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Anti-Müllerian Hormone, Follistatin Peptide and their Role in Polycystic Ovary Syndrome in Egyptian Women

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Abstract

Background and Objective: *Polycystic ovary syndrome (PCOS) is the most common cause of anovulation in women with infertility. In this study, the aim is assessing the role of Anti-Müllerian hormone (AMH), follistatin (FS) in diagnosis of PCOS.*

Materials and Methods*: This cross-sectional study was conducted in the Department of Obstetrics and Gynecology, Kasr El-Ainy, Cairo University from March 2016 to September 2017. Women with PCOS-related infertility based on Rotterdam criteria were considered eligible. Women who presented to infertility clinic due to either male factor or tubal infertility were considered as a control group. Serum levels of AMH and FS were measured in early follicular phase (days 2 or 3) of spontaneous menstrual cycle or progestin-induced uterine bleeding. Receiver operating characteristic (ROC) curves were used to assess diagnostic performance of these markers in women with PCOS.*

Results: *A total of 200 women were included in the study (100 in each group). Mean serum FS level was significantly higher in the PCOS group than in the non-PCOS group (1447.3 ± 141 pg/ml versus 845.5 ± 48 pg/ml, $P < 0.0001$). Mean serum AMH level was higher in PCOS group compared to non-PCOS group (3.547 vs. 3.205 ng/mL, $p > 0.05$). At a cut-off value of 969.3 pg/ml, sensitivity of FS in diagnosing PCOS was 70%, specificity was 70% and AUC is 0.76.*

Conclusion: *serum FS may act as a potential screening biomarker of PCOS regardless of age or BMI. Future studies are warranted to validate the diagnostic role and cost-effectiveness of this test.*

Keywords. *Polycystic Ovary Syndrome, Screening, Diagnosis, Anti-Mullerian hormone, Follistatin peptide alone, Egyptian women.*

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder among reproductive-aged women who present with infertility and menstrual disorders. PCOS is associated with long-term non-gynecologic complications including risk of type 2 diabetes and cardiovascular events. The diagnosis of PCOS is made using 2 out of 3 Rotterdam criteria, which include oligo- or anovulation, clinical or biochemical hyperandrogenism, or sonographic signs of polycystic ovary [1].

Follistatin (FS) is a β-subunit-specific high affinity binding protein that is secreted by granulosa cells. FS, at beginning perceived as a follicle-stimulating hormone (FSH) inhibitor, is an autocrine glycoprotein that is communicated in almost whole tissues of higher animals [2,3]. Over production of FS leads to infertility in mice4 and would subsequently be relied upon to arrest follicular growth [5]. FS has additionally been related with very touchy C-responsive protein (hsCRP) as a marker of inflammatory process in polycystic ovary syndrome [6]. FS functions coordinate ovarian follicular growth by binding activins. FS has been linked to PCOS either as a marker or as a part of pathophysiology of the disease [7]. Anti-Müllerian hormone (AMH) is a glycoprotein secreted from granulosa cells of the ovary and plays a role in follicular growth and development8. Assessment of circulating AMH reflects ovarian reserve and predicts response to hyperstimulation during in-vitro fertilization (IVF) cycles. Furthermore, it indicates antral follicle count, and may increase in women with PCOS [9].

The objective of this study was to establish a clear relationship between AMH and FS levels and PCOS, to evaluate diagnostic role of these markers and whether they impact treatment strategy in patients with PCOS.

Materials and Methods

Study Area

This cross-sectional study was conducted in the Department of Obstetrics and Gynecology, Kasr El-Ainy, Cairo University from March 2016 to September 2017.

Inclusion criteria

Women who attended infertility and reproductive endocrinology clinic for management of PCOS-related subfertility were considered eligible for the study. All patients were offered to meet with the

research coordinator and were thoroughly counseled to participate in the study. Inclusion criteria for the study include women aged between 18-35 years, confirmed diagnosis of PCOS based on European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine (ESHRE/ASRM) Rotterdam criteria 20031.

Exclusion criteria

Women were excluded if they have any associated chronic medical disorders or who decline participation in the study. An informed consent was obtained from women who agreed to participate in the study. Women belonging to the same age group who presented to infertility clinic due to either male factor or tubal infertility, but otherwise did not fulfill criteria for diagnosis of PCOS, were considered as a control group.

Experimental design

It was included 200 women divided as study group (group A) and control group (group B); 100 patients each. The final sample size was determined by sample size calculation calibrated at a power of 80% and error of 0.05, with consideration of 10% drop-off rate. All recruited women were managed per study protocol. This includes detailed history of age, weight, height, parity, gravidity, previous abortions, smoking, medical and surgical history. Physical examination was performed as clinically indicated.

