Original Article

Diagnostic Utility of Various Hormones across Different Polycystic Ovary Syndrome Phenotypes: A Cross-sectional Study

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Abstract

Background: Polycystic ovary syndrome (PCOS) presents a complex diagnostic challenge due to its heterogeneous nature. **Aim:** This study aimed to examine the diagnostic utility of various hormones across different PCOS phenotypes. **Settings and Design:** This cross-sectional study was carried out in 187 newly diagnosed PCOS women (18–40 years) attending the outdoor clinics of the department of endocrinology and obstetrics and gynaecology of a tertiary care centre in India. **Materials and Methods:** One hundred and eighty-seven PCOS women based on revised Rotterdam 2003 criteria were recruited. Ninety-four age-matched healthy females were taken as controls. All PCOS women were categorised into four phenotypes (A, B, C and D) based on the National Institute of Health (2012) criteria. Detailed clinical examination and hormonal investigations including testosterone, androstenedione, dehydroepiandrosterone sulphate (DHEAS) and anti-Müllerian hormone (AMH) were performed. **Statistical Analysis Used:** The receiver operating characteristic curve (ROC) was generated to find the diagnostic utility of various hormones by using SPSS version 26.0 software. **Results:** The largest PCOS group was phenotype A (33.15%, *n* = 61) followed by phenotype B (28.6%, *n* = 52), phenotype D (23.9%, *n* = 44) and phenotype C (16.3%, *n* = 30). In ROC analysis, AMH and testosterone (except phenotype D) were good diagnostic parameters for PCOS. AMH cutoffs varied from 4.4 to 5.6 ng/mL with sensitivities and specificities ranging from 86% to 97% and 85% to 100%, respectively, across all PCOS phenotypes. In the entire PCOS cohort, AMH at an optimal cutoff of 5.28 ng/mL had sensitivity and specificity of 87% and 97%, respectively, for the diagnosis of PCOS. Optimal testosterone cutoffs were 29.3, 25.1 and 23.1 ng/dL for phenotypes A, B and C, respectively, with reasonable sensitivities and specificities but not in phenotype D. Luteinising hormone (LH), follicle-stimulating hormone (FSH), LH/FSH ratio, androstenedione and DHEAS had low-to-moderate sensitivity across all phenotypes. **Conclusion:** AMH is a useful hormonal diagnostic marker for PCOS across all phenotypes.

Keywords: *Anti-Müllerian hormone, polycystic ovary syndrome phenotypes, testosterone*

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Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disease affecting women of reproductive age group with a prevalence of 5%–18%.[1] The exact pathogenesis of PCOS remains unclear, although insulin resistance and hyperandrogenism (HA) have been identified in approximately 50–80% and 40–80% of PCOS cases, respectively.[2] However, the pathophysiology in the rest of the individuals is still unknown. Among various diagnostic criteria proposed by the Androgen Excess Society and the National Institute of Health (NIH), the Rotterdam (2003) criteria[3] is the most commonly used for the diagnosis of PCOS. According to the Rotterdam criteria, any two of the three features such as oligo-anovulation, HA and/or polycystic ovarian morphology (PCOM) are required for diagnosis after exclusion of other causes of HA and ovulatory dysfunction (OD). Certain demerits of Rotterdam criteria include menstrual irregularity is common in the initial 3 years of menarche making it an unreliable marker. Moreover, some women may have regular menstrual cycles in spite of having anovulation. PCOM criteria on ultrasound (USG) is also controversial in adolescent girls. Further, the route of USG and interindividual operability impacts the accuracy of the scan as transvaginal USG is not possible among adolescent girls. Endocrine Society advises Liquid chromatography–mass spectrometry (LC–MS) based assay for estimation of testosterone which is not readily available. Hence, there is a need to find a better marker for the diagnosis of PCOS. In the last few years, anti-Müllerian hormone (AMH) has gained interest as a predictor of PCOM.[2] AMH is secreted from the antral follicles in the early follicular phase and has been found to be increased in PCOS women.[4] Previous studies showed an AMH cutoff level of 3.9 ng/mL was optimal for the diagnosis of PCOS[5,6] but other studies were not concordant.[7,8] Androgenic markers such as serum testosterone, androstenedione, dehydroepiandrosterone sulphate (DHEAS) and free androgen index (FAI) have been studied in PCOS, with variable results.[9]

NIH in 2012 further sub-classified PCOS into four phenotypes (A, B, C and D) based on the combinations of HA, OD and PCOM.[10] However, it remained unclear whether the same diagnostic criteria can be applied across all the PCOS phenotypes. The absence of a universal marker for diagnosing PCOS phenotypes adds to the confusion. There is also heterogeneity in AMH levels in different PCOS phenotypes with higher levels observed in phenotypes A and D.[11] Hence, this comprehensive study was undertaken with the aim to characterise the diagnostic performance of various markers, such as luteinising hormone (LH), follicle-stimulating hormone (FSH), LH/FSH ratio, AMH, testosterone, DHEAS, androstenedione and FAI in the NIH proposed PCOS phenotypes. To the best of our knowledge, there is no previous study which has comprehensively assessed all the above markers in different PCOS phenotypes.

