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ORIGINAL RESEARCH

# Diagnostic Performance of Anti-Müllerian Hormone, Luteinizing Hormone to Follicle-Stimulating Hormone Ratio, Testosterone, and Prolactin to Predict Polycystic Ovary Syndrome Among Sudanese Women

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**Background:** Polycystic ovary syndrome (PCOS) is one of the most important contributing factors to infertility. The diagnosis of PCOS is not an easy procedure, as the signs and symptoms are heterogeneous and of undefined etiology. There are only a few published studies that address the diagnostic performance of anti-Müllerian hormone in diagnosis of PCOS in sub-Saharan Africa including Sudan.

**Objective:** This study aims to assess anti-Müllerian hormone (AMH), luteinizing to follicle-stimulating hormone ratio (LH: FSH), total testosterone (TT), and prolactin (PRL) levels among PCOS. In addition, we determine if AMH can be used as a predictor of PCOS among Sudanese women.

**Methods:** There were 600 women enrolled in this observational cross-sectional study, 300 of whom had PCOS, and 300 of whom healthy women; PCOS was diagnosed using the Rotterdam criteria. On days 2–4 of the menstrual cycle, serum LH, FSH, AMH, TT, and PRL levels were measured for all participants. Diagnostic performance of these parameters for PCOS was determined by receiver operating characteristic (ROC) curve.

**Results:** Significantly higher means among PCOS regarding their BMI, AMH; LH: FSH ratio; TT; PRL, whereas significantly inverse in FSH compared with normal ovulatory women. On ROC analysis, AMH had the largest operating characteristic curve at cut-off >3.95 ng/mL; AUC = 0.999 with Youden's index 0.99%, followed by LH: FSH ratio at cut-off 0.749; AUC=0.932; Youden's index 0.813%, TT cut-off 0.82 mIU/L, AUC=0.852 with Youden's index 0.58, while PRL showed the lowest AUC=0.627 with cut-off 15.3 ng/mL, Youden's index was 0.18%, P. value<0.001.

**Conclusions:** Sudanese women with PCOS had higher serum AMH level, LH:FSH ratio, and TT level. Moreover, AMH level has better discriminative power and good diagnostic potency for the diagnosis of PCOS among Sudanese.

Keywords: PCOS women, AMH, LH:FSH ratio, TT, PRL, Sudan

#### Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder characterized by endocrine variation, the presence of ovarian cysts, and anovulation, all of which have a significant impact on a woman's life.<sup>1,2</sup> Disruption of reproductive hormones such as follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone (TT), and estrogen disrupts the normal menstrual cycle, resulting in oligomenorrhea and amenorrhea-like irregularities. PCOS

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is characterized by hyperandrogenism, menstrual irregularities, and ovarian cysts of varying sizes, despite significant individual variation. This multifactorial disorder improves initially in adolescents who are at high risk of developing multiple comorbidities, such as cardiovascular disorders, type II diabetes, obesity, infertility, endometrial dysplasia, and mental disorders.<sup>3,4</sup> The updated Rotterdam criteria, which were recently adopted by an international evidence-based PCOS guideline, are used to diagnose PCOS.<sup>5,6</sup>

AMH is also a key hormonal indication in the development and maturation of ovarian follicles in PCOS women.<sup>7</sup> AMH overproduction inhibits follicular growth, resulting in ovarian dysfunction. Inadequate folliculogenesis is caused by an accumulation of androgens that inhibit normal androgen production. During the early gonadotropin phase, the increased androgens stimulate the primordial follicles formation and antral follicles proliferation.<sup>8</sup> The hypothalamic GnRH secretion will stimulate the pituitary's gonadotropin hormone secretion. LH acts on the LH receptor to stimulate the production of androgen in ovarian theca cells, while FSH stimulates the follicle growth in ovarian granulosa cells by converting androgens to estrogens.<sup>9</sup>

It has been hypothesized that neuroendocrine system dysregulation leads to hypothalamic-pituitary-ovarian axis imbalance, resulting in overproduction of gonadotropin. The GnRH increase enhances LH over FSH, resulting in a significant increase in the LH:FSH ratio in PCOS.<sup>10,11</sup>

Due to the extensive heterogeneity of PCOS's clinical presentation, there are numerous reasons to believe that many PCOS-affected women have been misdiagnosed. In addition, the rising obesity epidemic may increase the prevalence of PCOS, as obesity may exacerbate the endocrine and metabolic profile of PCOS.<sup>12</sup> To our knowledge, there is a limited data in Sudan that considered the diagnostic efficacy of AMH, LH:FSH ratio, LH, and PRL level in diagnostic performance and predictors of PCOS among Sudanese women.

