Review Article
ISSN (Print): 2455-1244
ISSN (Online): 0971-913X
J Endocrinol Reprod. *Vol 26(3), 127-152, September 2022, DOI: 10.18311/jer/2022/30241*

Polycystic Ovary Syndrome (PCOS): An Overview and Our Experience

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Abstract

Polycystic Ovary Syndrome (PCOS) is the most common reproductive endocrine disorder in women of reproductive age. PCOS is characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovary morphology. The PCOS is known for more than 100 years; however, many areas of PCOS such as diagnosis, etiology, clinical features, and treatment are still debatable. This review aims to provide an overview of the historical evolution, diagnosis, biomarkers, and etiologic associations of PCOS as of today. A brief review of publications on PCOS and our research experience on PCOS are combined. All available biomarkers/associations implicated with PCOS, like androgens (testosterone, free androgen index, DHEAS, androstenedione, dihydrotestosterone), LH, 17-OH Progesterone, anti-Mullerian Hormone (AMH), inhibin B, leptin, insulin, interleukins, advanced glycation end product (AGE), bisphenol A (BPA), kisspeptin, melatonin, etc., besides genetic and epigenetic factors, associated with PCOS are briefed, along-with our research experience. The most acceptable consensus in naming the syndrome is Polycystic Ovary Syndrome (PCOS) and consensus diagnostic criteria presently followed are Rotterdam 2003 criteria with phenotypic classification (NIH 2012 criteria). Ideal androgen, method of estimation and its cut-off value is still a subject of controversy. DHT, an androgen, seems promising. The best available biomarker associated with PCOS could be AMH. Environmental contaminants such as bisphenol A and AGEs, and endogenous factors such as kisspeptin and melatonin have strong association with PCOS. Epigenetic alterations affecting various pathways (metabolic, steroid biosynthesis, ovarian function, AGE/RAGE, AMPK, inflammatory, etc.) and pathogenic variants of various genes (*INSR, IRS1, GHRL, LDLR, MC4R, ADIPOQ, UCP1, UCP2, UCP3, FTO, PCSK9, FBN3, NEIL2, FDFT1, PCSK9, CYP11, CYP17, CYP21, HSD17, STAR, POR, AKR1C3, AMH, AMHR2, INHBA, AR, SHBG, LHR, FSHR, FSH β, SRD5A, GATA4, THADA, YAP1, ERBB2, DENND1A, FEM1B, FDFT1, NEIL2, TCF7L2,* etc.) in some PCOS cases may be linked as underlying etiopathology. PCOS is a complex heterogeneous disorder, with genetic susceptibility besides environmental and epigenetic influences.

Keywords: Advanced Glycation End products, Androgens, Anti-Mullerian Hormone, Bisphenol A, Epigenetic Associations, Genetic Associations, Polycystic Ovary Syndrome

1. Introduction

Polycystic Ovary Syndrome (PCOS) is a complex reproductive disorder characterized by hyperandrogenism (hirsutism and/or high androgens), chronic oligo-ovulation, or anovulation (oligomenorrhoea or amenorrhea), and polycystic ovary morphology (polycystic and/or enlarged ovary). It is the most common reproductive endocrine disorder in women of reproductive age and its prevalence is reportedly between 8-15% of women of reproductive age and about 21% in high-risk women (e.g., infertility)[1–3]. Stein and Leventhal in 1935 first described the syndrome scientifically. They described clinical features associated with the condition as menstrual disturbance, infertility, and bilateral polycystic ovaries[4]. The characterization of the syndrome in past was challenging due to various defined criteria[2,5,6]. The worldwide commonly followed diagnostic criteria was Rotterdam criteria 2003[5]. This approach was modified in 2012 (NIH 2012 criteria) and classifies PCOS cases

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Article Received: 11.05.2022 Revised on: 04.09.2022 Accepted on: 03.10.2022

into four phenotypes, phenotypes A, B, C and D[7]. The pros and cons of various diagnostic criteria of PCOS can also be traced[8]. Presently, all three expert groups and others (international evidence-based guideline) have a consensus on following Rotterdam 2003 criteria with phenotypes i.e., NIH 2012 criteria[9].

Hyperandrogenism is one of the essential features of the syndrome, but none of the recommendations precisely defined, in particular the cut-off values of clinical or biochemical hyperandrogenism. Even international evidence-based guideline suggests for calculated free testosterone, or free androgen index, or calculated bioavailable testosterone using Liquid Chromatography–Mass Spectrometry (LCMS) or chromatography immunoassays methods; however, they were silent on cut-off levels. Rather, they recommend that laboratories should have their own reference range (cut-off value) based on levels from sizable normal women[10]. Similarly, recommendation for clinical hyperandrogenism is also vague. Committee suggests for a comprehensive history, and that physical examination should be completed for acne, alopecia, and hirsutism and, in adolescents, severe acne and hirsutism. For the assessment of hirsutism, the modified Ferriman Gallwey score (mFG) is preferably followed and a score ≥4-6 indicates hirsutism, depending on ethnicity[10].

Although hyperandrogenism is important for diagnosis, it is rarely observed or poorly associated in Asians[11–13]. About 50% of PCOS are overweight and some are underweight. Overweight PCOS women are prone to have infertility, insulin resistance, impaired glucose tolerance, and endometrial hyperplasia[14]. Hence, there is a pressing need to understand the underlying mechanism of PCOS phenotype-wise. Before assigning a case as PCOS, one should distinguish between PCOS-like conditions secondary to congenital adrenal hyperplasia (non-classical/adult onset/atypical), androgen producing tumor, exogenous androgen exposure, Cushing's disease, thyroid dysfunction, hyperprolactinemia, premature ovarian failure, etc.

Various biomarkers, other than androgens, such as AMH, LH, leptin, inhibin, etc., are implicated with PCOS. However, none yet has a high predictive value; hence, the diagnosis of PCOS solely depends on clinical judgment, and that may vary from person to person. There is a need to find out some promising diagnostic biomarkers for this syndrome. Identification of highly specific and sensitive biomarkers will help characterize the syndrome better. Based on the available literature, the target markers are AMH, DHT, leptin, kisspeptin, melatonin, etc.

The underlying etiology of PCOS in humans remains unexplored leading to difficulties in treating/ managing the disorder; in fact, no specific targeted treatment is available. Although in experimental animals PCOS can be produced through prenatal androgen or bisphenol A exposure, this (perinatal androgen-induced) cannot be mimicked in humans (most environmental pollutants are estrogenic); hence, unlikely to be considered an etiologic factor. The cause of PCOS in humans seems to be extremely heterogeneous and expected to be associated with epigenetic (influenced by environmental factors) and genetic factors. The genetic etiology of PCOS has not been completely established despite some association studies[15]. Environmental pollutants also may play some role as endocrine-disrupting chemicals and disrupt ovarian and metabolic function, causing PCOS-like abnormalities. Hence, it is important to explore the underlying etiopathology for genetic, epigenetic and environmental factors.

This review will provide an overview of the evolution of the syndrome with special reference to naming, diagnostic criteria, biomarker(s), and etiologic (genetic, epigenetic, and environmental) associations along with our research experience.

