با نام خالق ملت

Immunologic Aspects of PCOS

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Definition

- Polycystic ovary syndrome (PCOS) is the most prevalent endocrine disorder among premenopausal women.

  Yildiz, B.O.,et al. 2012. Human Reproduction

- The incidence of this syndrome varies, ranging from 6% to 20%
  - Depending on the diagnostic criteria applied, with higher prevalence in overweight or obese women and in specific ethnic groups.

    Lim, S.S., et al 2012. Human Reproduction

    Ding, T., et al 2017. OncoTarget

Definition

- PCOS is a complex and heterogeneous endocrinopathy
- Characterized by a constellation of symptoms and clinical features:
  - ❖ Hyperandrogenism (clinical or biochemical)
  - ❖ Ovarian dysfunction (menstrual irregularities)
  - ❖ Polycystic ovarian morphology.

- There are several diagnostic criteria for PCOS that utilize different combinations of these clinical traits.

- According to the Rotterdam criteria, the most widely used for the clinical diagnosis of PCOS is defined by at least **two of the three** aforementioned clinical features.

Rotterdam, E.A., 2004. Human Reproduction

- PCOS is considered the leading cause of anovulatory infertility.

  Franks, S., 2003. Endocrinology and Metabolism Clinics of North America

- PCOS is therefore clinically associated with subfertility or infertility.

- The deleterious impact of this pathology is not confined to reproductive function, and metabolic function is also frequently compromised.

- PCOS is closely linked to metabolic disorders such as obesity and insulin resistance (IR).

Gilbert, E.W., et al. 2018. Clinical Endocrinology (Oxf).

- A large proportion of women with PCOS :
  - ❖obese or overweight

Moran, C., 2012. The Internet Journal of Endocrinology

  - ❖exhibit IR with associated compensatory hyperinsulinemia.

Legro, R.S., 2004. Obstetrical and Gynecological Survey

Marshall and Dunaif, A., 2012. Fertility and Sterility.

Associated health conditions

- Metabolic complications
- Obesity
- Diabetes Mellitus
- Cardiovascular risk
- Neurological and psychological functions
- Cancer
  - ❖Endometrial and breast cancer
- Infertility

Barthelmess and Naz 2015

Etiology

- PCOS is a familial polygenic condition thought to be attributed to both genetic and environmental factors.

Franks S, et al. Hum Reprod. 1997
Franks S and McCarthy M. Rev Endocr Metab Disord. 2004
Vink JM, et al. J Clin Endocrinol Metab. 2006

- Several lines of evidence suggest that developmental, environmental, genetic, and epigenetic mechanisms are involved in the etiology of this endocrine disorder.

Fenichel, P.,et al, 2017. Annales d'Endocrinologie.
Escobar-Morreale, H.F., 2018. Nature Reviews Endocrinology