#### **Materials and Methods**

This observational cross-sectional, hospital-based study was conducted in the state of Khartoum– Sudan, between October 2020 and September 2021. Sudan's Ministry of Health in Khartoum approved the research protocol. This research was conducted in conformity with ethical principles of the responsible foundation and the Helsinki Declaration. Written informed consents were signed by all study participants.

In this study, there are 300 Sudanese women with PCOS as a case group compared with 300 fertile Sudanese women with non-PCOS as a control group enrolled to determine the cut-off value of common hormones (AMH, LH, LH:FSH ratio, TT, and prolactin) abnormality relevant to polycystic ovary syndrome.

Women age 20–39 years with confirmed PCOS. The European Society for Human Reproduction and Embryology (ESHRE) and the American Society of Reproductive Medicine (ASRM) have set criteria for the diagnosis of PCOS (Rotterdam 2003).<sup>7</sup> The Rotterdam criteria include oligo-anovulation (OA) (cycles greater than 35-day intervals or 8 cycles or less per year), hyperandrogenism (HA), and polycystic ovary morphology (PCOM) (ovarian volume of 10 mL and/or an antral follicle count [AFC] of more than 12 cysts of 2–9 mm in diameter, any two being enough for the diagnosis of PCOS).<sup>6</sup>

Fertile healthy women without PCOS, who had regular menstrual cycles (21 to 35 days), without polycystic ovary morphology on ultrasonography or hirsutism, comprised the control group. The women who were taking ovulation induction agents, anti-androgenic medications, oral contraceptives, or estrogenic glucocorticoids prior to the study were excluded. Medical history of gynecological (fertility, menstrual pattern, and hirsutism) and socio-demographic character-istics were collected using a validated questionnaire, followed by comprehensive general and pelvic examinations.

The weight was measured twice in accordance with protocol standards. After calibration, OMRON BF508l Body Fat Scales (China) were utilized. Participants were instructed to take out their bulky clothing and footwear. The calculated weights were within 0.1 kg, and their height was measured twice using a portable stadiometer (SECA-213 model, Germany). The BMI of Quetelet was calculated using the conventional formula (weight in kilograms divided by height in meter<sup>2</sup>). The WHO categories were used to describe BMI; normal (18.5–24.9 kg/m<sup>2</sup>), overweight (25.0–29.9 kg/m<sup>2</sup>), underweight (<18.5 kg/m<sup>2</sup>), and obese from (30 kg/m<sup>2</sup>) and higher. Ultrasonography was performed using Mindray (model: DP-50, Germany) during the follicular phase, and a vaginal transducer that confirmed or ruled out the presence or absence of ovarian cysts. If cysts were observed, the volume and number of small follicles in each ovary were determined.

Women were instructed to return between 2 and 5 days after spontaneous menstruation, or on a convenient date if they had amenorrhea. Between 8:00 and 9:00 a.m., while fasting, 5 mL of the venous blood was obtained in the plain container. Blood was centrifuged using a Hettich (D-78532 Tuttlingen, Germany) centrifuge; then, serum was extracted and stored at -20 degrees Celsius until the assay. According to the manufacturer's instructions, enzyme-linked immunosorbent assay (ELISA); ASYS (model: Expert Plus; type G020150; serial number: 28382; Austria) was used to measure the serum LH, FSH by indirect method; TT by competitive methods; PRL by direct method, and AMH by sandwich method with final fluorescent detection).

The data were coded and statistical analysis was carried out using the social package of statistical science version 26.0 (SPSS Inc., IBM, Chicago, IL, USA). For testing the normality of continuous data, the Kolmogorov–Smirnov was utilized. All data was skewed. The median with interquartile ranges were used to present continuous variables. Whereas qualitative data was expressed as a percentage (%). The relationship between PCOS and non-PCOS group was assessed by Mann–Whitney *U*-test and chi-square test. The predictive ability of the parameters and establishing the best cut-off values for predicting PCOS were examined using receiver operating characteristic curve analysis. The areas under the receiver operating characteristic curves (AUCs) were determined to identify the optimum parameters, and the ideal cut-off values were selected using the greatest Youden's index (sensitivity plus specificity-1), P. value considered significant at level $\leq 0.05$ .

#### Results

A total of 600 Sudanese adult women were enrolled. Their mean age was 28.9 (5.9) years. 300 had PCOS as a case group and 300 fertile women as a control group. Women with PCOS significantly increased in BMI compared with fertile women. Also, 76.6% (n=240) were overweight/obese based on their BMI (Table 1). Moreover, 52.3% (n=157) had menstrual cycle irregularity and 59.0% (n=177) had positive family history to PCOS (results not showed in table).