2. Historical Perspectives of PCOS

The landmarks in the history of PCOS are summarized in Table 1. The first description of PCOS can be traced to 1721 from a case description of the disorder by Vallisneri[16]. Vallisneri (from Italy) described a woman with infertility, obesity, and a large white shiny ovary that was compared with a pigeon egg. The second description of the disorder can be traced to Chereau in 1844[17]. Chereau (from France) described ovaries with the disorder as fibrous and sclerotic with hydropic follicles (sclerocystic ovary). Thereafter, various authors described ovarian pathology with the disorder as cystic degeneration of the ovary, hyperthecosis of the ovary, microcystic ovaries, etc[18–20]. All these older descriptions were focused on ovaries thus indicating ovarian pathology (either enlarged or polycystic or sclerotic) associated with the condition. However, the first scientific description of PCOS came from Stein and Leventhal in 1935 with the publication title "amenorrhea associated with

bilateral polycystic ovaries"[4]. Authors (from the USA) described seven cases of female infertility associated with bilateral enlarged polycystic ovary and menstrual disturbance (amenorrhoea or oligomenorrhoea) with a normal level of urinary 17-ketosteroids (to exclude congenital adrenal hyperplasia, androgen-producing tumor or obvious hyperandrogenaemia of any etiology) and gonadotropin (to exclude premature ovarian failure/menopause). The authors also described clinical findings like hirsutism, small breast, and small uterus. However, they did not give much importance to clinical/biochemical hyperandrogenism in their paper. The authors also reported restoration of menstruation and fertility after wedge resection of ovaries[4]. The importance of clinical hyperandrogenism, including its association with hyperthecosis, was discussed in detail initially by du Toit[21]. Later, the disease was linked with the inappropriate secretion of gonadotropins as key parameters for diagnosis for a short period of time and was later abandoned[22].

Since the first scientific description of PCOS by Stein and Leventhal in 1935 i.e., more than 85 years ago, there has been no consensus on the name or diagnostic criteria until recently. Now, the syndrome is known as polycystic ovary syndrome and a consensus in diagnostic criteria has been arrived at by all the three expert groups (NIH, Rotterdam and AES)[7,10]. However, the syndrome is still published either as polycystic ovary syndrome or polycystic ovarian syndrome. The syndrome/disease was popular with various names from time to time and region to region since its landmark naming as Stein Leventhal Syndrome in 1935. Before 1935 the disease was named sclerocystic ovary[18], hyperthecosis of the ovary[19], microcystic ovary[20] and, after popular Stein Leventhal Syndrome, as sclerotic polycystic ovary[24], polycystic ovary syndrome[25], polycystic ovarian diseases[26], polycystic ovary disease[27], polycystic ovarian syndrome,[28] ovarian micro-polycystic syndrome[29], etc. At present, the syndrome is most acceptably known as Polycystic Ovary Syndrome (PCOS) although some still prefer to call a polycystic ovarian syndrome.

3. Diagnostic Criteria of PCOS

The diagnostic criteria of PCOS were debatable, until recently, as recommendations were somehow different with different PCOS working groups (Table 2). The first scientific diagnostic criterion on PCOS came from a consensus declaration of National Institutes of Health

Table 1. Historical landmarks of polycystic Ovary Syndrome

Landmarks	Author	Year
First documented clinical description as case report of the disorder (obese woman with infertility and large white shiny ovary as like pigeon egg)	Vallisneri A[16]	1721
Fibrous and sclerotic ovary with hydropic follicle (sclerocystic ovary)	Chereau A[17]	1844
Cystic degeneration of ovary	Rokitansky C[18]	1855
Hyperthecosis of ovary	Bulius and Kretschmar[19]	1897
Microcystic ovaries	McGlinn JA[20]	1916
Amenorrhoea associated with bilateral polycystic ovaries/ Stein-Leventhal syndrome (First scientific description of PCOS)	Stein and Leventhal[4]	1935
Polycystic ovaries, menstrual disturbances and hirsutism: hyperthecosis (importance of clinical hyperandrogenism)	du Toit DAH[21]	1952
Sclerotic polycystic ovary	Davis CD, *et al*[23]	1956
Polycystic Ovary Syndrome	Keettel WC[24]	1957
Polycystic ovarian disease	Evans and Riley[25]	1958
Polycystic ovary disease	Lambeth and Kintner[26]	1959
Polycystic ovarian syndrome	Cook WS[27]	1965
Galactorrhoea and amenorrhea with polycystic ovaries	Lavric MV	1969
Linked with inappropriate secretion of gonadotropins	Yen SSC, *et al*[22]	1970
Ovarian Micro-polycystic syndrome	Vokaer R[28]	1977

(NIH) sponsored conference (The National Institute of Child Health and Human Development Conference of PCOS) in 1990; popularly known as NIH 1990 criteria[2]. The criteria are chronic anovulation (oligomenorrhoea/amenorrhoea) and hyperandrogenism (clinical i.e., hirsutism/biochemical i.e., high testosterone). Both criteria are required for diagnosis but need to exclude congenital adrenal hyperplasia/androgen-producing tumor. Thereafter came ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group diagnostic criteria in 2003; popularly known as Rotterdam 2003 criteria[5]. Criteria are oligo/anovulation, hyperandrogenism (clinical and/or biochemical), and Polycystic Ovary Morphology (PCOM). Any two criteria are required for the diagnosis but after exclusion of other endocrinopathies with known etiologies like congenital adrenal hyperplasia, premature ovarian failure, hyperprolactinemia, thyroid disorder, etc. Next came Androgen Excess PCOS (AE PCOS) society 2006 criteria (AE-PCOS 2006 criteria) which include hyperandrogenism (clinical/biochemical) and ovarian dysfunction (oligo/amenorrhoea) and/or polycystic ovary morphology (PCOM). Both the criteria are essential for PCOS diagnosis but after excluding disorders with known aetiologies and having similar characteristics such as oligo-ovulation and hyperandrogenism[6,29]. However, none of the criteria provided a cut-off value for either clinical or biochemical hyperandrogenism.

The NIH 1990 criteria seem inappropriate as they did not include polycystic ovary as criteria despite naming the syndrome as polycystic ovary syndrome (PCOS). The NIH 1990 criteria also exclude many PCOS cases; those without hyperandrogenism and others without ovulatory dysfunction. PCOS cases without hyperandrogenism are very common in southeast Asian countries[30,31]. The AE PCOS 2006 criteria are also inappropriate as it excludes all PCOS cases with normal androgen which are common PCOS phenotype in southeast Asia[30,31]. AE PCOS society in 2015 again modified their diagnostic criteria by agreeing with Rotterdam criteria[32,33]. Presently, all three expert groups and most related national societies are in agreement with Rotterdam 2003 criteria with phenotypic classification i.e., NIH 2012 criteria[7,9]. Serum 17-hydroxyprogesterone and Anti-Müllerian Hormone (AMH) are also important and useful for exclusion or determining a diagnosis of PCOS. Also, recommended for calculated free testosterone through LC-MS or calculated bioavailable testosterone through chromatography immunoassays methods or FAI as these are more sensitive.

