



بسم الله الرحمن الرحيم

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بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى

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ملاحظات: لا يوجد



## INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common endocrine metabolic disorders worldwide which affects approximately 7–10% of women during their reproductive age and characterized by hyperandrogenism, menstrual disturbance, chronic anovulation, polycystic ovaries, and infertility (*Kanafchian, 2017*).

According to the diagnostic criteria of Rotterdam in 2003, PCOS women should have at least two of the three criteria such as Oligo or anovulation, clinical hyperandrogenism and show a polycystic ovary on ultrasound (*Fauser, 2004*).

PCOS appears to be associated with an increased risk of metabolic aberrations, including insulin resistance and hyperinsulinemia, type 2 diabetes mellitus, dyslipidemia, and cardiovascular disease throughout womens' lifespan (*Teede, 2011*).

Obesity with a preponderance for abdominal fat accumulation is a common feature of PCOS. Anomalies in adipose tissue distribution and function contribute to the metabolic abnormalities such as insulin resistance and the generation of a proatherogenic inflammatory milieu (*Pazderska and Gibney, 2015*).

Insulin resistance and hyperinsulinemia are key findings in patients with PCOS, whether or not they are obese and about 70% of patients with PCOS are insulin-resistant (*Kanafchian, 2017*).

Recently, Lipocalin-2, also known as Neutrophil Gelatinase-Associated Lipocalin-2 (LCN2 or NGAL) has drawn the attention of many researchers, due to its implication in metabolic alterations and in the regulation of the immune response and cell homeostasis. Lipocalin-2 is a member of the Lipocalin superfamily comprised by small secreted proteins, and it is abundantly expressed in adipose tissue and liver (*De la Chesnaye et al., 2015*).

Lipocalin-2 is abundantly produced from adipocytes. The expression and secretion of this protein increases sharply after conversion of preadipocytes to mature adipocytes. Its expression can be induced by various inflammatory stimuli, including lipopolysaccharide and IL-1 $\beta$ . This evidence suggests that Lipocalin-2 may participate in inflammation-related disorders (*Law et al., 2010*). Lipocalin-2, is associated with obesity, obesity-related inflammatory processes and insulin resistance. Expression of LCN-2 was elevated by agents that promote insulin resistance and reduced by thiazolidinediones that decrease insulin resistance (*Yan et al., 2007*).

## **AIM OF THE WORK**

The aim of this study is to:

- ❖ Assess the association of Lipocalin-2 with polycystic ovary syndrome in a sample of the Egyptian female.
- ❖ Study the effect of metformin therapy on Lipocalin-2 level in female patients with polycystic ovary syndrome

## **Chapter (1)**

### **Polycystic Ovarian Syndrome**

#### **I. Epidemiology and prevalence (Ethnic variations)**

It is the commonest endocrine condition to affect women with an estimated prevalence of 10–15%. The prevalence of polycystic ovaries in the general population, as detected by ultrasound alone is 20–30%. In UK (between the ages of 18 and 25 years), polycystic ovaries are identified by ultrasound in 33%, and the prevalence of PCOS is 26%. **(Bellver et al., 2018)**

Factors that affect expression and presentation

- Racial differences in the color and distribution of hair
- Variations in hormone production and receptor activity
- Genetic variations in the control of insulin metabolism **(Escobar-Morreale, 2018)**

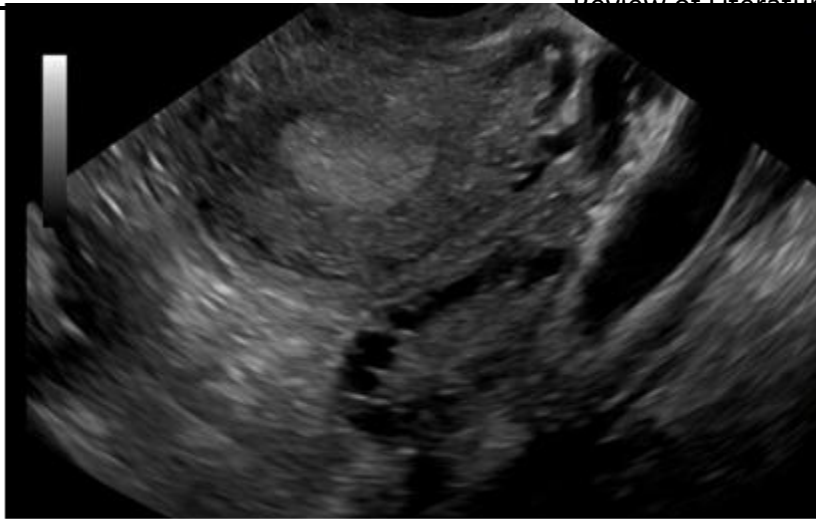
PCOS also appears to run in families, with approximately 50% of first degree female relatives being affected and an increased risk of metabolic problems in male relatives **(Teede et al., 2018)**.

