Nat. Volatiles & Essent. Oils, 2021; 8(4): 4523-4536

Serological markers of autoimmunity in women with polycystic ovarian syndrome

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Abstract:

Background: Polycystic ovary syndrome (PCOS) also called hyperandrogenic anovulation (HA) or Stein- Leventhal syndrome. Aim of the study: to evaluate serum levels of the common autoimmune markers, antinuclear antibodies (ANA), and anti-double-stranded DNA (dsDNA) in women with polycystic ovary syndrome (PCOS).

Methods: Study design: A prospective case control study. The included women were divided into 2 groups: group 1 included 50 women with PCOS according to Rotterdam Criteria (2003) (study group), and group 2 included 50 healthy, fertile, age matched women (control group). Transvaginal ultrasound performed to evaluate the ovaries and uterus. Blood samples were obtained from all included women, who were in the follicular phase (days 3–7 of spontaneous menses or progestin induced withdrawal bleeding) to determine serum levels of follicle-stimulating hormone, luteinizing hormone, thyroid-stimulating hormone, and for serological tests namely ANA and anti-dsDNA.

Results: In this study, there was significant difference between both groups regarding BMI in which the mean was 28.1±1.3 in study group while. 23.8±1.6 in control group. In women with PCOS, there was reduced gravidity and parity than the control group. In women of PCOS group (28%) had primary infertility, (40%) had secondary infertility, (32%) were fertile while (90%) were fertile in control group. The means of serum levels of LH, LH to FSH ratio, TSH were significantly higher in PCOS group than control group. The mean serum level of ANA was 1.01±0.79 IU/mL in PCOS group versus 0.58±0.44 IU/mL in control group with (P value 0.001) which is statistically significant. The mean serum level of anti-dsDNA was significantly higher in women of PCOS group when compared to those of control group (26.05±14.29 IU/mL versus 17.39±9.43 IU/mL respectively). The (P value 0.001) which is statistically significant.

Conclusion: There is an association between PCOS and autoimmune markers such as ANA and anti-dsDNA that might affect the clinical management of those women.

Keywords: Serological markers, autoimmunity, women, polycystic ovarian syndrome.

Introduction:

Polycystic ovary syndrome (PCOS) also called hyperandrogenic anovulation (HA) [1] or Stein- Leventhal syndrome. Polycystic ovary syndrome was first reported in modern medical literature by Stein and Leventhal who, in 1935, described seven women suffering from amenorrhea, hirsutism, and enlarged ovaries with multiple cysts [2].Polycystic ovarian syndrome (PCOS) is a common endocrine disorder, affecting women of reproductive age. The syndrome characterized by chronic oligo/anovulation and a variable combination of symptoms, including menstrual disturbances, obesity and hyperandrogenism. Based on the earlier National Institutes of Health (NIH) definition, PCOS is thought to occur in about 6%-8% of women worldwide, making it the most common reproductive disorder. However, when applying the new Rotterdam/the European Society for Human Reproduction and Embryology criteria, it is likely that the prevalence is even higher about 18% [3, 4].The highest reported prevalence of polycystic ovaries in a community survey was 52% in south Asian immigrants in Britain, of whom 49.1% had menstrual irregularity. South Asian people with an ovulatory PCOS have greater insulin resistance than an ovulatory white people with PCOS [5].Gonadotropin therapy is expensive and is associated with an increased risk of multiple pregnancy and ovarian hyperstimulation syndrome. So, patients monitored with ultrasonography and laboratory studies. Metformin frequently, but not universally, improves ovulation rates and pregnancy rates in women with PCOS. In vitro fertilization (IVF) is reserved for women with PCOS and unsuccessful gonadotropin therapy or those with other indications for thisprocedure [6].Antinuclear antibodies (ANA) are autoantibodies that bind to contents of the cell nucleus. In normal individuals, the immune system produces antibodies to foreign proteins (antigens) but not to human proteins (autoantigens). In some individuals, antibodies to human antigens are produced [7].The first evidence for antinuclear antibodies arose in 1948 when Hargraves, Richmond and Morton discovered the Lupus Erythematosus (LE) cell. These abnormal cells, which found in the bone marrow of persons who have systemic lupus erythematosus (SLE), categorized as polymorphonuclear leukocytes with phagocytosedwhole nuclei [8].There are many subtypes of ANAs such as anti-Ro antibodies, anti-Laantibodies, anti-Smith (anti-Sm) antibodies, anti-nuclear ribonucleoprotein(anti-nRNP) antibodies, anti- scleroderma 70(anti-Scl-70) antibodies, antidoublestrandedDNA (anti-dsDNA) antibodies, anti-histone antibodies, antibodies to nuclear pore complexes, anti-centromere antibodies and anti-sp100antibodies. Each of these antibody subtypes binds to different proteins or protein complexes within the nucleus [9]. Aim of the study: to evaluate serum levels of the common autoimmune markers, antinuclear antibodies (ANA), and anti-double-stranded DNA (dsDNA) in women with polycystic ovary syndrome (PCOS).