Table 2. Diagnostic criteria of Polycystic Ovary Syndrome

Parameters	NIH 1990* and 2012	Rotterdam 2003#	AES 2006+ and 2015
HA: Clinical and/or biochemical	HA	HA	HA
OA/OD/Ovarian dysfunction	OA	OD	Ovarian dysfunction (OD and/or PCOM)
PCOM	No	PCOM	Included with criterion 2
PCOS Diagnosis	Both Required	Any two required	Both required
Exclusion	Exclusion of other etiologies of androgen excess and anovulation	Exclusion of other etiologies of androgen excess and anovulation	Exclusion of other etiologies of androgen excess and anovulation
Remarks	Both criteria are necessary for diagnosis (PCOM was not considered as criteria)	Two of three criteria are necessary for diagnosis	Hyperandrogenism is must and 2nd and/or 3rd criteria is/are required for diagnosis
Modifications	NIH 2012: adopted Rotterdam 2003 criteria plus identification of phenotypes (vide Table 3)	Merged with NIH 2012 criteria	High AMH added as another optional criteria for 2nd criteria AMH value of >10 ng/ml absolute; >7 ng/ml likely; >5 ng/ml may be

* National Institute of Child Health and Human Development (NICHD)/NIH 1990 Guidelines
# European Society for Human Reproduction and Embryology and American Society for Reproductive Medicine (ESHRE/ASRM) or Rotterdam 2003 Guidelines
+ Androgen Excess Society (AES) 2006 Guidelines
HA (hyperandrogenism), OA (oligo-anovulation), OD (ovulatory dysfunction), PCOM (polycystic ovarian morphology)

The value of measuring levels of androgens other than these three in patients with PCOS is relatively low.

Present consensus in PCOS diagnostic criteria is the Rotterdam 2003 criteria with modifications in the form of phenotypic classifications i.e., NIH 2012 criteria[7,9]. This approach classifies PCOS cases into four phenotypes. These are phenotype A (hyperandrogenism, ovulatory dysfunction, and polycystic and/or enlarged ovary), phenotype B (hyperandrogenism and ovulatory dysfunction), phenotype C (hyperandrogenism and polycystic and/or enlarged ovary), and phenotype D (ovulatory dysfunction and polycystic and/or enlarged ovary; Table 3). Both NIH and AE PCOS societies rectified their mistake by adopting Rotterdam 2003 criteria and classifying them into 4 phenotypes[9,32]. However, none of the criteria precisely defined clinical or biochemical hyperandrogenism yet[9].

4. Hyperandrogenism

4.1 Clinical Hyperandrogenism

Manifestations of clinical hyperandrogenism are hirsutism, acne, androgenic alopecia, acanthosis nigricans, and virilization. Hirsutism is defined as excessive growth of terminal hair in women. Hirsutism severity is determined by using various visual scoring systems of hair growth, most commonly using the Ferriman and Gallwey scale, and a score of 9 or more is considered clinical hyperandrogenism[34]. However, none of the PCOS diagnostic criteria have provided a quantitative value (cut-off value) for clinical hyperandrogenism for the diagnosis of PCOS. The reasons are:

- Normative data in large populations are lacking; The assessment is subjective; Rarely do physicians follow the scoring method; Often treated well before the evaluation; Have ethnical variations; Less prevalent in adolescence[35].

Later, international evidence-based guidelines recommended using standardized visual scales when assessing hirsutism, such as the modified Ferriman Gallwey score (mFG) with a level ≥4-6 indicating hirsutism, depending on ethnicity[9].

Similarly, acne, androgenic alopecia, and acanthosis nigricans are potential markers for clinical hyperandrogenism; however, they were not incorporated with PCOS diagnosis because either not well studied or poor association[36] or conflicting results[37]. However,

Table 3. Phenotypic classifications of PCOS as per NIH 2012 criteria (extended Rotterdam 2003 criteria)/ International evidence-based guideline 2018

Type/Group	*Hyperandrogenism (HA)	**Ovulatory Dysfunction (OD)	***Polycystic Ovary Morphology (PCOM)
Phenotype A	Yes	Yes	Yes
Phenotype B	Yes	Yes	No
Phenotype C	Yes	No	Yes
Phenotype D	No	Yes	Yes

*Hyperandrogenism(clinical and/or biochemical)
NIH 2012 did not clarify quantitatively clinical and biochemical hyperandrogenism
International evidence-based guideline also did not clarify quantitatively biochemical hyperandrogenism; however, clarified partly clinical hyperandrogenism (≥4-6 mFG score for hirsutism) but not for alopecia or acne. Cut-off should be derived from laboratory data on normal women using calculated free T, or calculated bioavailable T or FAI
Clinical hyperandrogenism (hirsutism): Ferriman-Gallwey score ≥9
Modified Ferriman-Gallwey (mFG) score ≥8
mFG score ≥4-6 (international evidence-based guideline 2018
Biochemical hyperandrogenism: High testosterone (laboratory should derive from normal women)
High FAI (laboratory should derive from normal women)
**Ovulatory Dysfunction(oligomenorrhoea/amenorrhoea or oligo-ovulation/anovulation)
Oligomenorrhoea/oligo-ovulation: Menstrual cycle interval >35 days/<8 cycles/year
(if menarche <3 years before then >45 days)
Amenorrhoea/anovulation: No menstruation for >182 days
***Polycystic Ovary Morphology
Ovarian follicles of 2-9 mm in size with ≥ 20 follicles in one or both ovaries and/or Ovarian volume >10 ml in one or both ovary/ovaries on targeted (ovary) ultrasonography using 8 MHz transducer
Should not be used before 8 years completion of menarche

international evidence-based guidelines recommended using Ludwig visual score for assessing the degree and distribution of alopecia[9]. Virilization (increased muscle bulk, body hair, clitoromegaly, and deep voice) in PCOS females is unusual and mostly secondary to an androgen-producing tumor or congenital adrenal hyperplasia.

4.2 Biochemical Hyperandrogenism (High Androgens)

High androgens are regarded as one of the key features for the diagnosis of PCOS hence must be evaluated in all PCOS cases. The serum total testosterone and Free Androgen Index (FAI) are commonly used as androgen markers in addition to clinical hyperandrogenism[38]. AE-PCOS society recommends free testosterone (fT) through equilibrium dialysis techniques as it is more sensitive and discourages measuring other androgens. However, none of the PCOS diagnostic criteria has provided a quantitative value (cut-off value) for high androgens for the diagnosis of PCOS. The limitations of defining high circulating androgens are due to the inaccuracy and variability of the laboratory methods of measurement[39,40], wide variability in the normal population, normal ranges have not been well-established using well-characterized control populations, age (including adolescent and older females), and BMI have not been considered when establishing normal values for androgen levels[41,42], level alters easily following hormone use, etc. Free T or Free Androgen Index (FAI) are more sensitive methods of assessing hyperandrogenaemia[43]. Recommended methods for the assessment of FAI are the measurement of Sex Hormone-Binding Globulin (SHBG) and total testosterone. Hyperandrogenemia is conventionally measured as high testosterone (>0.6 ng/ml) or high free androgen index (>4½)[44–46]. However, high testosterone is rarely observed in Asians, including Indian women, particularly so in southern, western and eastern India[11,12]. FAI is also rarely used as a diagnostic marker due to assay complexity, cost and poor association[13]. Other androgens such as Dehydroepiandrosterone Sulphate (DHEAS), androstenedione, etc., are rarely studied for hyperandrogenemia markers. However, DHT measurement as a biomarker of hyperandrogenemia has been advocated to enhance diagnostic performance in PCOS[47,48]. Hirsutism is directly related to androgen that mainly acts on skin/hair follicles i.e., local DHT[49,50]. Moreover, the DHT estimation is comparatively simple (single test). Our experience suggests the significantly high value of serum DHT in PCOS women and can be recommended[51,52]. We observed mean DHT value of 584.27 pg/mL in PCOS women and 257.16 pg/mL in control women ($p<0.0001$) and area under ROC curve 0.895. Elevated serum levels of DHT (>462 pg/mL) can be introduced as hyperandrogenemia marker for PCOS in north Indian patients. However, international evidence-based guidelines recommended using calculated free testosterone through LC-MS or calculated bioavailable testosterone through chromatography immunoassays or FAI as these are more sensitive[9].