#### **II. Diagnosis**

##### **i. Rotterdam Criteria (2 out of 3)**

- The presence of clinical or biochemical features of hyperandrogenism

- Oligo-ovulation or anovulation (in other words a menstrual cycle disturbance)
- Polycystic ovaries on ultrasound:
  - The threshold for polycystic ovarian morphology (**PCOM**) is a follicle number per ovary of  $\geq 20$  (measuring 2–9 mm) and/or an ovarian volume  $\geq 10$  ml on either ovary, ensuring no corpora lutea, cysts or dominant follicles are present (**ESHRE 2018**).
  - Ultrasound should not be used for the diagnosis of PCOS in those with a gynecological age of  $< 8$  years ( $< 8$  years after menarche), due to the high incidence of multi-follicular ovaries in this life stage (**ESHRE 2018**).
  - In patients with irregular menstrual cycles and hyperandrogenism, an ovarian ultrasound is not necessary for PCOS diagnosis; however, ultrasound will identify the complete PCOS phenotype (**ESHRE 2018**).



**Figure (1):** Transvaginal ultrasound scan of polycystic ovary  
(*Dokras et al., 2018*)

Anti-mullerian hormone (AMH) may be a precise marker to detect PCO with a threshold serum concentration of  $>35$  pmol/l, but should not yet be used as an alternative for the detection of PCOM or as a single test for the diagnosis of PCOS (**ESHRE 2018**).

This definition of PCOS requires the exclusion of specific underlying diseases of the adrenal or pituitary glands (e.g. hyperprolactinemia, acromegaly, congenital adrenal hyperplasia, Cushing's syndrome, androgen-secreting tumors of the ovary or adrenal gland) which could predispose to similar ultrasound and biochemical features and also the exclusion of other causes of menstrual cycle irregularity secondary to hypothalamic, pituitary or ovarian dysfunction (**Ortiz-Flores et al., 2019**).

**Table (1): Another Definitions of PCOS**

Definition/year	Diagnostic criteria <sup>1</sup>
NIH/1990	Requires the simultaneous presence of: 1. Hyperandrogenism (clinical and/or biochemical) 2. Ovarian dysfunction
Rotterdam (ESHRE/ASRM)/2003	Requires the presence of at least two criteria: 1. Hyperandrogenism (clinical and/or biochemical) 2. Ovulatory dysfunction 3. Polycystic ovarian morphology <sup>2</sup>
AES/2006	Requires the presence of hyperandrogenism (clinical and/or biochemical) and either: 1. Ovulatory dysfunction 2. Polycystic ovarian morphology <sup>2</sup>
Androgen Excess and PCOS Society/2009	Requires the simultaneous presence of: 1. Hyperandrogenism (clinical and/or biochemical) 2. Ovarian dysfunction (ovulatory dysfunction and/or polycystic ovarian morphology <sup>2</sup> )

<sup>1</sup> It is important to state that as well as being well established by all the diagnostic criteria available PCOS diagnosis is an exclusion diagnosis of other disorders, such as NC-CAH, Cushing syndrome, acromegaly, hyperprolactinemia, hypothyroidism, premature ovarian failure, virilizing adrenal or ovarian neoplasm and a drug-related condition.

<sup>2</sup> The ultrasound definition of polycystic ovarian morphology is the presence of  $\geq 12$  follicles with a 2- to 9-mm diameter on the ovary. An ovarian volume  $> 10$  ml is also suggestive. Only one ovary consistent with polycystic ovarian morphology is sufficient for the diagnosis.

ESHRE = European Society for Human Reproduction and Embryology; ASRM = American Society for Reproductive Medicine.

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### **III. Pathogenesis:**

Polycystic ovaries develop when the ovaries are stimulated to produce excessive amounts of male hormones (androgens), particularly testosterone, by either the release of excessive luteinizing hormone by the anterior pituitary gland, high levels of insulin in the blood (hyperinsulinaemia) in women whose ovaries are sensitive to this stimulus or reduced levels of sex-hormone binding globulin (SHBG) resulting in increased free androgens (**Kabel, 2016**).

The syndrome acquired its name due to the common sign on ultrasound examination of multiple ovarian cysts which represent immature follicles. The follicles have developed from primordial follicles but the development has stopped at an early antral stage due to the disturbed ovarian function. The follicles may be oriented along the ovarian periphery appearing as a ‘string of pearls’ on ultrasound examination (**Lebbe, 2016**).