Materials and Methods

This cross-sectional study was carried out in 187 PCOS women of age group 18–40 years, attending the outdoor clinics of the department of endocrinology and obstetrics and gynaecology of a tertiary care centre from July 2021 to January 2023. Written informed consent was obtained from all participants and the study was conducted in accordance with the Declaration of Helsinki (2013). The study was approved by the Institutional Ethics Committee (No: 869/20.07.2021, M. K. C. G Medical College, Berhampur).

Newly diagnosed PCOS women meeting the revised Rotterdam 2003 criteria[3] were recruited. HA was defined by modified Ferriman–Gallway score (mFG score) ≥8 and hyperandrogenemia was defined as serum total testosterone ≥60 ng/dL.[12] Exclusion criteria encompassed other causes of HA such as non-classical congenital adrenal hyperplasia, Cushing's syndrome, hypothyroidism, hyperprolactinemia and androgen-secreting tumours. Drugs affecting glucose/lipid/androgen metabolism and insulin sensitivity such as glucocorticoids, oral contraceptive pills and antiandrogens within the past 3 months were excluded from the study. Two clinicians were involved in the diagnosis of PCOS cases, and this was confirmed by the corresponding author when there was any confusion in diagnosis.

Ninety-four age-matched healthy females with regular menstrual cycles without any features of HA were taken as controls. Both PCOS cases and controls were drawn from the same ethnicity. Comprehensive assessments, including anthropometric measures such as height, weight, body mass index (BMI) (kg/m^2), waist circumference, hip circumference, waist-to-hip ratio (WHR) as well as blood pressure, presence of acanthosis nigricans, hirsutism evaluation by modified Ferriman–Gallway score, were conducted following standard procedures. All PCOS cases were categorised into four phenotypes as per NIH in 2012 criteria[13] based on combinations of HA, OD and PCOM: Phenotype A (HA + OD + PCOM), Phenotype B (HA + OD), Phenotype C (HA + PCOM) and Phenotype D (OD + PCOM).

A fasting morning blood sample was collected from all participants for estimation of various biochemical and hormonal parameters. Biochemical investigations included fasting plasma glucose (FPG), glycated

haemoglobin (HbA1c), serum insulin and serum lipids such as total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). The hormonal investigations include thyroid-stimulating hormone (TSH), prolactin (PRL), LH, FSH, testosterone, sex hormone binding globulin (SHBG), DHEAS, androstenedione and AMH. Two-hour post-glucose plasma glucose (2 h PGPG) was estimated in all subjects by doing an oral glucose tolerance test with 75 g of anhydrous glucose. Frankly, diabetic cases were excluded from the study. Hormonal evaluation was done on days 3–5 of the spontaneous or progesterone-induced menstrual bleeding in all participants.

Plasma glucose and serum lipids were measured by using the biochemistry autoanalyser Autopak 300 APK (Siemens). A high-performance liquid chromatography method was used for estimation of HbA1C. Measurement of serum insulin, TSH, PRL, LH, FSH, testosterone, DHEAS and SHBG were done by chemiluminescence (CLIA) method in Advia Centaur CP machine (Siemens). AMH was measured in Snibe MAGLUMI 800 fully automated analyser by CLIA method. Androstenedione level was assessed by using enzyme-linked immunosorbent assay (ELISA) kit (Diametra) and analysed by using ELISA reader (Varioskan Lux thermo scientific reader) at 450 nm.

Homoeostatic model assessment of insulin resistance (HOMA-IR) was calculated by using the formula: fasting insulin (μIμ/mL) × FPG (mg/dL)/405. FAI was calculated by total testosterone/SHBG X100 (both in nmol/L).

Transabdominal USG was done during days 3–5 of the spontaneous or progesterone-induced menstrual bleeding by a single radiologist using the machine (GE LOGOQ F8 Expert) with curvilinear 6MHz probe. PCOM was diagnosed by the presence of 12 or more follicles of size 2–9 mm and/or ovarian volume ≥10 mL either unilaterally or bilaterally.[3]

Statistical analysis

The sample size for the present study was calculated by using OpenEpi software (Dean AG, Sullivan KM, Sir MM. OpenEpi: Open sources Epidemiologic Statistics for Public Health, Version 3.01) based on a previous study by Jena *et al*.[13] on PCOS. The minimum number of subjects required for the study was found to be 28 each for case and control to achieve a power of 90% and alpha value of 5%. Hence, we had planned the study to recruit at least 30 subjects from each phenotype and control.

Normality distribution was checked by using Kolmogorov–Smirnov test. Data (parametric) were expressed as mean ± standard deviation, whereas data (nonparametric) were expressed as median (P25–P75). Comparison between two groups was done by non-parametric test (Mann–Whitney *U*-test test) and parametric test (Student's *t*-test) whereas for more than two groups comparison non-parametric test (ANOVA test) and parametric test (Kruskal–Wallis test) were used. The receiver operating characteristic curve (ROC) was generated to find the diagnostic utility of various parameters. $P < 0.05$ was considered statistically significant. Statistical analysis was performed by using SPSS version 26.0 software (IBM Corp, Armonk, NY).

Results

In the current study of 187 women with PCOS, phenotype A constituted the largest group (33.15%, $n = 61$) followed by phenotype B (28.6%, $n = 52$), phenotype D (23.9%, $n = 44$) and phenotype C (16.3%, $n = 30$).