Furthermore, there was a significantly increased median of LH, PRL, TT, AMH, and LH:FSH ratio levels and inversed median FSH level among PCOS women compared with non-PCOS counterpart P-value <0.001 (Table 2). The ROC curves plotted for each hormone marker are shown in Figure 1. The optimum cut-off values are determined from the ROC curve analysis in Table 3. Moreover, among PCOS women most of them (n=297) had high level of AMH; 86.3% (n=259) had increased LH: FSH ratio; 71.0% (n=213) had hyperandrogenemia and 58.0% (n=174) had hyper-prolactinemia based on their cut-off values Table 2.

Moreover, among Sudanese PCOS women of reproductive age, the ROC curves pointed out that AMH had a better AUC (0.999) with cut-off 3.95 ng/mL (sensitivity = 99% and specificity = 100%); followed by LH: FSH ratio AUC

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Variables	PCOS n=300	Non-PCOS n=300	Total n=600	P- value
Age/ year				
Median (S.E.M) <sup>†</sup>	29.0 (90.3)	28.5 (0.3)	29.0 (0.2)	0.309*
Q1-Q3	25.0-33.0	24.0–32.7	25.0-33.0	
BMI kg/m <sup>2</sup>				
Median (S.E.M) <sup>†</sup>	28.4 (0.2)	22.8 (0.7)	24.3 (0.1)	0.000*
Q1-Q3	25.3–30.8	22.2–24.0	22.5–28.4	
Normal	70 (23.3) <sup>‡</sup>	292 (97.3)	362 (60.3)	0.000 <sup>§</sup>
Overweight	139 (46.3)	8 (2.7)	147 (24.5)	
Obese	91 (30.3)	0 (0.0)	91 (15.2)	

 Table I Age, Body Mass Index Among Study Group

Notes: \*P-value is obtained using Mann–Whitney U-test.  $^{+}Values$  are numbers and percentages;  $^{+}P$ -value is obtained using chi square test.

Abbreviations: S.E.M, standard error of mean.

Table 2         Luteinizing Hormone,	Follicle-Stimulating	Hormone,	Prolactin,	Total
Testosterone, Anti-Mullerian Ho	ormone Among Stu	dy Group		

Variables	PCOS n=300	Non- PCOS n=300	P- value	
LH mIU/L				
Median (S.E.M) <sup>†</sup>	8.9 (0.3)	4.2 (0.0)	< 0.001*	
Q1-Q3	5.8–13.5	3.3–5.3		
≤ 5.9	78 (26.0) <sup>‡</sup>	252 (84.0)	< 0.001 <sup>§</sup>	
> 5.9	222 (74.0)	48 (16.0)		
FSH mIU/mL				
Median (S.E.M) <sup>†</sup>	6.1 (0.1)	8.2 (0.1)	< 0.001*	
Q1-Q3	4.9–7.9	6.9–9.6		
PRL ng/mL				
Median (S.E.M) <sup>†</sup>	17.0 (0.6)	13.4 (0.4)	< 0.001*	
Q1-Q3	11.8–25.1	9.1–18.5		
≤ 15.3	126 (42.0) <sup>‡</sup>	180 (60.0)	< 0.001 <sup>§</sup>	
> 15.3	174 (58.0)	120 (40.0)		
TT mIU/L				
Median (S.E.M) <sup>†</sup>	1.84 (0.09)	0.39 (0.01)	< 0.001*	
Q1-Q3	0.70–3.90	0.20–0.64		
≤ 0.82	0.82 87 (29.0) <sup>‡</sup>		< 0.001 <sup>§</sup>	
>0.82	213 (71.0)	39 (13.0)		
AMH ng/mL				
Median (S.E.M) <sup>†</sup>	6.2 (0.1)	2.4 (0.04)	< 0.001*	
Q1-Q3	5.1–7.7	1.72–3.07		
≤ 3.95	≤ 3.95 3 (1.0) <sup>‡</sup>		< 0.001§	
> 3.95	297 (99.0)	0 (0.0)		
LH/FSH ratio				
Median (S.E.M) <sup>†</sup>	1.435 (0.07)	0.548 (0.00)	< 0.001*	
Q1-Q3	0.963-2.245	0.441–0.639		
≤ 0.749	4I (I3.7) <sup>‡</sup>	285 (95.0)	< 0.001§	
> 0.749	259 (86.3)	15 (5.0)		

Notes: <sup>†</sup>Median; S.E.M= Std. Error of Mean, Q1-Q3= Interquartile Ranges, <sup>‡</sup>Values are numbers and percentages; <sup>\*</sup>P-value were obtained using Mann–Whitney *U*-test; and <sup>§</sup>P were obtained using chi-square test.