Patients with PCOS have higher gonadotrophin releasing hormone (GnRH), which in turn results in an increase in LH/FSH ratio in females with PCOS. The majority of patients with PCOS have insulin resistance and/or obesity. Their elevated insulin levels contribute to or cause the abnormalities seen in the hypothalamic-pituitary-ovarian axis that lead to PCOS. Hyperinsulinemia increases GnRH pulse frequency, LH over FSH dominance, increased ovarian androgen production, decreased follicular maturation and decreased SHBG binding.

All these factors contribute to the development of PCOS (**Laganà et al., 2018**).

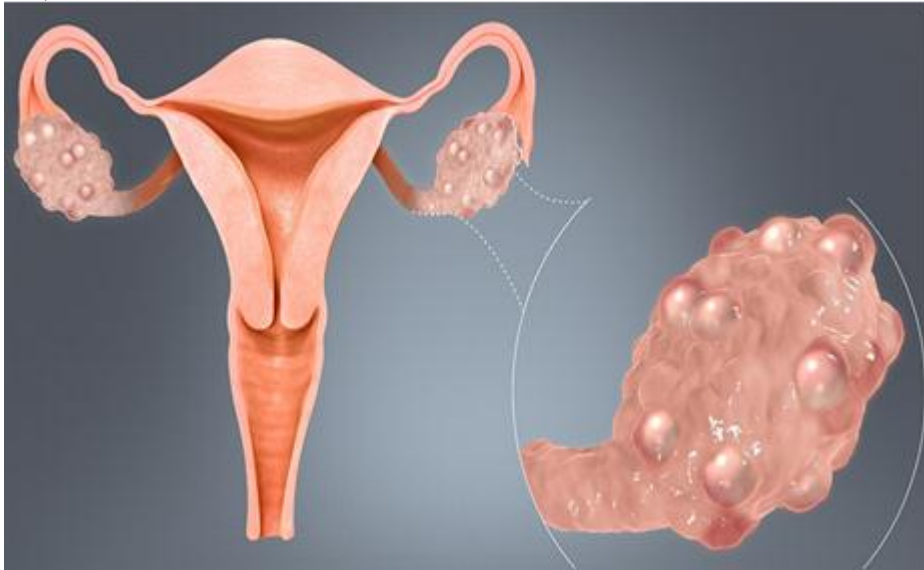
PCOS is characterized by a complex positive feedback of insulin resistance and hyperandrogenism. In most cases, it cannot be determined which of those two should be regarded to be the causative agent. Experimental treatment with either anti-androgens or insulin sensitizing agents improves both hyperandrogenism and insulin resistance (**Escobar-Morreale, 2018**).

Adipose tissue possesses aromatase, an enzyme that converts androstenedione to estrone and testosterone to estradiol. The excess of adipose tissue in obese patients causes them to have both excess androgens (which are responsible for hirsutism and virilization) and estrogens (which inhibit FSH via negative feedback) (**Stracquadanio, 2020**).

PCOS may be associated with chronic inflammation of the ovary which may induce conformational, endocrinal and metabolic changes which may predispose to PCOS. Several studies correlate the inflammatory mediators and oxidative stress with anovulation and other PCOS symptoms (**Lebbe, 2016**).

It was previously suggested that the excessive androgen production in PCOS could be caused by a decreased serum level of insulin-like growth factor binding protein-1 (IGFBP-1), in turn increasing the level of free IGF-1 which stimulates ovarian androgen production, but recent data concludes this

mechanism to be unlikely. PCOS has also been associated with a specific fragile X mental retardation 1 (FMR1) sub-genotype. Many studies suggested that women who have heterozygous-normal/low FMR1 have polycystic-like symptoms of excessive follicle-activity and hyperactive ovarian function (Shoukath et al., 2018).



**Figure (2):** Polycystic ovaries (*Bellver et al., 2018*)

#### *Menopause life stage and PCOS*

Postmenopausal persistence of PCOS could be considered likely with continuing evidence of hyperandrogenism. A diagnosis of PCOS post-menopause could be considered if there is a past diagnosis of PCOS, a long-term history of irregular menstrual cycles and hyperandrogenism and/or PCOM, during the reproductive years. Postmenopausal women presenting with new-onset, severe or worsening hyperandrogenism including hirsutism, require investigation

to rule out androgen-secreting tumors and ovarian hyperthecosis (Jeanes and Reeves, 2017).

#### **IV. Etiology:**

Some evidence suggests that patients have a functional abnormality of cytochrome P450c17, the 17-hydroxylase, which is the rate-limiting enzyme in androgen biosynthesis (Daan et al., 2016).