There was no significant difference in the mean age of all PCOS phenotypes compared to controls ($P = 0.541$). All PCOS phenotypes had significantly higher BMI, glycaemic profile (FPG, 2 h PGPG and HbA1C), lipid parameters (TC, TG and LDL), HOMA-IR, LH and AMH when compared to the healthy controls [Table 1]. WHR was significantly higher in phenotypes A and B whereas systolic blood pressure was significantly higher in phenotypes A, B and C than that of controls. HOMA-IR was 4–6 times higher in cases (highest in phenotype A followed by phenotype C, B and D) compared to controls. There was no statistically significant difference with respect to BMI, WHR, blood pressure, lipid parameters (TC, TG, HDL and LDL), glycaemic status (2 h PGPG and HbA1C), fasting insulin, FSH, DHEAS, androstenedione and PRL levels across different PCOS phenotypes [Table 1].

Phenotype A, which is a full-blown PCOS, had a significantly higher LH/FSH ratio compared to all other phenotypes. It had significantly higher levels of FPG, LH, testosterone and FAI than that of phenotypes C and D and had significantly higher levels of HOMA IR and AMH than that of phenotype D [Table 1]. No difference was found between phenotypes A and B except for LH/FSH ratio. Phenotype B had significantly higher levels of FPG, testosterone and FAI than that of phenotypes C and D whereas it had significantly higher levels of LH and AMH in comparison to phenotype D. Phenotype C had significantly higher levels of testosterone compared to phenotype D only.

Phenotype D was distinct in having significantly lower levels of testosterone than that of all other phenotypes. It had significantly lower levels of FPG, LH, FAI and AMH

Table 1: Comparison of baseline parameters across polycystic ovary syndrome phenotypes and controls

Parameters	Controls (*n*=94)	Phenotype A (*n*=61) (HA + OD + PCOM)	Phenotype B (*n*=52) (HA + OD)	Phenotype C (*n*=30) (HA + PCOM)	Phenotype D (*n*=44) (OD + PCOM)	*P*
Age (years)	23.7±4.1	24.8±4.3	23.6±4.3	24.3±4.4	24.3±4.9	0.514
Weight (kg)	58.0±8.9	64.2±10.8	63.3±10.8	62.2±9.1	62.7±11.0	0.003
BMI (kg/m^2)	23.1±3	26.0±3.5	25.9±3.7	25.4±2.2	25.2±3.4	0.000
WHR	0.8±0.07	0.9±0.1	0.8±0.1	0.8±0.1	0.8±0.1	0.000
SBP (mmHg)	114.0±5.82	118.9±10.2	118.4±9.3	119.8±10.6	117.0±10.3	0.002
DBP (mmHg)	75.0±5.38	76.4±7.4	75.2±6.7	76.1±6.3	76.2±6.8	0.648
FPG (mg/dL)	78.9±5.5	91.2±7.2	91.3±8.2	87.0±7.3¥,€	85.8±6.4¥,€	0.000
2HPGPG (mg/dL)	107.1±18.3	128.6±12.6	123.4±11.5	121.4±11.3	120.5±11.4	0.000
HbA1c (%)	5.3±0.2	5.6±0.3	5.5±0.3	5.5±0.3	5.5±0.2	0.000
Fasting insulin (mIU/L)	2.4±1.9	13.6±12.0	10.9±6.8	13.5±8.1	9.7±6.4	0.000
HOMA IR	0.5±0.38	3.1±2.5	2.5±1.6	2.9±1.7	2.1±1.4¥	0.000
TC (mg/dL)	132.6±24.01	188.3±32.6	191.7±40.0	179.0±41.4	172.2±34.4	0.000
TG (mg/dL)	114.6±22.6	154.8±59.1	187.1±89.4	173.1±97.6	161.6±93.5	0.000
HDL (mg/dL)	48.2±6.8	47.1±9.7	45.6±9.9	46.5±10.6	44.6±8.0	0.272
LDL (mg/dL)	61.5±21.8	110.3±33.0	108.7±36.5	97.8±34.9	95.3±29.2	0.000
TSH (mIU/L)	2.0±0.7	2.0±0.8	2.3±0.9	2.0±0.7	2.0±0.7	0.124
PRL (ng/mL)	10.4±3.5	15.3±23.1	12.1±7.2	12.7±8.1	11.9±6.9	0.172
LH (IU/L)	6.1±4.3	13.7±7.1	12.9±8.6	9.6±4.4¥	9.1±5.2¥,€	0.000
FSH (IU/L)	4.6±2.07	5.0±3.0	6.1±4.6	5.0±2.2	6.4±5.0	0.026
LH_FSH	1.4±0.97	4.2±4.1	2.7±2.2¥	2.0±0.9¥	2.0±1.5¥	0.000
Testosterone (ng/dL)	14.7±5.1	57.1±21.2	52.5±16.7	29.4±7.6¥,€	15.5±4.7¥,€,$	0.000
SHBG	52.7±15	55.1±23.3	50.8±23.4	47.5±21.3	40.2±25.9¥	0.005
FAI	1.1±0.5	4.4±3.1	4.6±3.1	2.7±1.8¥,€	2.3±2.2¥,€	0.000
DHEAS	175.1±70.9	208.6±71.0	219.8±109.2	208.7±69.3	193.3±93.0	0.016
Androstenedione (ng/mL)	2.8±1.2	4.5±2.2	4.4±2.0	4.7±2.5	4.3±2.0	0.000
AMH (ng/mL)	3.8±0.7	8.7±2.8	8.9±2.4	7.9±2.0	7.1±2.2¥,€	0.000

