Serological Autoimmune Markers in Women with Polycystic Ovary Syndrome

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Abstract: Objective: The aim of the current study was to investigate the association between polycystic ovary syndrome (PCOS) and certain serological markers of autoimmunity (namely anti-nuclear antibody [ANA], anti-double-stranded DNA [anti-dsDNA] and anti-histone antibodies] in a sample of Egyptian women. **Methods:** The study included two groups of women; the first group (Group I) included women presenting to the Infertility Outpatient Clinic at Ain Shams University Maternity Hospital, with a diagnosis of PCOS. An equivalent number of age-matched fertile women were included as a second control group (Group II). Blood samples were withdrawn for checking the levels of follicle stimulating hormone (FSH), luteinizing hormone (LH) and thyroid stimulating hormone (TSH). In addition, the samples were assayed for three non-organ-specific autoimmune antibodies, namely anti-nuclear antibodies (ANA), anti-double stranded DNA (anti-ds DNA) and anti-histone antibodies. **Results:** There was no significant difference between both groups regarding serum FSH level. The mean values of serum LH, serum LH-to-FSH ratio and serum TSH levels were significantly higher in women of group I [PCOS Group]. The mean serum levels of ANA, anti-dsDNA and ant-histone antibodies were significantly higher in women of group I. **Conclusion:** PCOS seems to be significantly associated higher serum levels of non-organ-specific autoantibodies. This may suggest an underlying autoimmune etiology in the pathogenesis of PCOS.

[Hassan M., Hussein M. and El-Wakil W. **Serological Autoimmune Markers in Women with Polycystic Ovary Syndrome.** *Life Sci J* 2014;11(1s):318-322] (ISSN:1097-8135). http://www.lifesciencesite.com. 57

Key word: PCOS – polycystic ovary syndrome – serological markers – autoimmunity – antinuclear antibody – anti-double stranded DNA antibody – anti-histone antibody

1.Introduction:

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women, affecting between 5% and 10% of women during the reproductive age [1]. The precise pathogenesis of PCOS is yet to be determined. The main underlying endocrinopathies related to PCOS are hyperandrogenism and impaired glucose tolerance secondary to insulin resistance [2-3]. Insulin resistance was reported to be not universal in all women with PCOS. Some authors stated that a considerable proportion of women with PCOS have insulin sensitivity comparable to healthy fertile women [4]. What confirms this latter fact is that some women with PCOS poorly respond to insulin sensitizers like metformin [5-6]. Therefore, mechanisms other than insulin resistance are implicated in the pathogenesis of PCOS. Autoimmunity has been proposed in the pathogenesis of PCOS since early 1990s. It has been demonstrated that women with PCOS have elevated serum concentrations of cytokines (e.g. tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6)) independent of obesity. Moreover, several authors have reported association between PCOS and certain autoimmune disorders, including autoimmune thyroiditis [7], Graves' disease [8], chronic lymphocytic thyroiditis [9], diabetes mellitus [10], the rare autoimmune disease Vogt-Koyanagi-Harada syndrome [11], and even autoimmune oophoritis and premature ovarian failure [12]. The link between PCOS and autoimmunity has been the question of recent studies and reviews [13-15]. The aim of the current study was to investigate the association between PCOS and certain serological markers of autoimmunity (namely anti-nuclear antibody [ANA], anti-double-stranded DNA [anti-dsDNA] and anti-histone antibodies] in a sample of Egyptian women.

2.Methods

The current case-control study was conducted at Ain Shams University Maternity Hospital during the period between March 2010 and August 2010. The study protocol was designed in accordance to the Declaration of Helsinki for the Ethical Principles of Medical Research, and was approved by the Ethical Research Committee at Obstetrics and Gynecology Department, Faculty of Medicine, Ain Shams University. Participating women had to sign an informed consent after thorough explanation of the purpose and procedures of the study. The study included two groups of women; the first group (Group I) included women presenting to the Infertility

