvement of insulin resistance in PCOS women decreases the level of hyperandrogenism (Baillargeon *et al.*, 2004).

1.2.3.5. Obesity

The effect of Obesity on PCOS and PCOS on obesity is complex, and strong evidence of an association is currently lacking. Although PCOS occurs in obese and lean

women, a systematic review and meta-analysis concluded that obesity was more prevalent in women with PCOS than women without PCOS (Lim *et al.*, 2012).

Women with PCOS are likelier to have upper-body fat distribution than weight-matched controls. More excellent abdominal or visceral adiposity is associated with greater insulin resistance, which could exacerbate PCOS's reproductive and metabolic abnormalities (Lord *et al.*, 2006).

It is known that obesity is associated with PCOS, but its causal role in this condition has yet to be determined. Very few studies report the association of BMI with menstrual irregularity. Few randomized controlled studies have been performed on lifestyle interventions, suggesting substantial reproductive and metabolic benefits (Moran *et al.*, 2009; Moran *et al.*, 2010).

1.2.3.6. Chronic Low- Grade Inflammation

Several studies have focused on the effects of chronic low_grade inflammation in PCOS. Some investigations have suggested that in some aspects, PCOS- related inflammation may depend on visceral adipose tissue (Garruti *et al.*, 2009).

This is a crucial point, considering that insulin resistance- related glucose ingestion in PCOS can induce an inflammatory response that increases nuclear factor-κB (NF- κB) activation and oxidative stress. It has been made clear that oxidative stress has an essential role in chronic low-grade inflammation and can be significantly augmented in PCOS by the expression of pro-inflammatory cytokines (Park *et al.*, 2009). It is conceivable to hypothesize that alteration in gene expression of pro-inflammatory cytokines, in turn, plays a role in the development of PCOS. Considerable evidence indicates that some polymorphisms in the inflammation- related genes encoding tumor necrosis factor- α (TNF- α), TNF receptor 2 (TNFR2), and interleukin- 6 (IL- 6) are associated with hyperandrogenism and PCOS. Along the same lines, inflammatory factors, including high sensitivity C- reactive protein (hsCRP), IL- 1β, IL- 18, and WBC count, have shown increased levels in PCOS (Escobar- Morreale *et al.*, 2003). On the other hand, more detailed studies showed WBC count to be one of the essential inflammatory variables affected by increased high body mass index (BMI) fatty acid. Moreover, obesity, metabolic syndrome, and insulin resistance have been reported to increase inflammatory mediators (Kim *et al.*, 2008).

Increasing evidence proposes that subclinical inflammation is a part of metabolic syndrome. Some features of metabolic syndrome, including visceral obesity, are correlated with low- grade inflammation. Several studies pointed out that different metabolic syndrome components correlate to inflammatory markers, including CRP, fibrinogen, and white cell count. High-sensitivity C-reactive Protein (hs-CRP) levels tend to increase in insulin resistance and obesity subjects. On the other hand, dyslipidemia, hypertension, low insulin sensitivity, and abdominal obesity increase CRP levels. In addition, glucose and macronutrient intake lead to inflammatory changes. In contrast, insulin displays anti- inflammatory effects (Paoletti *et al.*, 2006; Dulloo and Montani, 2012; Esser *et al.*, 2014).

1.2.4. Pathogenesis of PCOS

The exact mechanism of PCOS development is not yet completely elucidated; however, there are several hallmarks of abnormal function in women with PCOS. Pathophysiological abnormalities in gonadotropin secretion and ovarian folliculogenesis are well-known; however, steroidogenesis, abnormal or impaired insulin secretion or action, and abnormal adipose tissue function have also been described in PCOS. In the hypothalamus and pituitary, women with PCOS have increased gonadotrophin secretion of luteinizing hormone (LH), as well as increased LH pulse amplitude and frequency; increased LH pulses and overall increased day time secretion of LH are observed early during puberty in girls with hyperandrogenism, which may indicate that abnormalities of LH may be a primary defect in PCOS. This increased LH secretion stimulates androgen production in the ovarian theca cells, leading to hyperandrogenism in these females (Azziz, 2018).

Follicles within the ovary have also been noted to have increased resistance to follicle-stimulating hormone (FSH), which may contribute to the pathophysiology of PCOS. In most women with PCOS, LH to FSH ratios are inverted from average, with LH increasing, usually three times that of FSH. In addition, the ovaries excrete high levels of the anti-Mullerian hormone, a glycoprotein made in granulosa cells by preantral follicles, which may contribute to the disorder (Azziz *et al.*, 2016).

Studies indicate a relationship between the higher concentration of androgens and white blood cell count, so chronic low-grade inflammation may be mediated through

adiposity and androgens concentration. Further studies are needed to establish those relationships, especially the association of new polymorphisms of genes that play significant roles in the pathophysiologic mechanism implicated in the inflammatory process in PCOS. Adipose tissue releases more than 50 cytokines, acute-phase proteins, and other inflammatory mediators, which have an autocrine, paracrine, or systemic function and influence glucose metabolism, energy balance, and pro-inflammatory or anti-inflammatory activities. In PCOS, there is evidence of a low-grade chronic inflammation reflected by minor but significant increases in circulating levels of these mediators. These products have been linked to the development of metabolic and ovarian dysfunctions of the syndrome, such as insulin resistance, type 2 diabetes mellitus, cardiovascular risk factors, hyperandrogenism, and anovulation. Low-grade chronic inflammation in women with PCOS was initially thought to be related to a higher serum tumor necrosis factor-alpha (TNF-α) concentration, independently of Obesity (Gonzalez *et al.,* 1999).

However, a meta-analysis including nine studies has shown similar serum TNF-α levels in 726 women with PCOS compared with 328 controls. The same meta-analysis also found no significant differences in serum interleukin 6 (IL6) levels in women with PCOS control; however, evidence of publication bias favoring studies underestimating the differences in the mediator's levels between the groups was observed in that meta-analysis (Escobar-Morreale, Luque-Ramírez and González, 2011).

Other studies report increased concentrations of some circulating cytokines, such as IL18(Escobar-Morreale, Luque-Ramírez and González, 2011), IL1b, IL7, and IL17 (Knebel *et al.,* 2008); monocyte chemotactic protein 1 (MCP1); macrophage inflammatory protein-1alpha; macrophage migration inhibitory factor (Glintborg and Andersen, 2010); matrix metalloproteinases two and; WBC counts (Orio Jr *et al.*, 2005); soluble intercellular adhesion molecule 1; and soluble endothelial leukocyte adhesion molecule (Diamanti-Kandarakis *et al.,* 2006). Regarding serum CRP concentrations, increased levels in women with PCOS relative to those in healthy women after adjustment for BMI. They also noted that serum CRP concentrations in both PCOS and controls correlated positively with the degree of obesity and inversely with insulin sensitivity, although not with total testosterone concentrations (Kelly *et al.*, 2001). Thus, CRP elevations attributed to PCOS are obscured by the presence of obesity and are below the range to predict metabolic or cardiovascular risk (González, 2012).

One of the mechanisms of chronic low-grade inflammation in PCOS is related to the hypertrophy of adipocytes that cause compression phenomena in the stromal vessels, leading to adipose tissue hypoperfusion and, consequently, hypoxia. Adipose tissue hypoxia stimulates the activation of nuclear factor kappa B – a family of transcription factors that regulates the expression of many critical genes involved in inflammatory reactions, inducing the production and release of many mediators, such as TNF-α, ILs (IL1b, IL6, IL10, and IL18), transforming growth factor beta and interferon-gamma, factors of the complement cascade, Circulating Vascular Cell Adhesion Molecule-1 (sVCAM1), and MCP1. Results in the recruitment of macrophages into the adipose tissue, maintaining the inflammatory state, impairing adipose cell function, and leading to cell necrosis (Deligeoroglou *et al.*, 2012).

Furthermore, IL6 and IL1b stimulate the synthesis of CRP in the liver and promote the uptake of lipids into foamy macrophages within atherosclerotic plaques. Thus, cytokines and acute-phase proteins play a crucial role in the pathophysiological mechanisms of vascular endothelium and the development of atherosclerosis (González *et al.*, 2014a).

Moreover, in PCOS women, glucose ingestion seems to activate oxidative stress and induce the release of TNF alpha, IL6, and CRP (González *et al.*, 2014b). Similarly to obese subjects, lean women with PCOS present increased reactive oxygen species (ROS) generation compared with lean controls. ROS-induced oxidative stress is a known activator of Nuclear factor κ B (NF-kB), stimulating inflammatory reactions. These findings support that glucose ingestion promotes an obesity-independent pro-atherogenic inflammation with a systemic response in PCOS. Insulin resistance and hyperandrogenism may result from inflammation triggered by hyperglycemia and contribute to atherogenesis in PCOS (González, 2012; González *et al.*, 2014a).

1.2.5. Complications in Polycystic Ovary Syndrome

a. Reproductive Complications: Most patients with PCOS experience oligoanovulation, which results in subfertility associated with ovulatory dysfunction. Once they conceive, women with PCOS do not appear to demonstrate an increased risk for miscarriages or early pregnancy loss. However, they seem to be at increased risk for various obstetric

complications, including pregnancy-induced hypertension, gestational diabetes mellitus, and macrosomia (Zore *et al.*, 2017; Azziz, 2018).

b. Abnormal Glucose Tolerance (Impaired Glucose Tolerance or Type 2 diabetes): The overall risk of developing type 2 diabetes and impaired glucose tolerance (IGT) appears to be 3- to 7-fold higher in women with polycystic ovary syndrome (PCOS) compared with the average female population, and the onset of glucose intolerance in PCOS women seems to occur at an earlier age than in their healthy counterparts, usually already in the third–the fourth decade of life. Abdominal adiposity, unfavorable lipid profile, IGT, and insulin resistance, particularly in obese individuals and positive family history of diabetes are only some examples of risk factors for the development of type 2 diabetes carried by women with PCOS (Hudecova *et al.,* 2011).

c. Metabolic Syndrome: Several features of metabolic disturbances, insulin resistance and hyperinsulinemia have been observed in most women with PCOS. Therefore, Obesity and visceral Obesity are very common among women with PCOS, which is associated with metabolic syndrome (MetS). MetS is a cluster of endocrinopathy and metabolic disturbances, including hyperglycemia/insulin resistance, central obesity, dyslipidemia and hypertension. In this respect, some studies report that MetS is more common among women with PCOS due to the higher prevalence of insulin resistance, Obesity and visceral Obesity in these patients (Behboudi- Gandevani *et al.,* 2018).

d. High Blood Pressure: Data have been conflicting, but a large Kaiser Permanente study demonstrated that hypertension or elevated blood pressure was more than twice as common in women with polycystic ovary syndrome (27% vs. 12%) (Lo *et al.,* 2006).

e. Dyslipidemia: dyslipidemia is a prevalent metabolic abnormality in women with PCOS, with a prevalence of up to 70%. Insulin resistance is critical pathophysiology of PCOS, and dyslipidemia in women with PCOS may therefore be consistent with that found in the insulin-resistant state: decreased levels of high-density lipoprotein-cholesterol (HDL-C) and apolipoprotein (Apo) A-I, and increased levels of triglycerides (TG), ApoB and very low-density lipoprotein (Kim and Choi, 2013).

f. Nonalcoholic Fatty Liver Disease and Steatohepatitis: have been recognized as potential complications in women with polycystic ovary syndrome (Setji *et al.,* 2006).

g. Malignancy: The combination of oligo-anovulation and hyperinsulinemia places patients with PCOS at increased risk for endometrial hyperplasia and carcinoma. Consequently, endometrial biopsies should be considered liberally in patients with PCOS with a long-term history of untreated oligo-anovulation, particularly if endometrial thickness on ultrasonography is increased. Patients with PCOS may also be at increased risk for ovarian, but not breast, cancer (Zore *et al.,* 2017).

h. Obstructive Sleep Apnea (OSA): In several studies, the prevalence of OSA in women with PCOS has been considerably higher than in the general population. Although the pathophysiological mechanisms are not fully understood, obesity, insulin resistance and hyperandrogenemia, which are the main features of PCOS, are all proposed to play a role in the development of OSA in patients with PCOS (Helvaci *et al.,* 2017).

i. Cardiovascular Disease: Many studies demonstrate abnormal surrogate markers of cardiovascular disease in women with polycystic ovary syndrome. However, data about cardiovascular disease risk conflict with some studies suggesting an increased risk in women with polycystic ovary syndrome. Other studies have not found this difference in cardiovascular risk. While it is essential to recognize and treat cardiovascular risk factors in this population, additional cardiovascular risk and complications research is still needed to clarify the long-term risk (Setji, Lowry and Brown, 2006).

1.2.6. Diagnosis

1.2.6.1. Family History

The family history should include information regarding infertility, menstrual disorders and hirsutism in female relatives, early baldness in male relatives, and features of the metabolic syndrome, including obesity, glucose intolerance, diabetes, hypertension, cardiovascular disease and stroke (Harwood, Vuguin and DiMartino-Nardi, 2007).

1.2.6.2. Past Medical History

Past medical history should include birth, weight and the timing of pubarche, as both low birth weight and premature adrenarche have been identified as risk factors for PCOS. Obese patients should also be screened for the comorbidities of obesity, such as obstructive sleep apnea (OSA) symptoms, orthopedic problems, pseudotumor cerebral, diabetes mellitus, nonalcoholic steatohepatitis (NASH), depression and exercise intolerance (Harwood, Vuguin and DiMartino-Nardi, 2007).

1.2.6.3. Clinical Examination

In adults, PCOS is a syndromic disease, and patients are on a clinical spectrum; as such, it has been difficult to agree on diagnostic criteria. There have been three prior efforts to classify and diagnose PCOS. The most effort was a task force appointed by the Androgen Excess and PCOS Society (AE-PCOS) in 2006. This meeting led to the conclusion that PCOS needing (1) hyperandrogenism (hirsutism or hyperandrogenemia), (2) ovarian dysfunction (oligo-anovulation or polycystic ovary), and (3) exclusion of other androgen excess or related disorders (Azziz *et al.*, 2009).

The Rotterdam criteria were the first to account for the large spectrum of disorders that women may face and allow for the inclusion of many. Under the Rotterdam criteria, a woman only needs two of the three criteria, so she need not have polycystic ovaries, hyperandrogenism, or menstrual abnormalities. Now the Rotterdam criteria are the most commonly accepted and used. Because of the clinical variation seen in patients, there is a desire for evidence-based criteria; however, there is not currently an existing epidemiologic or basic research basis robust enough to support more conclusive diagnostic criteria; all 3 of the currently used criteria are based on expert opinion alone (Lizneva *et al.*, 2016).

1.2.6.4. Radiographic Studies

A diagnosis of PCOS rarely requires the use of ultrasound to confirm polycystic-appearing ovaries. As symptom management is the focus of PCOS, ultrasound adds little clinical value. However, an ultrasound may be warranted for investigating a pelvic mass, infertility, or pelvic pain. When possible, the ultrasound must be performed using an endovaginal ultrasound probe. Furthermore, obtaining an antral follicle count in each

ovary (all follicles 2 to 9 mm) is vital since the ultrasound diagnostic criteria for PCOS were established by reproductive endocrinologists rather than radiologists. Finally, there is a significant overlap between the diagnoses of polycystic-appearing ovaries and normal ovaries, with 30% to 50% of women younger than 30 having 12 or more follicles per ovary, which indicates that a polycystic-appearing ovary is not pathognomonic of PCOS (Dewailly *et al.,* 2014).

1.2.6.5. Laboratory Evaluation

In addition to history, physical examination, and pelvic ultrasonography, laboratory evaluation is also appropriate in a woman presenting with complaints consistent with PCOS. Before diagnosing patients with PCOS, it is essential to rule out other mechanisms for anovulation, as they frequently present with similar clinical symptoms. Conditions to be evaluated include hyperprolactinemia, thyroid disease, pituitary tumors inhibiting gonadotropin secretion, central inhibition (stress, weight loss, physical stress, eating disorders—these conditions typically result in amenorrhea, but lesser degrees of insult may result in intermittent anovulation). A general evaluation should include a prolactin level, thyroid-stimulating hormone level, dehydroepiandrosterone-sulfate, lipid panel, testosterone panel, glucose tolerance test and if clinically indicated, 24-hour urine-free cortisol screen for Cushing disease and 17-hydroxyprogesterone for non-classic Congenital Hyperplasia (table.1) (Azziz, 2018).

Documentation of ovulation with a serum progesterone level cycle days 22 to 24 is applicable. Rarely, the severe insulin-resistance syndrome may present with similar symptoms, in which case consultation with a specialist and an insulin level screen is recommended. An anti-Mullerian hormone level may be drawn as a surrogate for antral follicles but is not necessary for diagnosing PCOS (Rollene *et al.,* 2021).

1.2.6.6. Endometrial biopsy

Prolonged oligo-amenorrhea or amenorrhea, as well as the presence of hyperinsulinemia, puts women with PCOS at increased risk of endometrial hyperplasia, and endometrial tissue with an endometrial biopsy should be considered in women with a long history of untreated oligo-amenorrhea, particularly if an increased endometrial thickness is noted on ultrasound (Azziz, 2018).