Abbreviations: LH, luteinizing hormone; FSH, follicle stimulating hormone; PRL, Prolactin; TT, total testosterone; AMH, anti-Mullerian hormone;



Diagonal segments are produced by ties.

Figure I Receiver operating characteristic curve for discriminating between different hormones markers in diagnosing polycystic ovarian syndrome. \*p value <0.05 is considered significant.

Abbreviations: LH, luteinizing hormone; FSH, follicle stimulating hormone; AMH, anti-Mullerian hormone.

(0.932) with cut-off 0.749 (sensitivity = 86.3% and specificity = 95%); and prolactin had smaller AUC (0.627) with cut-off 15.3 (sensitivity = 62.7% and specificity = 60%). Furthermore, AMH and LH: FSH ratios had the highest Youden's index (0.99%, 0.813%, respectively). Both LH and TT had the same value of Youden's index (0.58%) Table 3.

#### Discussion

PCOS still does not have a clear cause, and subjective phenotypes make it hard to make a complete diagnosis. It is commonly known that PCOS is linked to insulin resistance. If this is combined with a delayed or imprecise diagnosis of PCOS, the prognosis for type 2 diabetes as well as cardiovascular disease may be worsened.<sup>13–15</sup> Moreover, evaluating PCOM via ultrasound of abdominal area can be challenging, especially in obese or virgin women. Consequently, simpler, more precise, and more quantitative diagnostic criteria are required.

In this study, a sample of Sudanese women was assessed for serum AMH, LH:FSH ratio, TT, and PRL. In addition, we determine if AMH can be used as a predictor of PCOS whatever the patient phenotype. Our study revealed that women diagnosed with PCOS had higher BMI measurements and hormonal indicator levels, except for FSH, which was observed with insignificant

Variables	AUC	S.E.M	P-value	95% CI: L- U	Sensitivity (%)	Specificity (%)	Youden's Index	Optimal cut-Off
АМН	0.999	0.001	0.000	0.997-1.00	0.99	1.00	0.99	3.95 ng/mL
LH:FSH ratio	0.932	0.012	0.000	0.908–0955	0.863	0.95	0.813	0.749
LH	0.872	0.015	0.000	0.843-0.900	0.74	0.84	0.58	5.9 mIU/mL
тт	0.852	0.016	0.000	0.821-0.883	0.71	0.87	0.58	0.82 mIU/L
PRL	0.627	0.023	0.000	0.583–0.672	0.58	0.6	0.18	15.3 ng/mL

Table 3 Performance of Hormones Marker as Predictors of PCOS from ROC Curve Analysis in Reproductive Age Women

Note: P-value significant at level≤ 0.05.

Abbreviations: AUC, Area under the curve; S.E.M, standard error of mean; CI (L-U), confidence internal lower- Upper bound.

differences compared with non-PCOS. The ROC curves mainly showed good specificity and sensitivity for AMH, LH:FSH ratio, LH, and TT. Serum AMH can also function within diagnostic parameters, as AMH concentration has demonstrated a significant correlation with PCOS, and reflect its severity.<sup>16,17</sup> Additionally, it is easily measurable at any point in a woman's cycle.<sup>18</sup> The results of this research revealed a statistically significant difference in AMH levels between the PCOS cases and controls. Similar to the findings of previous studies, the latter showed a threefold increase in AMH relative to controls.<sup>19,20</sup> In addition, study revealed that AMH more than 3.95 ng/mL was significantly associated with PCOS with a sensitivity of 99%; specificity 100% and Youden's index 0.99%. Tola et al found that AMH greater than 3.1 ng/mL has diagnostic efficiency in Sudanese PCOS women (sensitivity 96%; specificity 92%).<sup>21</sup> In Saudi population, the AMH diagnostic cut-off has been 3.19 ng/mL<sup>22</sup> Caucasians 4.7–5 ng/mL,<sup>23</sup> and 10 ng/mL in Japanese and Korean women.<sup>24,25</sup> This variation in ethnicity may be associated with variable AMH levels. Moreover, the threshold of AMH can be attributed to numerous variables, including ethnicity and methodological variations.<sup>26</sup> This may be the result of elevated androgen levels in women with PCOS disorder, leading to relative suppression of FSH production and abnormalities in the ovaries hormone. The finding of current study is consistent with the results described by Siebert et al.<sup>27</sup> In their study, Sahmay et al they found a significant difference in the level of FSH, LH, and LH:FSH ratio of PCOS women compared to controls.<sup>28</sup>

# Conclusion

In this study, AMH was found to be a useful diagnostic tool for PCOS. Future studies in various Sudanese regions should be conducted to validate the cut-off.

# **Data Sharing Statement**

This research article includes all data generated or analyzed during this study.

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# Disclosure

The authors report that there are no conflicts of interest in this work.

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