Statistically significant difference when compared to phenotype A=¥, phenotype B=€, phenotype C=$. Data expressed in mean±SD, Bold: Statistically significant difference when compared to controls. BMI=Body mass index, WHR=Waist-to-hip ratio, SBP=Systolic blood pressure, DBP=Diastolic blood pressure, FPG=Fasting plasma glucose, 2HPGPG=2-h postprandial glucose, HOMA IR=Homoeostasis model assessment of insulin resistance, TC=Total cholesterol, TG=Triglyceride, HDL=High-density lipoprotein, LDL=Low-density lipoprotein, TSH=Thyroid-stimulating hormone, FSH=Follicle-stimulating hormone, LH=Luteinising hormone, PRL=Prolactin, SHBG=Sex hormone-binding globulin, DHEAS=Dehydroepiandrosterone sulphate, FAI=Free androgen index, AMH=Anti-Müllerian hormone, HA=Hyperandrogenism, OD=Ovulatory dysfunction, PCOM=Polycystic ovarian morphology, HbA1c=Glycated haemoglobin, SD=Standard deviation

compared to phenotypes A and B and had significantly lower HOMA-IR than that of phenotype A [Table 1]. Hyperandrogenic PCOS group (phenotypes A, B and C) had significantly higher levels of FPG (P = 0.04), 2 h PGPG (P = 0.009), LH (P = 0.01), testosterone (P < 0.001), FAI (P = 0.002), AMH (P = 0.004) and significantly lower SHBG (P = 0.04) when compared to normoandrogenic group (phenotype D).

We conducted ROC analysis for the diagnosis of PCOS across all phenotypes by utilising various hormonal parameters such as LH, FSH, LH/FSH ratio, AMH, serum testosterone, androstenedione, FAI and DHEAS.

Among PCOS women with phenotype A, AMH had highest specificity of 100% and sensitivity of 86% at an optimal cutoff of 5.6 ng/mL for diagnosis of PCOS followed by serum testosterone with specificity of 98% and sensitivity of 88% at a cutoff of 29.3 ng/dL. FAI had good sensitivity of 98% with moderate specificity of 80.2% at a cutoff of 1.47. Other markers such as LH, FSH, LH/FSH ratio, androstenedione and DHEAS had low-to-moderate sensitivities for the detection of PCOS, as shown in Table 2 and Figure 1.

A similar trend was observed among PCOS women with phenotype B. AMH had highest specificity of 96.8% and sensitivity of 96% at an optimal cutoff of 5.2 ng/mL for diagnosis of PCOS followed by serum testosterone at a cutoff of 25.1 ng/dL had sensitivity and specificity of 94% and 95.7%, respectively, whereas FAI at 1.87 cutoff had good sensitivity and specificity of 98% and 92.6%, respectively. Other markers such as LH, FSH, LH/FSH ratio, androstenedione and DHEAS had low-to-moderate sensitivity for the detection of PCOS, as shown in Table 2 and Figure 1.

Table 2: Utility of various hormonal parameters for diagnosis of polycystic ovary syndrome in different phenotypes and whole cohort

Parameters	Phenotype-A			Phenotype-B			Phenotype-C			Phenotype-D			Whole cohort		
	Cutoff	Sensitivity (%)	Specificity (%)	Cutoff	Sensitivity (%)	Specificity (%)	Cutoff	Sensitivity (%)	Specificity (%)	Cutoff	Sensitivity (%)	Specificity (%)	Cutoff	Sensitivity (%)	Specificity (%)
AMH (ng/mL)	5.6	86	100	5.2	94.2	96.8	5.6	90	100	4.4	97.7	85.1	5.28	87.2	97.9
LH (IU/L)	9	82	84	9.87	65.4	88.3	7.2	70	69.1	7.92	56.8	74.5	8.67	94.6	35.11
FSH (IU/L)	5.87	31.5	82	6.32	32.7	87.2	6.3	30	87.2	6.3	36.4	87.2	6.32	30.5	87.2
LH_FSH ratio	1.4	83.6	63.8	1.5	73.08	67.02	1.4	76.7	63.8	1.5	59	67	1.4	75.4	65.83
Testosterone (ng/dL)	29.3	88.5	98.9	25.1	94	95.7	23.1	86.7	95	7.68	100	9.6	25.1	78.1	92.6
Androstenidione (ng/mL)	3.42	72	71	3.43	65.4	78.7	4.1	63.3	89.4	2.1	61.4	78.7	3.43	66.3	78.7
FAI	1.47	98.4	80.2	1.87	98.1	92.6	1.38	90	84	1.2	59	84	1.47	86.1	87.2
DHEAS (µg/dL)	197.4	59	74.47	204.5	55.8	76.6	228.49	46.7	84	167	50	75.5	196.78	55.1	73.4

AMH=Anti-Müllerian hormone, LH=Luteinising hormone, FSH=Follicle-stimulating hormone, FAI=Free androgen index, DHEAS=Dehydroepiandrosterone sulphate

Among PCOS women with phenotype C, AMH at an optimal cutoff of 5.6 ng/mL had highest sensitivity and specificity of 90 and 100%, respectively for diagnosis of PCOS. Serum testosterone at a cutoff of 23.1 ng/dL also had good sensitivity and specificity of 86 and 95%, respectively. Androstenedione at 4.1 ng/mL had lower sensitivity of 63% and specificity of 89%, respectively, for diagnosis. Other markers such as LH, FSH, LH/FSH ratio, FAI and DHEAS had low-to-moderate sensitivity for detection of PCOS, as shown in Table 2 and Figure 1.