Investigation of Serum hormones

Serum levels of AMH and Follistatin were measured using enzyme-linked immunosorbent assay (ELISA). AMH and FS were measured in early follicular phase (days 2 or 3) of spontaneous menstrual cycle or progestin-induced uterine bleeding.

Primary outcome of the study was to assess AMH and FS levels in women with PCOS. Secondary outcomes were to assess potential relationship between AMH/FS and women age, body mass index and previous ovarian surgery. This study was approved institutional review board of Department of Obstetrics and Gynecology, Kasr El-Ainy, Cairo University.

Statistical analysis

Descriptive presentation of data was made using mean/median, standard deviation for quantitative data and percentage for qualitative data. Analytical statistics were determined by normality tests (Kolmogorov-Smirnov or Shapiro-Wilk) for testing distribution of data, F-levene's Test for testing homogeneity of variances. Student T-test (for normally distributed data) and Mann Whitney U test (for non-parametric data) were used to compare two independent means for quantitative data. A crosstab and Chi square test were used to compare between different qualitative data. A P value < 0.05 is considered significant.

Receiver operating characteristic (ROC) curves were constructed to evaluate diagnostic performance of AMH in relation to PCOS. By plotting sensitivity against 1- specificity at each threshold level, and area under the curve (AUC) was computed. The AUC presents the probability of diagnosing PCOS and a value of 0.5 means that the test is no better than chance. SPSS version 19.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analyses.

Results

A total of 200 women were included in the study: 100 women with PCOS (Group A) and 100 women with non-PCOS related subfertility (group B) as the control group.

PCOS & Age: Tables 1,2&3 showed that There was no statistically significant difference regarding maternal mean age between the PCOS group (26.46 ± 0.452 years) and control group (26.54 ± 0.448 years). P-value was 0.90.

Ranks				
	Type of Patients	N	Mean Rank	Sum of Ranks
Age	PCOS	85	86.02	7312.00
	Control	87	86.97	7566.00
	Total	172		

Test Statistics[a]	
	Age
Mann-Whitney U	3657.000

Wilcoxon W	7312.000
Z	-.125
Asymp. Sig. (2-tailed)	.901
P-value 0.90	

Tables 1&2: Mann-Whitney test for the significance of Age between the studied groups

	Type of Patients		Statistic	Std. Error
Age	**PCOS**	Mean	26.46	.452
		Median	27.00	
		Variance	17.346	
		Std. Deviation	4.165	
		Interquartile Range	6	
		Skewness	.156	.261
	Control	Kurtosis	-.513	.517
		Mean	26.54	.448
		Median	27.00	
		Variance	17.437	
		Std. Deviation	4.176	
		Interquartile Range	5	
		Skewness	.137	
		Kurtosis	-.540	.258

Table 3: Descriptive comparison between the studied groups regarding Age

PCOS & Body mass index (BMI, Kg/m2): Similar, BMI was comparable between the 2 groups (PCOS group was 27.82 ± .39 Kg/m2 versus control group which was 28.46 ± .39 Kg/m2, P = 0.29).

PCOS & Ovarian Surgery: Tables 4&5 showed that There was no statistically significant difference regarding the prevalence of previous gynecologic surgery involving the ovaries (p-value 0.74) between the PCOS and control groups.

Chi-Square Tests					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.110[a]	1	.740		
Continuity Correction[b]	.002	1	.965		
Likelihood Ratio	.110	1	.740		
Fisher's Exact Test				.780	.482
Linear-by-Linear Association	.110	1	.741		
N of Valid Cases	172				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 6.42.

b. Computed only for a 2x2 table

Table 4: Pearson Chi-Square equation to test the significance of previous ovarian surgery between the studied groups

	Type of Patients	Total		
	PCOS	Control		
Ovarian_Suregery	NO	78	81	159
	YES	7	6	13
Total	85	87	172	

Table 5: Previous Ovarian surgery frequency of the two studied groups.

PCOS & Follistatin (FS): Tables 6 showed that The mean serum FS level was significantly higher in the PCOS group than in the non-PCOS group (1447.3 ± 141 pg/ml versus 845.5 ± 48 pg/ml, P < 0.0001).

