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The Role of Anti-ovarian Autoantibodies in Polycystic Ovary Syndrome

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ABSTRACT

Background: Autoimmunity has been proposed in the pathogenesis of polycystic ovary syndrome (PCOS) since early 1990s. Studies of anti-ovarian antibodies (AOA) that were performed so far yielded conflicting results.

Objective: This study aimed at evaluating the association between PCOS and AOA.

Patients and method: The study was conducted on 80 Egyptian women who were divided into two groups: cases group of 40 women with PCOS, and a control group of 40 healthy age-matched normally cycling fertile women. For both cases and control groups, serum level of AOA was measured using ELISA.

Results: Serum AOA level was significantly higher among cases than controls (p value < 0.05). No significant difference was found between cases and controls regarding their hormonal profile (estrogen, FSH, LH, and testosterone) except for the level of mid luteal progesterone which was significantly lower among cases (p value <0.05). No significant correlation was found between AOA and age, body mass index (BMI), FSH, LH, E2, mid luteal progesterone and testosterone in control group. However, a significant negative correlation between AOA and serum testosterone level was found in PCOS group.

Conclusion: Our results suggest that autoimmune ovaritis may be frequently associated with PCOS. Circulating AOA may represent a practical and suitable marker for diagnosis of PCOS.

Keywords: Autoimmunity, Anti-ovarian antibody, Polycystic ovarian syndrome, Pregnancy.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex disorder characterized by hyperandrogenism and chronic anovulation, leading to infertility in young women. Worldwide, PCOS affects 6–10% of women [1, 2]. Autoimmunity has been proposed in the pathogenesis of PCOS since early 1990s and many studies have reported combination between PCOS and many autoimmune disorders such as autoimmune thyroiditis, autoimmune oophoritis and premature ovarian failure. Many reviews have documented presence of many autoantibodies in PCOS patients such as: anti-nuclear (ANA), anti-histone, anti-thyroid, anti-spermatic and anti-islet cell antibodies [3].

The relation between ovarian autoimmunity and PCOS was previously evaluated and the detection of autoantibodies directed against various ovarian targets strongly supports the hypothesis of an autoimmune etiology of PCOS. In addition, it was shown by many studies that anti-ovarian antibody (AOA) could reduce fertilization rates, generate a poor response to gonadotropin stimulation, and could be responsible for implantation failures [4].

The aim of this study was to assess the association between PCOS and AOA in a sample of Egyptian women with PCOS compared to healthy controls.

SUBJECTS AND METHODS

Study participants:

This case-control study was conducted at Ain Shams University Obstetrics and Gynecology Hospital, Cairo, Egypt. It was composed of 80 patients who were divided into two groups: **Group 1 (patient group):** This group was composed of 40 women with PCOS diagnosed and identified according to the Rotterdam (2003) criteria [5]. All subjects with congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome, hyperprolactinemia, virilizing ovarian or adrenal tumors and those taking any hormonal treatment during the previous three months before the study were excluded.

Group 2 (control group): This group was composed of 40 healthy age-matched normally cycling fertile women visiting the outpatient clinics because of problems not related to PCOS, with no history of autoimmune diseases or hormonal therapy during the previous 3 months. Absence of PCOS was confirmed by measuring serum level of sex hormones (estrogen, progesterone, FSH, LH, and testosterone).

Methodology:

Both groups were subjected to the following:

(1) Detailed questionnaire, full medical history taking and clinical examination.

(2) Transvaginal ultrasound to evaluate the ovaries and uterus.

(3) Laboratory investigations including the following:

a. Serum level of sex hormones (estrogen, progesterone, FSH, LH, and testosterone) were measured by an automated electrochemiluminescence immunoassay system (Cobas e411 analyzer, Roche, Germany).

b. The presence of serum AOA was determined using a commercially available solid-phase enzyme immunoassay kit (anti-Ovarian Abs ELISA, EIA-293, DRG International, Inc. U.S.A.). Whole blood specimens were collected and allowed to clot, and the serum was separated by centrifugation and stored at -20°C. Sera were diluted 1:100 with dilution buffer (5 µL of serum + 495 µL of dilution buffer).