Table (1-1): Hormonal Testing in Women with Suspected Polycystic Ovary Syndrome (Azziz, 2018)

Hormonal Testing
Hyperandrogenism: **Total and free testosterone** **Dehydroepiandrosterone-sulfate (DHEA-S)**
Ovulatory dysfunction: **Progesterone cycle day 22-24** **Anti-Mullerian hormone (AMH)**
Excluding similar disorders: **Thyroid-stimulating hormone (TSH)** **Prolactin** **17-hydroxyprogesterone (17-OHP)** **Oral glucose tolerance test GTT** **Lipid panel** **24-h urine free cortisol**

1.2.7. Role of Interleukin-17 in Patients with Polycystic Ovary Syndrome

1.2.7.1. The IL-17 Family

There are six members in the interleukin 17 (IL-17) cytokine family, including IL-17A (commonly referred to as IL-17), IL-17B, IL-17C, IL-17D, IL-17E (also known as IL-25) and IL-17F. Among all the members, the biological function and regulation of IL-17A and IL17F are best understood. These two cytokines share the most substantial sequence homology. The genes encoding IL-17A and IL-17F are close on the same chromosome in both mice and humans, underscoring their shared patterns of expression

(Wang *et al.,* 2012). IL-17A and IL-17F mediate pro-inflammatory responses, with specific differences depending on the type and site of inflammation (Yang *et al.,* 2008; Ishigame *et al.,* 2009). IL-25 has the slightest sequence similarity with IL-17A, only 16% compared with 50% in the case of IL-17F. Correspondingly, IL-25 plays distinct roles in immunity, mainly regulating the T helper (Th2) response against helminthic parasites and allergic inflammation (Fort *et al.,* 2001; Owyang *et al.,* 2006; Fallon *et al.,* 2006). IL-17B, IL-17C and IL-17D have been shown to induce the production of pro-inflammatory cytokines, but their biological function is mainly unknown (Li *et al.,* 2000; Wu *et al.,* 2007; Yamaguchi *et al.,* 2007). Some studies by three groups highlighted the function of IL-17C in mucosal immunity and autoimmune responses (Ramirez-Carrozzi *et al.,* 2011; Song *et al.,* 2011; Chang *et al.,* 2011).

Also, IL-17 family cytokines mediate their biological functions via surface receptors on target cells. IL-17RA was the first identified IL-17 receptor, and four other IL-17R family members, IL-17RB, IL-17RC, IL-17RD and IL-17RE, were subsequently identified, primarily based on their sequence similarity with IL-17RA. Functional receptors for IL17 family cytokines often exist in heterodimers, with IL-17RA as a common subunit. For example, the receptor complex consisting of IL-17RA and IL-17RC recognizes IL-17A and IL-17F, whereas IL-17RA pairs with IL-17RB, followed by binding to IL-25 (Iwakura *et al.,* 2011; Chang and Dong, 2011). IL-17RE was identified as a receptor for IL-17C in complex with IL-17RA (Chang *et al.,* 2011; Ramirez-Carrozzi *et al.,* 2011; Song *et al.,* 2011).

1.2.7.2. IL-17A Producer Cells

Differentiation of Th17 cells, a distinct subpopulation of CD4+ T helper cells, are specialized by uniquely identifying differentiation and transcription factors. Tumor necrosis factor-α (TNF-α), IL-1β, transforming growth factor-β (TGF-β), and IL-6 can enhance Th17 differentiation (Wilson *et al.,* 2007). IL-17 does not inhibit Th1 or Th2 differentiation, so Th1 and Th2 cells typically dominate over Th17 cells. TGF-β can promote Th17 differentiation by suppressing the production of the inhibitory cytokines IFN-γ and IL-4. TGF-β synergizes with IL-6 to induce expression of the transcription factor RORγt, a key regulator of Th17 differentiation (Acosta-Rodriguez *et al.,* 2007;

Ivanov, Zhou and Littman, 2007). In humans, retinoic acid receptor-related orphan receptor-γt (RORγt), IFN-regulatory factor 4 (IRF-4), aryl-hydrocarbon receptor (AHR), and the transcription factor signal transducer and activator of transcription 3 (STAT3) expression are induced by IL-1β, IL-6, and IL-23 (Yang *et al.,* 2007; McGeachy and Cua, 2008; O'connor *et al.,* 2013).

Also, IL-17 cytokines are now known to be secreted by other cell types apart from CD8+ T cells, γδ T cells, natural killer T (NKT) cells, natural killer (NK) cells, monocytes, macrophages, dendritic cells (DC), microglia, neutrophils, eosinophil, astrocytes, and oligodendrocytes. Thus, cells of both the innate and the adaptive immune systems and non-immune cells produce IL-17A and IL-17F (Yamada, 2010).

1.2.7.3. IL-17 Signaling Pathways

Studies have demonstrated that IL-17 can activate nuclear factor κB (NF-κB) transcription factors in many cell types, including fibroblasts, macrophages, chondrocytes, intestinal epithelial cells, and colonic and pancreatic myofibroblasts (Korn *et al.,* 2007).

The NF-κB has been activated in response to IL-17D, IL-17E and IL-17F. IL-17 receptor (IL-17R) activates extracellular signal-regulated protein kinase (ERK1 and ERK2), stress-induced c-Jun N-terminal kinases (JNK-1 and JNK-2), and mitogen-activated protein kinases (p38 MAPKs) pathways. These signaling pathways result in the up-regulation of IL-6, IL-1 and NF-κB (Chang and Dong, 2011).

1.2.7.4. IL-17 Role in the Polycystic Ovary Syndrome

Polycystic ovary syndrome is an inflammatory condition. Several studies have found that Polycystic ovary syndrome patients have higher levels of circulating inflammatory molecules. It is unclear whether their increase is caused by Polycystic ovary syndrome or obesity, or abdominal adiposity (Chang and Dong, 2011). Interleukin-17 (IL-17) is a T cell-inferred inflammatory cytokine, delivered principally by Th17 cells, T cells, and neutrophils. IL-17 is also called IL-17A (Chaudhari *et al.,* 2016).

Also, IL-17 causes many cells to produce other pro-inflammatory cytokines and chemokines, which mediate immune responses. IL-17 is a potent inflammatory cytokine that causes tissue inflammation and inflammatory cell infiltration into target organs (Abbas, Alaaraji and Alâ, 2020). CD4 Th17 cells produce IL-17, a pro-inflammatory cytokine that stimulates the production of pro-inflammatory mediators (Mohammed *et al.,* 2021).

Furthermore, IL-17 can enhance neurite outgrowth by acting on distal neurites and sympathetic somata, indicating a new role for IL-17 in the neuroanatomical plasticity, which occurs with inflammation (Zangeneh, Naghizadeh and Masoumi, 2017; Sarhat, Rmaid and Jabir, 2020). This abnormal cytokine pattern plays a significant role in the immunopathogenesis of Polycystic ovary syndrome (Lomax *et al.,* 2011).

In adipose tissue macrophages, IL-17, plays a role in developing the inflammatory state by stimulating the secretion of IL- 1β, TNF- α, and IL- 6. IL- 17A can exert a role in inflammatory processes by affecting adipogenesis and glucose metabolism. Some studies have illustrated elevated IL- 17A and IL- 17F levels in PCOS patients. An increase in the TNF- α level can promote IL- 17A production. Moreover, IL- 17E levels decreased in PCOS subjects compared to healthy subjects. Furthermore, inflammation in the gingiva correlated with IL- 17A levels in PCOS patients. It has been reported that IL- 17A, as a pro-inflammatory cytokine, may be related to infertility in PCOS subjects. To date, there has not been much research on the effect of IL- 17 on PCOS. Nonetheless, it is expected that the combination of drugs from inflammatory cytokines will be taken on the path of treating this disease (Shin, Shin and Noh, 2009).

1.2.8. Role of IL-17 Gene Polymorphism in Patients with Polycystic Ovary Syndrome

Gene expression and SNP profiling tests have immense utility for the prognosis process and the discovery and exploration of therapeutic approaches. Indeed, developing more effective and reliable treatments for PCOS depends on identifying and decoding molecular signaling pathways that might be involved in the mechanisms of this syndrome (Matsui, 2013). With the current pace of advancement in the field of molecular pathology and the continued identification of pathways involved in the mechanisms of PCOS, it is reasonable to expect more use of specialized and unique panels of biomarkers for early

diagnosis and the development of exclusive treatments for different groups of women with PCOS. This trend is expected to lead to even faster identification of biomarkers, which will facilitate the development and use of patient-tailored treatments for women with PCOS (De Leo *et al.,* 2016).

Genotyping of SNPs can be done relatively quickly and at a reasonably low cost via polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP). However, although the results of PCR–RFLP are easy to interpret, this approach may not be the most suitable for genotyping because it involves extensive manual handling and longer pause times (Hashim and Al-Shuhaib, 2019). Polymorphisms can also be analyzed via amplification refractory mutation system–polymerase chain reaction (ARMS-PCR), which can be modified into a one-step procedure based on real-time PCR technology to reduce manual handling (Little, 1995). A third approach is identifying genetic variation in nucleic acid sequences using a post-PCR analysis method called high-resolution melting (HRM). The main advantage of HRM– PCR is its minimal manual handling, which significantly reduces the chance of error; however, the results of HRM–PCR are not as easy to interpret as those of other methods. Overall, it has been suggested that the most economical and least time-consuming approach is either ARMS-PCR or PCR–RFLP. Although these methods involve more handling than HRM-PCR, their results are far easier to interpret and analyze (Ehnert *et al.,* 2019).

Previous studies showed that PCOS is closely related to genetic factors. Studies have shown that the number of pro-inflammatory cells in the body is significantly increased during PCOS, suggesting that the immune system plays a role in PCOS. The human interleukin-17A (IL-17A) gene is located on chromosome 6p12.2 and encodes IL-17A, a major pro-inflammatory cytokine. IL-17A regulates diverse immune functions, such as promoting the production of other pro-inflammatory cytokines and cooperating with other cytokines. The rs2275913, a single nucleotide polymorphism (SNP) located at the promoter region of the IL-17A gene, is associated with the activation of the nuclear factor of activated T cell. Furthermore, it can significantly increase the level of IL-17A. In addition, several studies found that rs2275913 affects an individual's risk of developing different diseases. The occurrence of PCOS is closely related to the inflammation of the body. However, reports on the correlation between rs2275913 and PCOS are scarce. Although a case-control study conducted in Iran found that rs2275913 is associated with

PCOS, more studies from different regions are needed to confirm this finding (Zou *et al.,* 2022).

One of the important SNPs of IL-17A is rs2275913 (A/G). The A allele of this SNP tends to activate the nuclear factor of activated T cell (NFAT), an important transcription factor in IL-17A production, compared with the G variant form (Espinoza *et al.,* 2011).

A study among Iranian women reported that there might be an association between PCOS susceptibility and IL17A rs2275913 and IL32 rs9927163 SNPs. However, another Iranian study found no significant association between PCOS and the rs361525 polymorphism of the TNF gene. According to a meta-analysis of PCOS studies, the risk of this disorder is not associated with the –1031T/C, –308G/A, or –805C/T polymorphism of TNF (Hesampour *et al.,* 2019). Another study reported that there is probably no association between PCOS susceptibility and the –308G/A sequence variation of TNF (Liu *et al.,* 2016).

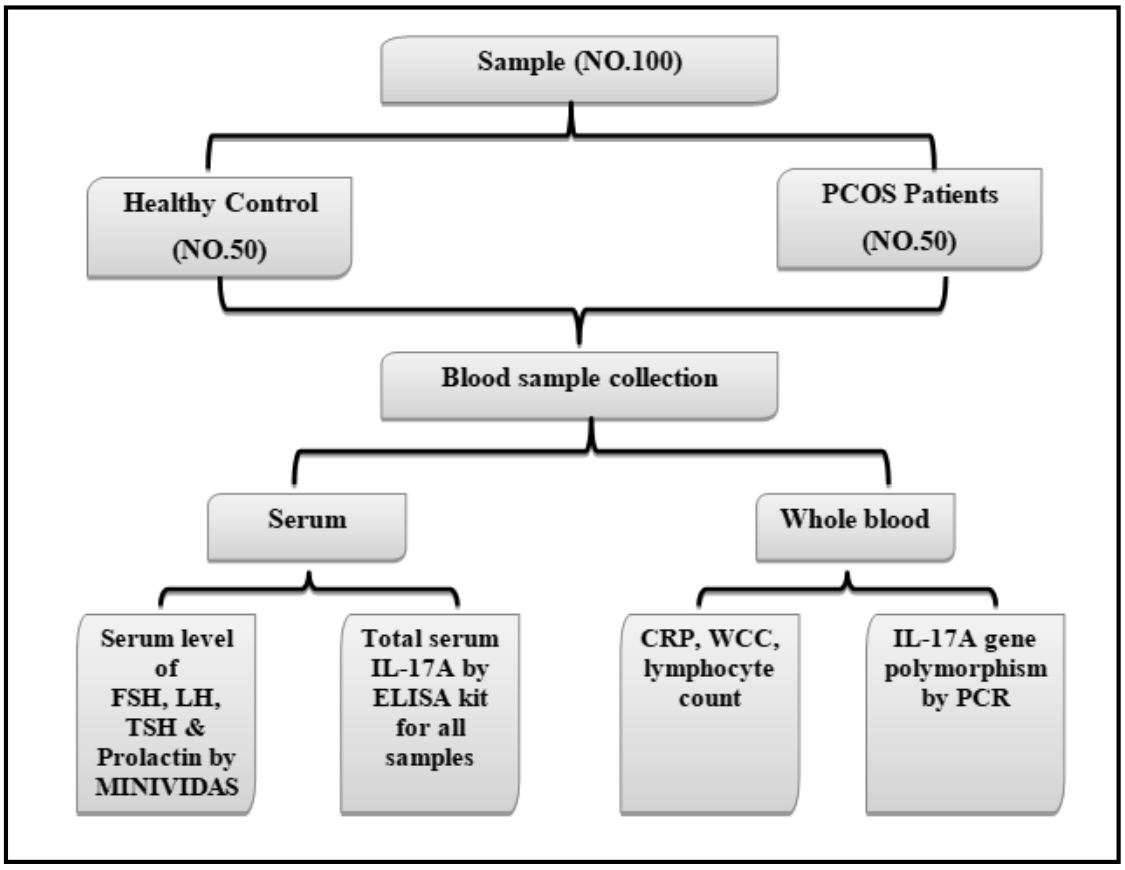
In addition, the genetic variations in inflammation-related genes, especially cytokines and their receptors, may increase the expression of IL-17. Some previous studies have reported that polymorphism of interleukin IL-1β, IL-6, IL-16 and IL-17A is correlated with the pathogenesis of inflammatory diseases (Kaur *et al.,* 2018).

Chapter Two
Materials
and
Methods

2. Study Design, Materials and Methods

2.1. Study Design

The case-control study was done in the Al-Najaf province from November 2021 to April 2022. Scheme of study design as below.



Scheme of study design

2.1.1. Subjects

The study included 100 subjects divided into two groups: The first group is the patient group, which consists of 50 patients with PCOS who attended the private clinic at a gynecological clinic in Najaf city, in AL-Sader Teaching Medical City and AL-Zahraa Teaching Hospital, and were diagnosed by specialized gynecologists according to The Rotterdam Consensus (2003), which requires the presence of at least two of the following characteristics: polycystic ovaries on ultrasound scan, menstrual irregularities and hyperandrogenism. The age group of the patients ranged from (13-48) years. The inclusion criteria for the patients are all with PCOS and no history of immunological disorders such as autoimmunity, immune deficiencies and cancer. The second group is

the control group, consisting of 50 healthy females with no history of pregnancy-related disorders and who had at least one delivery without complications. The control group included women with no family history of PCOS chosen. The exclusion criteria are patients with PCOS. The age group ranged from (14-49) years.

2.1.2. The Descriptive Variable of Subjects

A particular form has created for each subject's data and historical information, which includes age, history, menarche age, Body Mass Index (BMI), ultrasound, hormone analysis, CRP, White Cell Count (WCC), lymphocyte count, menses, occupation, hirsutism, acne appearance, family history of infertility, lack of physical exercise, high blood pressure, depression, sleep apnea, Cardiovascular disease and phone number.

2.1.3. Ethical Issue

Ethical approval has obtained from Karbala Medical College Ethical Committee. Also, verbal approval has been take from the patients before taking the sample**.** During sample collection, health measures and safety will be taken.

2.2. Materials

2.2.1. Instruments and Equipment

The main instruments and Equipment that used in this study were displayed in table (2-1).