Among PCOS women with phenotype D, serum AMH at an optimal cutoff of 4.4 ng/mL had highest sensitivity of 97% and specificity of 85% for diagnosis of phenotype D. Rest of the parameters had low-to-moderate sensitivity and specificity, as indicated in Table 2 and Figure 1.

AMH remained a robust tool for the diagnosis of PCOS across all phenotypes. When whole PCOS cohort was taken into consideration, AMH at a cutoff of 5.28 ng/mL had sensitivity and specificity of 87.2% and 97.9%, respectively, for diagnosing PCOS women. Testosterone at a cutoff of 25.1 ng/dL had sensitivity and specificity of 78.2% and 92.6%, respectively. Other markers such as FSH, LH, LH/FSH ratio, androstenedione and DHEAS had moderate-to-low sensitivity and specificity for detection of PCOS [Table 2 and Figure 1].

Discussion

In the present study, phenotype A is the most common followed by B, C and D phenotypes. The PCOS patients were more insulin resistant, had dysglycaemia and were likely overweight or obese compared to controls. Among all the hormones studied AMH and testosterone had best diagnostic utility for PCOS. AMH cutoffs varied from 4.4 to 5.6 ng/mL across the phenotypes with an optimal cutoff of 5.28 ng/mL in the entire cohort. Testosterone is a useful marker for diagnosis of PCOS with cutoffs varying from 23.1 to 29.3 ng/dL in phenotypes A, B and C but not in phenotype D.

In the present study of 187 young women with PCOS, phenotype A was the most common followed by phenotypes B, D and C aligning with previous studies.[14,15] Previous studies reported that the prevalence of normoandrogenic PCOS varies from 3.6% to 18%[14,15] but in our study, it was found to be 23.9%. This study also showed that HA and insulin resistance were predominant among PCOS phenotypes attributing to 76.4% and 63%, respectively. This is consistent with other studies showing insulin resistance and HA varying from 50% to 80% and 40–80%, respectively.[2]