Descriptive				
	Type of Patients		Statistic	Std. Error
Follistatin	**PCOS**	Mean	1447.3071	141.10334
		Median	1258.0000	
		Variance	1692362.889	
		Std. Deviation	1300.90849	
		Interquartile Range	722.25	
		Skewness	5.898	.261
		Kurtosis	43.896	.517
	Control	Mean	845.5724	48.77663
		Median	793.0000	
		Variance	206986.915	
		Std. Deviation	454.95815	
		Interquartile Range	532.90	
		Skewness	1.975	.258
		Kurtosis	7.288	.511

Table 6: Descriptive comparison between the studied groups regarding Follistatin

PCOS & Anti-Müllerian Hormone (AMH): Mean serum AMH level was higher in PCOS group compared to non-PCOS group (3.547 vs. 3.205 ng/mL, p > 0.05).

Confounding variables including age, BMI and incidence of early ovarian surgery were not significantly different between the two groups.

AMH & FST Correlation: AMH and FS levels showed weak positive association (p value > 0.05).

The diagnostic indices (sensitivity, specificity values) of Follistatin & AMH in the studied patients:

- ROC of FS level in patients with PCOS is illustrated in Figure 1. At a cut-off value of 969.3 pg/ml, sensitivity of FS in diagnosing PCOS was 70%, specificity was 70% and AUC is 0.76.
- On the other hand, mean serum AMH was not significantly different between PCOS group and control groups (3.5 ± 0.2 ng/ml versus 3.2 ± 0.2 ng/ml, respectively, P = 0.13)

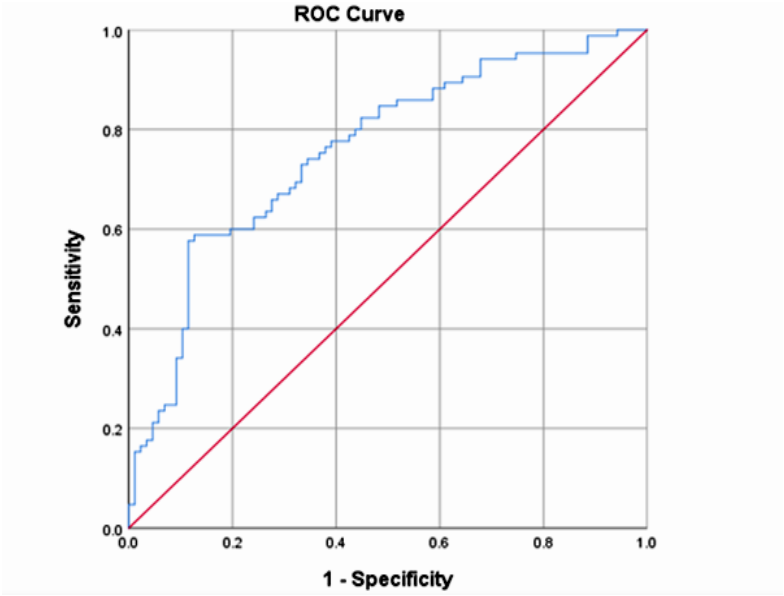

Figure 1: The ROC curve represents the sensitivity and specificity of Follistatin

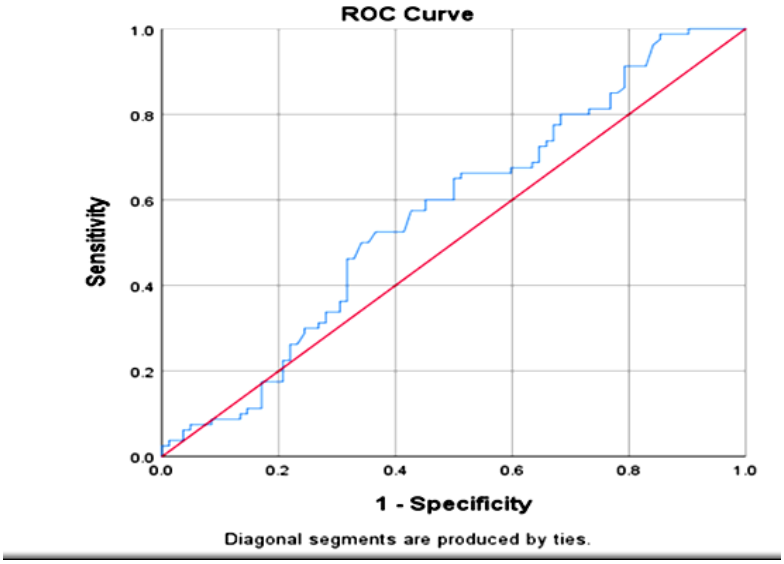

Figure 2: The ROC curve represents the sensitivity and specificity of AMH

Figure 2 shows ROC of AMH level in patients with PCOS; AUC is 0.57 indicating a poor test performance in diagnosing PCOS (P = 0.131).

