

Assessment of Autoimmune Thyroid Markers in Euthyroid Egyptian Patients with Polycystic Ovary Syndrome

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Abstract: Introduction: Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women in child bearing period. A condition that causes irregular menstrual periods because monthly ovulation is not occurring and levels of androgens in women are elevated. The condition occurs in about 5 to 10 percent of women. Most, but not all, women with PCOS are overweight or obese, and they are at higher than average risk of developing diabetes and obstructive sleep apnea. Most women with PCOS are able to lead a normal life without significant complications. Objectives: The aim of this study was to assess the presence of thyroid antibodies and their levels in sera of euthyroid women with PCOS in Egypt. Patients and Methods: This study was conducted on 45 euthyroid women with PCOS and 18 healthy women as a control group in the Outpatient Clinics of Endocrinology Unit, Ain Shams University Hospital. PCOS was defined according to the revised 2003 Rotterdam criteria. Thyroid function was evaluated by measurement of serum TSH and FT4 levels by chemiluminescence immune assay. Antithyroid peroxidase and anti-thyroglobulin antibodies (anti-TPO and anti-TG, respectively) were detected as markers for thyroid auto-immunity by enzyme-linked immunosorbent assay (ELISA). Results: Women with PCOS had significantly higher levels of anti-TPO in comparison to controls (27 ± 10 and 21 ± 10 IU/mL, respectively; $p < 0.05$) and no significant difference was found in serum levels of anti-TG, TSH, or FT4 between the two groups. Patients with PCOS had a non significant higher prevalence of positive results for anti-TG and/or anti-TPO in comparison to controls (40% and 22.2%, respectively; $p > 0.05$), anti-TPO alone (28.9% and 16.7%, respectively; $p > 0.05$) and anti-TG alone (22.2% and 11.1%, respectively; $p > 0.05$). No significant associations were found between the assayed antibodies and thyroid hormones. Conclusions: It is important to screen for thyroid autoimmune markers in euthyroid women with PCOS. And that furthers studies are needed to confirm the relation between thyroid auto-antibodies and PCOs in such euthyroid patients.

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1. Introduction:

Polycystic ovary syndrome (PCOS) is considered to be the most common cause of anovulatory infertility in women in reproductive age. It is characterized by menstrual dysfunction, anovulation, and signs of hyper-androgenism (1).

Although the exact etiopathophysiology of this condition is unclear, PCOS can result from abnormal function of the hypothalamic-pituitary-ovarian (HPO) axis. A key characteristic of PCOS is inappropriate gonadotropin secretion, which is more likely a result of, rather than a cause of, ovarian dysfunction. In addition, one of the most consistent biochemical features of PCOS is a raised plasma testosterone level (2).

PCOS is also associated with peripheral insulin resistance and hyper-insulinemia, and obesity amplifies the degree of both abnormalities (3).

Autoimmune thyroid disorders are characterized by the presence of thyroid auto-antibodies (Abs), particularly anti-thyroid peroxidase (TPO) and anti-thyroglobulin (TG) auto-Abs.

Thyroid autoimmunity (TAI) is the most common autoimmune disorder in women of reproductive age, with a prevalence varying between 5 and 15%. It is five- to ten-times more common in women than in men and can be presented without thyroid dysfunction, thus remaining undiagnosed (4). Some diseases of the thyroid gland, such as hypothyroidism and hyperthyroidism are associated with fetal loss. More studies have confirmed this association and linked TAI with recurrent abortions. The mechanism involved in the association between TAI and pregnancy loss is not clear. It is postulated that the presence of thyroid Abs reflects a generalized activation of the immune system and a heightened autoimmune state against the fetal-placental unit (4).

2. Objectives

The aim of the study was to compare the levels of thyroid auto-antibodies in a group of Egyptian euthyroid women having PCOS with a control group of women in reproductive age to determine whether PCOS patients were at a greater risk of thyroid autoimmune diseases or thyroid dysfunction.