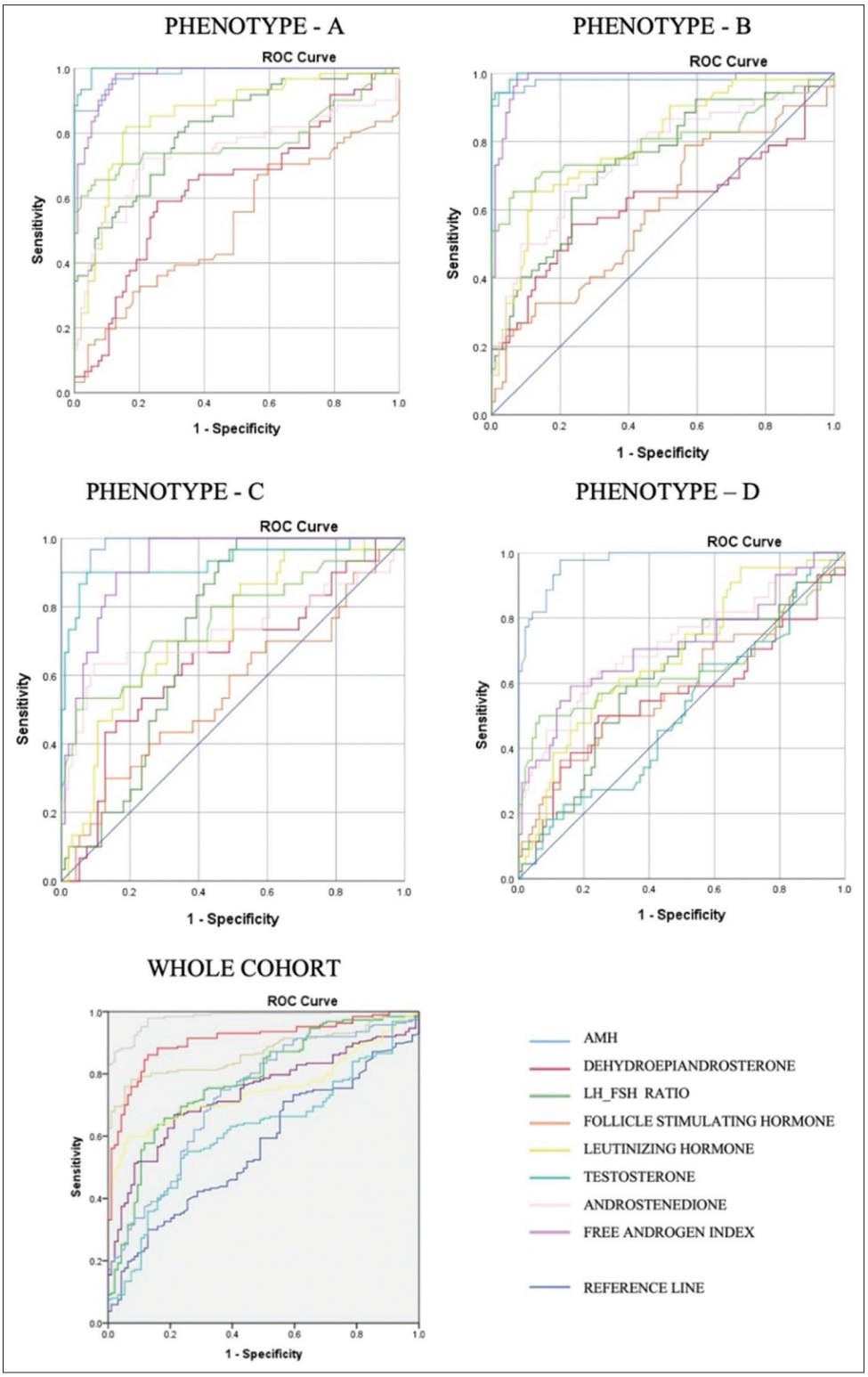

Figure 1: Receiver operating characteristic curves of different hormonal parameters for diagnosis of polycystic ovary syndrome in different phenotypes and whole cohort

All PCOS phenotypes had significantly higher BMI, insulin resistance (HOMA IR) and metabolic parameters such as FPG, 2-h postprandial glucose, lipids (TC, TG and LDL) and AMH compared to the control group. These results were in agreement with other studies.[14,16] In contrary to other studies[16,17] where obesity, insulin resistance and metabolic syndrome were more common in phenotype A as compared to other phenotypes, the present

showed that these metabolic parameters were uniformly increased across PCOS all phenotypes suggesting a higher risk of adverse metabolic and cardiovascular outcomes.

The diagnosis of PCOS is mostly based on revised Rotterdam 2003 criteria. The main pitfalls of Rotterdam criteria are reliance on subjective interpretations. Although insulin resistance is central to the pathogenesis of PCOS, it has not been included as a diagnostic criterion. Studies trying to find a single marker for detection of PCOS have not been successful. In the present study, all the hormones were studied comprehensively for their diagnostic utility in various PCOS phenotypes. To the best of our knowledge, this is the first study to assess all the conventional androgen markers such as testosterone, androstenedione, FAI, DHEAS and SHBG besides AMH across different phenotypes. AMH and testosterone (except phenotype D) had the highest diagnostic accuracy for PCOS with area under the curve of 0.98–0.99 and 0.92–0.99, respectively, among all the studied hormones. AMH has been shown in the present study to be consistently elevated in all PCOS phenotypes compared to controls and at a cutoff of 5.28 ng/mL in the entire cohort had highest sensitivity 87.2% and specificity 97.9%. Similar to the present study, Mahajan and Kaur[18] reported AMH at a cutoff of 5.03 ng/mL had a lower sensitivity of 70.6% and specificity of 79.9% for the diagnosis of PCOS. However, others have reported lower cutoffs of AMH at 4.1 ng/mL and 3.44 ng/mL by Halder *et al*.[19] and Saxena *et al.,*[20] respectively, with variable sensitivities and specificities. Malhotra *et al*.[21] reported AMH cutoff of 6.06 ng/mL for diagnosis of PCOS with high sensitivity of 91.4% and specificity of 90.7% probably due use of ELISA. Among Caucasians Pigny *et al*.[22] and Tremellen and Zander-Fox[23] found optimal AMH cutoffs of 5.6 ng/mL and 5.07 ng/mL, respectively, for diagnosis of PCOS, similar to the present study. However, much higher AMH cutoffs were reported by several authors from Asia. Song *et al*.[24] and Li *et al*.[25] found the AMH cutoffs of 10 ng/mL and 8 ng/mL for diagnosis of PCOS in Korean and Chinese women, respectively. The variable cutoffs of AMH across studies may be related to ethnic diversity, studied age group and phase of menstrual cycle besides the assay methods used for estimation of AMH. Very few studies looked into AMH in phenotype A but none in all four phenotypes. In phenotype A, Halder *et al*.[19] and Gursu *et al*.[26] found an optimal AMH cutoffs of 5.17 ng/mL and 6.09 ng/mL, respectively, for diagnosis. AMH is a glycoprotein belonging to the transforming growth factor-beta superfamily and is produced by the granulosa cells of the preantral and small antral follicles. AMH is known for its correlation with ovarian antral follicle count and has been proposed as a diagnostic surrogate for PCOM in PCOS.[2] The 2023 International Evidence-based Guidelines for PCOS recommends for using AMH for defining PCOM in adults with either irregular menses or HA.[2] The present study explored the utility of AMH across all the PCOS phenotypes reaffirming its use as an alternative to PCO morphology.