Method:

This prospective case-control study was carried out in department of obstetrics and gynecology of Al-Imamein Al-Kadhimein Medical City, Baghdad, Iraq. The study was conducted over a period of twelve months starting from the first of February 2015 to the end of January 2016.The study purpose and procedures were explained to all enrolled women, and verbal consent was obtained from all women before enrolling them in the study.The included women were divided into 2 groups: group 1 included 50 women diagnosed with PCOS according to the 2003 Rotterdam Criteria and were recruited from the infertility clinic(study group) and group 2 included 50 fertile control women seeking contraception in the outpatient clinic without having PCOS (control group).

Inclusion criteria:

1. Age (18-31) years.
2. No medical or hormonal treatments for at least three months.
3. All women had normal thyroid function tests.
4. All women had normal prolactin level.
5. The included women were in the follicular phase (days 3–7 of spontaneous menses or progestin-induced withdrawal bleeding).

Exclusion criteria:

1. A history of medical diseases like hyperthyroidism, hyperprolactinemia, or chronic hypertension.
2. Any hormonal treatment during the previous 3 months before the study or any medication affecting ANA and anti-dsDNA levels, such as antipsychotics (e.g., chlorpromazine, haloperidol, and clozapine).
3. Drug-induced lupus associated with pyrazinamide or sulfadiazine.
4. Aromatase inhibitors (e.g., letrozole and anastrozole), which increase the incidence of autoimmune disorders such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA).

For all included women a 5-ml venous blood sample taken in the morning. The venous blood sample allowed to clot then centrifuged and the supernatant serum separated and stored frozen in aliquots at -

20C. The withdrawn serum samples were assayed for the levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), thyroid stimulating hormone (TSH), anti-nuclear antibodies (ANA) and anti-double stranded DNA (anti-ds DNA) antibodies.Serum ANA levels measured by immunometric enzyme immunoassay using AESKULISA ANA-8S KIT/Germany. The antigens to ANAs are bound to microwells and antibodies against these antigens, if present in diluted serum, bind to the respective antigen. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase conjugated antihuman IgG immunologically detects the bound patient antibodies forming a conjugate/antibody/antigen complex. Results given as optical density quotient. Sera that showed an ANA level ≥ 1.0 IU/ml considered positive.The anti-dsDNA antibodies measured by enzyme-linked immunosorbent assay (ELISA) using AESKULISA dsDNA-G KIT/Germany. Human recombinant dsDNA is bound to microwells. Antibodies to this antigen, if present in diluted serum or plasma, bind to the respective antigen.Washing of the microwells removes unspecific serum components. Horseradish peroxidase-conjugated antihuman IgG immunologically detects the bound patient antibodies forming a conjugate/antibody/antigen complex.Results given as International units per milliliter. Sera that showed an anti-dsDNA antibody level > 35 IU/ml considered positive, while those, which showed a level between 15 IU/ml and 35 IU/ml, considered borderline and a level less than 15 IU/ml considered negative.**Statistical analysis** done by SPSS 22, frequency and percentage used for categorical data, mean and SD for continuous data. Chi-square used for assessed association between categorical variables, T-test used for assessed difference between mean of continuous variables. P-value less or equal to 0.05 is consider significant.

Results:

Women (100) were included in the study. Included women divided into 2 groups: group 1 (n=50) women with PCOS, and group 2 (n=50) healthy, fertile, age-matched control women. Table (1) shows the age distribution and BMI of the PCOS group and the control group: The mean age of the PCOS group was 26.0±3.5 while in the control group 27.1±2.7 and (P value 0.357) which is statistically not significant.The mean of the body mass index (BMI) was higher in women of PCOS group 28.1±1.3 than in women of control group 23.8±1.6 and is statistically highly significant.Gravidity decreases in women of PCOS group in comparison to control group. The P value (0.0001) which is statistically highly significant.This means that parity decreases in women of PCOS group in comparison to control group. The P value (0.0001) which is statistically highly significant.In PCOS group, while none of women of control group had three abortions. The P value was 0.076, which is statistically not significant.16 patients (32%) were fertile in

PCOSgroup while 45 patients (90%) were fertile in control group. P value was 0.0001 which is statistically highly significant.

