International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2018): 7.426

Evaluation of Hormonal and Autoimmunity Serological Markers in Iraqi Women with Polycystic Ovarian Syndrome

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Abstract: ***Antinuclear antibodies (ANAs) and double-stranded DNA (dsDNA) antibodies are well-established markers of autoimmunity, which are used in clinical practice in the diagnosis of autoimmunologic diseases. There is a suspected role of autoimmunologic processes and elevated levels of these antibodies in women with polycystic ovarian syndrome. Our study aimed to evaluate serum levels of the common autoimmune markers, antinuclear antibodies (ANA), and anti-double-stranded DNA (dsDNA) as well as some hormones in women with polycystic ovary syndrome (PCOS). This prospective case control study was conducted in Karama Teaching Hospital /Baghdad/Iraq during the period from the 1st of January 2016 to the end of December 2016 on 80 women who were divided into (2) groups: group (1) included 40 women with PCOS according to Rotterdam Criteria (2003) (study group), and group (2) included 40 healthy, fertile, age matched women (control group). Transvaginal ultrasound was 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 of this study showed a significant difference between both groups regarding BMI. 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 and (32%) were fertile, while (90%) of the control group were fertile. The means of serum levels of LH, LH to FSH ratio and TSH were significantly higher in PCOS group than the control group. The mean serum level of ANA and anti-dsDNA were significantly higher in PCOS group when compared with their levels in the control group. It can be concluded that there is an association between PCOS and autoimmune markers such as ANA and anti-dsDNA which might affect the clinical management of those women.***

Keywords: Polycystic ovarian syndrome, Autoimmunity, Serological markers, Antinuclear antibody, Antidouble stranded DNA antibody

1. 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 is 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 PCOS is a heterogeneous collection of signs and symptoms that gathered together form a spectrum of a disorder with a mild presentation in some but a severe disturbance of reproductive, endocrine and metabolic function in others [5].

Anovulation in women with PCOS is characterized by inappropriate gonadotropin secretion. Specifically, alterations in gonadotropin-releasing hormone (GnRH) pulsatility lead to preferential production of luteinizing hormone (LH) compared with follicle-stimulating hormone (FSH) [2].

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 [6].

ANAs are found in many disorders, as well as some healthy individuals. These disorders include: systemic lupus erythematosus (SLE), rheumatoid arthritis, Sjögren's syndrome, scleroderma, polymyositis, dermatomyositis, primary biliary cirrhosis, drug induced lupus, autoimmune hepatitis, multiple sclerosis, discoid lupus, thyroid disease, antiphospholipid syndrome, juvenile idiopathic arthritis, psoriatic arthritis, juvenile dermatomyositis, idiopathic thrombocytopaenic purpura, infection and cancer. These antibodies can be subdivided according to their specificity, and each subset has different propensities for specific disorders [7, 8].

Anti-dsDNA antibodies are a group of anti-nuclear antibodies and their target antigen is double stranded DNA. They are highly diagnostic of systemic lupus erythematosus (SLE) and are implicated in the pathogenesis of lupus nephritis [9].

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Anti-double stranded DNA (anti-dsDNA) antibodies are a useful tool for the diagnosis of systemic lupus erythematosus and represent one of the criteria of the American College of Rheumatology (ACR) for the classification of SLE. Several studies have shown a correlation between disease activity and anti- dsDNA antibody levels in SLE, particularly in patients with renal involvement making detection of such antibodies relevant in SLE monitoring [10, 11].

2. Patients and Methods

This prospective case-control study was carried out in the department of obstetrics and gynecology of, Karama Teaching Hospital Baghdad, Iraq during the period from from the 1st of January 2016 to the end of December 2016.

The 80 women in this study were divided into 2 groups: group 1 included 40 women diagnosed with PCOS according to the 2003 Rotterdam Criteria and were recruited from the infertility clinic(study group) and group 2 included 40 fertile control women seeking contraception in the outpatient clinic without having PCOS (control group).

Patients were chosen by simple selection criteria and we used a questionnaire form to evaluate many of dependent variables such as age, parity and abortion, history of infertility, body mass index, medical history and any family history of autoimmune diseases.