Testosterone and androstenedione were reported to be useful as the diagnostic markers of hyperandrogenemia in PCOS previously. Although the gold standard test for the estimation of testosterone is LC–MS, it is not available everywhere and is cumbersome. Studies have shown that testosterone as measured by RIA extraction method had comparable results with LC–MS estimation and a lower cutoff of 35 ng/dL had been proposed for detection of hyperandrogenemia in hospital-based studies.[27] In our study, testosterone at a cutoff of 25.1 ng/dL had a specificity of 92.6% and sensitivity of 78.1% for diagnosing PCOS cases using immunoassay. Similar results were seen in a study where testosterone cutoff of 30 ng/mL had 80.8% sensitivity and 81.3% specificity.[19] This could be true as high testosterone is rarely observed in Asian women with PCOS.[28] Different ethnicities might have different testosterone cutoffs for diagnosis of PCOS. Hence, there is a need for ethnicity-specific cutoffs for diagnosis of hyperandrogenemia in PCOS subjects. The traditional cutoff for testosterone at 60 ng/dL for hyperandrogenemia has low sensitivity of 45% with good specificity of 95%.[12] This underscores the need for revising serum testosterone cutoffs in the diagnosis of PCOS. Across the hyperandrogenic phenotypes, testosterone showed good sensitivity (86.7%–94%) and specificity (95%–98.9%) at cutoffs ranging from 23.1 to 29.3 ng/dL, for diagnosis of PCOS in concordance with previous studies.[19,29]

Although some studies reported that androstenedione may have higher sensitivity compared to testosterone for detecting hyperandrogenemia;[9,30] the present study is in contradiction to those studies. Androstenedione at levels ranging from 3.42 to 4.1 ng/mL had moderate sensitivity (63.3%–72%) and specificity (71%–89.4%) whereas FAI ranging from 1.38 to 1.87 showed reasonable sensitivity (90%–98.4%) and specificity (80.2%–92.6%) for diagnosis of PCOS across hyperandrogenic phenotypes. Similar results were shown in previous studies.[31] Since free testosterone was not estimated using equilibrium dialysis, it cannot be commented upon. DHEAS performed poorly for the detection of PCOS across all phenotypes, as shown in previous studies.[32,33]

The major strength of our study is that we have analysed the diagnostic utility of all the conventional androgen

markers as well as AMH across all PCOS phenotypes in a good number of PCOS women. However, we have few limitations. In our study, we have not measured free testosterone for detection of hyperandrogenemia. Besides the USG was done transabdominally for detection of PCOM instead of ideal transvaginal route due to lack of consent from the studied population.

Conclusion

In the current study, AMH emerged as a consistent and may be considered as a useful hormonal diagnostic marker for PCOS across all phenotypes, exhibiting high sensitivity and specificity. However, this needs further validation in larger studies.

Authors contribution

PRK: Conceptualised the study, designed the research, managed recruitment, performed statistical analysis, manuscript editing and proofreading. RT: Contributed to recruitment, performed statistical analysis and participated in manuscript writing. DKD: Contributed in recruitment and proof reading. DP: Performed statistical analysis.

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Conflicts of interest

There are no conflicts of interest.

Data availability statement

The corresponding author of this manuscript is willing to share the data supporting the results of this manuscript upon reasonable request.

References

1. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, *et al*. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Hum Reprod 2018;33:1602-18.
2. Teede HJ, Tay CT, Laven JJ, Dokras A, Moran LJ, Piltonen TT, *et al.* Recommendations from the 2023 international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Eur J Endocrinol 2023;189:G43-64.
3. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004;19:41-7.
4. Pigny P, Merlen E, Robert Y, Cortet-Rudelli C, Decanter C, Jonard S, *et al*. Elevated serum level of anti-mullerian hormone in patients with polycystic ovary syndrome: Relationship to the ovarian follicle excess and to the follicular arrest. J Clin Endocrinol Metab 2003;88:5957-62.
5. Butt MS, Saleem J, Aiman S, Zakar R, Sadique I, Fischer F. Serum anti-müllerian hormone as a predictor of polycystic ovarian syndrome among women of reproductive age. BMC Womens Health 2022;22:199.
6. Salmassi A, Mettler L, Hedderich J, Jonat W, Deenadayal A, von Otte S, *et al*. Cut-off levels of anti-mullerian hormone for the prediction of ovarian response, *in vitro* fertilization outcome and ovarian hyperstimulation syndrome. Int J Fertil Steril 2015;9:157-67.
7. Dewailly D. Diagnostic criteria for PCOS: Is there a need for a rethink? Best Pract Res Clin Obstet Gynaecol 2016;37:5-11.
8. Sahmay S, Aydin Y, Oncul M, Senturk LM. Diagnosis of polycystic ovary syndrome: AMH in combination with clinical symptoms. J Assist Reprod Genet 2014;31:213-20.
9. Kanbour SA, Dobs AS. Hyperandrogenism in women with polycystic ovarian syndrome: Pathophysiology and controversies. Androgens Clin Res Ther 2022;3:22-30.
10. Lizneva D, Suturina L, Walker W, Brakta S, Gavrilova-Jordan L, Azziz R. Criteria, prevalence, and phenotypes of polycystic ovary syndrome. Fertil Steril 2016;106:6-15.
11. Bhide P, Kulkarni A, Dilgil M, Dhir P, Shah A, Gudi A, *et al*. Phenotypic variation in anti-Mullerian hormone (AMH) production per follicle in women with polycystic ovary syndrome (PCOS) and isolated polycystic ovarian morphology (PCOM): An observational cross-sectional study. Gynecol Endocrinol 2017;33:801-6.
12. Bui HN, Sluss PM, Hayes FJ, Blincko S, Knol DL, Blankenstein MA, *et al*. Testosterone, free testosterone, and free androgen index in women: Reference intervals, biological variation, and diagnostic value in polycystic ovary syndrome. Clin Chim Acta 2015;450:227-32.
13. Jena D, Choudhury AK, Mangaraj S, Singh M, Mohanty BK, Baliarsinha AK. Study of visceral and subcutaneous abdominal fat thickness and its correlation with cardiometabolic risk factors and hormonal parameters in polycystic ovary syndrome. Indian J Endocrinol Metab 2018;22:321-7.
14. Sachdeva G, Gainder S, Suri V, Sachdeva N, Chopra S. Comparison of the different PCOS phenotypes based on clinical metabolic, and hormonal profile, and their response to clomiphene. Indian J Endocrinol Metab 2019;23:326-31.
15. Głuszak O, Stopińska-Głuszak U, Glinicki P, Kapuścińska R, Snochowska H, Zgliczyński W, *et al*. Phenotype and metabolic disorders in polycystic ovary syndrome. ISRN Endocrinol 2012;2012:569862.
16. Carmina E, Lobo RA. Comparing lean and obese PCOS in different PCOS phenotypes: Evidence that the body weight is more important than the rotterdam phenotype in Influencing the Metabolic Status. Diagnostics (Basel) 2022;12:2313.
17. Al-Jefout M, Alnawaiseh N, Al-Qtaitat A. Insulin resistance and obesity among infertile women with different polycystic ovary syndrome phenotypes. Sci Rep 2017;7:5339.
18. Mahajan N, Kaur J. Establishing an anti-müllerian hormone cutoff for diagnosis of polycystic ovarian syndrome in women of reproductive age-bearing Indian ethnicity using the automated anti-müllerian hormone assay. J Hum Reprod Sci 2019;12:104-13.
19. Halder A, Kumar H, Sharma M, Jain M, Kalsi AK, Pandey S. Serum anti-Müllerian hormone: A potential biomarker for polycystic ovary syndrome. Indian J Med Res 2023;158:397-406.
20. Saxena U, Ramani M, Singh P. Role of AMH as diagnostic tool for polycystic ovarian syndrome. J Obstet Gynaecol India 2018;68:117-22.
21. Malhotra N, Mahey R, Cheluvaraju R, Rajasekaran K, Patkar D, Prabhakar P, *et al*. Serum anti-mullerian hormone (AMH) levels among different PCOS phenotypes and its correlation with clinical, endocrine, and metabolic markers of PCOS. Reprod Sci 2023;30:2554-62.