Table (2-1): Instrument and Equipment

Instrument and Equipment	Company	Source
Autoclave	**Hirayama**	**Japan**
Biological Cabinet Class II	**Labconco, logic**	**USA**
Burner	**Amal**	**Turkey**
Deep freeze refrigerator	**New Brunswick**	**UK**
Disposable syringe (5ml)	**Medi**	**China**

Distillator	**GFL**	**Germany**
EDTA tube	**Zhongfan-medical**	**China**
Electrophoresis unit	**Biometra**	**Germany**
ELISA automated washer	**Paramedical**	**Italy**
ELISA reader and printer	**Paramedical**	**Italy**
Eppendrof tubes (0.2& 1.5 ml)	**Sterellin Ltd**	**UK**
Face mask	**PRO.care**	**China**
Filter paper	**MEHECO**	**China**
Gel documentation	**Biometra**	**Germany**
Gel Tube	**AFCOVAC**	**Jordan**
Glass Cylinders (250,500&1000 ml)	**Isolab**	**Germany**
Glass Flasks	**Isolab**	**Germany**
Gloves	**AEGIS**	**Malaysia**
Hematology analyzer	**Sysmex**	**Japan**
High-speed cold Microcentrifuge	**Hettich**	**Germany**
Incubator	**Bender**	**Germany**
Micropipette (different size)	**Eppendorf**	**Germany**
Micropipette tips (different sizes)	**Human**	**Germany**
Mini spin centrifuge	**Eppendorf**	**Germany**
MINI VIDAS®	**BioMérieux**	**France**
Nanodrop	**Eppendorf**	**Germany**
PCR system	**Biometra**	**Germany**
Refrigerator	**Kiriazi**	**Egypt**

Sensitive balance	**AND**	**Japan**
Thermo mixer	**Eppendorf**	**Germany**
UV visible	**Analytic Jena**	**Germany**
Vortex	**Thermo Scientific**	**Singapore**

2.2.2. Chemical and Biological Material

The main chemicals that are used in this study were in Table (2-2).

Table (2-2): Chemical and Biological Materials with their Manufacturing Company and Country of Origin

Chemicals and Biological Materials	Manufacturing Company	Country
Absolute Ethanol	**Scharlab**	**Spain**
Agarose	**Pronadisa**	**Spain**
Ethidium bromide	**Intron**	**Korea**
Tris-Borate-EDTA Buffer (TBE buffer)	**HiMedia**	**India**

2.2.3. ELISA Kit Used in the Study

Tests of ELISA used in this study were demonstrated in Table (2-3).

Table (2-3): ELISA Kits used in The Study

ELISA Kit	Manufacturing Company	Country
Human IL17A (Interleukin 17 A) ELISA Kit	**Elabscience®**	**USA**

2.2.3.1. ELISA Kit Content of Human IL-17A

The kit of ELISA contents are presented inTable (2-4).

Table (2-4): Kit Components and Storage of IL-17A

Item	Specifications	Storage
Micro ELISA Plate (Dismountable)	96T: 8Wells ×12 strips 48T: 8Wells ×6 strips 24T: 8Wells ×3 strips 96T*5:5 plate, 96T	-20°C, six months
Reference Standard	96T: 2 Vials 48/24T: 1Vial 96T*5: 10 Vial	
Concentrated Biotinylated Detection Ab (100×)	96T:1 Vial, 120µL 48/24T: 1 Vial, 60µL 96T*5: 5 Vials, 120µL	
Concentrated HRP Conjugate (100×)	96T:1 Vial, 120µL 48/24T: 1 Vial, 60µL 96T*5: 5 Vials, 120µL	-20°C(Protect from light), 6 months
Reference Standard & Sample Diluent	96T/48/24T: 1Vial, 20mL 96T*5: 5 Vials, 20mL	2-8 °C, 6 months
Biotinylated Detection Ab Diluent	96T/48/24T 1Vial, 14mL 96T*5: 5 Vials, 14mL	
HRP Conjugate Diluent	96T/48/24T: 1Vial, 14mL 96T*5: 5 Vials, 14mL	
Concentrated Wash Buffer (25×)	96T/48/24T: 1Vial, 30mL 96T*5: 5 Vials, 30mL	
Substrate Reagent	96T/48/24T: 1Vial, 10mL 96T*5: 5 Vials, 10mL	2-8 °C (Protect from light)
Stop Solution	96T/48/24T: 1Vial, 10mL 96T*5: 5 Vials, 10mL	2-8 °C
Plate Sealer	96T/48/24T: 5 Pieces 96T*5: 25 Pieces	
Product Description	1copy	
Certificate of Analysis	1copy	

2.2.4. DNA Extraction Kit

Table (2-5) demonstrated contents of DNA extraction kit.

Table (2-5): Kit Components of DNA Extraction

Label	Contents (100 Columns)	Manufacturing Company	Country
RBC Lysis Buffer	135 mL	FAVORGEN Biotech Corp.	Taiwan
FATG Buffer	30 mL		
FABG Buffer	40 mL		
W 1 Buffer	45 mL		
Wash Buffer* (Concentrate)	25 mL		
Elution Buffer	30 mL		
FABG Mini Column	100 Pieces		
Collection Tube	200 Pieces		
User Manual	1		
Preparation of Wash Buffer by adding ethanol (96 ~ 100 %)			
*Ethanol volume for Wash Buffer		100 mL	

2.2.5. Polymerase Chain Reaction Kits

The kit of PCR contents of gene amplification was demonstrated in Table (2-6).

Table (2-6): Reagent and Equipment Required for PCR

Reagent and Equipment of PCR	Volume	Company	Country
GoTaq® G2 Master Mixes	2 × 1.25 ml	Promega	USA
Nuclease free water	2 × 1.25 ml		
Forward primer	30 nmol	Macrogen	South Korea
Reverse primer	30 nmol		
DNA Markers	500 µL	abm® Applied BiologicalMaterials	Canada

2.2.6. Instrument and Equipment of RT-PCR

The main instruments and Equipment that used in this study were listed in Table (2-7).

Table (2-7): Instrument and Equipment of RT-PCR

Instrument and Equipment	Company	Source
AURA TM PCR Cabinet	EuroClone	Italy
Balance	Kernpfb	Germany
Biopette Variable Volume 0.5-10 µL Volume 2-20 µL Volume 10-100 µL Volume 100-1000 µL	Labnet	Germany
Combi-spin	Biosan	Native
Electrophoresis	CBS, Scientific	USA
Incubation	Jrad	Chain
Micro spin 12, High-speed Mini-centrifuge	Bio San	Germany
Microwave	Gosonic	Chain
Mini-Power Supply 300V, 2200V	Supplier	Chain
Multi Gene Opti Max Gradient Thermal Cycler	Labnet	USA
RT-PCR	Sacace	Italy
UV transmission	Vilber lourmat	France
V-1 plus, Personal Vortex for tubes	Digsystem	Germany

Water distilater	**Rovsun**	**Chain**

2.2.7. Chemical and Biological Materials of RT-PCR

The main chemicals that are used in this study were illustrated in Table (2-8).

Table (2-8): Chemical and Biological Materials with their Manufacturing Company and Country of Origin

Material	Cat #	Company
2X Go Taq® G2 Master Mix	**0000437101**	**Promega / USA**
Agarose	**8100.11**	**Conda / USA**
Ladder 50 plus bp	**24072**	**Intron / Korea**
Red safe staining solution	**21141**	**Intron / Korea**
SNP ID: 20X Assay Working	**rs2275913**	**Thermo Fisher Scientific / USA**
TBE buffer 10 X	**IBS.BT004**	**Conda / USA**

2.3. Methods

2.3.1. Sample Collection

Blood samples were drawn 10 ml from the veins of 100 subjects (patients and control) by using a disposable syringe and a sterilization technique. Three ml of blood were dispensed into 2 EDTA tubes (1.5 ml of blood into each tube) for the hematological and molecular test and stored at -20 °C for PCR to determine the presence or absence of IL-17A gene polymorphism in studying of the samples of the blood.

The 7 ml of the remaining blood was allowed to clot after that serum was separated by centrifugation at 3000 rpm for 10 minutes. Serum was collected in an Eppendorf tube and then divided into 2 groups, one group stored at -20 °C for an ELISA test to determine the concentration of IL-17A. The remaining group was used to determine the LH, FSH, TSH and prolactin concentrations by Mini Vidas®

2.3.2. Estimation of IL-17A

2.3.2.1. Test Principle of IL-17A

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate in this kit has been pre-coated with an antibody specific to Human IL-17A. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for human IL-17A and Avidin-Horseradish Peroxidase (HRP) conjugate is added successively to each microplate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain human IL-17A, biotinylated detection antibody and Avidin-HRP conjugate will appear blue. Adding a stop solution terminates the enzyme-substrate reaction, 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-17A. You can calculate the concentration of human IL-17A in the samples by comparing the OD of the samples to the standard curve.

2.3.2.2. Reagent Preparation

1. All the reagents were brought to room temperature (18-25C^{o}) before use.

2. Wash buffer was prepared by adding 30 ml of Buffer to 720 ml of distilled water to form 750 ml of wash buffer.

3. Standard working solution**:** Centrifuge the standard at 10,000g for 1 min. After N1.0 mL of Reference Standard and Sample Diluent has been added, let it stand for 10 min and invert it gently several times. After it had completely dissolved, I thoroughly mixed it with a pipette. This reconstitution produces a working solution of 2000 pg/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 2000, 1000, 500, 250, 125, 62.5, 31.25, 0 pg/mL.

Dilution method: 7 EP tubes were taken, and 500 μL of Reference Standard and Sample Diluent were added to each tube. Pipetted 500 μL of the 2000 pg/mL working solution

into the first tube and mixed it to make a 1000 pg/mL working solution. Pipetted 500 µL of the solution from the former tube into the latter one according to these steps (Figer 2-1).

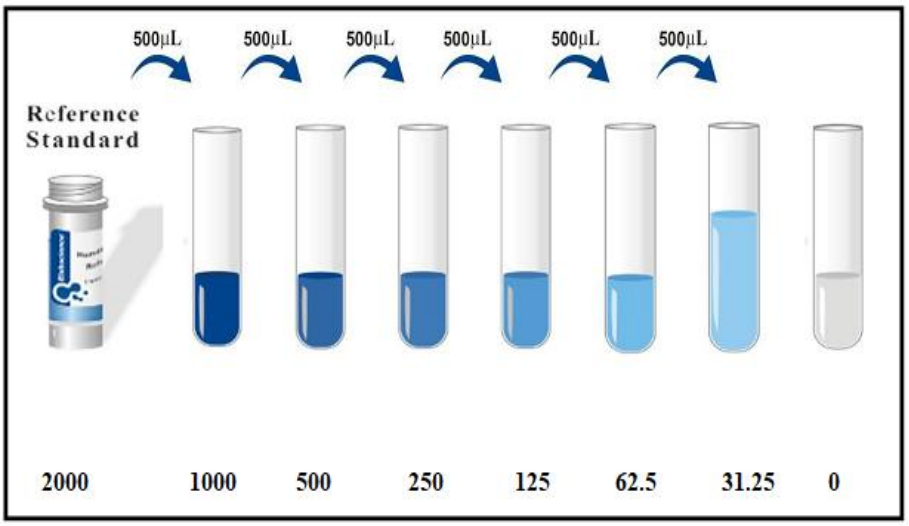


Figure (2-1): Steps of the Duplication Dilution.

4. Biotinylated Detection of Ab working solution, each well needs 100 µl for 96 wells prepared more than 9600 µl.

5. Concentrated Horseradish peroxidase (HRP) Conjugate working solution: It also needs 100 µl per well, so it was prepared slightly more than the calculated amount.

2.3.2.3. Assay Procedure

1. Sample: 100µL of Standard, Blank, or Sample was added per micro ELISA plate well. The blank well was filled with Reference standards and Sample diluent. After that, solutions were mixed gently, covered on the plate with sealer, and then incubated for 90 minutes at 37°C and made wash to remove unbound Reagent.

2. Biotinylated Detection Ab: The liquid of each well was removed, and immediately 100µL biotinylated Detection Ab working solution was added to each well and covered with the plate sealer incubated for 1 hour at 37°C.

3. Wash: All plate wells were aspirated and washed, repeated three times. The wash was done by filling each well with Wash Buffer (approximately 350 µL) by using a squirt bottle.

4. HRP Conjugate:100µL of HRP Conjugate working solution was added to each well, covered with the plate sealer, and then incubated for 30 minutes at 37°C.

5. Wash: The wash process was repeated five times in step 3.

6. Substrate Reagent: 90 µL of Substrate Solution was added to each well and covered with a new Plate sealer, then incubated for about 15 minutes at 37°C.

7. Stop: 50 µL of Stop Solution was added to each well. Then, the color turns yellow immediately.

8. OD Measurement: for determining the optical density (OD value) of each well at once, a microplate reader set at 450 nm was used.

2.3.2.4. Results Calculation

The ELISA results were calculated based on the readings for each standard and the sample's optical density. Then create a standard curve by plotting the OD value for each standard on the y-axis against the concentration on the x-axis and draw a best-fit curve through the points on the graph in the excel office program.

1. First, calculate the OD value for each standard and sample, then construct the standard curve.

2. Determine the amount of IL17A in each sample by locating the OD according to their site in the curve.

2.3.3. Hormone Evaluation

2.3.3.1. Test Principle of (FSH, LH, Prolactin and TSH)

The VIDAS® (FSH, LH, Prolactin and TSH) assay is an enzyme-linked fluorescent immunoassay (ELFA) performed in an automated instrument. The instrument controls all assay steps and assay temperature. A pipette tip-like disposable device, the Solid Phase Receptacle (SPR), serves as a solid phase for the assay and a pipetting device. The SPR® was coated at manufacture with mouse monoclonal anti-(FSH, LH, Prolactin or TSH) antibodies. The VIDAS hormone assay configuration prevents nonspecific reactions with the SPR. Reagents for the assay were located in the sealed Reagent Strips. The sample is transferred into the well containing an anti-hormone antibody conjugated

with alkaline phosphatase. The sample/conjugate mixture was cycled in and out of the SPR, and the hormone will bind to antibodies coated on the SPR and to the conjugate forming a "sandwich." Wash steps and remove unbound conjugate. A fluorescent substrate, 4-methylumbelliferyl phosphate, is cycled through the SPR. Enzymes remaining on the SPR wall catalyzed the conversion of the substrate to the fluorescent product 4-methylumbelliferone. The optical scanner in the instrument measures fluorescence intensity; it is proportional to the hormone concentration in the sample. When the VIDAS hormone assay is completed, the results were analyzed automatically by the instrument, and a report is printed for each sample.

2.3.3.1.1. Assay Procedure for FSH

1. Necessary components have removed from the kit, and all unused components returned to storage at 2-8°C.

2. Components have allowed to reach room temperature (approximately 30 minutes).

3. One "FSH" strip and one "FSH" SPR have used for each sample, control, or calibrator to be tested. The storage pouch has carefully resealed after removing the required SPRs.

4. The test has identified by the "FSH" code on the instrument. The calibrator must be identified by "S1" and tested in duplicate. If the control is to be tested, it should be identified by "C1".

5. If needed, the "FSH" Reagent Strips have labeled with the appropriate sample identification numbers.

6. The calibrator, control, and sample have mixed using a vortex-type mixer (for serum or plasma separated from the pellet).

7. The calibrator, control, and sample test portion for this test was 200 µl.

8. The "FSH" Reagent Strips and SPRs have inserted into the appropriate position on the instrument.

9. The assay processing has initiated as directed in the Operator's Manual. All the assay steps performed automatically by the instrument.

10. The vials have been enclosed and returned to the required temperature after pipetting.

11. The assay has completed in approximately 40 minutes. After the assay was completed, the SPRs and strips removed from the instrument.

12. The used SPRs and strips have disposed of in an appropriate recipient.

2.3.3.1.2. Assay Procedure for LH

1. Necessary components were removed from the kit, and all unused components returned to storage at 2-8°C.

2. Components have allowed to reach room temperature (approximately 30 minutes).

3. One "LH" strip and one "LH" SPR have used for each sample, control, or calibrator to be tested. The storage pouch has carefully resealed after removing the required SPRs.

4. The test has identified by the "LH" code on the instrument. The calibrator must be identified by "S1" and tested in duplicate. If the control needs to be tested, it should be identified by "C1".

5. If needed, the "LH" Reagent Strips have labeled with the appropriate sample identification numbers.

6. The Calibrator, Control, and sample have mixed using a vortex-type mixer (for serum or plasma separated from the pellet).

7. The calibrator, control, and sample test portion for this test was 200 µl.

8. The "LH" Reagent Strips and SPR®s have inserted into the appropriate position on the instrument.

9. The assay processing has initiated as directed in the Operator's Manual. All the assay steps performed automatically by the instrument.

10. The vials have reclosed and returned to the required temperature after pipetting.

11. The assay have completed in approximately 40 minutes. After the assay was completed, the SPRs and strips removed from the instrument.

12. The used SPRs and strips have disposed of in an appropriate recipient.

2.3.3.1.3. Assay Procedure for Prolactin

1. Necessary components removed from the kit, and all unused components returned to storage at 2-8°C.

2. One "PRL" strip and one "PRL" SPR have used for each sample, control, or calibrator to be tested. The storage pouch has carefully resealed after removing the required SPRs.

3. The "PRL" code has identified the test on the instrument. The calibrator must be identified by "S1" and tested in duplicate. If the control is to be tested, it should be identified by "C1".

4. If needed, the "PRL" Reagent Strips have labeled with the appropriate sample identification numbers.

5. The calibrator, control and samples have mixed using a vortex-type mixer (for serum or plasma separated from the pellet).

6. The calibrator, control, and sample test portion for this test was 200 µl.

7. The "PRL" Reagent Strips and SPRs have inserted into the appropriate position on the instrument.

8. The assay processing has initiated as directed in the Operator's Manual. All the assay steps performed automatically by the instrument.

9. The vials have reclosed and returned to the required temperature after pipetting.

10. The assay have completed in approximately 40 minutes. After the assay was completed, the SPRs and strips removed from the instrument.