Inclusion criteria were (18-31) years age, no medical or hormonal treatments for at least three months, normal thyroid function tests, normal prolactin level and the included women were in the follicular phase (days 3–7 of spontaneous menses or progestin-induced withdrawal bleeding.

Exclusion criteria were: A history of medical diseases like hyperthyroidism, hyperprolactinemia, or chronic hypertension, 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, drug-induced lupus associated with pyrazinamide or sulfadiazine and aromatase inhibitors (e.g., letrozole and anastrozole), which increase the incidence of autoimmune disorders such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). From all studied women, 5 ml venous blood was taken in the morning. The venous blood was allowed to clot, then centrifuged and serum samples were stored frozen in aliquots at -20C. The 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 were measured by immunometric enzyme immunoassay using AESKULISA ANA-8S KIT/Germany.

The anti-dsDNA antibodies were measured by enzyme-linked immunosorbent assay (ELISA) using AESKULISA dsDNA-G KIT/Germany.

Results are given as International units per milliliter. Sera that showed an anti-dsDNA antibody level > 35 IU/ml were considered positive, while those which showed a level between 15 IU/ml and 35 IU/ml were considered borderline and a level less than 15 IU/ml was considered negative.

Statistical Analysis

Analysis of data was carried out using the available statistical package of SPSS-22 (Statistical Packages for Social Sciences- version [22].

Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values).

The significance of difference of different means (quantitative data) were tested using Students-t-test for difference between two independent means.

The significance of difference of different percentages (qualitative data) were tested using Pearson Chi-square test (X2-test) with application of Yate's correction or Fisher Exact test whenever applicable. Statistical significance was considered whenever the P value was equal or less than 0.05.

3. Results

A total of 80 women were included in the study. Included women were divided into 2 groups: group A (n=40) women with PCOS, and group B (n=40) healthy, fertile, age-matched control women.

Table (1) shows comparison of endocrine function between PCOS group and the control group:

The mean value of serum LH level in PCOS group was 11.1±1.6, while in control group was 4.1±2.1 (P value 0.0001), which is statistically highly significant. The mean value of serum FSH level in PCOS group was 4.95±0.81, while in the control group was 5.05±1.52 (P value 0.714) which is statistically non-significant. The mean value of serum LH to FSH ratio in PCOS group was 2.242±0.210 which is higher than the control group (0.811±0.233) (P value 0.0001) so, it is statistically highly significant. The mean value of serum TSH level in PCOS group was 2.13±0.38 higher than the control group 1.12±0.18 (P value 0.0001), which is statistically highly significant.

Table 1. Association of prolactin level with pregnant women age

	PCOS	Controls	P value
LH (mIU/ml)	11.1±1.6 (7.60-13.10)	4.1±2.1 (2.25-7.03)	0.0001*
FSH (mIU/ml)	4.95±0.81 (3.35-7.15)	5.05±1.52 (4.15-7.25)	0.714
LH/FSH Ratio	2.242±0.210 (1.359-2.505)	0.811±0.233 (0.392-1.501)	0.0001*
TSH (MIU/ml)	2.13±0.38 (1.38-2.65)	1.12±0.18 (0.93-1.55)	0.0001*
*Significant difference in between means using Student-t-test for two independent means at 0.05 level			

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The mean serum level of ANA was 0.97±0.74 and range was (0.11-3.15) IU/ml in PCOS group while the mean serum level of ANA was 0.61±0.48 and range was (0.10-2.05) IU/ml in the control group. The (P value 0.011) which is a statistically significant result.

In our study, 13 patients (32.5%) were positive for ANA and 27 patients (67.5%) were negative for ANA in PCOS group, while 4 women (10%) were positive for ANA and 36 women (90%) were negative for ANA in the control group. The (P value 0.0287), which is a statistically significant finding as shown in table [2].

This means that there is significant relation between ANA level and polycystic ovary syndrome.