3. Patients and Methods:

3.1. Study Participants

This case-control study was performed between March and November 2014 in Cairo, Egypt. Women who are clinically and biochemically euthyroid with signs of hyperandrogenism and/or oligomenorrhea were included in this study. PCOS was defined according to the revised 2003 Rotterdam criteria (5), which require the presence of at least two of the following indicators:

i. Ovulatory disturbance, mainly oligomenorrhea or amenorrhea.

ii. hyperandrogenism as defined either clinically by hirsutism, or severe acne/seborrhea, and/or biologically by elevated levels of total or free testosterone;

iii. And polycystic ovaries at ultrasonography (6).

Controls were females in reproductive age with regular menstrual cycles, no signs of hyperandrogenism, normal ovaries on pelvic ultrasound examination, and normal serum levels of free testosterone.

The exclusion criteria for this study were the presence of other medical conditions that cause irregular menstrual cycles and androgen excess such as hyperprolactinemia, hypothyroidism, and hyperthyroidism. We also excluded women who were taking oral contraceptives or corticosteroids as well as patients who did not fulfill Rotterdam criteria.

The study group included 45 euthyroid women with PCOS, whereas 18 euthyroid normally ovulating women were studied as a control group.

A detailed history was taken that included current age, age at menarche, history of menstrual irregularity, acne, hirsutism, infertility, obstetric history, thyroid disorders, history of similar disorders in the family, contraceptive methods, and the current medications. All participants recruited voluntarily in the study and signed the informed consent form. At time of collecting samples none of the participants had mentioned the administration of iodine supplements; however, all of them were using iodinated salt in the diet.

3.2. Samples

Six milliliters of venous blood were collected under complete aseptic precautions using clot activator tube in the morning between the second and the fifth day of menstrual cycle. After complete

clotting, samples were centrifuged at 1000 xg for 15 minutes, and sera were aliquoted and stored frozen at -20°C prior to assay. Hemolysed samples were discarded, repeated freezing and thawing was avoided.

3.3. Methods

3.3.1. Assay of TSH and FT4 by chemiluminescence Immune Assay:

This is done on a fully automated Cobas e411 (Roche Diagnostics GmbH, Sandhofer Strasse, Mannheim, Germany) using instrument's manufacturer reagents. TSH assay is a sandwich immunoassay using a biotinylated TSH antibody and a ruthenium-labeled TSH-specific antibody for capture and detection, respectively. Meanwhile FT4 assay is a competitive chemiluminescent immunoassay using a specific anti-T4 antibody labeled with a ruthenium complex for capture and detection. Chemiluminescence is the detection signal. TSH standard reference range is 0.27-4.2 μ IU/mL; intra-assay coefficient of variation [CV], 1.5%-8.6%; and inter-assay CV, 1.8%-8.7%. Free thyroxine (FT4) reference range is 0.93-1.7 ng/dL; intra-assay CV, 1.4%-2.9%; and inter-assay CV, 2.7%-6.6%.

3.3.2. Assay of Anti-TG and Anti-TPO by ELISA:

The assays of auto-antibodies were performed using the anti-TG and anti TPO ELISA kits supplied by Genway Biotech Inc. (Nancy Ridge Drive, San Diego, CA, USA). The two assays are solid phase quantitative ELISA based on sandwich principle. The microtiter strips are pre-coated with human thyroglobulin (TG) in the first assay and recombinant thyroid peroxidase (TPO) in the second assay; to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material, horseradish peroxidase (HRP) labeled anti-human IgG conjugate is added. This conjugate binds to the captured TG-specific antibodies and the captured TPO-specific antibodies, in the first and second assays, respectively. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of TG specific IgG antibodies in the specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint color. Absorbance at 450 nm is read using an ELISA microwell plate reader. Results are deduced from a calibration curve deduced from standard results for each assay obtained in the same run and are expressed in IU/mL. Antithyroid peroxidase antibody (anti-TPO) reference value is < 35 IU/mL; intra-assay CV, 2.5%-7.0%; and inter-assay CV, 7.1%-24.4%, and anti-thyroglobulin antibody (anti-TG); reference value is < 20 IU/mL; intra-assay CV, 4.6%-5.6%; and inter-assay CV, 5.9%-8.7%.