5. Other Biomarkers

5.1 AMH

Researchers are exploring the role of AMH in the causation of the disease and also evaluating its ability as a surrogate diagnostic marker for the syndrome. AMH is a glycoprotein produced by the granulosa cells of developing ovarian follicles[53]. The amount of AMH produced by the ovary depends on the number of developing follicles. The level of AMH in circulation can be the marker of the number of functioning follicles present which is usually found to increase in PCOS. Thus high serum level of AMH is expected in PCOS[54]. AMH level remains constant during phases of a menstrual cycle as well as following exogenous estrogen intake, an important advantage over gonadotropin and gonadal hormones[55].

A high level of AMH in PCOS is reported by many and recommended for diagnostic use[31,56,57] but no consensus on the cut-off value among studies[56,58] that varies from 4.7 ng/mL to >5 ng/mL[56,59,60] or even 10 ng/mL in Japanese and Korean women[61,62]. We observed a median AMH value of about 8.5 ng/mL in PCOS (3½ times more than control; $p<0.001$) and a maximum (10.2 ng/mL) with phenotype D[63,64]. High value (>5.2 ng/mL) was observed in more than 85% cases and unaffected by age (within the reproductive age group), BMI, hirsutism (FG score) or androgens (Spearman's correlation)[63]. Sensitivity of AMH was reported in various publications between 49% and 74% when the specificity was set at 92%; however, others reported higher sensitivity (over 80%) with a little lower specificity[31,58,63–66]. The AMH estimation is comparatively simple, sensitive, and at present the best available biomarker associated with PCOS. However, international evidence-based guidelines state that with

improved standardization of assays and established cut off levels or thresholds based on large scale validation in populations of different ages and ethnicities, AMH assays will be accurate in the detection of PCOM but presently should not yet be used as a single test for the diagnosis of PCOS or as an alternative for the detection of PCOM[9].

5.2 Luteinizing Hormone (LH)

LH/FSH ratio was previously considered the diagnostic marker of the syndrome and was routinely measured in every patient[22]. LH, as well as LH/FSH ratio, are significantly elevated in women with PCOS as compared with control[67,68]. Elevated LH concentrations can be observed in approximately 60% of women with PCOS[69] whereas the LH/FSH ratio may be elevated in up to 95% of subjects[68]. LH levels may be influenced by BMI (higher in lean PCOS). The clinical utility of the LH/FSH ratio in the diagnosis of PCOS remains doubtful due to inter-observer variability and poor reproducibility in the assessment of the LH/FSH ratio[70,71]. In our study on the prediction model for PCOS using multivariable binary logistic regression final weighted score for LH was statistically not significant and hence not a good marker[63]. This is also viewed by all three societies and none recommended using LH or LH/FSH ratio as a marker.

5.3 Leptin

Leptin is a hormone secreted by the adipose tissue of the body and the level of leptin is proportional to the body fat[72-74]. It regulates food intake and thus food and energy balance of the body via the hypothalamus of the brain. Leptin is also known as the satiety hormone. Leptin resistance is common with obesity and thus cannot inhibit hunger.

Various studies have found the role for leptin in reproduction[75-78]. Animals with a deficiency of leptin, like in ob^{-}/ob^{-} are found to have central hypogonadism[75,76]. When leptin is supplemented to these animals, hypogonadism improves[77]. On the other hand, when leptin is administered in normal prepubertal mice, it accelerates puberty. Leptin levels also were found to be high in PCOS women[78]. Hyperleptinemia in PCOS women has been shown in some studies[78]. However, we did not find any difference ($p>0.05$) in leptin value in total PCOS cases (20.3 ng/mL) *vs* control (12.9 ng/mL) but we observed a significant difference ($p=0.0018$) between high and normal BMI PCOS cases (ongoing work)[80]. The AUC of ROC was 0.66, indicating poor association with total PCOS, although there was a good link of PCOS with high BMI (AUC, 0.83).

5.4 Inhibins

Inhibins are heterodimeric glycoproteins. There are two forms of inhibin i.e., inhibin A and Inhibin B. The granulosa cells of the ovary synthesize inhibin A (luteinized/secretory phase granulosa cells) and inhibin B (non-luteinized/follicular phase granulosa cells)[81]. Alpha and beta A (α-βA) subunits compose inhibin A whereas alpha and beta B (α-βB) subunits compose inhibin B[82]. Both the types of inhibin have diverse and different biological functions. Inhibin B inhibits the secretion of FSH from the anterior pituitary. It also has local paracrine action in the ovary. Its biological function in regulating ovulation is not well understood. Inhibin B level correlates with ovarian activity and, therefore, may be associated with PCOS[83]. There may also be a relationship between high LH and high Inhibin B in some PCOS cases[84]. Our study did not find any difference in the level of inhibin B between PCOS and control (ongoing study)[80].

5.5 Insulin

PCOS is characterized by insulin resistance and compensatory hyperinsulinemia, which increases the risk of impaired glucose tolerance and type 2 diabetes mellitus (T2DM)[85,86]. Studies have shown that 30-40% of PCOS women have impaired glucose tolerance and 10% of them develop T2DM[87–90]. Women with PCOS frequently have obesity as well as insulin insensitivity[91–93] but lean women with PCOS have the same sensitivity to insulin as controls[94–96]. Studies also have shown defects in insulin secretion in PCOS families[97]. Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) is used frequently to assess insulin resistance[98]. Various studies also have shown the role of insulin in the synthesis of androgen in the ovaries[99,100]. Insulin stimulates the production of ovarian androgens and reduces the synthesis of hepatic SHBG, thus increasing the levels of total and bioavailable androgens[101,102].

PCOS women with hyperandrogenemia have a higher resistance to insulin than PCOS women with normal androgen levels[103,104]. Insulin acts in synergy with LH to produce androgen by activating signaling pathways through its receptor in women with PCOS[105-107]. Insulin also stimulates the proliferation of theca cells in rats[108,109]. Our study on PCOS also detected high fasting

insulin levels in PCOS cases, in particular, phenotype D than control indicating a role in phenotype D (ongoing work)[80]. This is also supported by the AUC of ROC as 0.92 in phenotype D (very strong association)[80].