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A mix of ovarian proteins was bound to the surface of the wells of the ELISA plate. The standards and diluted samples were added into the wells and then incubated. Anti-ovarian antibodies present in the diluted serum bound to the ovarian proteins and are thus immobilised on the plate. After several washing steps, we added the horseradish peroxidase (HRP) coupled with polyclonal antibodies directed against the fragment crystallizable (Fc) part of human immunoglobulins. The unbound conjugate was washed away, and the 3, 3', 5, 5'tetramethylbenzidine substrate (TMB) was added.

The reaction between HRP and TMB substrate formed a color. The reaction was stopped by adding 0.25 M sulphuric acid (H2SO4). Absorbance of the standards and samples was measured with a microplate reader at wavelength 450 nm and a reference measurement was done with wavelength 630 nm. A standard curve was drawn by using the measured absorbance values of the standards. The levels of AOA in blood samples were determined from the standard curve in U/mL.

Ethical approval:

An informed consent was received from all PCOS and healthy women included in the present study**. The study protocol was approved by The Research Ethical Committee of Faculty of Medicine, Ain Shams University.**

Statistical analysis

It was performed using the following tests: 1. Comparison between two independent groups for non-parametric data by applying Wilcoxon Rank Sum test. 2. Ranked Spearman correlation test to study the possible association between each two variables among each group for non-parametric data. The significance of the p-value: $p > 0.05$: non-significant, $p < 0.05$: significant, $p < 0.01$: highly significant. 3. Diagnostic validity test: A receiver operating characteristic curve (ROC curve) was constructed and area under the curve (AUC) was calculated to obtain the most sensitive and specific cutoff.

RESULTS

Results of the present study showed that serum AOA level was significantly higher among cases than controls. No significant difference was detected between patients and controls regarding their age, BMI and hormonal profile (FSH, LH, E2 and testosterone) except for the level of mid luteal progesterone which was significantly lower among cases **(Table 1)**.

Table (1): Comparison between patient and control groups regarding age, BMI and laboratory data (*Z value= statistic for Wilcoxon Rank Sum Test, *N = number of subjects).

	Cases (N=40)			Controls (N=40)			*Z value	P value
	Median	25 Perc	75 Perc	Median	25 Perc	75 Perc		
Age	25.5	22	30	26	24	30	-0.585	0.559
BMI	34.5	30	39	36	29	38	-0.082	0.935
FSH	5.725	3.9	7.43	5.54	3.98	6.61	-0.26	0.795
LH	5.575	3.78	7.26	5.075	3.88	6.7375	-0.645	0.519
E2	27.675	23.3875	32.355	30.66	22.91	34.0975	0.9	0.368
Progesterone	7.285	3.29	8.365	8.26	6.93	9.555	-2.767	**0.006**
Testosterone	0.32	0.105	0.4125	0.41	0.14	0.555	-1.412	0.158
AOA	7.4	6.7	8	6.6	6.5	7.3	-3.209	**0.001**

Analysis for the correlation between serum AOA level and personal data and hormonal profile in control and PCOS groups was done. No significant correlation was found between AOA and age, BMI, FSH, LH, E2, mid luteal progesterone or testosterone in control group. However, a significant negative correlation between AOA and serum testosterone level was found in PCOS group (p value<0.05) **(Table 2)**.

Table (2): Correlation between serum AOA level and personal data and hormonal profile in control and PCOS groups (r*= Ranked Spearman's Correlation Coefficient)

	AOA in Control group		AOA in PCOS group	
	r*	P value	r*	P value
Age	-0.107	0.511	-0.024	0.884
BMI	0.094	0.564	-0.036	0.825
FSH	0.018	0.912	-0.126	0.44
LH	0.045	0.784	-0.054	0.742
E2	0.266	0.097	0.143	0.378
Progesterone	-0.026	0.875	-0.221	0.171
Testosterone	-0.068	0.679	-0.391	**0.013**

A multiple regression analysis was done in this study using PCOS as the dependent variable and other data (age, BMI, FSH, LH, E2, mid luteal progesterone and testosterone) as the independent variables. The analysis showed that age, BMI, FSH, LH, E2, and testosterone levels did not significantly predict the presence of PCOs. However, serum AOA = 0.161, p<0.01) and mid luteal progesterone levels (Reg. coef. = -0.041, p<0.05) did significantly predict the presence of PCOS **(Table 3)**.

Table (3): Multiple regression analysis for variables in PCOS (*Reg. Coef = Regression Coefficient).