PCOS is a genetically heterogeneous syndrome in which the genetic contributions remain incompletely described. PCOS is an inherently difficult condition to study genetically because of its heterogeneity, difficulty with retrospective diagnosis in postmenopausal women, associated subfertility, incompletely understood etiology, and gene effect size (Barber et al., 2017).

Studies of family members with PCOS indicate that an autosomal dominant mode of inheritance occurs for many families with this disease. The fathers of women with PCOS can be abnormally hairy; female siblings may have hirsutism and oligomenorrhea; and mothers may have oligomenorrhea. Research has suggested that in a large cohort of women with PCOS, a family history of type 2 diabetes in a first-degree family member is associated with an increased risk of metabolic abnormality, impaired glucose tolerance, and type II diabetes (Cavalcante et al., 2019).

An important link between PCOS and obesity was corroborated genetically for the first time by data from a case-

control study in the United Kingdom that involved 463 patients with PCOS and more than 1300 female controls. The investigators demonstrated that a variant within the FTO gene (rs9939609, which has been shown to predispose to common obesity) was significantly associated with susceptibility to the development of PCOS (**Barber et al., 2008**).

Wickenheisser et al reported that CYP17 promoter activity was 4-fold greater in cells of patients with PCOS. This research suggests that the pathogenesis of PCOS may be in part related to the gene regulation of CYP17 (**Wickenheisser et al., 2000**). However, in a study that assessed candidate genes for PCOS using microsatellite markers to look for association in 4 genes— CYP19, CYP17, FST, and INSR —only 1 marker near the INSR gene was found to be significantly associated with PCOS. The authors concluded that a susceptibility locus for PCOS (designated PCOS1) exists in 19p13.3 in the INSR region, but it cannot be concluded that the INSR gene itself is responsible (**Tucci et al., 2001**).

Subsequent studies have found additional associations, such as those of 15 regions in 11 genes previously described to influence insulin resistance, obesity, or type 2 diabetes. Individuals with PCOS were found more likely to be homozygous for a variant upstream of the PON1 gene and homozygous for an allele of interest in IGF2. Interestingly, the PON1 gene variant resulted in decreased gene expression, which could increase oxidative stress. The exact result of the

IGF2 variant is unclear, but IGF2 stimulates androgen secretion in the ovaries and adrenal glands (**Fenjanchi Rahnema et al., 2020**).

In study by Goodarzi et al, the leucine allele was found to be associated with protection against PCOS, as compared to the valine allele at position 89 in SRD5A2. The leucine allele is associated with a lower enzyme activity (**Goodarzi et al., 2006**). When the results of this study are combined with those of an observational study by Vassiliadi et al, based on urinary steroid profiles in women with PCOS, further support can be found for an important role for 5-alpha reductase in the pathogenesis of this syndrome (**Vassiliadi et al., 2009**).

In a genome-wide association study for PCOS in a Han Chinese population, 3 strong regions of association were identified, at 2p16.3, 2p21, and 9q33.3. The polymorphism most strongly associated with PCOS at the 2p16 locus was near several genes involved in proper formation of the testis, as well as a gene that encodes a receptor for luteinizing hormone (LH) and human chorionic gonadotropin (HCG). This polymorphism was also located 211kb upstream from the FSHR gene, which encodes the follicle-stimulating hormone (FSH) receptor (**Mykhalchenko et al., 2017**).

## **V. The spectrum of clinical manifestations of polycystic ovarian syndrome**

- Hyperandrogenism (hirsutism, acne, alopecia)
- Menstrual disturbance (unpredictable irregular heavy cycles/Amenorrhea/oligo-menorrhea)
- Infertility (Anovulatory infertility)
- Obesity (**Muhunthan, 2018**)

Polycystic ovaries can exist without clinical signs of the syndrome, expression of which may be precipitated by various factors, most predominantly an increase in body weight (**Jeanes and Reeves, 2017**).

*N.B. Health professionals should consider ethnic variation in the presentation and manifestations of PCOS, including: (ESHRE 2018)*

- *A relatively mild phenotype in Caucasians*
- *Higher body mass index (BMI) in Caucasian women, especially in North America and Australia*
- *More severe hirsutism in Middle Eastern, Hispanic and Mediterranean women*
- *Increased central adiposity, insulin resistance, diabetes, metabolic risks and acanthosis nigricans in South East Asians and Indigenous Australians*
- *Lower BMI and milder hirsutism in East Asians*
- *Higher BMI and metabolic features in Africans*

### **❖ Hyperandrogenism (hirsutism, acne, alopecia)**

Ovarian hyperandrogenism is thought to have genetic origins with amplification in some by hyperinsulinemia