5.5 Inflammatory Markers, Including Interleukins

Researchers have found that PCOS is associated with a low level of chronic inflammations[110,111]. In vitro studies have shown that inflammatory factors such as IL4, IFNγ, etc., are responsible for up-regulation of androgen production in the theca cells of the ovary[112]. This phenomenon raises the possibility that inflammation may be the direct cause of hyperandrogenism in PCOS[113]. The pro-inflammatory cytokines, such as interleukin activate the HPA axis and control adrenal steroidogenesis[114,115]. We studied PCOS cases with IL4 and interferon γ. We found a statistically significant difference between PCOS cases and control, but maximum in phenotype D ($p<0.0001$) with AUC of ROC as 0.88 indicating association[80]. However, we did not observe any difference in interferon γ (undetected in both control and PCOS cases)[80].

5.6 Advanced Glycation End Products (AGEs)

Advanced glycation end products (AGEs) are produced non-enzymatically by the interaction of the carbonyl group of carbohydrates with the amino groups of proteins either inside or outside the body[116,117]. AGEs are formed inside the body or preformed AGEs are taken directly through the ingestion of fast food, processed food, or by smoking[118,119]. AGEs exert their effect by inducing oxidative stress by altering enzyme activity by inducing cytotoxic pathways and by damaging nucleic acids[120-122]. AGEs also cause insulin insensitivity by modifying the activity of protein kinase C. AGEs act either through receptor-dependent or receptor-independent pathways. AGE receptor is present in the cell membrane, the extracellular matrix or circulation. The cell membrane receptor is called RAGE. The circulatory receptor is called soluble receptor for AGEs (sRAGE)[123]. The circulatory receptors bound to the AGEs and prevent their binding to RAGE, thus ameliorating their inflammatory effects on body tissues[125].

AGEs have been linked with the pathogenesis of some diseases such as diabetes, hypertension, renal diseases, Alzheimer's disease, and aging[120-122]. Recently, AGEs have been implicated in the pathogenesis of PCOS[106,126]. In various studies, AGEs have been found raised in the serum of PCOS patients[120,127]. Researchers have found increased serum levels of AGEs and increased expression of miRNA of pro-inflammatory RAGE in the ovarian tissue of PCOS women, which affects androgen synthesis and follicle maturation[106,126]. A positive correlation of AGEs in PCOS women has been found with androgens[128]. In addition, the increased immunohistochemical location of AGEs in polycystic ovaries suggests a possible direct action of AGEs on ovarian function. In general, endogenous and exogenous AGEs can play a role in the pathogenesis of PCOS[129]. Dietary changes and the use of gastric lipase inhibitors can reduce the level of AGEs, serum testosterone, as well as oxidative stress in PCOS women[130]. We also have found statistically highly significant ($p<0.001$) differences in mean AGE level between PCOS (12 ng/mL) and control (4.8 ng/mL), more so with overweight (BMI>25 kg/m^2) and phenotype A PCOS cases. AUC of ROC analysis also indicates a strong association; the value varies from 0.88 (phenotype A) to 0.9 (PCOS with high BMI cases). We have also observed a positive correlation with testosterone, DHT, BMI, bisphenol A, and leptin but not with estrogen, progesterone, insulin, AMH, LH, and DHEAS.

5.7 Bisphenol A (BPA)

Bisphenol A (BPA) is commonly used in the plastic industry as a plasticizer. Plastic containers are now used very commonly in our daily life and, therefore, exposure to bisphenol in humans is continuous[131,132]. It is a known endocrine disrupter and an estrogen-mimicking substance. Studies have found that BPA is associated with obesity, changes in puberty, and ovulatory dysfunction[132]. BPA level is elevated in women with ovulatory dysfunction[131–135] as well as with PCOS[136,137]. BPA interferes with steroidogenesis, folliculogenesis, and ovarian morphology[133,134,138]. Rodent studies indicate that BPA enhances ovarian androgen production in vitro and induces insulin resistance in vivo[138]. In experiments using rat PCOS-like phenotype can be developed by exposing BPA in utero or neonatal period. Animals also develop later dysregulated insulin and glucose metabolism[140,141]. In vitro theca cell culture with BPA synthesizes more testosterone[139-142]. BPA, being a potent SHBG binder, displaces androgens, thereby increasing the levels of free androgens[143]. Androgens inhibit BPA clearance in the liver, leading to increased serum levels of BPA[144]. Our own study finds a high level (>245 ng/mL) in over 60% of PCOS cases and an AUC of ROC of 0.84. Spearman's

correlation analysis also finds a significant correlation between BPA and androgens (testosterone, free androgen index, dihydrotestosterone, etc)[80]. Logistic regression analysis finds 8X more PCOS prediction compared to controls when BPA levels are high.

5.8 Kisspeptin

Kisspeptin (KISS) is a neuropeptide encoded by the KISS1 gene and acts via its receptor, KISS1R. Kisspeptin is a ligand of the G-protein coupled receptor, GPR54, which stimulates GPR54 activity leading to an increase in LH level. Kisspeptin was discovered as a suppressor of human malignant melanoma in 1996 and a useful marker for distinguishing metastatic melanomas from non-metastatic melanomas[145]. Kisspeptin was also isolated from the human placenta in 2001 as a metastasis inhibitor, thus called metastin[146]. Deactivating mutations of the KISS1R gene may lead to hypogonadotropic hypogonadism and mutations in its activation result in central precocious puberty. Kisspeptin/GPR54 signaling appears to be a key regulator of reproduction[147] and defects may lead to hypothalamic alterations in the pulsatile secretion of gonadotropin-releasing hormone (GnRH) resulting in hypersecretion of luteinizing hormone (LH) by the pituitary[148]. Various studies reported a high level of serum kisspeptin in women with PCOS than in controls, in particular with normal BMI[149,150]. We are also working on serum kisspeptin levels in PCOS and found a significant difference ($p=0.0051$) from control women. However, we did not observe any correlation between androgens, estrogen, or LH. Our observation supports kisspeptin's role in the pathophysiology of PCOS directly in ovarian granulosa cells[151].

5.9 Melatonin

Melatonin is an indolamine hormone mainly secreted from the pineal gland at night or in darkness. Melatonin is also synthesized at the gastrointestinal tract, skin, retina, bone marrow, and lymphocytes[152] besides reproductive organs, like the granulosa cells, oocytes, and cytotrophoblasts[153]. It is associated with the regulation of the sleep-wake cycle. Melatonin has various different pharmacological properties such as antioxidant, immunomodulatory, anti-angiogenic, and oncostatic effects[154]. Melatonin inhibits hypothalamo-pituitary-gonadal axis[155]. Melatonin acts via its receptors (transmembrane G-protein-coupled) such as melatonin receptor 1 and melatonin receptor 2[156].