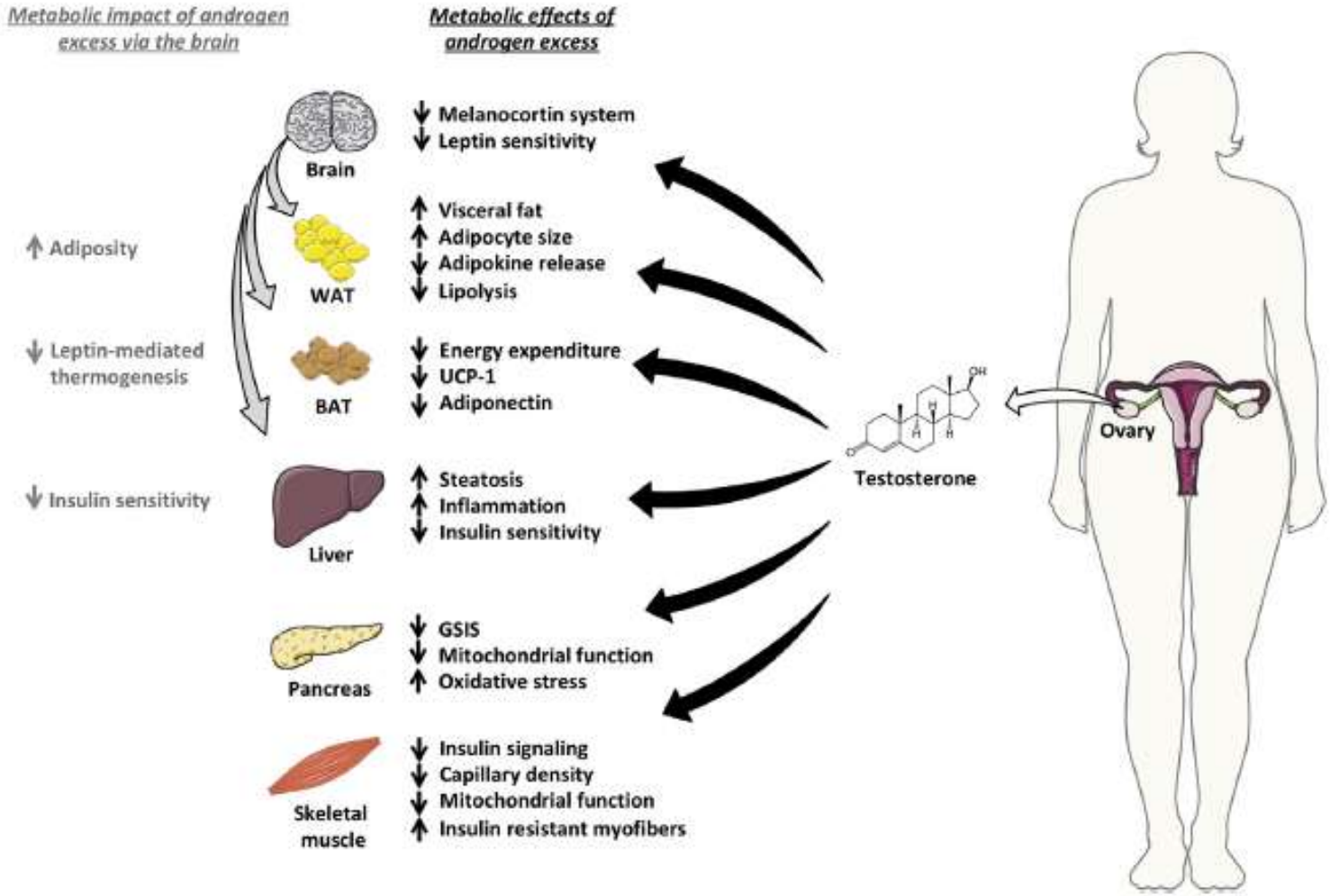

Figure 2: Metabolic impact of androgen excess in PCOS. In women with PCOS, androgen excess has a detrimental impact on different metabolic tissues, including the adipose tissue (white and brown), liver, pancreas, and skeletal muscle. Androgen excess also impairs systemic metabolism via the brain, primarily increasing adiposity and reducing insulin sensitivity. The figure was created using tools provided by Servier Medical Art (https://smart.servier.com).

Sanchez-Garrido and Tena-Sempere 2020

Immunology

- Obesity has recently been classified as a status of **low-grade inflammation** due to the excessive production of cytokines, adipokines, and other reactants.

Repaci A, et al. Mol Cell Endocrinol. 2011

- These markers include TNF-α, IL-6, IL-1, IP-10, CRP and IL-18.

Repaci A, et al. Mol Cell Endocrinol. 2011
Orio F, et al. J Clin Endocrinol Metab. 2005
Alexander RW. N Engl J Med. 1994
Amato G, et al. Obstet Gynecol. 2003

- They act as inflammation mediators to maintain inflammation in adipose tissue.

Gordon S. Nat Rev Immunol. 2003

- It is thought that the constant release of these mediators is what initiates insulin resistance, type 2 diabetes, and other metabolic complications.

Repaci A, et al. Mol Cell Endocrinol. 2011

- It is also thought that this inflammation in PCOS could be causative of the common metabolic and cardiovascular difficulties.

CRP, a common marker of inflammation, is produced by adipose tissue in response to pro-inflammatory cytokines.

Castell Jv et al. FEBS Lett. 1989

High levels of CRP are strongly correlated with the risk of cardiovascular complications.

Ridker PM, et al. Circulation. 2003

It has been well-established that women with PCOS have increased levels of CRP when compared to healthy subjects (up to 96% greater, and 102% when BMI was matched).

Kelly CC, et al. J Clin Endocrinol Metab. 2001
Escobar-Morreale HF, et al. Fertil Steril. 2011

There is also a relationship between PCOS and IL-18, another pro-inflammatory cytokine.

IL-18 is associated with IR and metabolic complications, and has been found to correlate with testosterone levels in women with PCOS.