Discussion

In this study, serum FS levels were shown to be significantly higher among patients with PCOS who present with fertility issues regardless of BMI, age and previous pelvic surgery. Normally, serum FS changes in response to energy deprivation and women with hypothalamic amenorrhea due to exercise tend to have altered FS level [10]. A previous study showed that serum FS is increased in PCOS patients with significant increase among obese women. FS was negatively correlated with FSH [11]. The analysis herein, FS showed promising diagnostic performance and it may be considered for further studies to establish a single screening tool for PCOS independent of the other diagnostic criteria with acceptable sensitivity and specificity. In the present study, high AUC values indicate were reported at a cut-off value of 969 pg/ml.

Norman et al. (2001) strongly supports the results of the present study as they reported higher levels of follistatin and lower activin levels in the circulation of women with PCOS [12]. Liao et al. (2000) reported preliminary results in follistatin gene mutations in PCOS. They did not discover a mutation in the coding region of the Chinese population, yet there might be changes in follistatin in other ethnic populaces or in reality changes in the regulation of the follistatin gene [13].

Another study had the same results with our study done by Eldar-Geva et al. (2001) They reported that follistatin was expanded by 80% - 90% in PCOS patients, autonomous of obesity [14]. In addition, Philips et al. (2000) announced PCOS as the most significant variable that freely expanded follistatin and it has been demonstrated that essentially all circulating follistatin in women is activin bound [15]. As per Norman et al. (2001) there is no significant relationship amongst follistatin and BMI, and the serum follistatin level is higher and activin level is lower in PCOS which also supports our findings. Follistatin levels might be a valuable instrument to study pathological conditions in women with PCOS [12].

AMH has been well-established as a test of ovarian reserve. However, the results did not support its role as a diagnostic tool of PCOS. However, AMH has been investigated as a prediction tool in women in PCOS and some evidence may support its role as a predictor of menstrual disorders [16]. In patients with infertility, AMH may have a role in predicting response to ovulation induction cycles, response to menopausal gonadotropins dose, and risk of ovarian hyperstimulation syndrome [17]. However, the results did not show that AMH is suitable as a screening tool for PCOS. Nevertheless, a cut-off value of 4.82 ng/ ml may be used to likely exclude the diagnosis (as specificity 80% and sensitivity 20%). The study is supported by sample size calculation powered to the primary outcome. However, further

prospective studies are required to assess diagnostic performance of FS as a screening test in women with PCOS using Rotterdam criteria as an index test.

The results of our study are in concurrence with prior studies, for instance, Li et al. (2010) detailed that serum AMH concentration were elevated in adolescent Chinese women with PCOS, however the serum AMH estimations offered a generally poor diagnostic power with a sensitivity of 61.7% and a specificity of 70% at a cut-off of 8 ng/ml. They proposed that the low sensitivity and specificity in their investigation was inferable from the lower predominance of hyperandrogenism, insulin resistance and obesity in their associate attributable to racial contrasts [18].

Dewailly et at., (2014) reported that although serum anti-Müllerian hormone concentrations associated with the ultrasonographically assigned ovarian volume and count of antral follicle, the diagnostic benefit of estimation of these levels in women with the polycystic ovary disorder is dubious, which is similar to the results of our study [19].

Conclusion

In conclusion, FS may present a future screening tool in PCOS regardless of age or BMI. However, future studies are required to validate these findings and analyze cost-effectiveness of this approach in comparison to standard practice.

Significance Statement

This study discovers that the mean level of serum FS was significantly higher in the PCOS group than in the non-PCOS group (1447.3vs. 845.57pg/mL, respectively; $p < 0.05$). The mean serum AMH level was higher in the PCOS group than in the non-PCOS group but statistically Non-Significant (3.547 vs. 3.205ng/mL, respectively; $p > 0.05$), that can be beneficial for screening and diagnosing PCOD

In the current study FS can be suitable as a single screening tool for PCOS, independent of the other diagnostic criteria with both acceptable sensitivity and specificity, the accuracy is remarkable, also AMH is not suitable as a single screening tool for PCOS, independent of the other diagnostic criteria and may be used to exclude the condition but not to prove it (as specificity 80% and sensitivity 20%). Future studies should use universally accepted methods for AMH measurements and international standards should be established. If a high sensitivity and specificity is confirmed by others, AMH may replace US examination of the ovaries in PCOS diagnosis. The mean age, BMI and incidence of earlier ovarian surgery were not significantly different among the polycystic ovary syndrome and normal groups.

This study will help the researcher to uncover the critical areas of PCOS that many researchers were not able to explore. Thus, a new theory on screening and diagnosing PCOS may be arrived at.

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