Item	Reg. Coef.*	T	p	Sig.	F-Ratio	P	Sig.
(Constant)	-0.175	-0.275	0.784	NS			
AGE	-0.003	-0.241	0.81	NS			
BMI	0	-0.012	0.99	NS			
AOA	0.161	2.726	**0.008**	**HS**			
E2	-0.005	-0.739	0.462	NS			
FSH	-0.013	-0.285	0.776	NS			
LH	0.034	0.85	0.398	NS			
Testosterone	-0.166	-0.743	0.46	NS			
Progesterone	-0.041	-2.046	**0.045**	**S**			
					2.879	**0.008**	HS

NS: P-value >0.05; S: P-value <0.05; HS: P-value <0.001

The best diagnostic efficacy (67.5%) for serum AOA level was at the cutoff level of 6.7 U/ml **(Table 4 and Figure 1)**.

Table (4): Diagnostic Validity Test for serum AOA level in PCOS **(**TN: true negative, FP: false positive, TP: true positive, FN: false negative, %Sp: %specificity, %Sn: %sensitivity, %PN: negative predictive value, %PP: positive predictive value, %Eff: %efficacy).

AOA	TN	FP	TP	FN	%Sp	%Sn	%PN	%PP	%Eff
6.7	**25**	**15**	**29**	**11**	**62.5**	**72.5**	**69.4**	**65.9**	**67.5**

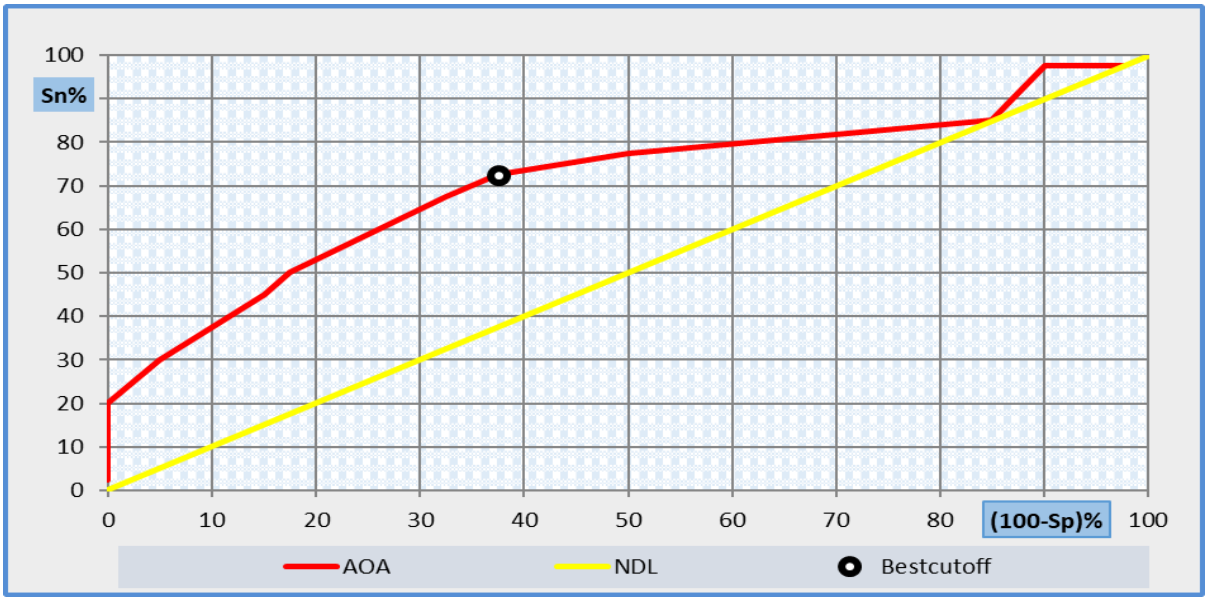

Fig. (1): ROC showing the diagnostic performance of serum AOA level for discriminating patients with PCOS from those without PCOS (Sn=sensitivity, Sp=specificity, NDL=non diagnostic line)