3.4. Statistical Analysis

Values were reported as mean \pm standard deviation (SD). Mean values were compared using Student's t test and differences in positive results between groups were tested using Chi squared or Fisher exact test. The association of both anti-TPO and anti-TG with TSH and FT4 were assessed using Pearson's correlation test. A p value < 0.05 was considered statistically significant; $p < 0.01$ highly significant; and $p > 0.05$ not significant. All statistical analysis was done by using IBM PASW version 18.

4. Results:

Results are presented in tables (1-3) and figure (1). Table (1) shows that patients with PCOS are significantly younger in comparison to controls ($t = 4.157$, $p < 0.001$). The mean age of the patients with PCOS and controls was 22.24 ± 4.7 years (range, 16-31) and 27.89 ± 5.4 years (range, 19-36), respectively. It also shows a statistically significant higher BMI in PCOS in comparison to controls with $t = 2.505$ and $p = 0.015$.

Comparison between patients and controls revealed that 7 patients (15.6%) had family history of hypothyroidism, hyperthyroidism, or goiter among which two patients had positive results for anti-TPO and one had just positive results for anti-TG. Among controls, only one woman (5.6%) had a family history of hypothyroidism and positive results for both anti-TPO and anti-TG.

The difference of the prevalence of thyroid auto-antibodies among studied groups are shown in Table (2) and Figure (1). Our results showed that positive

results for anti-TG and anti-TPO were more prevalent in women with PCOS. From 45 euthyroid patients with PCOS, 13 (28.9%) patients had positive results for anti-TPO, in comparison to three women (16.7%) in the control group; though not significantly different ($p = 0.522$). Positive results for anti-TG was seen in 10 (22.2%) patients and 2 women (11.1%) in the control group ($p = 0.482$).

Positive results for presence of auto-antibodies (anti-TPO and/or anti-TG) was noticed in 18 (40%) patients and only 4 women (22.2%) in the control group ($p = 0.246$).

Table (3) shows that serum levels of anti-TPO were significantly higher in women with PCOS in contrast to the controls ($p = 0.029$); however, serum levels of anti-TG, TSH, and FT4 of the patients with PCOS and the controls did not differ significantly.

No significant correlation was revealed between anti-TPO and TSH ($r = 0.065$, $p = 0.615$) or between anti-TPO and FT4 ($r = -0.102$, $p = 0.425$) in patients. No significant correlation was revealed between anti-TG and TSH ($r = -0.126$, $p = 0.324$) or between anti-TG and FT4 ($r = -0.104$, $p = 0.418$) in patients.

Table 1: Statistical Comparison of the Demographics between Patients and Controls using Student's t test

	Controls (n=18)	PCOS (n=45)	t	p
Ages (Yrs)	27.89 \pm 5.4	22.24 \pm 4.7	4.157	0.00
BMI (Kg/m ²)	24.2 \pm 2	25.9 \pm 2.5	2.505	0.015

$p < 0.01$, < 0.001 : Highly significant difference

Table 2: Statistical Comparison of the Intervening Variables between Patients and Controls Using Fisher Exact test