11. The used SPRs and strips have disposed of in an appropriate recipient.

2.3.3.1.4. Assay Procedure for TSH

1. Necessary components removed from the kit, and all unused components returned to storage at 2-8°C.

2. Components allowed to reach room temperature (approximately 30 minutes).

3. One "TSH" strip and one "TSH" SPR have used for each sample, control, or calibrator to be tested. Ensure the storage pouch has carefully resealed after removing the required SPRs.

4. The test has identified by the "TSH" code on the instrument. The calibrator must be identified by "S1" and tested in duplicate. If the control needs to be tested, it should be identified by C1.

5. If needed, the "TSH" Reagent Strips have labeled with the appropriate sample identification numbers.

6. The calibrator, control and samples have mixed using a vortex-type mixer (for serum or plasma separated from the pellet).

7. The calibrator, control, and sample test portion for this test was 200 µl.

8. The "TSH" Reagent Strips and SPRs have inserted into the appropriate position on the instrument.

9. The assay processing has initiated as directed in the Operator's Manual. All the assay steps performed automatically by the instrument.

10. The vials have reclosed and returned to the required temperature after pipetting.

11. The assay have completed in approximately 40 minutes. After the assay was completed, the SPRs and strips were remove from the instrument.

12. The used SPRs and strips have disposed of in an appropriate recipient.

2.3.4. Molecular Study

2.3.4.1. Genomic DNA Extraction

1. A 300 µl of whole blood have pipetted into a 1.5 ml microcentrifuge tube (which had not provided).

2. A 3X the RBC Lysis Buffer sample volume was added and mixed by inversion (no vortexing).

3. The mixture was leaved at room temperature for 10 minutes. (noted that the mixture has becomed deep-red and transparent after incubation).

4. The 1.5 ml tube was applied to the centrifuge at 3,000 x g for 5 minutes, and the supernatant was removed entirely.

5. An 100 µl of RBC Lysis Buffer was added to the pellet, and the cells were resuspe by pipetting.

6. A 200 µl of FABG Buffer was added to the mixture and mixed well by vortexing

7. The mixture was leaved at room temperature for 10 minutes (during incubation, the mixture was inverted every 3 minutes)

8. The required Elution Buffer was preheated in a 70 °C water bath.

9. A 200 µl of absolute ethanol was added to the sample and mixed well by pulse vortex.

10. A FABG Column was place in a Collection Tube, and then the sample mixture was transfered to FABG Column and centrifuged at 14,000 rounds per minute for 1 minute. The Collection Tube was discarded, and the FABG Column was placed in a new Collection Tube.

11. A 400 µl of W 1 Buffer was added to the FABG Column and centrifuged for 30 seconds at 14,000 rounds per minute. The flow-through has discarded, and the FABG Column was placed back in the Collection Tube.

12. A 600 µl of Wash Buffer was add to the FABG Column and centrifuged for 30 seconds at 14,000 round per minute speed. The flow-through has discarded, and the FABG Column was placed back in the Collection Tube.

(The ethanol was added to Wash Buffer when first opened)

13. An additional centrifugation was applied for 3 minutes at 14,000 rounds per minute.

14. The dry FABG Column was placed in a new 1.5 ml microcentrifuge tube.

15. An 100 µl of preheated Elution Buffer to the membrane center of FABG Column

16. The FABG Column was incubated at 37 °C for 10 minutes in an incubator.

17. The DNA was eluted by centrifugation for 1 minute at 14,000 rounds per minute.

2.3.4.2. Detection of DNA Extraction Efficiency

a. Agarose gel electrophoresis 1.5 % was adopted to confirm the presence and integrity of the extracted DNA.

b. Nanodrop use to detect nucleic acid concentration. The photometric measurement of nucleic acids is based on the intrinsic absorptivity properties of nucleic acids (DNA). When an absorption spectrum has measured, nucleic acids absorb light with a characteristic peak at 260 nm.

2.3.4.3. Primer Pair of IL-17A Gene Preparation

The primer pair (Macrogen/Korea) was dissolved using sterile ddH2O in this study (table 2-9). The 10-pmol/µl stock primer solution was mix with 90 µl of ddH2O to prepare a working stock.

Table (2-9): Primer Pair of IL-17A Gene

IL-17A SNP	Primer sequence (5′→3′)	Reference
IL-17A(rs2275913)	**F: 5'-GCCAAGGAATCTGTGAGGAA-3'**	**(Hesampour *et al.*, 2019)**
	R: 5'-TGCCTGCTATGAGATGGACA-3'	

2.3.4.4. Polymerase Chain Reaction (PCR) Reaction Mixture

PCR master mix preparation was done according to Promega protocol (Go Taq®Promega Green Master Mix, LOT: 0000505764) (as shown in tables 2-10). To prevent nucleic acid contamination, the PCR master mix components were added to a UV PCR cabinet.

Table (2-10): Contents of the Reaction Mixture

Contents of the Reaction Mixture	Volume
Forward primer (10 pmol/µl)	**2 µl**
Reverse primer (10 pmol/µl)	**2 µl**
Master Mix	**12.5 µl**
Nuclease Free water	**3.5 µl**

Template DNA	**5 µl**
Total volume	**25 µl**

2.3.4.5. Polymerase Chain Reaction (PCR) Conditions

Specified primer pairs and conventional polymerase chain reaction (PCR) were employed to amplify a target DNA. To obtain a PCR product, the process typically comprises three successive phases (denaturation, annealing, and elongation) that were repeated cycle after cycle (amplicon). The size of PCR product (5µL) was determined on a 1.5 percent (w/v) agarose gel by electrophoresis in 1 percent (w/v) TBE buffer and observed with ethidium bromide after staining with the dye. The size of the product was evaluated by using the sizer 100 bp DNA ladder (abm®/Canada). The conditions for the polymerase chain reaction were liste in Table (2-11).

Table (2-11): PCR Conditions for Amplification of *IL-17A Gene*

Step	Temperature	Time	Number of cycles
Initial Denaturation	**95 °C**	**5 Minutes**	**1 cycle**
Denaturation	**95°C**	**30 Seconds**	**29 cycles**
Annealing	**54°C**	**60 Second**	
Extension	**72°C**	**60 Seconds**	
Final Extension	**72°C**	**5 Minutes**	
Hold	**4 °C**	∞	

2.3.4.6. Agarose Gel Electrophoresis

All technical and preparatory requirements for agarose gel electrophoresis for DNA detection and analysis were be according to (Bartlett and Stirling,1998). 1.5 gram agarose powder in 100 ml TBE buffer (90 ml DW was add to 10 ml of TBE buffer 10 X, the final concentration was 1 X and pH 8). The mixture was then boiled until transparent, cooled to 50°C, and ethidium bromide 0.5 g/ml was added. The agarose was gently put into an equilibrated. Gel try with two combs placed at each end, and the gel tray was sealed at both ends. After 30 minutes, the agarose solidified. The combs and seal were gently removed. The comb created wells for DNA samples. This process was follow by a ladder of DNA markers (five microliters) into one well of each agarose gel well. The electrophoresis chamber was fill with 1 X TBE buffer until the gel tray was covered. For one hours, the electric current was 80 volts. The amplified PCR products were identified and observed using agarose gel electrophoresis and ethidium bromide staining. A gel documentation system was used to determine the electrophoresis outcome. Positive results were identified when the sample's DNA band base pairs were equivalented to the target product size (Bartlett and Stirling, 1998).

2.3.4.7. Performing RT-PCR

1. A 40X Custom SNP Genotyping Assay has diluted to a 20X working solution.

2. The 20X Assay Working solution has vortexed and centrifuged.

3. TaqMan® Genotyping Master Mix has thoroughly mixed by swirling the bottle.

4. The thawed frozen samples were resuspended by vortexing, and then the tubes were centrifuged briefly.

5. The number of reactions was calculated for each assay.

6. The contents of the RT-PCR reaction mixture were indicated in the table below (2-12).

Table (2-12): Contents of the RT-PCR Reaction Mixture

Component	20 µL (Final volume)
20X Assay Working	**0.5 µL**
2X TaqMan® Master	**10 µL**
DNA Sample Volume	**4 µL**
Nuclease-free	**5.5 µL**

7. Before transferring it to the optical reaction plate for thermal cycling, the reaction mix for each assay was prepared.

8. The required volumes of 2X TaqMan® Genotyping Master Mix and 20X Genotyping Assay were pipetted into a sterile tube.

9. The tube was capped.

10. The tube was vortexed briefly to mix the components.

11. The tube was centrifuged briefly to spin down the contents and eliminate air bubbles from the solution.

12. The used thermal cycling conditions were specified in the following Table (2-13).

Table (2-13): RT-PCR Conditions for Detecting *IL-17A Gene* Polymorphism.

Steps	Predesigned SNP		
	Temp.	Duration	Cycles
Enzyme activation	**95°C**	**10 minutes**	**HOLD**
Denaturation	**95°C**	**15 seconds**	**40**
Annealing/ Extension	**60°C**	**1 minute (scanning)**	

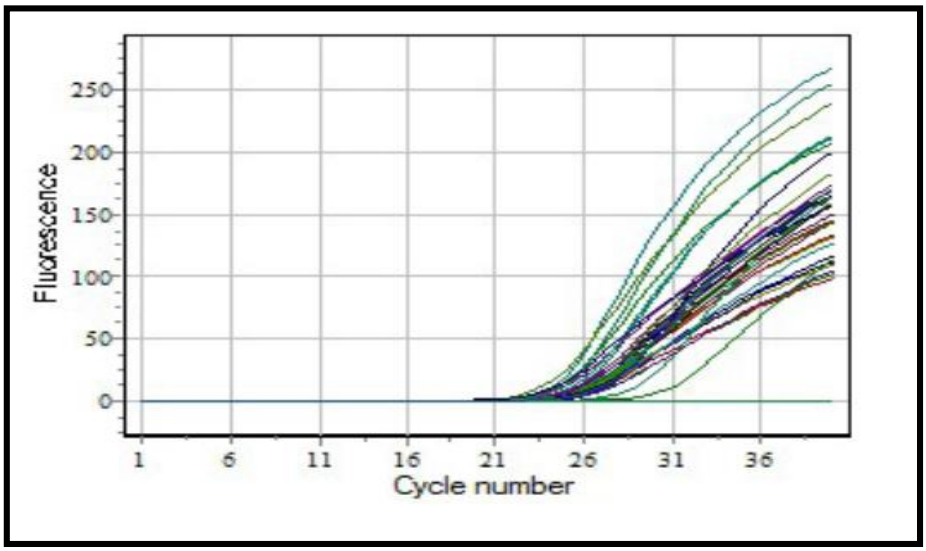


Figure (2-2): FAM Channel Curves (Allele G)

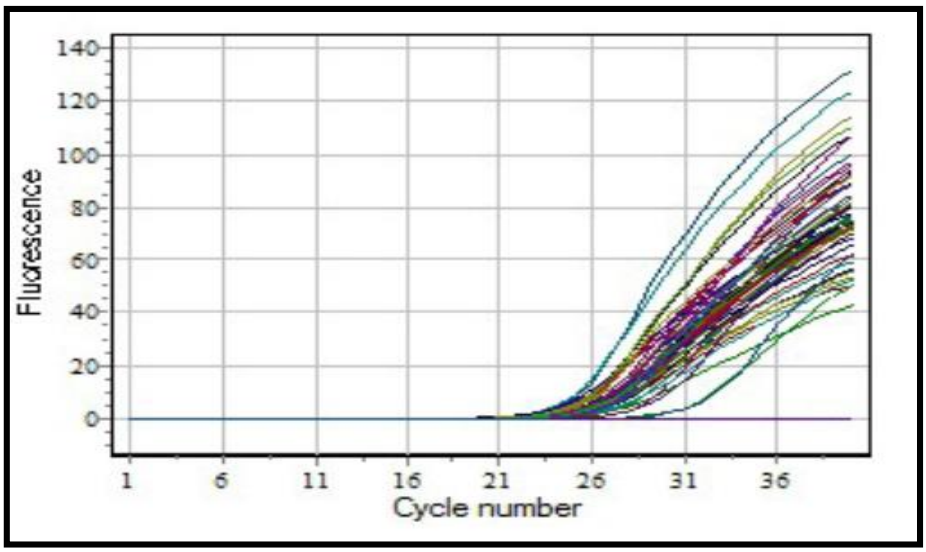


Figure (2-3): Hex Curves (Allele A)

2.3.5. Statistical Analysis

Statistical analysis were performed by using SPSS statistical package for Social Sciences (version 20.0 for windows, SPSS, Chicago, IL, USA). Quantitative data were represented as mean, SD and range. To test differences between the two groups, Student's t-test was used. Median and IQR (Inter Quartile Range) were used to describe IL-17A as the distribution was non-normal (Kolmogorov-Smirnov test). The Mann-Whitney test was used to study the difference between the two studied groups. Pearson correlation test was used to test the relation between quantitative data. ROC test (Receiver Operator

Characteristic) was done to test the sensitivity and specificity of the IL-17A cut-off point differentiating the patient group. A *P-value* of < 0.05 was considered statistically significant.

Chapter Three

Results

3. Results

3.1. Mean Age, Menarche and BMI of the PCOS Patients and Control

One hundred individuals, divided into 50 patients clinically diagnosed with PCOS and 50 controls, attended the gynecology clinics in Najaf province. The demographic characteristics of the subjects are presented in table (3-1).

The ages of patients ranged from (13-48) with a mean value (27.80) and a standard deviation (8.16) while the ages of the control ranged from (14-49) with a mean value (26.88) and a standard deviation (9.33) with (*P*=0.620) regarding age.

The menarche ages of patients ranged from (11-13) with a mean value (11.92) and a standard deviation (0.75) while the menarche ages of the control ranged from (10-12) with a mean value (11.70) and a standard deviation (0.54). There was no significant difference between the control and patient groups (*P*=0.091) regarding menarche age.

In Polycystic ovarian syndrome patients, the body mass index (BMI) has a mean value (27.75) and a standard deviation (4.97), while the BMI of the control with mean value (24.08) and a standard deviation (3.36). There were significant differences in BMI between patients and control (*P*=0.005).

Table (3-1): Distribution of Age, Menarche and BMI Characteristics of the Studied Subjects.

Variables	Group				*P value*
	Patient		Control		
	Mean	SD	Mean	SD	
Age	27.80	8.16	26.88	9.33	0.620**
Menarche	11.92	0.75	11.70	0.54	0.091**
BMI	27.75	4.97	24.08	3.36	**0.005***

***P value* is significant, ***P value* is nonsignificant (Student's t-test), SD: Standard Deviation**

3.2. Marriage, Infertility, Ultrasound, Menses, Occupation, Hirsutism and Acne in the Studied Subjects

In Polycystic ovarian syndrome patients, the number of married females was 37 (74.0%), and nonmarried females were 13 (26.0%) while in the control group, the number of married females was 50 (100.0%) while the nonmarried females were 0 (0.0%). There was a significant difference between PCOS patients and control (*P*=0.005), as clarified in a Table (3-2).

In Polycystic ovarian syndrome patients, the number of suspected infertile women was 28 (56.0%), and the number of fertile women reached 22 (44.0%), while in the control group, the number of infertile women was 0 (0.0%) and the fertile women 50 (100%). There was a significant difference between PCOS patients and control (*P*=0.005).

The individuals in the study underwent an ultrasound examination. It was found that the patient group with PCOS had ovarian cysts was 45 (90.0%), while the patients without ovarian cysts were 5 (10.0%). According to the control group, the women with ovarian cysts were 0 (0.00%), and those without ovarian cysts were 50 (100%). There was a significant difference between PCOS patients and control (*P*=0.005).

It was found that patients with PCOS who had regular menses were 7 (14.0%), irregular menses were 32 (64.0%), amenorrhea menses was 1 (2.0%), hypermenorrhea menses were 2 (4.0%), and oligomenorrhea menses were 8 (16.0%). In the control group for the same study, it was found that the women who had regular menses were 41 (82.0%), irregular menses were 9 (18.0%), amenorrhea menses were 0 (0.0%), hypermenorrhea menses were 0 (0.0%), and oligomenorrhea menses were 0 (0.0%). There was a significant difference between PCOS patients and control (*P*=0.005).

In Polycystic ovarian syndrome patients, the number of housewives was 36 (72.0%), and employers were 14 (28.0%), while in the control group, the number of housewives was 30 (60.0%), while the employer was 20 (40.0%). There was no significant difference between PCOS patients and control (*P*=0.291).

In Polycystic ovarian syndrome patients, the number of hirsutism women was 27 (54.0%), and the number of non-hirsutism women reached 23 (46.0%), while in the control group, the number of hirsutism women was 0 (0.0%) and non-hirsutism women 50 (100%). There was a significant difference between PCOS patients and control (*P*=0.005).

In Polycystic ovarian syndrome patients, the number of women with acne were 24 (48.0%), and the number of women without acne reached 26 (52.0%), while in the control group, the number of women with acne were 4 (8.0%) and women without acne 46 (92.0%). There was a significant difference between PCOS patients and control (*P*=0.005).

Table (3-2): Distribution of Marriage, Infertility, Ultrasound, Menses, Occupation, Hirsutism and Acne Characteristics in the Patients and Control Groups.