The concentration of melatonin in ovarian follicles is higher than that of plasma suggesting its role in ovarian function[157]. Studies have shown higher melatonin levels in blood in PCOS patients compared to healthy women and could be used as a marker for the prediction of PCOS[158,159]. Elevated melatonin levels in serum of PCOS patients were found to be positively correlated with testosterone levels and LH/FSH ratio[159]. Melatonin treatment also promotes follicular maturation and ovulation through the protection of follicles against oxidative stress leading to follicular atresia[159]. We have also observed a significantly ($p<0.0001$) higher median value of melatonin in PCOS (121 pg/mL) than in control (40 pg/mL). However, we did not find any correlation with androgens or gonadotropins but inverse correlation was observed with estrogen. Various SNPs of melatonin receptors (rs2119882, rs10830963) are reported to be associated with PCOS[160–162]. However, we did not find any pathogenic/likely pathogenic variants of melatonin receptors in our PCOS study (WES of 51 phenotype A PCOS cases).

6. Genetics Associations

PCOS is frequently (20-40%) observed in first-degree female relatives of the general population[163]. Dutch twin's study also observed a heritability of 0.79 thus suggesting the influence of genetic factors in the development of PCOS[164]. The genetic factors contributing to etiology of PCOS were found at 72%[164]. The genetic influence of PCOS is supported by twins and family clustering[164–167]. Hyperandrogenemia and insulin resistance, a common association in PCOS, more frequently exist in families of women with PCOS[168]. Similarly, 17-OH progesterone above basal normal level is often associated with PCOS, indicating an enzymatic defect in steroid biosynthesis[169,170] even in the carrier state[171]. The clinical features of non-classic congenital adrenal hyperplasia, a common autosomal recessive disorder due to mutations in steroidogenic enzyme genes (*CYP21A2, CYP11B1, CYP11B2, CYP11A1*, *CYP17A1*, *HSD3B2*, *POR, StAR, MC2R, MRAP, etc.*), predominantly reflect androgen excess rather than adrenal insufficiency. Reddy *et al.*[172] reported CYP11A1 (tttta)(n) repeat polymorphism as a potential molecular marker for PCOS risk. Adolescent and adult women usually present with menstrual abnormality, hirsutism, and infertility[173]. Higher prevalence has been reported in Turkey (33%), France (23%), Portugal (18%), Greece (9%), India (6%), etc[174–178]. The phenotypic

spectrum for mutations in the cytochrome P450 oxidoreductase (*POR*) gene has been expanded to include amenorrhea, infertility, and low sex steroid hormone levels[179]. Partial loss of function missense mutations in the Steroidogenic Acute Regulatory protein (*StAR*) gene have been associated with non-classic lipoid adrenal hyperplasia; mutations in the ACTH receptor (*MC2R/ ARMC5*) gene or the melanocortin 2 receptor accessory protein (*MRAP*) gene are associated with phenotypes similar to non-classic lipoid adrenal hyperplasia[180]. Other genes for which association with PCOS have been replicated include *FBN3, HSD17B6, INSIG2, TCF7L2, MC4R, POMC, ACVR2A, FEM1B, FTO, ADIPOQ, etc*[181–184]. Various researchers carried out genome-wide association studies and reported associations with *LHCGR,FSHR*, *THADA, DENND1A, YAP1*, *RAB5B, SUOX,* etc[185–189]. Day *et al*[190]. reported significant associations with *ERBB4, FSHB, RAD50,* and *KRR1* genes. Although GWAS identified many hypothetical PCOS susceptibility genes their contribution is negligible[167,191]. In Han Chinese women, genome-wide association studies reported 11 genetic loci associated with PCOS, and these loci are found in regions where gonadotropins, insulin signals, reproductive hormones, and T2DM[187,192] and some of the variants were also detected in European women and may be necessary for PCOS etiology, regardless of ethnicity[188]. Although an association of PCOS with diabetes mellitus and obesity has been indicated, the mechanism involved is still unexplained[193].

During the last few years, growing evidence is pouring on etiopathogenetic associations of AMH gene/receptors with PCOS rather than being merely a marker[194,195]**.** In vitro experiment on granulosa cells from the ovary of anovulatory PCOS shows 75-folds higher production of AMH in comparison to granulosa cells of normal ovaries. This indicates increased serum AMH in PCOS, reflecting an intrinsic dysregulation of the granulosa cells[196]. This is supported by the finding of AMH and AMHR (AMHR2 in particular) pathogenic variants with PCOS[197].

PCOS-linked genes listed in the OMIM database are *PCOS1, FOXL2, CAPN10, SHBG,AKR1C3, FBN3, GATA6, SRD5A1, SRD5A2, AR, SULT2A1, H6PD, 17beta-HSD3, INS, INSR, IGF2, IRDN, IL18, ADIPOQ*, *AMH, LHB, FSHR, CYP19A1, CYP11A1, CYP17A1, HSD11B1, HSD3B2, STAR, CORTRD1, etc.* Other genes frequently associated with PCOS are *C9orf3, DENND1A, ERBB3/ RAB5*, *TOX3, SRD5A2, SRD5A1, HMGA2*, *THADA, SOD2, ERRB4, YAP1, GATA4/NEIL2, ZBTB16, FSH-β, FTO, SIRT1, etc*[197–200].

We are working on PCOS since several years and our initial whole exome sequencing results identify pathogenic/likely pathogenic/novel variants in obesity and insulin-related genes like *UCP1* (c.680C>T), *UCP2* (c.262C>T), *IRS1* (c.2674A>G) and *GHRL* (c.214C>A, n=5) in eight PCOS patients with high BMI and high fasting insulin level[201] and steroid biosynthesis pathway genes like *CYP21A2* (c.1174G>A, c.955C>T, c.428T>A), *STAR* (c.158G>T), *POR* (c.1000G>A, c.751G>A), *HSD17B6* (c.118G>A) and *AKR1C3* (c.613T>G) in ten cases of phenotype A/D PCOS with normal BMI, and insulin level[202]. We have also detected pathogenic and likely pathogenic variants for *AMH, AMHR2, INHBA, AR, SHBG, LHR, FSHR, FSH β, SRD5A, GATA4, THADA, YAP1, ERBB2, DENND1A, FEM1B, FDFT1, NEIL2, TCF7L2, INSR, LDLR, MC4R, ADIPOQ, UCP3, FTO, PCSK9, THADA, FBN3, NEIL2, FDFT1, PCSK9, CYP11, CYP17, etc.* genes in 51 PCOS WES study (ongoing study). These genes can be categorized as metabolic, steroid biosynthetic, gonadal function-related, and other genes. We have also observed multiple pathogenic/likely pathogenic variants of more than one gene in many PCOS cases thus indicating polygenic etiology in most PCOS cases.

Literature on gene expression study detects differentially expressed genes on metabolism and cell division/apoptosis with PCOS[203–206]. Characterization of these genes showed that retinoic acid synthesis and Wnt signal transduction altered in the PCOS theca cell. In addition, the transcription factor GATA6, which regulates the promoter activity of CYP17 and CYP11A, was increased in the PCOS compared to normal theca cells. A study with 119 known ovarian genes from women with PCOS showed differential expression compared to standard control ovarian samples[207]. Those differentially expressed genes were involved in various biologic functions, such as cell division/apoptosis, regulation of gene expression and metabolism. Another study showed that high-quality morphologically indistinguishable oocytes of women with and without PCOS have different gene expression profiles[208]. Those differentially expressed genes were associated with chromosome alignment and segregation during mitosis and/or meiosis.