Table (1): association between variables and PCOS, control cases.

		PCOS		contrl		
		No	**%**	**No**	**%**	
Age (years)	<20	2	4.0	-	-	0.357
	20---24	14	28.0	10	20.0	
	25---29	24	48.0	27	54.0	
	=>30	10	20.0	13	26.0	
BMI (Kg/m2)	Normal (18.5-24.9)	-	-	39	78.0	0.0001*
	Overweight (25-29.9)	46	92.0	11	22.0	
	Obese (=>30)	4	8.0	-	-	

		PCOS		Control		P-value
		No	**%**	**No**	**%**	
Gravidity	0	10	20.0	-	-	0.0001*
	1	11	22.0	3	6.0	
	2	15	30.0	21	42.0	
	3	12	24.0	17	34.0	
	4	2	4.0	9	18.0	
	0	15	30.0	-	-	0.0001*

Parity	1	15	30.0	5	10.0	
	2	16	32.0	27	54.0	
	3	4	8.0	15	30.0	
	4	-	-	3	6.0	
Abortion	0	32	64.0	33	66.0	0.076
	1	12	24.0	17	34.0	
	2	4	8.0	-	-	
	3	2	4.0	-	-	

		PCOS		Control		
		No	**%**	**No**	**%**	
Infertility	Primary	14	28.0	5	10.0	0.0001*
	Secondary	20	40.0	-	-	
	Fertile	16	32.0	45	90.0	
Infertility duration (years)	2	11	32.4	5	100	
	3	14	41.2	-	-	
	4	4	11.8	-	-	0.0001
	5	4	11.8	-	-	
	>5	5	14.7	-	-	

P-value ≤ 0.05 (significant).

Comparison of endocrine function between PCOS group and control group.

Table (2): shows Comparison of endocrine function between PCOS group and control group.

	PCOS	Controls	P value
LH (mIU/ml)	10.4±1.7 (7.20-13.40)	4.4±1.5 (2.40-7.54)	0.0001*
FSH (mIU/ml)	5.1±0.9 (3.70-7.50)	5.2±1.0 (4.03-7.40)	0.445
LH/FSH Ratio	2.058±0.214 (1.449-2.510)	0.853±0.255 (0.396-1.496)	0.0001*
TSH (MIU/ml)	2.1±0.4 (1.40-2.70)	1.3±0.2 (0.91-1.70)	0.0001*

P-value ≤ 0.05 (significant).

Comparison and distribution of serum level of ANA between PCOS group and control group.

Table (3): Comparison of serum level of ANA between PCOS group and control group.

ANA (IU/ml)	PCOS	Controls

Mean±SD	1.01±0.79	0.58±0.44
Standard Error of Mean	0.111	0.062
Range	0.09-3.10	0.11-2.10
Percentile 05th	0.18	0.15
$_{25}$th	0.31	0.26
50th (Median)	0.72	0.50
$_{75}$th	1.66	0.71
95th	2.40	1.75
99th	3.10	2.10
P value	0.001*	

P-value ≤ 0.05 (significant).

Table (4): The distribution of ANA and dsDNA levels in women of PCOS and control groups.

		PCOS		control		
		No	**%**	**No**	**%**	
ANA (IU/ml)	Negative (<1)	30	60.0	46	92.0	0.0001*
	Positive (=>1)	20	40.0	4	8.0	

dsDNA (IU/ml)	Negative (<15)	12	24.0	21	42.0	0.0001*
	Borderline (15-35)	22	44.0	27	54.0	
	Positive (>35)	16	32.0	2	4.0	
dsDNA (IU/ml)	Negative (<=35)	34	68.0	48	96.0	0.0001*
	Positive (>35)	16	32.0	2	4.0	

P-value ≤ 0.05 (significant).

Table (5): Comparison of serum level of dsDNA between PCOS group and control group.

dsDNA (IU/ml)	PCOS	Controls
Mean±SD	26.05±14.29	17.39±9.43
Standard Error of Mean	2.021	1.333
Range	4-53	1-51.12
Percentile 05th	5.0	6.0
25th	15.0	11.0
50th (Median)	26.0	16.0
75th	38.0	22.0
95th	51.0	34.0

99th	53.0	51.12
P value	0.001*	

P-value ≤ 0.05 (significant).