DISCUSSION

In the current study, the association between AOA and PCOS among Egyptian women was investigated. Forty women with a diagnosis of PCOS were included, along with 40 healthy age-matched normally cycling fertile women as a control group. Presented results showed that serum AOA level was significantly higher among cases than controls. Our findings agrees with the results of research done by **Fenichel *et al.*** [6] and **Ali *et al.*** [7] in which they found statistically significant higher serum levels of AOA in women with PCOS than for the control group, using ELISA technique. In addition, another research reported histological findings in a case of PCOS consistent with autoimmune oophoritis [8]. **Lonsdale *et al.*** [9] reported ovarian and adrenal antibodies, and lymphocytic infiltration of ovaries in two patients with PCOS. **Van Gelderen and Gomes dos Santos** [10] showed antibodies to human ovarian sections and granulosa cells by immunofluorescence in 4/8 patients with PCOS. On the other hand, **Rojansky *et al.*** [11] reported a lack of ovarian antibodies in 30/31 women with PCOS. In addition, a case control study was conducted in which enzyme immunoassay was used to assess the presence of AOA and the authors found that the prevalence of ovarian antibodies in serum was similar among the controls and PCOS (25%) [12]. The discrepancy in the results of different research work and studies in assessing the presence of AOA in PCOS patients may be due to many possible causes. It could be due to the use of different antigenic substrates in these studies. For example, **Van Gelderen and Gomes dos Santos** [10] used granulosa cells as antigen substrate, **Fenichel *et al.*** [6] used a human ovary extract, **Rojanski *et al.*** [11] used ovarian follicle theca interna and **Luborsky *et al.*** [12] used microsomal antigens for their study. Multiple antigenic targets seem to have a role in ovarian autoimmunity,

including cellular and oocyte related antigens. Studies that only evaluated one target antigen may leave women with ovarian autoimmunity unnoticed. Other possible reasons for the discrepancy in the results are antigen preparation and criteria for study and comparison groups. Also, the methodology of the assay has a major impact on the results of different research groups [13].

In the present study also, the control and PCOS groups were compared regarding their hormonal profile (estrogen, progesterone, FSH, LH, and testosterone) and no significant difference was found between cases and controls except for the level of mid luteal 21 progesterone which was significantly decreased among cases. The low serum mid luteal 21 progesterone in PCOS cases agrees with the pathology of the ovaries in this syndrome. Normally, progesterone is produced by the corpus luteum after ovulation occurs. In PCOS, oligo and/or anovulation lead to decreased progesterone synthesis. However, some studies have different results regarding serum level of testosterone and LH hormones in PCOS and control groups. For example, **Luborsky *et al.*** [12] mentioned that serum level of testosterone was significantly higher in women with PCOS than control group ($P<0.001$). In addition, **Hassan *et al.*** [14] stated that mean value of serum LH was significantly higher in women with PCOS patients than control group ($P<0.001$).

An analysis for the correlation between serum AOA level and personal data and hormonal profile in control and PCOS groups was added in this study. No significant correlation was found between AOA and age, BMI, FSH, LH, E2, mid luteal progesterone and testosterone in control group. However, a significant negative correlation between AOA and serum testosterone level was found in PCOS group.

A multiple regression analysis was performed in the current study using PCOS as the dependent variable and other data (age, BMI, FSH, LH, E2, mid luteal progesterone and testosterone) as the independent variables. This analysis showed that PCOS can be predicted by serum levels of AOA and mid luteal progesterone. These findings enforce the hypothesis of an autoimmune mechanism in the pathogenesis of PCOS. In addition, this agrees with the common finding of low serum levels of mid luteal progesterone in PCOS cases due to oligo and/or anovulation present in this syndrome[15].

To assess the diagnostic validity of AOA in PCOS, ROC curve was constructed to obtain the most sensitive and specific cutoff for this test. We found that the best diagnostic accuracy (67.5%) for serum AOA is at the cutoff level of 6.7 U/ml.

Taken together, this study clearly reveals the presence of an autoimmune reaction against ovarian tissue in female patients with PCOS, suggesting the possibility of an autoimmune mechanism in the pathogenesis of PCOS. Further attempts to characterize reproductive tissue autoantigens that act as targets for AOA should be encouraged. In addition, AOA assays should be optimized to detect all different antibody isotypes to increase the sensitivity of the assays. This may help in implementing the serum AOA level assay as a diagnostic and/or prognostic test for PCOS.

CONCLUSION

Our results suggest that autoimmune ovaritis may be frequently associated with PCOS. Circulating AOA may represent a practical and suitable marker for diagnosis of PCOS.

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