Outpatient Clinic at Ain Shams University Maternity Hospital, with a diagnosis of polycystic ovary syndrome (PCOS). Diagnosis of PCOS was based on the Rotterdam PCOS Consensus of the European Society of Human Reproduction and the American Society of Reproductive Medicine (ESHRE/ASRM), as presence of at least two of the following three criteria: hyperandrogenism (biochemical or clinical [in the form of hirsutism and/or acne]); chronic anovulation (oligo/amenorrhea); sonographic features of polycystic ovary; after exclusion of other etiologies (e.g. congenital adrenal hyperplasia, androgen-secreting tumors and Cushing syndrome) [1]. The sonographic feature of PCOS included the presence of ≥ 12 follicles in each ovary measuring 2-9 mm in diameter and/or increased ovarian volume (> 10 ml), regardless of follicle distribution or ovarian stromal echogenicity. One ovary fulfilling these criteria was sufficient to define positive sonography for PCOS. All included infertile women had a normal serum prolactin and thyroid stimulating hormone (TSH) levels. Women who had any acute condition, and those who had received any hormonal treatment during the previous 3 months or any medications that would affect serum levels of the assayed antibodies (antipsychotics, pyrazinamide, sulfadiazine, or aromatase inhibitors) were excluded. An equivalent number of age-matched fertile women were included as a second control group (Group II). For all included women a 5-ml venous blood sample was taken. Blood sample was taken in the cycle day 2 or 3 (in women with regular menstrual cycles) or in day 2 or 3 of the induced withdrawal bleeding (in amenorrheic women). Withdrawal bleeding was induced in amenorrheic women with oral progestin (medroxyprogesterone acetate [Provera®, Pharmacia Pharmaceuticals, Pfizer Egypt], 5 mg tablets) twice per day for 5 days after excluding pregnancy by a negative serum pregnancy test. The venous blood sample was allowed to clot, then centrifuged and the supernatant serum was separated and stored frozen in aliquots at -20°C. All samples were assayed at the same time after recruiting all women. The withdrawn serum samples were assayed for the levels of follicle stimulating hormone (FSH), luteinizing hormone (LH) and thyroid stimulating hormone (TSH). In addition, the samples were assayed for three non-organ-specific autoimmune IgG antibodies, namely anti-nuclear antibodies (ANA), anti-double stranded DNA (anti-ds DNA) and anti-histone antibodies.

ANA Assay

The ANA assay is a quantitative immunometric enzyme immunoassay for the quantitative screening on antinuclear antibodies (ANA screen, ORG 538, Orgentec Diagnostika GmbH, Mainz, Germany). This assay collectively detects, in one well, ANAs against SS-A (Ro), SS-B (La), RNP-70, Sm, RNP/Sm, Scl-70, centromere B, and Jo-1. Sera that showed an ANA level ≥ 10 IU/ml were considered positive [16].

Anti-dsDNA Antibody Assay

The anti-dsDNA antibody assay was an indirect solid phase enzyme-linked immunosorbent assay (ELISA) for the quantitative measurement of IgG class autoantibodies against dsDNA in human serum (anti-dsDNA ORG 604, Orgentec Diagnostika GmbH, Mainz, Germany. Sera that showed an anti-dsDNA antibody level ≥ 75 IU/ml were considered positive, while those which showed a level between 30 and less than 75 were considered borderline [16].

Anti-histone Antibody Assay

The anti-histone assay was an indirect solid phase enzyme-linked immunosorbent assay (ELISA) for the quantitative measurement of IgG class autoantibodies to histone in human serum (anti-histone ORG 507; Orgentec Diagnostika GmbH, Mainz, Germany). Sera that showed an anti-histone antibody level ≥ 40 IU/ml were considered positive [16].

Sample size justification

Sample size was calculated using EpiInfo® version 6.0, setting the power at 80% and the two-sided confidence level at 95%. Data from previous study [17] showed that at least one autoimmune antibody was positive in sera of 40.7% of women with PCOS, in contrast to 14.8% of control women. Calculation according to these values produced a minimal sample size of 50 cases in each group.

Statistical Methods

Statistical analysis was performed using SPSS® for Windows version 15.0. Difference between two independent groups was analyzed using independent student's t-test (for numeric variables) or chi-squared test (for categorical variables). Correlation between two metric variables was calculated using Pearson's correlation coefficient. Significance level was set at 0.05.