Variables		Group				*P value*
		Patient		Control		
		Count	%	Count	%	
Marriage	Yes	37	74.0%	50	100.0%	**0.005***
	No	13	26.0%	0	0.0%	
Infertility	Fertile	22	44.0%	50	100.0%	**0.005***
	Infertile	28	56.0%	0	0.0%	
US	Yes	45	90.0%	0	0.0%	**0.005***
	No	5	10.0%	50	100.0%	
Menses	Regular	7	14.0%	41	82.0%	**0.005***
	Irregular	32	64.0%	9	18.0%	
	Amenorrhea	1	2.0%	0	0.0%	
	Hypermenorrhae	2	4.0%	0	0.0%	
	Oligomenorrhea	8	16.0%	0	0.0%	
Occupation	Housewife	36	72.0%	30	60.0%	0.291**
	Employer	14	28.0%	20	40.0%	
Hirsutism	Yes	27	54.0%	0	0.0%	**0.005***
	No	23	46.0%	50	100.0%	
Acne	Yes	24	48.0%	4	8.0%	**0.005***
	No	26	52.0%	46	92.0%	

***P value* is significant, ***P value* is nonsignificant (Chi-Square test), US: Ultrasound**

3.3. Family History of Infertility, Lack of Exercise, Hypertension, Depression, Sleep Apnea and CVD Counts in Patients and Control

In table (3-3), Polycystic ovarian syndrome patients with a family history of infertility were 4 (8.0%), and the patients that did not have a family history of infertility 46 (92.0%). In the control group, the number of women with a family history of infertility was 0 (0.00%), while the number of women that did not have a family history of infertility reached 50 (100%). There was no significant difference between PCOS patients and control (*P*=0.117).

Polycystic ovarian syndrome patients with a lack of exercise were 17 (34.0%), and those without a lack of exercise were 33 (66.0%). In the control group, the number of women who lacked exercise was 0 (0.0%), while the number of women without lack exercise reached 50 (100%). There was a significant difference between PCOS patients and control (*P*=0.005).

Polycystic ovarian syndrome patients with hypertension 3 (6.0%), and the patients that did not have hypertension 47 (94.0%). In the control group, the number of women

with hypertension was 0 (0.0%), while the number of women that did not have hypertension reached 50 (100%). There was no significant difference between PCOS patients and control (*P*=0.242).

In Polycystic ovarian syndrome patients, the number of depressed women was 11 (22.0%), and the number of non-depressed women reached 39 (78.0%), while in the control group, the number of depressed women was 0 (0.0%) and non-depressed women 50 (100%). There was a significant difference between PCOS patients and control (*P*=0.001).

In PCOS patients, the number of women with sleep apnea was 9 (18.0%), and the number of women that did not have sleep apnea reached 41 (82.0%), while in the control group, the number of women with sleep apnea was 0 (0.0%), and the number of women that did not have sleep apnea were 50 (100%). There was a significant difference between PCOS patients and control (*P*=0.003).

In PCOS patients, the number of women with cardiovascular disease (CVD) was 0 (0.0%), and the number of women that did not have CVD reached 50 (100%), while in the control group, the number of women with CVD were 2 (4.0%) and the number of women that did not have CVD were 48 (96.0%). There was no significant difference between PCOS patients and controls (*P*=0.242).

Table (3-3): Family History of Infertility, Lack of Exercise, Hypertension, Depression, Sleep Apnea and CVD Characteristics of The Studied Subjects.

Variables		**Group**				***P value***
		Patient		**Control**		
		Count	**%**	**Count**	**%**	
Family history of infertility	Yes	4	8.0%	0	0.0%	0.117**
	No	46	92.0%	50	100.0%	
Lack of exercise	Yes	17	34.0%	0	0.0%	**0.005***
	No	33	66.0%	50	100.0%	
Hypertension	Yes	3	6.0%	0	0.0%	0.242**
	No	47	94.0%	50	100.0%	
Depression	Yes	11	22.0%	0	0.0%	**0.001***
	No	39	78.0%	50	100.0%	
Sleep apnea	Yes	9	18.0%	0	0.0%	**0.003***
	No	41	82.0%	50	100.0%	
	Yes	0	0.0%	2	4.0%	0.242**

Cardiovascular disease (CVD)	No	50	100%	48	96.0%	

***P value* is significant, ***P value* is non significant (Chi-Square test)**

3.4. The Mean of FSH, LH, TSH, Prolactin, WBC and Lymphocyte of Patients and Control Groups

The mean of follicle-stimulating hormone (FSH) in PCOS cases was (7.11) and in control was (5.71). The SD of FSH in PCOS cases was (10.88) and in control was (3.34). However, the association was nonsignificant (*P*=0.402) between PCOS patients and control, as shown in table (3-4).

Also, the mean of luteinizing hormone (LH) in PCOS cases was (5.46) and in control was (4.53). The SD of LH in PCOS cases was (6.75) and in control was (3.88) However, the association was nonsignificant (*P*=0.429) between PCOS patients and control.

However, the mean of stimulating thyroid hormone (TSH) in PCOS cases was (1.86) and in control was (2.79). The SD of TSH in PCOS cases was (1.03) and in control was (2.03). There was a significant difference in TSH between patients and control (*P*=0.004).

Also, the mean of prolactin in PCOS cases was (25.24) and in control was (20.93). The SD of prolactin in PCOS cases was (15.57) and in control was (10.75) However, the association was nonsignificant (*P*=0.111) between PCOS patients and control.

On the other hand, the mean white blood cell count (WBC) in PCOS cases was (7.12) and in control was (7.57). The SD of WBC in PCOS cases was (1.94) and in control was (1.92) However, the association was nonsignificant (*P*=0.230) between PCOS patients and control. Although, the mean of lymphocytes in PCOS cases was (31.81) and in control was (33.67). The SD of lymphocytes in PCOS cases was (7.50) and in control was (8.78). However, the association between PCOS patients and control was nonsignificant (*P*=0.231).

Table (3-4): Determination of FSH, LH, TSH, Prolactin, WCC and Lymphocyte Characteristics of the Studied Subjects.

Variables	**Group**				***P value***
	Patient		**Control**		
	Mean	**SD**	**Mean**	**SD**	

Follicle Stimulating Hormone (FSH)	7.11	10.88	5.71	3.34	0.402**
Luteinizing Hormone (LH)	5.46	6.75	4.53	3.88	0.429**
Thyroid-stimulating Hormone (TSH)	1.86	1.03	2.79	2.03	**0.004***
Prolactin	25.24	15.57	20.93	10.75	0.111**
White cell count (WCC)	7.12	1.94	7.57	1.92	0.230**
Lymphocyte	31.81	7.50	33.67	8.78	0.231**

***P value* is significant, ***P value* is nonsignificant (Student's t-test), SD: Standard Deviation**

3.5. Serum level of IL-17A in Patients and Control Groups

The mean IL-17A serum level in PCOS cases was (11.46) and in control was (9.53). The SD in PCOS cases was (9.50) and in control was (18.99). However, the association was significant (*P*=0.005) between PCOS patients' serum IL-17A and control, as demonstrated in the table (3-5).

Table (3-5): Mean Differences of IL-17A Among Patients and Control.

Variables	**Group**				***P value***
	Patient		**Control**		
	Mean	**SD**	**Mean**	**SD**	
IL-17A	11.46	9.50	9.53	18.99	**0.005***

***P value* is significant (Mann-Whitney test), SD: Standard Deviation**

3.6. C-Reactive Protein Count in PCOS Patients and Control Groups

In PCOS patients, the number of positive C-Reactive Protein (CRP) women was 12 (24.0%), and the number of negative CRP women reached 38 (76.0%), while in the control group, the number of positive CRP women was 0 (0.0%) and negative CRP women 50 (100%). There was a significant difference between PCOS patients and control (*P*=0.005), as shown in table (3-6).

Table (3-6): C-Reactive Protein Count in Studied Groups.

Variables		Group				*P value*
		Patient		Control		
		Count	%	Count	%	
C- Reactive Protein (CRP)	Positive	12	24.0%	0	0.0%	**0.005***
	Negative	38	76.0%	50	100.0%	

***P value* is significant (Chi-Square test)**

3.7. Correlation Between Study Markers in PCOS Patients and Control

The Correlation between the studied markers is clarified in Table (3-7) and the figures (3-1,2,3,4,5,6).

Table (3-7): Correlation Between IL-17A, FSH, LH, TSH, Prolactin, WCC and Lymphocytes in Patients and Control.

Correlations								
		Control						
Patients		IL-17A	FSH	LH	TSH	Prolactin	WCC	Lymph.
IL-17A	r		-0.130	-0.022	-0.149	-0.143	0.037	0.060
	P		0.370	0.880	0.302	0.322	0.798	0.678
	N		50	50	50	50	50	50
FSH	r	-0.134		0.723	0.447	0.124	0.005	0.081
	P	0.353		0.000*	0.001*	0.392	0.971	0.574
	N	50		50	50	50	50	50
LH	r	-0.137	0.898		0.438	0.214	0.122	0.053
	P	0.343	**0.005***		0.001*	0.136	0.398	0.712
	N	50	50		50	50	50	50
TSH	r	0.190	-0.035	-.0001		-0.009	0.123	0.123
	P	0.186	0.811	0.997		0.952	0.395	0.396
	N	50	50	50		50	50	50
Prolactin	r	-0.028	-0.175	-0.090	-0.081		-0146	0.163
	P	0.848	0.223	0.536	0.574		0.313	0.258
	N	50	50	50	50		50	50
WCC	r	0.047	-0.212	-0.094	0.051	0.183		-0.250
	P	0.746	0.138	0.515	0.725	0.202		0.080
	N	50	50	50	50	50		50
Lymph.	r	0.014	-0.060	-0.084	0.081	0.399	-0.317	
	P	0.922	0.679	0.563	0.574	**0.004***	**0.025***	
	N	50	50	50	50	50	50	

***P value* is significant, ***P value* is nonsignificant (Pearson's correlation test), r=Correlation Coefficint, N=number, P= p value**

3.7.1. Correlation Between LH and FSH in PCOS Patients

Figure (3-1) showed the correlation between LH and FSH levels for PCOS patients. There was a significant correlation between LH and FSH (r= 0.898, *P*=0.005).

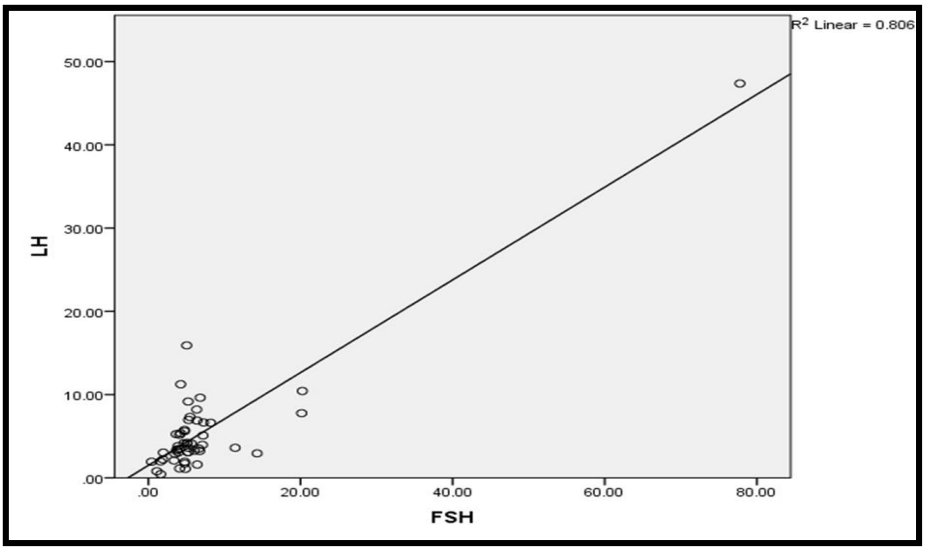


Figure (3-1): Correlation between LH and FSH in patients group. Pearson correlation coefficient r=0.898, *P-Value* 0.005

3.7.2. Correlation Between Lymphocytes and prolactin in PCOS Patients

Figure (3-2) showed the correlation between lymphocyte count and prolactin level for PCOS patients. There was a significant correlation between lymphocyte count and prolactin level (r = 0.399, *P*=0.004).

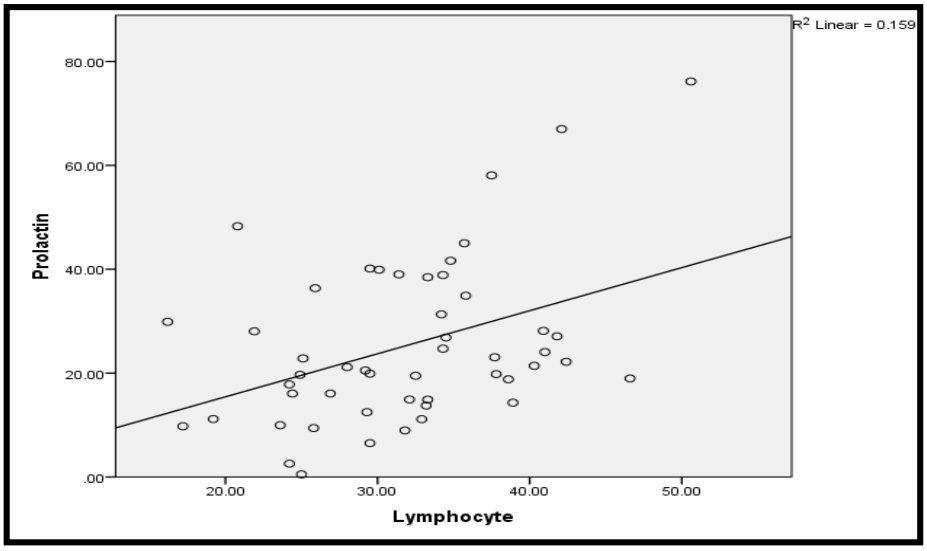


Figure (3-2): Correlation Between Lymphocytes and Prolactin in Patients Group. Pearson Correlation Coefficient (r=0.399, *P-Value* 0.004).

3.7.3. Correlation Between Lymphocytes and WCC in PCOS Patients

Figure (3-3) showed the correlation between lymphocyte count and WCC for PCOS patients. There was a significant correlation between lymphocyte count and WCC (r= -0.317, *P*=0.025).

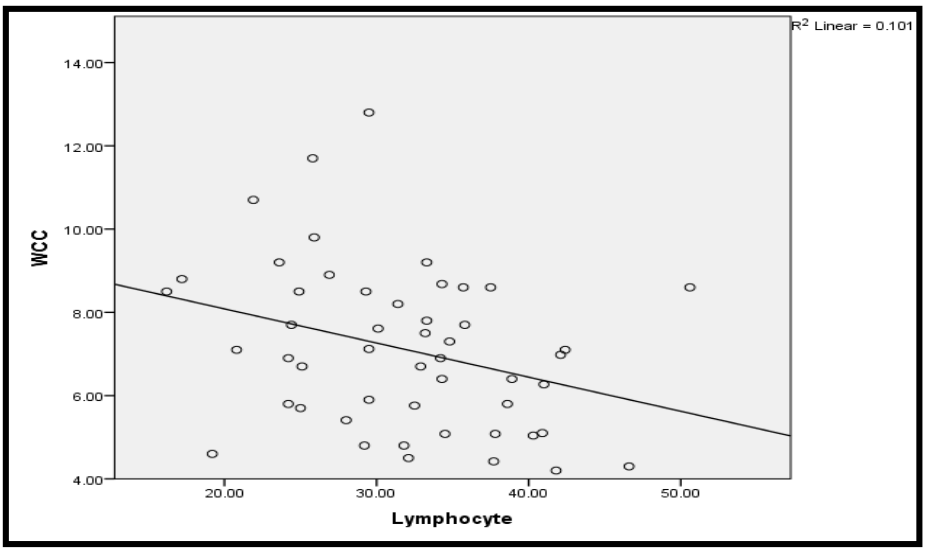


Figure (3-3): Correlation Between Lymphocytes and WCC in Patients Group. Pearson Correlation Coefficient (r = -0.317, *P-Value 0.025*).

3.8. PCR-Based Detection of SNP

3.8.1. Genomic DNA Extraction

The quality and integrity were be checked by agarose gel electrophoresis before performing the PCR reaction, as shown in figure (3-4). All DNA extracts showed single bright band.

3.8.2. *IL-17A Gene* Amplification

The gene segment that contains the possible single nucleotide polymorphism within the IL-17A gene region was amplified from extracted DNA for each sample of patients with PCOS and healthy control. Using a specific primer, the polymerase chain reaction was performed under optimum conditions; then, the PCR product was electrophoresis on agarose gel 1.5%. The results were a single clear band with a molecular size of 400 bp. The size of the amplicon was determined by comparison with the DNA ladder 100 bp, as shown in figure (3-5).