Escobar-Morreale HF, et al. J Clin Endocrinol Metab. 2004

Increased levels of MCP-1, MIP-1α, WBC, IL-6, TNF-α, and oxidative stress are additional markers of inflammation found in women with PCOS.

Escobar-Morreale HF, et al. Fertil Steril. 2011

González F, et al Steroids. 2009.

Glintborg D,et al. Clin Endocrinol (Oxf).2009

Sabuncu T, et al. Clin Biochem.2001

Ebejer K and Calleja-Agius J. Gynecol Endocrinol. 2013

It is thought that this increase in specific cytokines (CRP, IL-6, and TNF-α) is mostly attributed to obesity, and not solely to PCOS .

Samy N, et al Dis Markers. 2009

- Inflammation is found in PCOS patients who are obese as well as non-obese.
- Women with PCOS of normal weight have a higher buildup of fat in the visceral area compared to other parts of the body.

Kirchengast S and Huber J. Hum Reprod. 2001

- This distribution of visceral adiposity in non-obese women has been shown to be correlated with increased insulin resistance and is probably a causative factor of low-grade inflammation in these patients.

Sathyapalan T, Atkin SL. Mediators Inflamm. 2010

- These data suggest that obesity does not need to be present in a PCOS patient to experience low-grade inflammation.

- These findings bring up a vital question that should be further examined:
- is the inflammation caused by PCOS, or a result of obesity/other metabolic problems?
- To our knowledge, there has been no study examining the effect of anti-inflammatory treatment in women with PCOS.
- The response of anti-inflammatory drugs in PCOS patients needs to be examined.

Barthelmess and Naz Front Biosci (Elite Ed). 2015

- Women with PCOS often exhibit low levels of progesterone, causing anovulatory complications.
- During a normal cycle, estrogen promotes the increased production of IL-6 during the follicular phase, which is later inhibited by progesterone in the luteal phase.

Angstwurm MW, et al. Cytokine. 1997

- The absence of progesterone in PCOS patients may lead to over-stimulation of the immune system, inducing autoantibodies.

Petríkova J, et al. Eur J Intern Med. 2010

- Combined oral contraceptive pills contain progesterone; this daily dose could help reduce the expression of pro-inflammatory cytokines ,while simultaneously causing a decrease in testosterone levels in women with PCOS.

Butts Cl et al. Int Immunol. 2007
Banaszewska B, et al. Ginekol Pol. 2011

- OCPs provide a double-edged sword when administered - an improvement in hormonal balance as well as a reduction of inflammation

PLOS ONE

RESEARCH ARTICLE

Biomarker Profiles in Women with PCOS and PCOS Offspring; A Pilot Study

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- Hyperandrogenic PCOS women (HA-PCOS, n = 34)
- Normoandrogenic PCOS women (NA-PCOS, n = 34
- Non-PCOS reference population (n = 32)
- PCOS offspring (n = 14, age 6±8 years),
- A paedriatic reference population (n = 30).

- Clustering profile of adipocytokines (IL-1b, IL-6, IL-13, IL-17, IL-18, TNF-α, adiponectin, adipsin, leptin, chemerin, resistin, RBP4, DPP-IV/sCD26, CCL2/MCP-1)
- Growth factors (PIGF, VEGF, sVEGF-R1)
- Soluble cell adhesion molecules (sICAM-1/sCD54, sVCAM-1/sCD106)
- Other inflammatory related proteases (MMP-9, S100A8, Cathepsin S).
- Differences in median biomarker concentrations between groups, and associations with the free androgen index (FAI; Testosterone/SHBG x100).

Table 2. Biomarker concentrations in PCOS women and non-PCOS reference group.