7. Epigenetics Associations

Epigenetics is the study of heritable changes in gene expression and activity that is not caused by DNA sequence alterations. This includes DNA methylation and post-translational histone modification[209]. Epigenetic mechanisms play an important role in the control of gene expression by organizing the nuclear architecture of chromosomes, restricting or facilitating transcription factor access to DNA, and preserving a memory of past transcriptional activities[210]. Epigenetics explains how the genome and environment work in tandem[211,212]. DNA methylation is a natural tool on cytosine bases at CpG island promoter sequences and inactivates genes[213]. Epigenetic modification regulates gene transcription, X-chromosome inactivation, and cellular development and differentiation[214]. Inappropriate epigenetic reprogramming during gametogenesis and early embryogenesis has been identified as contributor to many common diseases with fetal origins such as PCOS[215,216]. Additionally, epigenetic alterations have been observed as non-random X-chromosome inactivation in PCOS women, evidencing that epigenetics may modulate the effect of the androgen receptor gene located on the X chromosome[217,218]. The role of epigenetics in PCOS is supported by studies on primates where intrauterine exposure to testosterone induces PCOS phenotype in the female offspring[215,219,220]. During development, adverse prenatal conditions may influence persistent epigenetic changes like imprinting of genes or increased, decreased levels of DNA methylation on CpG sites, which can lead to under or over-expression of genes and alteration of molecular pathways which may lead to a risk of development of PCOS during later part of life[216]. DNA methylation is the principal mechanism of epigenetics so far known to date. PCOS-like features also can be produced in small mammals by exposing their mothers to pesticides, androgens, bisphenol A, etc., during their pregnancy[221,222]. These indicate that PCOS might have an epigenetic basis. In humans, aberrant gene methylation (CEBPB, IL-6, IR, etc) has been reported in patients with PCOS[223,224]. Hypermethylation in the PPARG1 promoter and hypomethylated in the NCOR1 and HDAC3 promoter were reported in hyperandrogenic granulosa cells of PCOS[225]. PCOS women display dysfunction of subcutaneous adipocytes in addition to altered adipose tissue expression of PPARG, LEPR, TWIST1, CCL2, etc genes[226].

Epigenetic changes in fetal life are also implicated in the developmental origins of PCOS.[227] Early prenatal testosterone-treated adult female rhesus monkeys exhibit LH hypersecretion, ovarian hyperandrogenism, oligoanovulation, and PCO; they also demonstrate insulin resistance[166,222]. Prenatally testosterone-treated sheep also demonstrate LH hypersecretion, persistent follicles, and insulin resistance[228]. In mouse, PCOS phenotypes is seen in F1 generation female offspring following androgen exposure as well as in F2 generation offspring without androgen exposure during pregnancy, suggesting that intrauterine epigenetic programming is independent of androgens and can be genetically advanced[229].

In humans, studies also have shown a link between weight gain during pregnancy and the delivery of a baby who later developed PCOS[230]. A potential mechanism that can produce this effect is the epigenetic process[231,232]. Epigenetic alteration of various genes linked with PCOS are *LHCGR, YAP1, FOXO3* (hypomethylation), *CYP19A, PPARGC1A, PPARG* (hypermethylation), *ncRNAs* (miR-93/GLUT4, miR-320/ERK1/2, miR-21/LATS1; lncRNA H19, lncRNA SRA, lncRNA GAS5) &*miRNA* (miRNA21, miRNA93, miRNA-320)[233].

Our experience on the epigenetics in PCOS also confirms its role, mainly through alterations in methylation, global DNA (methylation DNA ELISA), global RNA (methylation RNA ELISA) as well as gene-wise (850K methylation array) epigenetic investigations. We observed hypermethylation in phenotype A (p=0.004) but absent in phenotype D which is either hypomethylated or normal in peripheral blood of PCOS women[80]. We have also observed differential (p=0.0015) global RNA hypomethylation in blood in comparison to control. We observed differential methylation (hypermethylation) in the CCL4L1 (cytokine/chemokine) gene in phenotype D PCOS in comparison to control besides differentially methylated (hypomethylated) promoter of ENSG00000271778/lncRNA gene in phenotype A PCOS in comparison to control. We have observed differential methylation of various pathways in phenotype A PCOS cases with high BPA and/or AGEs. Pathways involved are diabetes mellitus (30%), oocyte meiosis and maturation (26%), glucagon signaling (11%), insulin secretion or resistance (11%), steroidogenesis (7%), AMPK signaling (7%), AGE-RAGE signaling (4%), GnRH secretion (4%), etc. Differentially methylated genes commonly involved are *INSR, IRS1, GHRL, ADIPOQ, FTO, CYP, GnRH, NF kappa, TNF, AGE/RAGE, AMPK,* aldosterone, E2,

prolactin, progesterone, apoptosis, etc. These findings are in accordance with WES findings of major pathogenic variants. Most of the publications on genome-wide methylation profiling in PCOS are from granulosa cells/other ovarian tissue and very few on peripheral blood[234–239]. Promoter methylation of YAP1 gene (hypomethylated) in ovary granulosa cells of PCOS patients promotes the YAP1 expression, which plays a key role in the pathogenesis of PCOS[240].

8. Co-morbidity/Complication with PCOS

8.1 Reproductive

The commonest reproductive complication of PCOS is anovulatory infertility[241]. PCOS women are prone to have early abortions due to low progesterone and high androgens. Pregnancy complications like gestational diabetes, preeclampsia, preterm birth, etc., are also more frequent with PCOS. Maternal complications are also common, particularly hyperandrogenic PCOS[242] women with PCOS is prone to have ovarian hyperstimulation syndrome (life threatening condition) during ovulation induction with gonadotropin, in particular those with high AMH level[243]. PCOS women also have increased risk for endometrial cancer, and could be due to obesity, diabetes, anovulation and ovulation induction[244].

8.2 Metabolic

PCOS is often associated with insulin resistance and hyperinsulinemia, more often with phenotype A[245,246]. PCOS is also associated with impaired glucose tolerance and type 2 diabetes as well as vascular endothelial dysfunction and metabolic cardiovascular syndrome[247–249] However, publication on long term follow up reported no increased risk for stroke or ischaemic heart disease in PCOS women, even at post-menopause[250].

9. Management

There is no specific treatment for PCOS and presently all measures are directed to overcome various symptoms. Lifestyle adjustments should be the first-line management to improve reproductive, metabolic, cardiovascular, and psychosocial symptoms[251]. These focuses on diet modification, weight management and physical exercise besides meditation. A session at counselling on importance of lifestyle adjustment on combating PCOS should be explained for better motivation. Severe cases may require silencing of ovarian function for a brief period with oral contraceptive pill (combined with lowest estrogen) with/without addition of metformin (in particular with obesity/other metabolic problems; also helps in weight reduction)[252]. For the management of anovulatory infertility with no other factors, lifestyle intervention is recommended but if it fails, ovulation induction using clomiphene citrate or letrozole with/without metformin may be recommended. If it fails then gonadotrophins and thereafter laparoscopic surgery (wedge resection of ovary) may be recommended as next line of management. However, many women with PCOS fail to conceive despite all measures. The role of antiandrogens in the treatment of hirsutism or Bariatric surgery to improve fertility in PCOS is controversial[253,254].