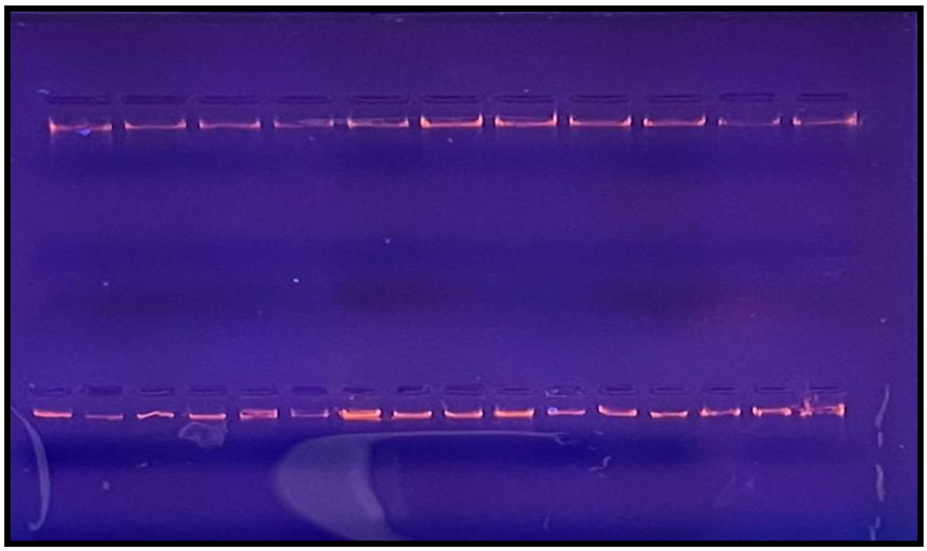


Figure (3-4): Evaluation of DNA Extract Quality and Integrity. 1.5% Agarose Gel Electrophoresis of Genomic DNA in 80 Volts at 15 Minutes.

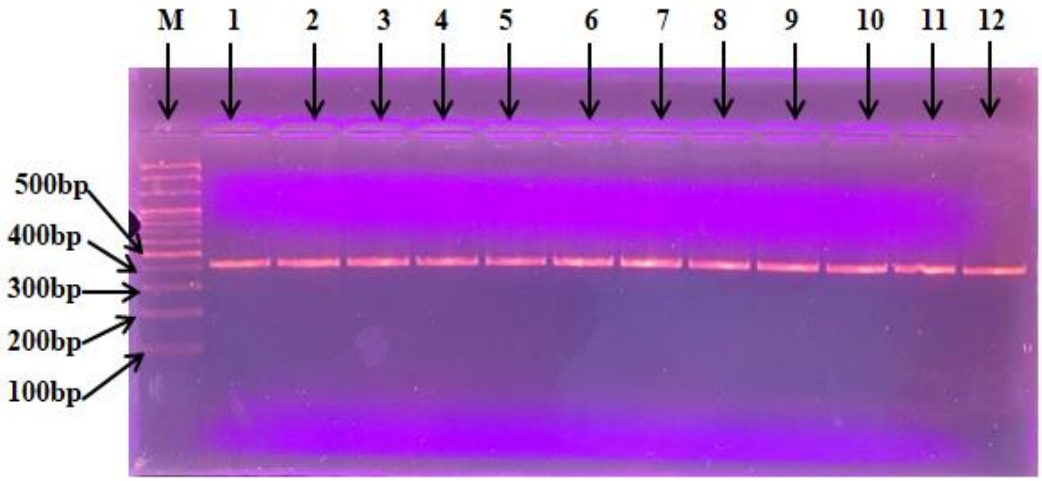


Figure (3-5): Gel Electrophoresis for PCR Product of IL-17A Gene. 400bp with DNA Ladder 100bp (M) on Agarose Gel 1.5% in 80 Volts, 1 Hour and Detection of the Result by UV Documentation System.

3.8.3. Detection of *IL-17A Gene* (rs2275913) Polymorphism in Patients and Control by RT-PCR

Genetic polymorphism of the *IL-17A gene* (rs2275913) was observed with three genotypes (AG, GG, and AA) in PCOS patients and control. The "G" allele was elevated in control compared with patients (67% vs. 59%, respectively), whereas the "A" allele was elevated in patients compared with control (41% vs. 33%, respectively), as shown in the Table (3-8). In the alleles, there was no significant difference between the group studied (*P*=0.307). The AG genotype was found to be at a higher frequency in patients compared with control (50.0% vs. 42.0%, respectively), the GG genotype was found to be at a higher frequency in control compared with patients (46.0% vs. 34.0%, respectively) and the AA genotype was elevated in patients compared with control (16.0% vs. 12.0%, respectively) as shown in the Table (3-8). There was no significant difference in the genotype with the group studied (*P*=0.421).

Table (3-8): Genotypes and Allele Frequency Distribution of *IL-17A Gene* Polymorphism in Patients and Control.

Variables		Group				*P value*
		Patient		Control		
		Count	%	Count	%	
Alleles	G	59	59%	67	67%	0.307*
	A	41	41%	33	33%	
IL-17A genotypes	AG	25	50.0%	21	42.0%	0.421*
	GG	17	34.0%	23	46.0%	
	AA	8	16.0%	6	12.0%	

***P value* is nonsignificant (P> 0.05) (Chi-Square test).**

3.8.4. Association Between IL-17A Genotypes and Alleles with IL-17A Serum Level in PCOS Patients

The study showed a nonsignificant association between IL-17A genotypes (*P*=0.873) and alleles frequency (*P*=0.959) with IL-17A serum level in PCOS patients, as presented in table (3-9).

Table (3-9): Association Between IL-17A Genotypes and Alleles with IL-17A Serum Level in Patients Group (N=50).

Variables		IL-17A		*P value*
		Median	IQR	
IL-17A genotypes	AG	10.21	13.44	0.873*
	GG	7.12	19.01	
	AA	14.72	17.56	
Alleles	A	10.23	15.65	0.959*
	G	7.12	19.01	

***P value* is nonsignificant (P> 0.05), (Kruskal-Wallis test), IQR: Interquartile range**

3.9. Receiver Operating Characteristic Curves (ROC)

Curve analysis showed the cutoff value of (3.784) for IL-17A with an Area of (0.662). According to the AUC, the IL-17A was a predictive marker for diagnosing

PCOS, as shown in Table (3-10). The sensitivity and specificity of the immune marker were clarified in Table (3-11).

Table (3-10): Diagnostic Utility of Area Under the Curve in Figure (3-9).

Area Under the Curve					
Variable(s)	**Area**	**SE**	***P value***	**Asymptotic 95% Confidence Interval**	
				Lower Bound	**Upper Bound**
IL-17A	0.662	0.055	**0.005***	0.554	0.771

***P value* is significant, SE: Standard Error**

Table (3-11): Cutoff Value, Sensitivity and Specificity in Differentiating Patients from the Control Groups.

Variable(s)	Cutoff value	Sensitivity	Specificity
IL-17A	3.784	68.0%	54.0%

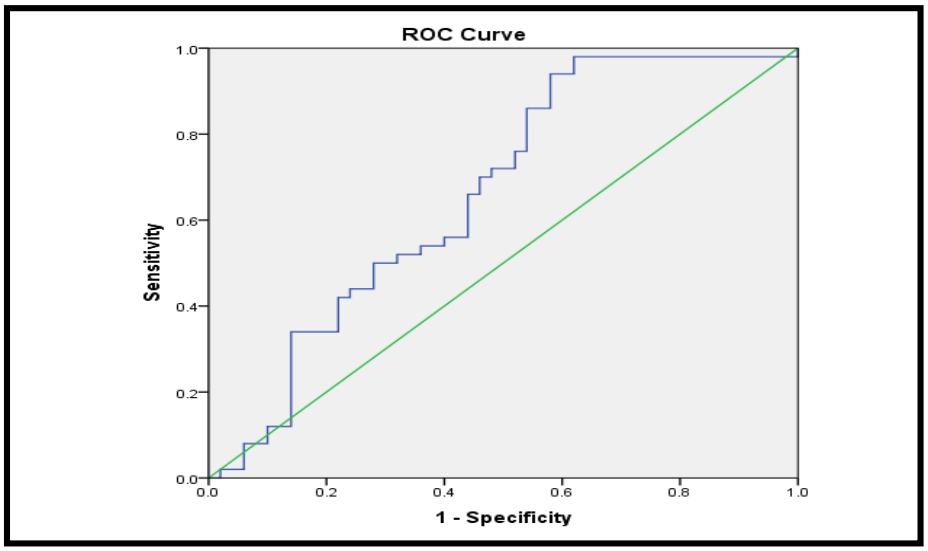


Figure (3-6): Receiver Operating Characteristic Curve of IL-17A for Diagnosis of PCOS.

Chapter Four

Discussion

4. Discussion

4.1. Demographic Data of PCOS Patients and Control

4.1.1. Mean Age, Menarche and BMI of the PCOS Patients and Control

The mean age of the patient group in the present study was 27.8. It is consistent with other studies, such as a study conducted by Diamanti‐Kandarakis *et al.* (2007), in which the mean age was 27.52 years, a study by Ramezani Tehrani *et al.* (2021) in which the mean age was 27.9 years, the study by Shen *et al.* (2015) in which mean age was 27.04 and a study by Javedani *et al.*(2015). They reveal that the mean (±SD) ages of PCOS and control groups were 28.9 (±2.8) and 27.9 (±2.7) years, respectively, with (*P*=0.104). Study by Moini *et al.*, (2015) showed From 125 PCOS patients and 117 healthy women, the mean age was 27.85 in the PCOS group and 30.82 in normal subjects. Also Al-Musawy, Al-Saimary and Flaifil (2018) reported non-statistically significant differences between the patients and control groups regarding age.

In the present study, the menarche ages of patients with mean values (11.92) and a standard deviation (0.75) while the menarche ages of the control had a mean value (11.70) and a standard deviation (0.54). There was no significant difference between the control and patient groups (*P*=0.091). This finding was similar to the study conducted by Huang *et al.* (2010), in which the PCOS patient mean value was (12.0) while the control means value was (12.5) and to the study by Han *et al.* (2015), in which PCOS patient's mean (±SD) values was (12.6±1.6) while control the mean (±SD) was (12.6±1.1) with (*P*=0.860). On the contrary, the study by Tabassum *et al.* (2021) found that the menarche age of the PCOS group in 11-14 years was 67(67.0%) and older than 14 years was 33(33.0%) while in the control the menarche ages in 11-14 years were 200(100.0%) and >14 was 0(0.0%) (*P*=0.000).

The study showed that PCOS patients had a body mass index (BMI) with a Mean value (27.75) and a standard deviation (4.97) while the BMI of control with a Mean value (24.08) and a standard deviation (3.36). There were significant differences in BMI between patients and control (*P*=0.005). Various studies have shown the prevalence of obesity among PCOS, ranging from 42% to 62.5%. Sangabathula *et al.*,(2015) reported mean BMI as (34.3). At the same time, Joshi *et al.* (2017) and Alakananda, Prasad and

Goel, (2015) reported that BMI was (27.4) and (25.5), respectively. There is wide variability in the prevalence of overweight (BMI 25 to 30 kg/m2) and obese (BMI >30 kg/m2) women in PCOS populations across different countries. The proportion of women with PCOS who are overweight but not obese ranges from 10% in Italy to 37% in Kuwait. The highest prevalence of obesity is reported in studies conducted in the United States and Australia, with 61% to 76% of women with PCOS considered obese (Glueck *et al.,* 2005; Ching, Burke and Stuckey, 2007). The differences between these studies and the present study might be due to ethnic differences or small samples size.

4.1.2. Marriage, Infertility, Ultrasound, Menses, Occupation, Hirsutism and Acne in the Studied Subjects

Regarding marriage, the study showed PCOS patients, the number of married females was 37 (74.0%), and nonmarried females 13 (26.0%); in the study by Tabassum *et al.*, (2021), a total of 100 PCOS cases and 200 healthy control cases were enrolled in the number of married females was 41 (41.0%). Nonmarried females were 59 (59.0%). In another study by Mirza *et al.,* (2014), a total of 150 individuals participated in the study, of which 75 were diagnosed with PCOS cases and 75 control; the number of married females was 37 (49.3%), and unmarried females were 38 (50.7%). The study by Sayyah-Melli *et al.,* (2015) included 742 PCOS patients, and 798 control groups were married women with PCOS (20.9%) and control (37.3%). (In the present study, inclusion criteria for control were just married, fertile, healthy women)

Of 50 cases of PCOS included in the present study, 37 were married. 28 (56.0%) presented with infertility. This result agreed with the study by Alakananda, Prasad and Goel (2015), who demonstrated that out of 66 cases of PCOS, 21 were married. 21, 18 presented with infertility, 11 as primary (52.38%) and seven as secondary infertility (33.33%). A study accomplished by Joshi, Yonzon and Tandukar, (2017) found that 46% of patients were married, and 43% complained of infertility. Ramanand *et al.*, (2013) revealed that in the study on 120 PCOS women, 47 were married, and 44.68% of married women complained of infertility. (In the current study, inclusion criteria for control were just married, fertile, healthy women).

Polycystic ovarian morphology was defined as the presence of 12 or more follicles, measuring between 2 and 9 mm, throughout the entire ovary follicle number per

ovary (FNPO) and an ovarian volume (OV) >10 cm^3 (Lujan *et al.*, 2013). In this study, the individuals underwent an ultrasound examination, and it found that the patients with PCOS who had ovarian cysts were 45 (90.0%), while the patients without ovarian cysts were 5 (10.0%). This result agreed with the study by Alakananda, Prasad and Goel, (2015) among 66 women with PCOS ultrasound picture of polycystic ovaries found in 59 (89.39%) patients and the study by Moghetti *et al.*, (2013) among 137 women with PCOS diagnosed by Rotterdam criteria found PCO morphology in 89%. Much controversy has arisen from reports of unusually high polycystic ovaries in healthy women of reproductive age using the ultrasound-based criteria supported by the Rotterdam consensus (Duijkers and Klipping, 2010; Johnstone *et al.*, 2010; Kristensen *et al.*, 2010).

Regarding menstrual cycle, in the current study, it was found that patients with PCOS who had regular menses were 7 (14.0%), irregular menses were 32 (64.0%), amenorrhea menses was 1 (2.0%), hypermenorrhea menses were 2 (4.0%), and oligomenorrhea menses were 8 (16.0%). In the control group for the same study, it was found that the women who had regular menses were 41 (82.0%), irregular menses were 9 (18.0%), amenorrhea menses were 0 (0.0%), hypermenorrhea menses were 0 (0.0%), and oligomenorrhea menses were 0 (0.0%). Several studies, such as a study by Hussein and Alalaf (2013), found that oligomenorrhea and amenorrhea were significantly more common among the PCOS women at 74.5% and 11.3%, respectively, while in the non-PCOS group, the rate was 23.4% and 2.3% respectively (P=0.000). Also, a study by Nahar *et al.* (2017) reported that 74.0% had oligomenorrhea, 21.0% had secondary amenorrhea, and 5.0% had regular menstrual cycles. In a study completed by Timpatanapong and Rojanasakul (1997), only 59.0% had oligomenorrhoea, and 37.0% had regular cycles. In addition, a study by Christodoulopoulou *et al*., (2016) took 309 women with PCOS in Athens. In total, 72.2% suffered from menstrual cycle disorders. Among them, 58.3% of women had a cycle that exceeded 35 days, 5.2% had a cycle that lasted less than 26 days, and 8.7% suffered from amenorrhea. In another study by Sangabathula and Varaganti (2017), out of 100 cases, 87% had oligomenorrhea, 7% presented with amenorrhea, regular cycles were observed in 6% and 13% presented with menorrhagia. In the study by Tabassum *et al.* (2021) in which, a total of 100 PCOS cases had regular cycle 1 (1.0%), irregular were 45 (45%) and delayed/late were 54 (54.0%), while 200 healthy control in which regular cycle was 192 (96.0%), irregular were 8

(4.0%) and delayed/late was 0 (0.0%) (*P*=0.000). These differences might be due to low sample size and medication.

The current study showed that the PCOS patients, the number of housewives were 36 (72.0%) and employer were 14 (28.0%), while in the control group, the number of a housewife was 30 (60.0%) while the employer was 20 (40.0%). There was no significant difference between PCOS patients and control *(P*=0.291). These result agreed with several studies, such as a study by Hussein and Alalaf (2013), which revealed the number of a housewife in PCOS patients were 89 (84.0%) and employed 17 (16.0%), while in the control group, housewife were 182 (85.0%) and employed were 32 (15.0%). In the study by Tabassum *et al.*, (2021) in which, a total of 100 PCOS cases students 60 (60.0%), house maker 35 (35.0%), unemployed 2 (2.0%) and employed 3 (3.0%), while 200 healthy control include students 79 (39.5%), house maker 98 (49.0%), unemployed was 0 (0.0%) and employed was 23 (11.5%) (*P*=0.000). In the study by Sayyah-Melli *et al.* (2015), the rate of high school and higher education in the cases and control were significant (*P*<0.001). Sixty percent of the PCOS group and 53% of the control had jobs. The rate of university education in cases and control was also significant (*P*<0.001).

In the current study, In PCOS cases, the number of hirsutism women was 27 (54.0%), and the number of non-hirsutism women reached 23 (46.0%), while in the control group, the number of hirsutism women was 0 (0.0%) and non-hirsutism women 50 (100%). There was a significant difference between PCOS patients and control (*P*=0.005). These results agreed with several studies, such as Alakananda, Prasad and Goel (2015), in which out of 66 cases of PCOS included in the study, 45 (68.18%) had hirsutism. In the study by Ramanand *et al*. (2013), clinically, (44.16%) of women had hirsutism. The prevalence of hirsutism in the study by Nahar *et al.* (2017) was (69%); however, significantly higher than the findings of Yousouf *et al.*, (2012) (31%) and Joshi, Yonzon and Tandukar, (2017) (32.5%).