3.Results

Fifty women with a diagnosis of PCOS (according to the eligible criteria) were included as group I, along with 50 fertile women as group II. Table-1 shows characteristics of included women. There were no significant differences between women of both groups regarding the age. The mean weight and BMI were significantly higher in women of group I [PCOS Group]. There was no significant difference between both groups regarding serum FSH level. The mean values of serum LH, serum LH-to-FSH ratio and serum TSH levels were significantly higher in women of group I [PCOS Group].

Table-1 Difference between Groups regarding Characteristics and Basal Serum Hormonal Levels

	Group I [PCOS Group] (n=50)	Group II [Control Group] (n=50)	*P*
Age (years)	28.9 ± 5.6	27.1 ± 3.5	0.057
Weight (kg)	76.8 ± 3.8	73 ± 4.3	<0.001
BMI (kg/m^2)	28.1 ± 1.1	26.1 ± 0.9	<0.001
Serum FSH (mIU/ml)	6.9±1.4	7.3±1.2	0.128
Serum LH (mIU/ml)	10.2±2.1	5.2±1.4	<0.001
Serum LH:FSH Ratio	1.5±0.3	0.7±0.12	<0.001
Serum TSH (μIU/ml)	1.5±0.6	1.1±0.4	0.002

Data presented as mean ± SD; Analysis using Independent Student's t-Test;
BMI body mass index [calculated as the weight (in kilograms) divided by squared height (in meters)];
FSH follicle stimulating hormone; LH luteinizing hormone; TSH thyroid stimulating hormone

The mean serum levels of ANA, anti-dsDNA and ant-histone antibodies were significantly higher in women of group I [PCOS Group]. The proportion of women who had positive markers (according to the set cutoff values in the *'methods'* section) were significantly higher in women of group I [PCOS Group] (Table-2).

Table-2 Difference between Groups regarding Serum Levels of Autoimmune Markers

	Group I [PCOS Group] (n=50)	Group II [Control Group] (n=50)	*P*
Serum ANA (IU/ml)	9.0 ± 5.1	5.4 ± 2.5	<0.001*
Positive Serum ANA	19 (38%)	4 (8%)	<0.001**
Serum Anti-dsDNA (IU/ml)	56.3 ± 25.6	26 ± 10.7	<0.001*
Positive Serum Anti-dsDNA	15 (30%)	0 (0%)	<0.001**
Borderline Serum Anti-dsDNA	29 (58%)	19 (28%)	0.045**
Anti-Histone (IU/ml)	43.5 ± 19.5	26.4 ± 8.4	<0.001*
Positive Anti-Histone	29 (58%)	4 (8%)	<0.001**

Data presented as mean ± SD * Analysis using Independent Student's t-Test
** Analysis using Chi-squared Test ANA anti-nuclear antibodies
Anti-dsDNA anti-double-stranded DNA antibodies

There were no significant correlation between serum levels of ANA, anti-dsDNA and anti-histone antibodies and any of the other variables (age, weight, BMI, serum FSH, LH, LH-to-FSH ratio). Only serum TSH was significantly and positively correlated to the serum ANA level in both groups [r=0.955, *p*<0.001; and r=0.906 *p*<0.001; respectively] (Table-3).

Table-3 Correlation between Serological Markers and Measured Variables in Included Women in Both Groups

		Serum ANA		Serum Ant-ds-DNA		Serum Anti-Histone	
		Group I	Group II	Group I	Group II	Group I	Group II
Age	*r*	0.068	0.208	0.067	-0.220	0.014	0.003
	p	0.637	0.148	0.646	0.124	0.923	0.985
Weight	*r*	-0.183	0.149	0.043	0.063	0.025	0.167
	p	0.203	0.300	0.766	0.664	0.865	0.247
BMI	*r*	-0.002	0.091	-0.049	-0.070	-0.024	0.050
	p	0.987	0.532	0.738	0.631	0.870	0.730
Serum FSH	*r*	-0.278	-0.148	-0.047	0.001	0.105	0.133
	p	0.051	0.306	0.747	0.998	0.467	0.357
Serum LH	*r*	-0.201	-0.144	0.041	0.015	-0.202	0.147
	p	0.161	0.319	0.780	0.918	0.159	0.309
Serum LH:FSH Ratio	*r*	0.072	-0.090	0.078	0.002	-0.284	0.089
	p	0.617	0.535	0.592	0.987	0.055	0.539
Serum TSH	*r*	0.955	0.906	0.190	-0.035	0.027	0.159
	p	<0.001	<0.001	0.186	0.811	0.854	0.270

r Pearson's correlation coefficient;
BMI body mass index [calculated as the weight (in kilograms) divided by squared height (in meters)];
FSH follicle stimulating hormone; LH luteinizing hormone; TSH thyroid stimulating hormone;
ANA anti-nuclear antibodies; Anti-ds-DNA anti-double-stranded DNA antibodies