Table 2: Comparison of serum level of ANA between PCOS group and control group

ANA (IU/ml)	PCOS	Controls	ANA (IU/ml)
Mean±SD	0.97±0.74	0.61±0.48	Mean±SD
Standard Error of Mean	0.117	0.075	Standard Error of Mean
Range	0.11-3.15	0.10-2.05	Range
Percentile 05th	0.17	0.16	Percentile 05th
25th	0.29	0.28	25th
50th (Median)	0.75	0.49	50th (Median)
75th	1.45	0.38	75th
95th	2.35	1.77	95th
99th	3.07	2.19	99th
P value	0.011*		P value
*Significant difference in between means using Student-t-test for two independent means at 0.05 level			

Table 3: The distribution of ANA and dsDNA levels in women of PCOS and control groups

		PCOS		Controls		P value
		No	%	No	%	
ANA (IU/ml)	Negative (<1)	27	67.5	36	90.0	0.0287*
	Positive (=>1)	13	32.5	4	10.0	
dsDNA (IU/ml)	Negative (<15)	9	22.5	17	42.5	0.0013*
	Borderline (15-35)	18	45.0	22	55.0	
	Positive (>35)	13	32.5	1	2.5	
dsDNA (IU/ml)	Negative (<=35)	30	75.0	38	95.0	0.0283*
	Positive (>35)	10	25	2	5.0	
*Significant difference in proportions using Pearson Chi-square test at 0.05 level.						

Table (2) shows the relation between prolactin level and pregnant parity with chi-square test (0.942). There was no significant relation between prolactin level and pregnant parity among different parity groups (P-value 0.625).

Table 4: Association of prolactin level with gestational age at examination

Prolactin level	Gestational age at examination (weeks)				Total	Chi Square Test	P-value
	24-27	28-30	31-36	≥37			
negative	12	11	11	16	50	2.184	0.535
positive	23	23	23	31	50		
Total	11	12	12	15	100		

4. Discussion

Simple, reliable and rapid tests for diagnosis of PROM are needed. Since there is no unique and non invasive. As a common hormonal disorder, PCOS is an important syndrome affecting women 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 [12].

There are several systemic or organ-specific diseases of auto immunologic origin which can attack the ovaries as targets. In the present study, we selected well-established markers of autoimmunity (ANA and anti-dsDNA) that are used in clinical practice for diagnosing autoimmunologic diseases [12, 13].

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 revealed that there is no significant difference between both groups regarding serum level of FSH (P value was 0.714). Hassan M et al 2014 and Ahmed K. Makled et al 2015 agree with our findings [15, 13].

In our study the serum TSH level in PCOS group ranging from (1.38-2.65) µIU/ml while (0.93-1.55) 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 [16].

Our study showed that 13 patients (32.5%) had positive serum level of ANA and 27 patients (67.5%) had negative serum level of ANA in PCOS group while in control group, 4 patients (10%) had positive serum level of ANA and 36 patients (90%) had negative serum level of ANA (P value 0.0287). From these results our study revealed that the serum level of ANA was significantly higher in the PCOS group than the control group.

In the study of Reimand K. et al 2001 who 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 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 [17].

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Hassan M et al 2014 proved that PCOS was associated with elevated serum levels of ANA in which (38%) of PCOS group had positive serum level of ANA while (8%) were positive for control group (P value <0.001) which is consistent with our study [15].

However, these results disagree with Hefler-Frischmuth K et al 2010 who discovered that serum levels of ANAs were similar between the two groups in a study of 109 women with PCOS and 109 age-matched healthy controls [18].

Bahareh Hamedi et al 2014 studied the relationship between serum levels of ANAs 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 ANAs which disagree with our study [12].

Our study showed that 10 patients (25%) had positive serum level of dsDNA and 30 patients (75%) had negative serum level of dsDNA in PCOS group while in control group, 2 patients (5%) had positive serum level of dsDNA and 38 patients (95%) had negative serum level of dsDNA (P value 0.0283). From these results, 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 controlgroup in a study of 109 women with PCOS and 109 age-matched healthy controls [18]

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 [19].

In the current study there is an abnormally elevated level of autoimmune markers (ANA and anti-dsDNA) in women with PCOS. This indicates abnormally exaggerated immune responses in these women and this might raise the idea of using some drugs as low dose aspirin, lower molecular weight heparin, and steroids during the course of ovulation induction in PCOS women.

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