Moreover, in the present study of PCOS patients, the number of women with acne was 24 (48.0%), and the number of women without acne reached 26 (52.0%). In contrast, in the control group, the number of women with acne was 4 (8.0%) and women without acne 46 (92.0%). There was a significant difference between PCOS patients and control (*P*=0.005). These results agreed with a study by Christodoulopoulou *et al.*, (2016) in which (36%) of the samples had androgenetic alopecia, and (56.4%) had acne among 309

patients of PCOS. However, Moini and Eslami, (2009) found acne in (25.64%) and alopecia in (24.54%) in a study of 549 women with 273 PCOS patients. A total of 100 patients of PCOS were included in the study of Nahar *et al.*, (2017), who reported 31 (31.0%) had acne and a study by Sangabathula and Varaganti, (2017), found out of the 100 studied patients, 21 (21.0%) had acne. The differences in these results may be due to the small volume of samples in this study.

4.1.3. Family history of Infertility, Lack of Exercise, Hypertension, Depression, Sleep Apnea and CVD in Patients and Control

In our study, there was no significant association between a family history of infertility and PCOS patients compared with healthy control in this study. PCOS patients with a family history of infertility 4 (8.0%), and the patients that did not have a family history of infertility 46 (92.0%). In the control group, the number of women with a family history of infertility was 0 (0.00%), while the number of women that did not have a family history of infertility reached 50 (100%). These results are slightly lower than those found in the study by Shan *et al.* (2015). They reported that 41(14.4%) of PCOS patients had a family history of infertility and 244 (85.6%) did not, while in the control group, only 4 (0.7%) had a family history of infertility and 576 (99.3%) had not, with (P=0.000). The present study result is incompatible with Hussein and Alalaf, (2013). They found there were 106 women in the PCOS group and 214 in the non-PCOS group, the family history of PCOS patients was 40 (37.7%), which is slightly higher than in the non-PCOS was 67 (31.3%). In addition, a study by Al-Musawy, Al-Saimary and Flaifil, (2018) demonstrated that out of 73 PCOS cases, 50 (68.5%) had a history of infertility, and out of 73 fertile control 0 (0.0%) had a history of infertility.

Also, the present study showed a significant association (P=0.005) between lack of exercise and PCOS patients compared with healthy control. PCOS patients were with a lack of exercise 17 (34.0%), and the patients that did not have a lack of exercise were 33 (66.0%). In the control group, the number of women who lacked exercise was 0 (0.0%), while the number of women without lack exercise reached 50 (100%). This result was agreed with several studies, such as the study by Shan *et al.* (2015), in which lack of exercise PCOS cases were 159 (27.4%) and the patients that did not have a lack of exercise were 421 (72.6%), while the control in the same study with lack of exercise were 43 (15.1%) and those without lack of exercise 242 (84.9%). Lack of physical exercise,

leading to uneven distribution of body fat, is a significant risk factor for centripetal obesity. One study advises proper diet and regular physical exercise to obese PCOS patients to alleviate symptoms like excessive hair and irregular menstruation (Le Donne *et al.*, 2012).

Hypertension develops in some women with PCOS during their reproductive years, and sustained hypertension may develop in later life. In women with the disorder, it reduces vascular compliance, and vascular endothelial dysfunction was noted in most but not all (Wesen, 2008). In addition, the current study showed that the patients with PCOS with hypertension 3 (6.0%) and the patients that did not have hypertension 47 (94.0%). In the control group, the number of women with hypertension was 0 (0.0%), while the number of women that did not have hypertension reached 50 (100%). There was no significant difference between PCOS patients and control (P=0.242). These results disagreed with another study conducted by Chen *et al.* (2007), in which the high bioavailable testosterone levels (free androgen index: ≥ 19%) in women with PCOS increased the risk of elevated blood pressure (SBP ≥ 130 mm Hg and/or DBP ≥ 85 mm Hg) with (P=0.029) after adjustment for age, anthropometric measures, and metabolic profiles. Furthermore, the study by Joham *et al.*, (2015) in which Hypertension prevalence was 5.5% (95% CI: 3.3–7.7) in women reporting PCOS and 2.0% (95% CI: 1.6–2.3) in women not reporting PCOS (P <0.001). Other studies agreed with the current result, such as the study by Meyer, McGrath and Teede, (2005), which demonstrated no difference in 24-hour ambulatory BP between women with PCOS and control despite evidence of increased arterial stiffness and endothelial dysfunction and Zimmermann *et al.*, (1992) also did not find differences in mean BP on 24-hour ambulatory BP between PCOS and control despite decreased insulin sensitivity in the PCOS group.

The present study showed the number of depressed women was 11 (22.0%) and the number of non-depressed women reached 39 (78.0%), while in the control group, the number of depressed women was 0 (0.0%) and non-depressed women 50 (100%). There was a significant difference between PCOS patients and control (P=0.001); this result is compatible with the study by Chaudhari, Mazumdar and Mehta (2018). The prevalence of anxiety disorders was (38.6%), and the prevalence of depressive disorders was (25.7%). A study by Cinar *et al.*, (2011) reported that (28.6%) of PCOS women versus (4.7%) of control women had clinical depression scores indicating an 8.1-fold increased risk of depression in PCOS (P=0.001). Also, a study accomplished by Anitha,

SubhaRevathi and Kalaivani (2017) revealed that depression scores were significantly increased in the PCOS group compared to the control ($P < 0.001$). PCOS patients with menstrual irregularities have been linked to higher rates of depression. These irregularities mean these women have brief fertile periods, and emotional changes like anger, irritability, and signs of stress are seen in these periods; the emotional changes are linked to the hormonal changes in those fertile periods (Balikci *et al.*, 2014).

The prevalence of obstructive sleep apnea (OSA) in the general population varies considerably between studies, mainly due to differences in the populations studied, study designs and the methods and criteria used to diagnose OSA. In the present study, the number of women with sleep apnea was 9 (18.0%), and the number of women that did not have sleep apnea reached 41 (82.0%), while in the control group, the number of women with sleep apnea was 0 (0.0%), and the number of women that did not have sleep apnea were 50 (100%). There was a significant difference between PCOS patients and control (P=0.003). Vgontzas *et al.* (2001) assessed the prevalence of OSA in 53 premenopausal women with PCOS compared to 452 control women. The authors found that PCOS women were 30 times more likely to have OSA than control and that the difference between the two groups remained significant even after controlling for BMI. In an independent study published in the same year, a comparison of 18 overweight women with PCOS with 18 age- and weight-matched control showed that PCOS women were significantly more likely to suffer from symptomatic OSA (based on an apnea-hypopnea index (AHI) >5 and the presence of excessive daytime sleepiness) than control women (44.4% vs. 5.5%) (Fogel *et al.*, 2001). Finally, survey assessments of the prevalence of sleep apnea risk (using the Berlin questionnaire) in a cohort of 40 women with PCOS revealed that three of four women were at high risk for sleep apnea. About two third of these PCOS women had poor sleep quality as assessed by the Pittsburgh Sleep Quality questionnaire, and 45% had chronic daytime sleepiness as defined by the Epworth Sleepiness Scale. Remarkably, less than 8% of these 40 women with PCOS cohort were free of sleep complaints (Tasali, Van Cauter and Ehrmann, 2006).

In women with PCOS, the role of hyperandrogenism in Cardiovascular disease (CVD) development has been challenging to distinguish from the effect of obesity and IR. CVD in PCOS may be related to IR, as cardiac and endothelial dysfunction, more significant carotid intima-media thickness test (cIMT), and dyslipidemia are associated with IR in adults (Robinson *et al.*, 1996; Yaralı *et al.*, 2001; Orio *et al.*, 2004; Vural *et*

al., 2005). The current study showed no significant difference between PCOS patients and control (*P*=0.242). This result was agreed with a study by Lakhani *et al.* (2000), which reported beneficial internal carotid artery flow parameters consistent with reduced vascular tone in women with PCOS, another cohort of 309 women with PCOS, and 343 control with a mean (±SD) follow-up time of (23.7) (±13.7) years, women with PCOS had no increase in adverse cardiovascular outcomes in midlife even though they weighed more compared to control, for example, adjusted HR for MI and stroke were 0.74 [(0.32–1.72); *P*=0.48]and 1.05 [(0.28–3.92); *P*=0.94], respectively (Iftikhar *et al.*, 2012). Additionally, a study by Zhao *et al.* (2016), a meta-analysis of 5 case-control and 5 cohort studies of over 100,000 women aged 20–74 years, with 7–40 follow-up years, reported no significant association was observed between PCOS and MI [OR 1.01 (0.68–1.51); *P*=0.95]. In contrast, a large national registry study from Denmark by Rubin *et al.* (2019), which included women with a median age of 29 who were followed for a median of 11 years, found that women with PCOS had a greater risk of incident CVD compared to control. The CVD event rates were (22.6 vs. 13.2) per 1000 patient-years for women with PCOS versus control [fully adjusted hazard ratio (HR) 1.6 (1.5-1.6), $p < 0.001$. Moreover, a population-based retrospective study of 2566 Australian women with PCOS hospitalized from 1997–2011 with 25,660 randomly selected age-matched control also demonstrated that women with PCOS had more diagnoses of cardiovascular events (Hart and Doherty, 2015).

4.2. Laboratory Data among the Studied Patients and Control Group

4.2.1. The mean of FSH, LH, TSH, Prolactin, WBC and Lymphocyte

Regarding follicle-stimulating hormone (FSH), there was a nonsignificant association (*P*=0.402) between PCOS patients and healthy control in the current study. The Mean of (FSH) in PCOS cases was (7.11) and in healthy control was (5.71). The SD of FSH in PCOS cases compared with health control was (10.88 vs. 3.34 respectively). This result is consistent with other studies, such as Anwary, Alfazzaman and Begum,

(2010) study in which fifty subfertile women suffering from PCOS were recruited for evaluation in which serum FSH range was 2.30-13.10 mlU/ml (mean±SD 6.10±1.94). In only one patient (2%), serum FSH level was low(<2.8 mlU/ml) and average (2.8-21.0) in the rest 49 (98%) patients, and another study by Desforges-Bullet *et al.,* (2010) in which 22 PCOS women had serum FSH was range 2.8–9 IU/L (mean 5.9) and control range 4.8–10.6 lU/L (mean 7.3) with (*P*=0.06). The present study disagreed with a study by Mehde and Resan, (2014) in which sixty patients with PCOS and thirty healthy control participated in this study FSH in PCOS cases with (mean±SD) (5.03±0.77), and control had (mean±SD) (6.00±0.95) with *(P*=0.045). Determinations of FSH, however, are characterized by many difficulties. One obvious problem is the inconvenience of the required blood draw on day 2 or 3 of menses. The second issue of concern is the degree of cycle-to-cycle fluctuation in baseline FSH levels (Hehenkamp *et al.,* 2006).

Regarding luteinizing hormone (LH) level, in this study, the Mean of (LH) in PCOS cases was (5.46) and in healthy control was (4.53). The SD of LH in PCOS cases was (6.75) and in healthy control was (3.88). However, the association was nonsignificant (*P*=0.429) between PCOS patients and the control. A study by Anwary, Alfazzaman and Begum (2010) reported that serum LH was normal (1.1-14.7 mIU/ml) in 22 (44%) women and raised (>14.7mIU/ml) in 28 (56%) cases. The mean (±SD) serum LH level was (15.02±3.66 mIU/ml) (range 6.70-25.50). Another study by Mehde and Resan (2014), in which LH in PCOS cases with mean±SD (12.68±3.57) and control had mean±SD (6.96 ±0.99) with (*P*=0.0008). The mean LH level was observed at (12.79±7.1 mIU/ml) in a study by Begum (2009) and Nahar *et al.* (2017). Other studies by Timpatanapong and Rojanasakul (1997), Codner *et al.* (2007) and Kumar *et al.* (2016) found that serum LH was (8.68±5.5 mIU/ml, 8.1±3.0 mIU/ml and 9.3±5.0 mIU/ml respectively). Because of the pulsatile nature of their release, a single test failure to detect an increased LH/FSH ratio, as well as its lack of specificity, has led to the recommendation that LH/FSH ratios should not be included in the diagnostic criteria for PCOS (Alnakash and Al-Taee, 2007)

Concerning thyroid stimulating hormone (TSH), the present study showed a significant association (*P*=0.004) between PCOS patients compared with healthy control. The mean of TSH in PCOS cases was (1.86) and in healthy control was (2.79). The SD of TSH in PCOS cases was (1.03) and in healthy control was (2.03). This result agreed with some studies, such as a study by Anwary, Alfazzaman and Begum (2010), in which the mean (±SD) of TSH serum level was (2.35±0.82 µIU/ml) (range 0.94-4.20); 49 (98%)

women had average level (0.4-4.0 µIU/ml) and 1 (2%) had raised value (>4.0 µIU/ml) and a study by Christodoulopoulou *et al*. (2016) whose found FSH serum level about (2.39±1.63). Also, in a study by Nahar *et al.* (2017), the mean serum TSH was 3.4±1.28 µIU/ml. Islam, Pathan and Ahmed (2015) found hypothyroidism in 11.4% of PCOS patients.

Regarding prolactin level, the mean of prolactin in PCOS cases was (25.24) and in healthy control was (20.93). The SD of prolactin in PCOS cases was (15.57) and in healthy control was (10.75). However, the association between PCOS patients and control was nonsignificant (*P*=0.111). This result agreed with a study by Nahar *et al.* (2017) in which the mean serum prolactin level was found to be (315.15±80.5 µIU/ml) with a reference range of (204-412 µIU/ml) and a study by Anwary, Alfazzaman and Begum (2010), in which serum prolactin was normal (1.9-25.0 ng/ml) in 43 (86%) and raised (>25 ng/ml) in 7 (14%) women; mean (±SD) was (23.52±46.96 ng/ml) (range 5.60-315.18), but Begum (2009) found it higher (415.15±180.5) in another group of Bangladeshi women and study by Mehde and Resan (2014) found a highly significant increase in serum prolactin (*P*>0.001). Islam, Pathan and Ahmed (2015), in another Bangladeshi study, observed hyperprolactinemia in 18.6% of cases of PCOS.

In addition, there was no significant association (*P*=0.230) between white blood cell (WBC) counts in PCOS patients compared with healthy control in this study. The Mean of WBC in PCOS cases was (7.12) and in healthy control was (7.57). The SD of WBC in PCOS cases was (1.94) and in healthy control was (1.92). This result agreed with some studies, such as a study by Patel *et al.,* (2017) in which the mean of WBC in PCOS cases was (7.95±1.50) and in control was (6.87±2.84) with (*P*=0.156). On the contrary, a study by Orio *et al.* (2005) evaluated 150 women with PCOS and 150 control matched for age and BMI. Median WBC in the PCOS and control groups was (7,260 and 5,220 cells/mm^3, respectively), with (*P*<.0001), and in a study by Herlihy *et al.,* (2011), the mean WCC was higher in the PCOS group compared with the non-PCOS group (8.9×10^9/l vs. 7.4×10^9/l *P*=0.002). There was a leucocytosis (WCC>11×10^9/l) present in 19% of the PCOS group compared with 1% in the non-PCOS group (*p*<0.001). In a large national cohort study, WBC was a predictor of coronary heart disease mortality independent of smoking and other traditional risk factors (Brown, Giles and Croft, 2001).

Regarding lymphocyte count, the mean of lymphocytes in PCOS cases was (31.81) and in healthy control was (33.67). The SD of lymphocytes in PCOS cases was (7.50) and in healthy control was (8.78). However, the association was nonsignificant (*P*=0.231) between PCOS patients and control groups. The current study result is compatible with a study by Yilmaz, Duran and Basaran (2016) in which the mean lymphocyte count was (31.04±6.42) in the patient group and (34.4±6.69) for the control group with (*P*=0.036). On the contrary, a study by Almaeen *et al.* (2022), in which the mean lymphocyte count was (38.77±10.74) in the patients' group and (46.99 ±1.63) in control with significant association (*P*=0.000). Significant elevation of lymphocytes and monocytes was also found in a study by Orio *et al.* (2005).

4.2.2. C- Reactive Protein in PCOS Patients and Control Groups

Several inflammatory biomarkers, such as the C-reactive protein, were employed to predict low-grade inflammation in PCOS (Kelly *et al.*, 2001). The present study showed a highly significant association between CRP and PCOS patients compared with healthy control. The number of positive CRP women was 12 (24.0%), and the number of negative CRP women reached 38 (76.0%), while in the control group, the number of positive CRP women was 0 (0.0%) and negative CRP women 50 (100%) with (*P*=0.005). These results agreed with several studies indicated to highly significant CRP levels in PCOS patients versus control (Kelly *et al.*, 2001, Boulman *et al.*, 2004 Blumenfeld, 2019).