Discussion:

As a common hormonal disorder, PCOS is an important syndrome-affecting woman of reproductive age with various components of metabolic and cardiovascular type. The syndrome carries important health implications throughout the life. Apart from its metabolic and cardiovascular complications, the field of gynecology often faces reproductive issues of the syndrome [(10)].Our study showed that (28%) of PCOS group had primary infertility and (40%) had secondary infertility while in the control group, (10%) had primary infertility and none of the control group had secondary infertility. This is in agreement with Roos N et al 2011 in which they concluded that oligo-ovulation or anovulation in women with polycystic ovary syndrome is a major cause of infertility, and such women might require ovulation induction or assisted reproductive technology to become pregnant [(11)].Our study showed that PCOS group had less chance to become pregnant in which (20%) were nulligravida while none of the control group were nulligravid. This is in agreement with N. A. Bagegni et al 2010 who proved that nulligravidity in PCOS group 78(60%) while in control group 20 (15%) [(12)].In our study there is significant relation between PCOS group and reduced parity in comparison with control group (P value 0.0001). This finding is in agreement with M. Mikolaet al 2001 who showed that PCOS patients were more often nulliparous than controls (76 versus 42%) (P < 0.001) [(13)].In our study, women of PCOS group had BMI more than control group where about (92%) of PCOS group were overweight and (8%) were obese while (22%) of control group were overweight with a (P value 0.0001) which is statistically significant indicates the role of obesity in polycystic ovary syndrome.Ferdousi Begum 2009 concluded that BMI >25 was 67% among PCOS group while 19% among controls (P<0.001) which is statistically significant. This agrees with our study [(14)].In our study there is statistical difference between PCOS group and control group regarding the serum LH level (P value 0.0001) and LH to FSH ratio (P value 0.0001) which is statistically significant. This is in consistent with Ferdousi Begum 2009 who proved that serum LH concentrations are significantly elevated in PCOS women as compared to controls(P<0.05) [(14)].Our study agrees with Niken and Kanadi study on 105 women with PCOS who revealed that 66.7% are with increasing LH / FSH ratio [(15)].Our study revealed that there is no significant difference between both groups regarding serum level of FSH

(P value was 0.445). Ahmed K. Makledet al 2015 agree with our findings [16].In our study the serum TSH level in PCOS group ranging from (1.40-2.70) µIU/ml while (0.91-1.70) µIU/ml in the control group with (P value 0.0001) which is statistically significant. This finding agrees with Janssen OE et al 2004 who found that PCOS patients had a higher mean TSH level (P<0.001) and a higher incidence of TSH levels above the upper limit of normal (PCOS 10.9%, controls 1.8%; P<0.001) which is statistically significant [17].In the study of Reimand K. et al 2001 who investigated the prevalence of autoimmune derangements in 108 women with reproductive failure' [primarymenstrual cycle disturbances, PCOS, endometriosis, luteal phase insufficiencyand unexplained infertility] in comparison to 392 control women, The detection of ANA was found in 7 PCOS patients (19.4%) versus (3.6%) incidence in detection of ANA in the control group of 392 women (p<0.005) which agrees with our study [18].In the study of SamsamiDehaghani A et al 2013 when 35 patients with PCOS (21-38 years old) and 35 fertile healthy women (25-35 years old) as the control group were recruited in the study, There is 3 out of the 35 patients (8.6%) were positive for ANAs in the PCOS group while none of the controls were positive. This finding is in consistent with our study [19].Our study is in consistent with Ahmed K. Makledet al 2015 who reported that the serum ANA level was significantly higher in PCOS group than in control group with (P value<0.001) which is statistically significant [16].Our study revealed that the serum level of dsDNA was significantly higher in the PCOS than the control group. This agrees with Hefler-Frischmuth K et al 2010 who revealed that women with polycystic ovary syndrome (PCOS) had significantly elevated serum levels of anti-double-stranded DNA (anti-dsDNA) antibodies than the control group in a study of 109 women with PCOS and 109 age-matched healthy controls [20].Our findings are in agreement with Hassan M. et al 2014 when proved that 15 patients (30%) of PCOS group were positive for serum level of anti-ds DNA while none of the control group had anti-ds DNA (P value<0.001) which is statistically significant [21].Samsami DA et al 2014 study agrees with our results in that patients with PCOS had significantly higher levels of anti-dsDNA compared to control group (p = 0.001) which is statistically significant [22].However, BaharehHamedi et al 2014 studied the relationship between serum level of dsDNA and PCOS in 102 women with PCOS and 100 healthy controls. He proved that no significant differences were detected between cases and controls in the level of dsDNA which disagree with our study [10].

Conclusion:

There is an association between PCOS and autoimmune markers such as ANA and anti-dsDNA that might affect the clinical management of those women.

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