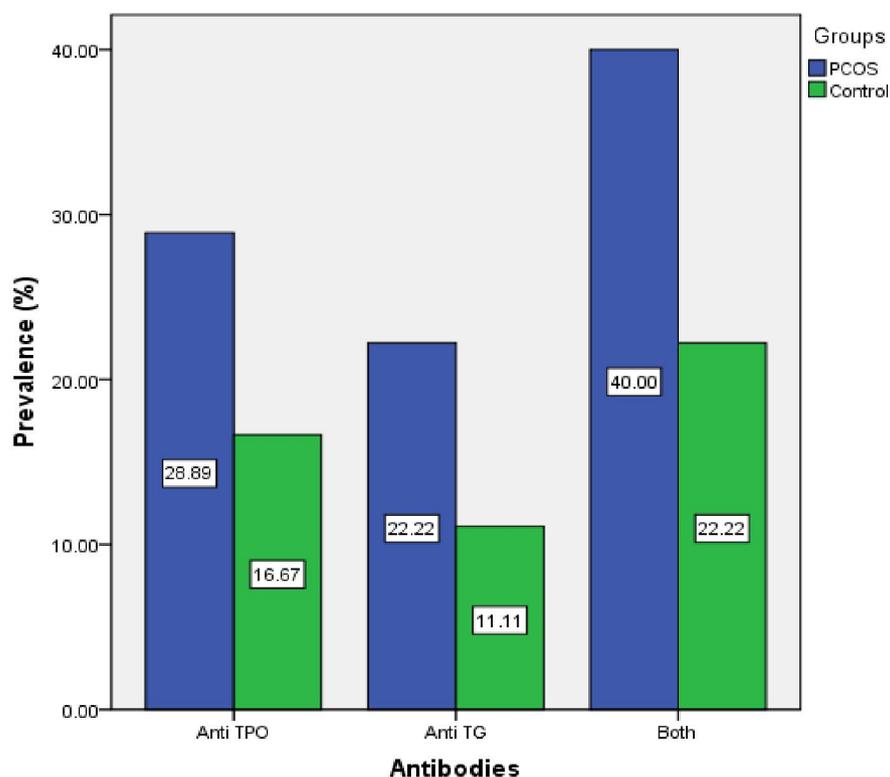
	Controls (n=18)	PCOS (n=45)	p Value
Family history of thyroid disease	1 (5.6%)	7 (15.6%)	0.421
Positive Anti TG	2 (11.1%)	10 (22.2%)	0.482
Positive Anti TPO	3 (16.7%)	13 (28.9%)	0.522
Positive auto-antibodies (Anti TPO and/or Anti TG)	4 (22.2%)	18 (40%)	0.246

Data are presented as frequency. $p > 0.05$: Non-significant difference.

Table 3: Statistical Comparison of the Studied Thyroid Markers between PCOS Patients and Controls

	PCOS (n=45)		Control (n=18)		p Value
	Mean	\pm Standard Deviation	Mean	\pm Standard Deviation	
Free T4(ng/dL)	1.14	0.20	1.17	0.23	0.673
TSH(μ IU/mL)	2.20	0.99	2.33	0.79	0.612
Anti TPO(IU/mL)	27	10	21	10	0.029
Anti TG(IU/mL)	15	7	12	7	0.124

Data are presented as mean \pm SD. $p > 0.05$: Non-significant difference; $p < 0.05$: Significant difference;



Stacked Bar chart showing the difference in the prevalence of positive thyroid auto-antibodies among studied groups.

Abbreviations: PCOS: polycystic ovary syndrome; TSH: thyroid stimulating hormone; anti-TPO: anti thyroid peroxidase antibody; anti-TG: antithyroglobulin antibody., BMI: body mass index;

Figure 1: Prevalence of Antithyroid Peroxidase and Antithyroglobulin Antibodies Positivity in Patients with Polycystic Ovary Syndrome and Controls

5. Discussion:

Patients with PCOS often have defective progesterone secretion which leads to an increased estrogen to progesterone ratio. Estrogen can increase the expression of interleukin-6 in T cells and the absence of inhibitory action of progesterone may lead to over-stimulated immune system and makes these patients more prone to autoimmune disorders (7). An increased level of circulating C-reactive protein (CRP) has been demonstrated in patients with PCOS, which is considered a kind of low-grade inflammation (8).

Also Hefler-Frischmuth, 2010, showed that some serologic markers of autoimmunity were elevated in patients with PCOS, and various systemic and organ-specific auto-antibodies have been recognized in patients with PCOS (9). Thyroid hormones have an important role before and during pregnancy and to our knowledge, there was no study that had evaluated the thyroid autoimmunity in patients with PCOS in Egypt; hence, it seemed necessary to conduct a study concerning this syndrome.