	HA-PCOS (n = 34)	NA-PCOS (n = 34)	non-PCOS (n = 32)	P-value
IL-13 (pg/ml)	4.5 [0.9–19.5]	5.4 [1.5–29.2]	0.9 [0.9–6.6]	**0.043**
IL-18 (pg/ml)	95 [52–135]	75 [47–103]	87 [61–116]	0.53
CCL2/MCP-1 (pg/ml)	101 [83–139]	111 [82–136]	112 [80–152]	0.25
PIGF (pg/ml)	35 [18–46]	28 [14–42]	28 [15–44]	0.33
VEGF (ng/ml)	0.4 [0.3–0.5]	0.3 [0.1–0.5]	0.4 [0.2–0.7]	0.34
MMP-9 (μg/ml)	1.8 [1.2–3.1]	1.3 [0.8–2.2]	1.7 [0.8–2.4]	0.16
sVEGF-R1 (ng/ml)	1.4 [1.2–1.7]	1.5 [1.4–1.9]	1.4 [1.1–1.6]	0.053
S100A8 (ng/ml)	2.5 [1.2–4.5]	0.9 [0.3–4.6]	2.6 [0.7–5.3]	0.19
Adipsin (ng/ml)	0.4 [0.2–0.4]	0.3 [0.2–0.4]	0.3 [0.2–0.4]	0.31
Leptin (ng/ml)	3.0 [1.4–7.9]	0.4 [0.3–1.1]	1.9 [0.7–2.9]	**<0.001**
Resistin (ng/ml)	38 [27–54]	31 [24–39]	35 [30–45]	**0.017**
RBP4 (μg/ml)	43 [39–49]	41 [37–44]	41 [36–45]	0.10
DPP-IV/ sCD26 (μg/ml)	0.8 [0.7–1.0]	0.9 [0.7–1.0]	0.7 [0.6–0.8]	**0.005**
sICAM1/ sCD54(μg/ml)	0.3 [0.3–0.4]	0.3 [0.3–0.4]	0.3 [0.3–0.4]	0.97
sVCAM (μg/ml)	3.7 [3.2–4.5]	3.8 [3.2–4.5]	4.0 [3.2–5.1]	0.42
Cathepsin S (ng/ml)	13 [10–15]	11 [9–12]	11 [10–14]	**0.009**
Adiponectin (μg/ml)	131 [111–219]	258 [192–362]	233 [204–285]	**<0.001**

Values represent median concentrations [interquartile ranges]. P-values were calculated with ANOVA on logtransformed values for difference between all groups. PCOS: polycystic ovary syndrome, HA: hyperandrogenic, NA: normoandrogenic, IL: interleukin, CCL2/MCP-1: monocyte chemoattractant protein-1, PIGF: placental growth factor, VEGF: vascular endothelial growth factor, MMP-9: matrix metallopeptidase 9, RBP-4: retinol-binding protein 4, DPP-IV/ sCD26: dipeptidyl peptidase IV, sICAM: soluble intercellular adhesion molecule 1, sVCAM: soluble vascular cell adhesion molecule 1. IL-1b, IL-6, IL-17, TNF-α and chemerin are not shown as the majority of samples (>57%) were undetectable measurements evenly distributed amongst the study population.

Daan et al. Plos One 2016

Table 4. Baseline characteristics of PCOS offspring and reference group.

		PCOS offspring (n = 14)	Reference group (n = 30)	P-value
Complications during pregnancy				0.08
	None	8 (57)	25 (86)	
	Hypertensive complications	5 (36)	2 (7)	
	Gestational diabetes	1 (7)	-	
	Infection	1 (7)	2 (7)	
Mode of delivery				0.05
	Vaginal spontaneously	7 (50)	23 (79)	
	Caesarean section	5 (36)	6 (21)	
	Assisted vaginal delivery	2 (14)	-	
Gestational age at delivery (weeks)		40.1 [37.5–40.4]	40.4 [39.4–41.2]	0.19
Preterm delivery				0.15
	Yes	1 (7)	-	
	No	13 (93)	29 (100)	
Birth Weight (grams)		3295 [3061–3651]	3600 [3200–3940]	0.27
Neonatal complications				
	Small for gestational age	1 (7)	1 (3)	0.15
	Large for gestational age	2 (14)	2 (7)	0.43
Sex				0.98
	Male	6 (43)	13 (43)	
	Female	8 (57)	17 (57)	
Age at screening (years)		7.0 [6.6–8.1]	7.8 [7.6–7.9]	0.09
BMI (kg/m^2)		15.7 [14.5–16.8]	14.9 [14.5–16.0]	0.12

Daan et al. Plos One 2016

Table 5. Median biomarker concentrations in PCOS offspring and reference group.