Recent, genomic data analysis indicates that many of PCOS cases (about 20% in our study) can be linked to steroid biosynthesis pathway genes (pathogenic/likely pathogenic variants) and this group i.e., non-classical congenital adrenal hyperplasia can be treated specifically with corticosteroid. In coming years targeted personalized therapy depending on underlying genetic/epigenetic etiology will be in practice to overcome this syndrome.

10. Summary

PCOS is the most common reproductive endocrine disorder in women of reproductive age. PCOS is characterized by hyperandrogenism (clinical and/or biochemical; first criterion), ovulatory dysfunction (oligo and/or anovulation; second criterion), and polycystic ovary morphology (polycystic and/or enlarged ovary; third criterion). At present the only followed PCOS diagnostic criteria is Rotterdam 2003 criteria with phenotypic sub-classifications i.e., NIH criteria 2012. This approach classifies PCOS cases into four phenotypic groups viz., phenotype A (all three criteria), phenotype B (first two criteria), phenotype C (first and last criteria), and phenotype D (last two criteria). The commonest phenotype of PCOS is phenotype A. Phenotype D PCOS is common at a younger age with normal BMI and BPA, and associated mostly with higher AMH, fasting insulin and IL4. The PCOS is associated with genetic factors, often with multiple genes and epigenetic

factors influenced via environmental factors (Figure 1). Environmental pollutants play some role as Endocrine-Disrupting Chemicals (EDC) and disrupt ovarian as well as metabolic function thus causing PCOS-like abnormalities. BPA, a widely used estrogenic plasticizer, is one such EDC that is associated with genesis of PCOS. Similarly, AGEs are also associated with PCOS. Among genetic causes, various genes are associated viz., ghrelin, insulin, insulin receptor, steroid biosynthesis enzymes, *AMH, AMHR2, FSH, FSHR, GATA4, LHCGR, THADA, DENND1A, YAP1*, *RAB5B, SUOX, NEIL2*, etc. Epigenetic changes in fetal life are also implicated in the developmental origins of PCOS as evident with PCOS-like phenotypes induced experimentally through prenatal exposure with various agents like androgens, BPA, AGEs, etc in rhesus monkeys, sheep, rats, mice, etc. Hypomethylation (ENSG00000271778/lncRNA) or hypermethylation (CCL4L1) of various genes and

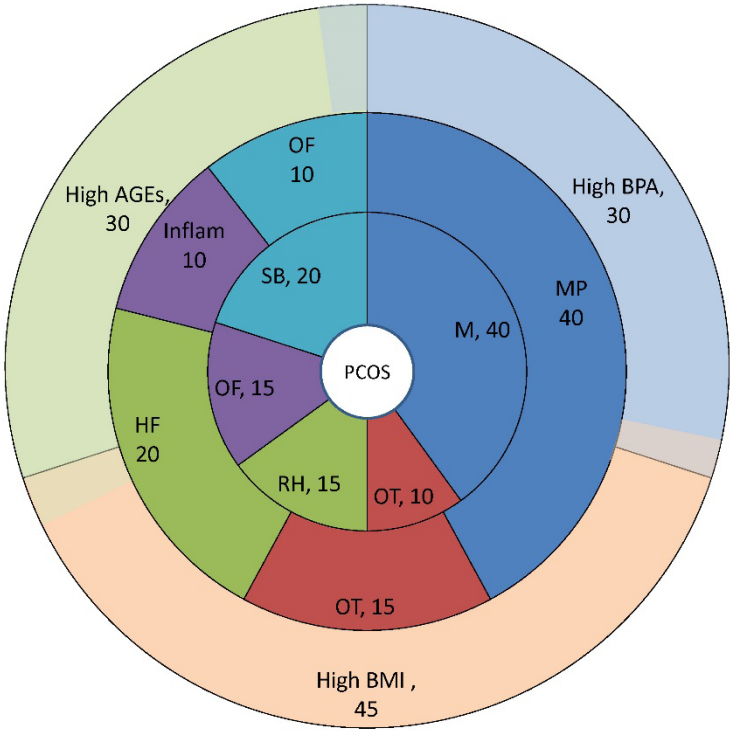

Figure 1. Associations of genetic (51 cases), epigenetic (29 cases), and environmental (80-100 cases) factors with PCOS from our study.

Genetic associations

M (metabolic; in 40%) genes: INSR, IRS1, GHRL, LDLR, MC4R, ADIPOQ, UCP1, UCP2, UCP3, FTO, PCSK9, THADA, FBN3, NEIL2, FDFT1, PCSK9, etc

SB (steroid biosynthesis; in 20%) genes: CYP11, CYP17, CYP21, HSD17, STAR, POR, AKR1C3, etc

OF (ovarian function; in 15%) and RH (reproductive hormone; in 15%) genes: AMH, AMHR2, INHBA, AR, SHBG, LHR, FSHR, FSH β, SRD5A, etc

OT (other; in 10%) genes: GATA4, THADA, YAP1, ERBB2, DENND1A, FEM1B, FDFT1, NEIL2, TCF7L2, etc

Epigenetics associations

MP (metabolic; in 40%) pathways: diabetes mellitus, glucagon signaling, insulin synthesis/secretion, adipocyte function, etc

HF (hormone functions; in 20%: synthesis/secretion/action) pathways: cortisol, aldosterone, E2, prolactin, progesterone, GnRH, etc

OF (ovarian function; in 15%) pathways: oocyte maturation, mitosis, etc

Inflammatory (in 10%) pathways: NF kappa, TNF, etc

OT (other; in 15%) pathways: aging, AGE/RAGE, apoptosis, AMPK, etc

Environmental associations

BPA (bisphenol A): high in 30% of cases

AGEs (advanced glycation end product): high in 30% of cases

BMI (body mass index): high in 45% of cases

pathways (diabetes mellitus, insulin secretion/resistance, oocyte development, glucagon signaling, steroidogenesis, AMPK signaling, AGE-RAGE signaling, etc.) are associated with PCOS.

11. Conclusions

PCOS is characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovary morphology. PCOS consensus diagnostic criteria recommended by international committee on PCOS guideline is Rotterdam 2003 criteria with phenotypic sub-classifications (A to D) i.e., NIH 2012 criteria. Among biomarkers calculated free testosterone, or calculated bioavailable testosterone or FAI is recommended and AMH is promising. However, yet no consensus derived on cut-off levels and need more research data before any recommendation. The commonest phenotype of PCOS is phenotype A. Phenotype D (without hyperandrogenism) seems to be different as more prevalent at a younger age with normal BMI, and more frequently associated with high AMH and fasting insulin. The underlying etiology of PCOS seems to be extremely heterogeneous and associated with genetic factors, often involvement of multiple genes and epigenetic factors influenced by environmental factors, in particular bisphenol A and AGEs. There is no specific treatment for PCOS at present, and mostly directed to treat symptoms. Soon, we will be in a position to treat PCOS specifically according to underlying etiopathology viz., corticosteroid for non-classical CAH presenting as PCOS, epigenetic modification, etc. In coming years targeted personalized therapy depending on underlying genetic/epigenetic etiology will be in practice to overcome this syndrome.

12. References

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