In the present study, serum levels of anti-TPO were significantly higher in those women with PCOS

in contrast to the controls. A non-significant higher prevalence of thyroid auto-antibodies in patients with PCOS in comparison to the controls was noted. Out of 45 euthyroid patients with PCOS, 18 (40%) had positive results for thyroid auto-antibodies (anti-TG and/or anti-TPO) in comparison to the controls with 4 (22.2%) positive result in 18 participants. The exact prevalence of PCOS in Egyptian population is unknown; however, based on the diagnosis rate of new cases, it has become obvious that PCOS is a common cause of pregnancy complications among Egyptian women in reproductive age.

Our findings are very close to those of Janssen et al. (10) study in Germany where elevated levels of anti-TPO or anti-TG were detected in 47 (26.9%) of 175 patients with PCOS in comparison to only 14 (8.3%) out of 168 controls. While another study by Sinha et al. (11) reported positive results for anti-TPO in 22.5% of 80 patients with PCOS in contrast to 1.25% positive results of 80 controls. Another study by Ozdemir et al. (12) found that 37.8% of 107 patients with PCOS had positive anti-TPO or anti-TG. The small differences in the percentage between

studies could be attributed to different value of cutoff point and the size of studied groups.

A 2012 study showed that women with PCOS had a 65% increase in thyroid peroxidase antibodies, and a 26.6% increase in the incidence of goiter, when compared to age-matched subjects (13) and these findings were also similar to ours.

Another 2013 analysis found that in a total of 6 studies involving 1605 women, there was an increased prevalence of autoimmune thyroiditis, increased serum TSH, increased anti TPO antibodies, and anti TG antibodies in women with PCOS when compared to control groups (14). In addition to the higher incidence of autoimmune thyroid disease in women with PCOS, Ott and colleagues postulated that women suffering from PCOS-related infertility who also had high anti-TPO levels were significantly more likely to be resistant to Clomid (15).

Our results are also in agreement with another cross-sectional study from Syria that was conducted in euthyroid patients with PCOS and showed that from 56 euthyroid patients with PCOS, 11 (19.6%) patients had positive results for anti-TPO, in comparison to only one woman (3.3%) in the control group ($\chi^2 = 4.3$, $p = 0.037$). Positive results for anti-TG was seen in 12 (21.4%) patients and 1 woman (3.3%) in the control group ($\chi^2 = 4.9$, $p = 0.026$) (16). Their results revealed positive auto-antibodies (anti-TPO and/or anti-TG) in 16 (28.6%) patients and only 1 (3.3%) control (16). They also showed that serum levels of anti-TPO were significantly higher in PCOS patients in comparison to controls. This gives importance to the investigation of thyroid autoimmunity in this group of patients. Patients with anti-TPO and anti-TG are more likely to develop thyroid dysfunction later in life. Kachuei *et al.* (13) from Iran agree much with our study regarding the significantly higher levels of serum anti-TPO in patients with PCOS than in controls (216 ± 428 vs. 131 ± 364 IU/mL; $p = 0.04$); and that serum levels of anti-TG did not show any significant difference between groups.

In the present study, TSH and FT4 levels did not differ significantly between patients with PCOS and controls while some studies such as Janssen *et al.* (10) reported that TSH levels were higher in patients with PCOS; this may be explained by considering that we only included euthyroid participants in our study. These findings require more investigations to understand the underlying association between PCOS and thyroid autoimmunity and the mechanism by which this common condition alters pregnancy outcomes.

5.1. Limitations

The trend of the difference in the non significant results was promising but statistical results were compromised by limited candidates' number.

Moreover, data concerning family and personal medical history were obtained by asking participants as medical files were not available. .

5.2. Conclusions

This study shows that PCOS was associated with presence of positive thyroid auto-antibodies in the sera of Egyptian patients; so that, these patients could be at increased risk of thyroid disorders (and fetal loss?). Thyroid autoimmunity markers should be requested in patients with PCOS who decide to get pregnant even when there is no evidence of overt thyroid dysfunction.

5.3. Recommendations

Further prospective studies are needed to confirm the relation between thyroid auto-antibodies and PCOs in such euthyroid patients.

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