4.3. Serum Level of IL-17A in Patients and Control Groups

In the present study, the Mean of IL-17A serum level in PCOS cases was (11.46) and in healthy control was (9.53), SD in PCOS cases was (9.50) and in healthy control was (18.99). However, the association was significant (*P*=0.005) between PCOS patients' serum IL-17A and control, as shown in table (3-5)**.** This result agreed with several studies, such as a study by Balta *et al.* (2016), in which the IL-17A level was significantly higher in PCOS patients compared to the control (*P*<0.05). Also, the study by Özçaka *et al.* (2013) showed that the two groups with PCOS had higher concentrations of IL-17A. In contrast, another study by Zangeneh, Naghizadeh and Masoumi (2017) showed that the serum level of IL-17A in women with PCOS was significantly lower than in the control group. Also, a study by Hesampour *et al.* (2019) in which IL-17A levels were

undetectable in most cases and control. Like some other cytokines, the short serum half-life of IL-17A might account for the unsuccessful detection of this cytokine in samples (Najafi *et al.*, 2014).

4.4. Correlation Between Study Markers in PCOS Patients and Control

4.4.1. Correlation Between LH and FSH in PCOS Patients

The normal gonadotrophin axis is disturbed in PCOS women; therefore, LH levels increase, and FSH levels decrease, leading to a reversal of the LH/FSH ratio between LH level and FSH level for PCOS patients (Balen *et al.*, 2003). The present study showed a significant correlation between LH and FSH (P=0.005). This result disagreed with a study by Banaszewska *et al.* (2003), who reported an abnormal LH/FSH ratio when it is greater than 2 and 4.5% of PCOS women having an elevated ratio. However, they have shown that the mean LH/FSH ratio was not statistically significant in his study between the PCOS and non-PCO. Another study conducted in Saudi Arabia also has reported that regardless of age and weight factors, Saudi patients with PCOS have higher levels of LH/FSH; but have lower levels of FSH compared to controls, and they could not find a significant increase in LH levels (Fakhoury *et al.*, 2012).

4.4.2. Correlation Between Lymphocytes and prolactin in PCOS Patients

There was a significant correlation between lymphocyte count and prolactin (r=0.399, P=0.004). This result agreed with the study by SM and Al-Allaff (2022), which showed strong positive correlation coefficients between WBC with neutrophils, lymphocytes and monocytes (0.797, 0.790, 0.712, respectively) and finally, leptin and prolactin (0.474). The findings revealed that the hormone prolactin affects some immune parameters in women with PCOS.

4.4.3. Correlation Between Lymphocytes and WCC in PCOS Patients

There was a significant correlation between lymphocyte count and WCC (r=0.317, *P*=0.025). The result agreed with a study by Shi *et al.* (2013) in which the total WBC counts and lymphocyte counts were elevated in PCOS subjects (P<0.01). Higher lymphocyte counts contributed to higher total WBC counts in PCOS women compared to age-matched control. When BMI adjusted the data, the difference in WBC and lymphocyte counts between patients and control remained significant. Moreover, a study by SM and Al-Allaff (2022) showed strong positive correlation coefficients between WBC with lymphocytes (0.790). On the contrary, a study by Tola, Yalcin and Dugan (2017) reported a positive predictive effect of WBCs and a negative predictive effect of lymphocytes on insulin resistance in women with PCOS. Notably, a study showed that increased inflammatory biomarkers in women with PCOS were equal in lean and obese women compared with BMI-matched women who had no PCOS (Keskin Kurt *et al.,* 2014).

4.5. IL-17A Gene (rs2275913) Polymorphism in Patients and Control

Genetic polymorphism of the IL-17A gene (rs2275913) was observed with three genotypes (AG, GG, and AA) in PCOS patients and control. The "G" allele was elevated in control compared with patients, whereas the "A" allele was elevated in patients compared with control. In the alleles, there were no significant differences with the group studied (*P*=0.307). The AG genotype was found to be at a higher frequency in patients compared with control, the GG genotype was found to be at a higher frequency in control compared with patients, and the AA genotype was elevated in patients compared with control. The genotype showed no significant differences with the group studied (*P*=0.421). This result disagreed with a study by Hesampour *et al.* (2019), showed that the distribution of (rs2275913) SNP in IL-17A was different between Iranian women with PCOS and healthy women, GG genotype and G allele were found in a higher proportion in the PCOS group compared with the control group, which was considered the first study to report the relationship between IL-17A (rs2275913) and PCOS. Furthermore, a study by Zou *et al.* (2022) in which the distribution of (rs2275913) in the control and PCOS groups was statistically different. The multivariate logistic regression model showed that (rs2275913) could increase the risk of PCOS occurrence.

4.6. Association Between IL-17A Genotypes and Alleles with IL-17A Serum Level in PCOS Patients

The study showed a nonsignificant association between IL-17A genotypes and alleles with IL-17A serum level in PCOS patients (*P*=0.873) and allele frequency (*P*=0.959). These results disagreed with the study by Zou *et al.* (2022) in which, among PCOS patients, patients carrying the (rs2275913) A allele increased serum IL-17A levels. It could lead to more disturbed hormone levels and a more severe clinical manifestation. Furthermore, a study by Hesampour *et al.* (2019) showed that IL-32 serum level was not significantly different between the two studied groups and the serum level of IL-17A was not detectable.

4.7. Receiver Operating Characteristic Curves (ROC)

Curve analysis showed the cutoff value of (3.784) for IL-17A with AUC (0.662). According to the AUC, the IL-17A was a predictive marker for diagnosing PCOS. The sensitivity and specificity of the immune marker were 68.0% and 54.0%, respectively. This result agreed with other studies, such as a study by Balta *et al.* (2016), in which ROC curve analysis, PCOS can be predicted by the use of IL-17A, IL-23 and IL-33 which at a cutoff value of 8.37 pg/mL (44 % sensitivity, 83 % specificity), 26.75 pg/mL (36 % sensitivity, 64 % specificity) and 14.28 pg/mL (83 % sensitivity, 39 % specificity), respectively.

Conclusions and Recommendations

Conclusions:

1. The GG genotypes and G allele of *IL-17A Gene* (rs2275913) polymorphism could protect PCOS women in the Iraqi population.

2. Interleukin-17A (IL-17A) serum level had a highly significant relationship with control compared with PCOS patients; therefore, Interleukin-17A may be used as a predictive marker for diagnosing PCOS.

3. IL-17A serum level was not associated with genotypes and alleles frequency of IL-17A gene (rs2275913) polymorphism in PCOS.

4. There were significant association between TSH and PCOS.

Recommendations:

1. Further studies with larger samples are required to better understand the effect and association of IL-17A gene (rs2275913) polymorphism with PCOS patients.

2. Other studies are required to take the association between other SNPs of the IL-17A gene and PCOS.

3. Studying the role of other immunological markers in PCOS patients.

4. Other studies with large sample size should be conducted to confirm the significant association of IL-17A serum level with PCOS.

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Appendix

Appendix I: PCOS patients' and healthy control questionnaires

Patients and Control Data:

- Age (year)
- Menarche age
- Duration of marriage
- Duration of infertility
- Body Mass Index (BMI) (Kg/m2)
  Height (m)
  Weight (kg)
- Menses
  Regular
  Irregular
  Amenorrhea
  Hyper menorrhea
  Oligomenorrhea
- Occupation
  Housewife
  Occupied

-Hirsutism
- Acne appearance
- Family history of infertility
- Lack of physical exercise
- High blood pressure
- Depression
- Sleep apnea
- Cardiovascular disease

Lab. Data:

- Follicle stimulating hormone (FSH) (mIU/ml)
- Luteinizing hormone (LH) (mIU/ml)
- Thyroid-stimulating hormone (TSH) (mIU/ml)
- Prolactin (Pro) (ng/ml)
- CRP
- White Cell Count (WCC)
- Lymphocyte count

Appendix II: ELISA instrument

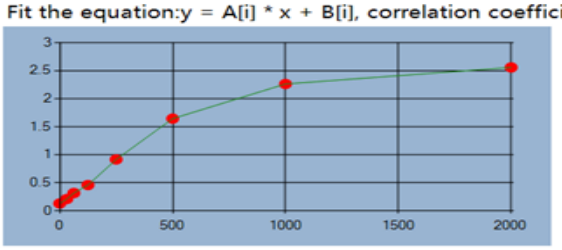
Appendix III : ELISA curve for IL-17A

IL-17A Standard curve line

Measurement method: End point method

Calculation method:Non-linear regression

Fit the equation:y = A[i] * x + B[i], correlation coefficient =0.990

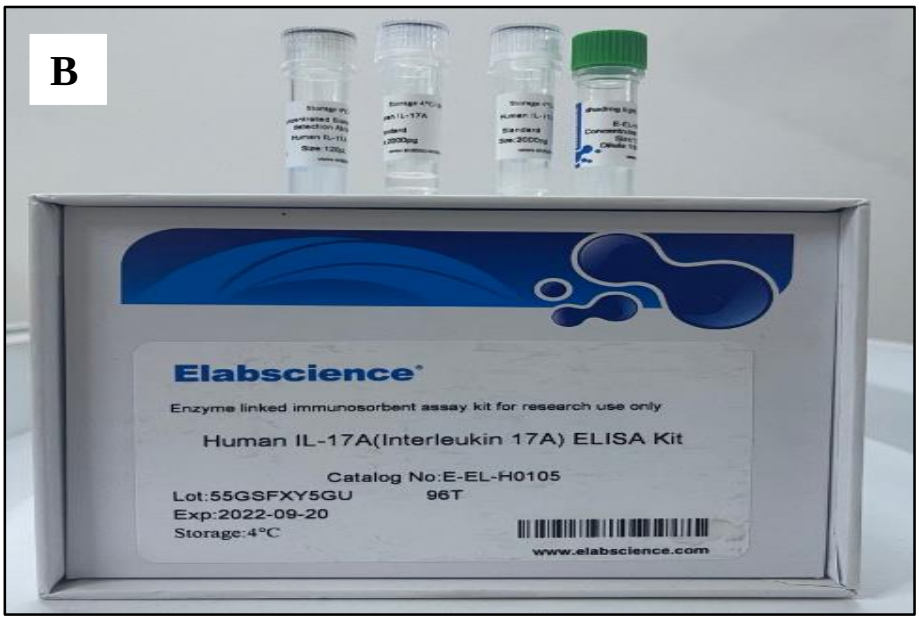


ABS	Concentration
0.132	0.000
0.211	31.250
0.318	62.500
0.460	125.000
0.917	250.000
1.645	500.000
2.263	1000.000

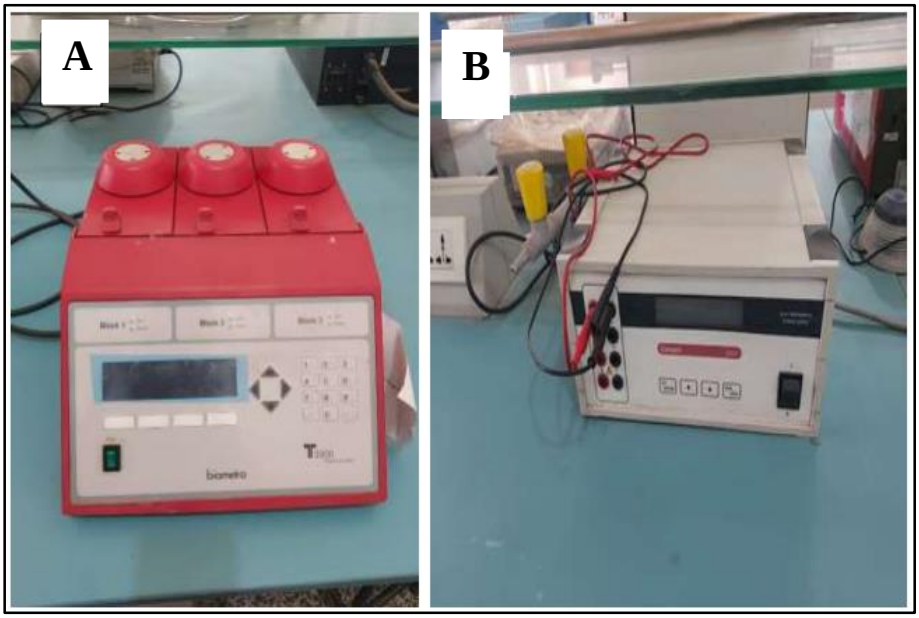
Appendix IV : ELISA kit for serum IL-17A (A and B)

A

B



Appendix V : (A) PCR instrument, (B) Gel electrophoresis and (C) Biological safety cabinet



Appendix VI : RT-PCR instrument

الخلاصة

متلازمة تكيس المبايض (PCOS) هي أكثر اضطرابات الغدد الصماء شيوعًا بين النساء في سن الإنجاب والتي تتميز باضطرابات الدورة الشهرية والسمات السريرية أو الكيميائية الحيوية لفرط الأندروجين. تترافق متلازمة تكيس المبايض مع العديد من الأمراض المصاحبة بما في ذلك السمنة ومقاومة الأنسولين ومرض السكري من النوع الثاني، بالإضافة إلى اضطرابات المزاج وتوقف التنفس أثناء النوم (OSA) وأمراض القلب والأوعية الدموية (CVD). هدفت الدراسة الحالية إلى تحديد تأثيرتعدد الانماط الجينية *IL-17A* (rs2275913) على متلازمة تكيس المبايض (PCOS) بالإضافة إلى الكشف عن مستوى IL-17A في الدم بأستخدام تقنية الايلايزا.

هذه الدراسة عبارة عن دراسة الحالات والشواهد واعداد المشاركات في هذه الدراسة مقسمة إلى مجموعتين مجموعة الاصحاء (عدد= ٥٠) ومجموعة المرضى (عدد= ٥٠) مسجلين في مدينة الصدر التعليمية الطبية ومستشفى الزهراء التعليمي خلال الفترة من تشرين الثاني ٢٠٢١ إلى نيسان ٢٠٢٢.

تم جمع الدم الكامل من كل مشارك مع ملاحظة أنه تم استخدام المصل لتحديد مستويات IL-17A في المصل لجميع العينات بواسطة تقنية الايلايزا بينما تم استخدام الدم الكامل لتحديد عدد خلايا الدم البيضاء والخلايا اللمفاوية واستخلاص الحمض النووي. وباستخدام Sysmex XN-350 احد أجهزة تحليل الدم الآلي التفاضلية تم الكشف عن عدد خلايا الدم البيضاء وعدد الخلايا اللمفاوية. واكتشاف تعدد الانماط *لجين* (rs2275913) *IL-17A* بواسطة تقنية الوقت الحقيقي لتفاعل البوليميريز التسلسلي (RT-PCR).

أظهرت نتيجة هذه الدراسة وجود علاقة ذات دلالة إحصائية بين زيادة مستوى المصل IL-17A ومرضى متلازمة تكيس المبايض مقارنة مع الاشخاص الاصحاء وكان المتوسط الحسابي IL-17A في المرضى (١١,٤٦) والمتوسط الحسابي للاشخاص الاصحاء (٩,٥٣). كما أن مستوى IL-17A يعتبر علامة تنبؤية لتشخيص متلازمة تكيس المبايض.

فيما يتعلق بتعداد خلايا الدم البيضاء والخلايا اللمفاوية ، كان هناك ارتباط غير مهم بين مرضى متلازمة تكيس المبايض والاشخاص الاصحاء مع (P = 0.230) لكريات الدم البيضاء (P = 0.231) للخلايا الليمفاوية.

كما أظهرت الدراسة الحالية تعدد الأنماط الوراثي*لجين* *IL-17A* (rs2275913) والذي لوحظ مع ثلاثة طرز وراثية (AG، GG ، و AA) في مرضى متلازمة تكيس المبايض ومجموعة السيطرة. ارتفع الأليل "G" في مجموعة السيطرة مقارنةً بالمرضى (٦٧٪ مقابل ٥٩٪ على التوالي) حيث ارتفع الأليل "A" في المرضى مقارنةً بمجموعة السيطرة (٤١٪ مقابل ٣٣٪ على التوالي). في الأليلات لم يكن هناك فرق معنوي مع مجموعة الدراسة (P=0.307). تم العثور على النمط الجيني AG ليكون بتكرار أعلى في المرضى مقارنة مع مجموعة السيطرة (٥٠,٠٪ مقابل ٤٢,٠٪ ، على التوالي) ، وجد أن النمط الجيني GG كان بتكرار أعلى في مجموعة السيطرة مقارنة بالمرضى (٤٦,٠٪ مقابل ٣٤,٠٪ ، على التوالي). وارتفع النمط الجيني AA في المرضى مقارنة مع مجموعة السيطرة (١٦,٠٪ مقابل ١٢,٠٪ ، على التوالي). في التركيب الوراثي ، لم يكن هناك فرق معنوي مع

مجموعة الدراسة (P=0.421). بالإضافة إلى ذلك ، وجدت هذه الدراسة ارتباطًا غير مهم بمستوى مصل IL-17A مع الأنماط الجينية وتكرار أليل *لجين IL-17A* (rs2275913) في مرضى متلازمة تكيس المبايض.

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دراسة دور تعدد الاشكال الجيني للانترلوكين A-17 ومستوى المصل في النساء اللواتي يعانين من متلازمة تكيس المبايض

رسالة مقدمة الى مجلس كلية الطب/جامعة كربلاء وهي جزء من متطلبات نيل درجة الماجستير في الاحياء المجهرية الطبية/الاحياء المجهرية

من قبل الطالبة

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