	PCOS offspring (n = 14)	Reference group (n = 30)	P-value	Adjusted P-value	FDR
IL-13 (pg/ml)	8.5 [0.9–37.7]	16.5 [5.0–77.6]	0.14	0.06	0.26
IL-18 (pg/ml)	134 [85–196]	139 [107–188]	0.64	0.73	0.78
CCL2/MCP-1 (pg/ml)	113 [88–138.]	134 [100–159]	0.18	0.22	0.47
PIGF (pg/ml)	37 [32–59]	31 [16–69]	0.17	0.29	0.53
VEGF (ng/ml)	0.3 [0.1–0.6]	0.2 [0.1–0.5]	0.59	0.65	0.74
MMP-9 (µg/ml)	1.0 [0.6–2.2]	0.2 [0.2–0.5]	**0.001**	**0.003**	**0.02**
sVEGF-R1 (ng/ml)	1.4 [1.1–1.7]	1.6 [1.1–2.0]	0.47	0.46	0.71
S100A8 (ng/ml)	2.7 [0.7–3.1]	0.0 [0.0–0.1]	**<0.001**	**<0.001**	**<0.001**
Adipsin (ng/ml)	0.3 [0.1–0.3]	0.3 [0.2–0.3]	0.71	0.96	0.96
Leptin (ng/ml)	0.1 [0.0–0.5]	0.0 [0.0–0.3]	0.31	0.63	0.74
Resistin (ng/ml)	26 [21–32]	22 [19–36]	0.53	0.18	0.44
RBP4 (µg/ml)	35 [32–37]	35 [32–40]	0.20	0.05	0.26
DPP-IV/sCD26 (µg/ml)	2.2 [1.8–2.7]	2.5 [2.0–3.1]	0.19	0.08	0.27
sICAM1/sCD54(µg/ml)	0.5[0.4–0.6]	0.5 [0.4–0.6]	0.50	0.50	0.71
sVCAM (µg/ml)	7.7 [6.5–10.3]	6.9 [5.7–9.4]	0.18	0.16	0.44
Cathepsin S (ng/ml)	12 [10–13]	11 [11–13]	0.42	0.31	0.53
Adiponectin (µg/ml)	273 [215–312]	258 [239–325]	0.70	0.61	0.74

Daan et al. Plos One 2016

In this preliminary investigation :

- significant differences in adipocytokines between women with or without hyperandrogenic PCOS and non-PCOS controls, mostly influenced by BMI.
- Leptin and adiponectin showed the strongest correlation with the FAI in adult women with PCOS.

- In PCOS offspring other inflammatory biomarkers (MMP-9, S100A8) were increased, suggesting that these children may exhibit increased chronic low-grade inflammation.

Additional research is required to confirm results of the current exploratory investigation.

Daan et al. Plos One 2016

To evaluate serum levels of anti-nuclear antibody (ANA) among Indian women with PCOS.

- **89 eligible women** who consented were enrolled. All these women along with **87 age-matched**, healthy controls underwent, clinical (menstrual history, anthropometry, hirsutism scoring), biochemical, hormonal assessment and serum ANA estimation. OGTT after overnight (8–12 h) fast with 75 g oral glucose load was done for 1 h, 2 h glucose and insulin measurements.

- The mean age of cases and controls was comparable
- The prevalence of ANA positivity was significantly higher among women with PCOS (18.4% vs. 2.29%; $p < .001$).

Higher prevalence of ANA positivity among women with PCOS, being a marker of autoimmunity, suggests a possible role of autoimmunity in causation of PCOS and needs further elucidation.

Rashid A.2018

- 39 PCOS patients and 23 age-matched controls were enrolled.
- The profiles of Th1 (IFN-γ) and Th2 (IL-4) cytokines of $CD3^+$ T lymphocyte subsets were analyzed by flow cytometry.
- The proportion of Th1 cells and Th1/Th2 ratio were significantly higher in PCOS patients than those in controls, accompanied by elevated T, LH, LH/FSH, FINS, HOMA-IR index and reduced E_2/T.
- The Th1/Th2 ratio was increased when BMI and WC were enhanced in PCOS.
- Moreover, the significant difference of Th1/Th2 ratio was observed between WC subgroups of PCOS.

Conclusions:

- Th1 type immunity is predominant in systemic immunization of PCOS patients.
- Th1/Th2 immune imbalance is connected with obesity, especially abdominal obesity, and may be one of the underlying mechanism for the pathogenesis of PCOS.

Gong P. Gynecol Endocrinol 2018

CONCLUSION

- PCOS is becoming a more prevalent disorder among women of reproductive age with lifelong complications.
- One of the most challenging aspects of this syndrome is its ambiguous diagnostic criteria and vast complexity of characteristics.
- In the future, more research in the genetics and pathophysiology of PCOS is needed to determine preventative risk factors as well as successful treatment modalities for this syndrome.


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