22. Pigny P, Gorisse E, Ghulam A, Robin G, Catteau-Jonard S, Duhamel A, *et al*. Comparative assessment of five serum antimüllerian hormone assays for the diagnosis of polycystic ovary syndrome. Fertil Steril 2016;105:1063-9.e3.
23. Tremellen K, Zander-Fox D. Serum anti-Mullerian hormone assessment of ovarian reserve and polycystic ovary syndrome status over the reproductive lifespan. Aust N Z J Obstet Gynaecol 2015;55:384-9.
24. Song DK, Hong YS, Sung YA, Lee H. Insulin resistance according to β-cell function in women with polycystic ovary syndrome and normal glucose tolerance. PLoS One 2017;12:e0178120.
25. Li L, Chen X, Mo Y, Chen Y, Wenig M, Yang D. Elevated serum anti-mullerian hormone in adolescent and young adult Chinese patients with polycystic ovary syndrome. Wien Klin Wochenschr 2010;122:519-24.
26. Gursu T, Eraslan A, Angun B. Comparison of body mass index, anti-mullerian hormone and insulin resistance parameters among different phenotypes of polycystic ovary syndrome. Gynecol Obstet Clin Med 2022;2:164-70.
27. Salameh WA, Redor-Goldman MM, Clarke NJ, Mathur R, Azziz R, Reitz RE. Specificity and predictive value of circulating testosterone assessed by tandem mass spectrometry for the diagnosis of polycystic ovary syndrome by the National institutes of health 1990 criteria. Fertil Steril 2014;101:1135-41.e2.
28. Ramanand SJ, Ghongane BB, Ramanand JB, Patwardhan MH, Ghanghas RR, Jain SS. Clinical characteristics of polycystic ovary syndrome in Indian women. Indian J Endocrinol Metab 2013;17:138-45.
29. Ye W, Xie T, Song Y, Zhou L. The role of androgen and its related signals in PCOS. J Cell Mol Med 2021;25:1825-37.
30. O'Reilly MW, Taylor AE, Crabtree NJ, Hughes BA, Capper F, Crowley RK, *et al*. Hyperandrogenemia predicts metabolic phenotype in polycystic ovary syndrome: The utility of serum androstenedione. J Clin Endocrinol Metab 2014;99:1027-36.
31. Grassi G, Polledri E, Fustinoni S, Chiodini I, Ceriotti F, D'Agostino S, *et al*. Hyperandrogenism by liquid chromatography tandem mass spectrometry in PCOS: Focus on testosterone and androstenedione. J Clin Med 2020;10:119.
32. Boucher H, Robin G, Ribière L, Martin C, Espiard S, Catteau-Jonard S. Is it useful to measure DHEAS levels in PCOS? Ann Endocrinol (Paris) 2024;85:95-9.
33. Moran C, Arriaga M, Arechavaleta-Velasco F, Moran S. Adrenal androgen excess and body mass index in polycystic ovary syndrome. J Clin Endocrinol Metab 2015;100:942-50.