4.Discussion

The current study showed that PCOS was associated with elevated serum levels of three non-organ-specific autoantibodies (ANA, anti-dsDNA and anti-histone antibodies). This finding was reported by a number of previous studies. Reimand et al. investigated the prevalence of autoimmune derangements in 108 women with 'reproductive failure' [primary menstrual cycle disturbances, PCOS, endometriosis, luteal phase insufficiency and unexplained infertility], in comparison to 392 control women. The assayed markers included ANA, smooth muscle (SMA), parietal cell (PCA), thyroid microsomal (TMA), mitochondrial (AMA) antibodies. They found that 40.7% of the patients' sera and 14.8% of the control sera contained at least one type of autoantibodies; and that ANA and SMA were the most frequently detected antibodies [17]. In a very similar study to the current one, Hefler-Frischmuth et al. investigated the serum levels of ANA, anti-dsDNA, anti-histone and anti-nucleosome antibodies in 109 women with PCOS in comparison to 109 control women. They found that serum levels of anti-histone and anti-nucleosome antibodies were significantly higher in the PCOS group, while those for ANA and anti-dsDNA were comparable in both groups [16]. The association between PCOS and autoimmunity did not come out of thin air. Several case reports and reviews showed association between PCOS and many autoimmune diseases, particularly autoimmune thyroid diseases [7-8]. High prevalence of autoimmune thyroiditis was found in women with PCOS [18]. It was reported that an exaggerated TSH and a blunted free T4 response during thyrotropin-releasing hormone (TRH) testing, indicative of latent hypothyroidism, were found in a group of PCOS patients. Elevated basal or TRH-induced TSH levels were also found in PCOS patients. Acanthosis nigricans, a finding common in patients with insulin resistance and also in PCOS, has been reported to have a positive correlation with hypothyroidism [7]. Antiovarian antibodies of all isotypes (immunoglobulin [IgG, IgA, IgM]) were significantly higher in PCOS patients [17]. Anti-ovarian autoantibodies localized to the granulosa cells were detected PCOS patients. In analogy to thyroid and adrenal endocrinopathies characterized by hypersecretion of hormones, the authors speculated on a possible pathogenic mechanism of PCOS involving stimulatory antibodies. PCOS patients were also reported to have an increased prevalence of lupus erythematosus. Association between autoimmune disorders of the thyroid and PCOS does not necessarily imply that both have underlying genetic or autoimmune pathway. It was shown that chronic anovulation and the relative low progesterone and high estrogen and androgen levels might accelerate the autoimmune response, leading to increased likelihood of autoimmune thyroiditis. Estrogens are known to increase interleukin (IL)-4 expression in TH2 cells, IL-1 in monocytes, IL-6 in T-cells and interferon gamma in TH1 cells. During normal menstrual cycles in young women, IL-6 is elevated in the follicular phase and decreased in the luteal phase, and inversely correlated to progesterone levels. Hence, the immune stimulatory activity of estrogens seems to be counteracted by progesterone. As patients with PCOS have no or nearly no progesterone because of anovulatory cycles, the immune system in these patients seems to be overstimulated, which may propagate autoimmune disease [7]. On the other hand, mild (and even subclinical) hypothyroidism may cause deterioration of PCOS by stimulating conversion of androstenedione to testosterone or estradiol and decreasing metabolism of sex hormones [7,19]. This latter finding was in accordance with the significantly higher TSH levels encountered in women with PCOS in the current study.

In conclusion, PCOS seems to be significantly associated higher serum levels of non-organ-specific autoantibodies. This may suggest an underlying autoimmune etiology in the pathogenesis of PCOS.

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1/24/2014