# Serum immune profiling of patients with polycystic ovary syndrome

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## Abstract

**Background**: Polycystic ovary syndrome (PCOS), the most prevalent hormonal disorder in females, is characterized by low levels of progesterone, which causes increased estrogen levels leading to production of various anti and auto-antibodies. This study aimed to estimate and compare levels of anti-nuclear antibodies (ANA), anti-thyroid peroxidase (anti-TPO), anti-thyroglobulin (anti-TG), and anti-islet cell antibodies (anti-ICAb) in patients with PCOS and healthy controls. **Methods**: The present comparative study included 82 subjects divided into two groups with 41 individuals in each group. Group I included healthy subjects, while Group II included patients diagnosed with PCOS. Blood samples were collected to determine serum levels of ANA, anti-TPO, anti-TG, and anti-ICAb using commercially available ELISA kits. Data were analyzed by using SPSS 20.0. **Results**: Two (4.8%) subjects in Group II had ANA, but none of the other healthy individuals had these auto-antibodies. Levels of anti-TPO were higher in Group II (6.01 IU/ml) than in Group I (5.98 IU/ml). Levels of anti-TG and anti-ICAb were higher in Group I (19.86 and 32.49 IU/ml, respectively) than in Group II (19.78 and 26.07 IU/ml, respectively). **Conclusion**: Levels of ANA and anti-TPO were higher in patients with PCOS than in controls. By contrast, levels of anti-TG and anti-ICAb were higher in controls than in patients with PCOS.

*Keywords: amenorrhea, antibodies, oligomenorrhea, polycystic ovary syndrome*

## Introduction

Polycystic ovary syndrome (PCOS) was first reported in 1935 by American gynecologists.[1] Approximately 6–10% of women worldwide have PCOS,[2] and about 5–10% of women in Pakistan have this disease.[3] In women during reproductive age, PCOS may result in oligomenorrhea, anovulation, menorrhagia, and infertility. Presumptive diagnosis of PCOS is made by assessing the clinical signs and symptoms of hyperandrogenism and confirmed by polycystic ovaries on ultrasonography;[4,5] in polycystic ovary, each ovary contains 12 follicles with a diameter of 2–9 mm.[6,7] A woman with two out of three clinical signs, such as oligo-anovulation, hyperandrogenism, and polycystic ovaries, is diagnosed as PCOS.[8]

The majority of PCOS patients are overweight (BMI = 25 and <30 kg/m$^2$) to obese (BMI = 30 kg/m$^2$); however, up to one-third of these patients may be normal or even underweight.[9] Excessive hair growth and acne occur in up to two-thirds of patients; androgenic alopecia is not very common (<10%) in PCOS.[10] The characteristic features of PCOS include increased serum luteinizing hormone (LH), high LH/FSH (follicle stimulating hormone) ratio, and increased amplitude and frequency of pulsatile LH secretion.[11–13]

The hypothalamus secretes gonadotrophin releasing hormone (GnRH), which causes the release of LH and FSH. These hormones bind ovarian cells that release estrogen and inhibin, which regulate the menstrual cycle.[14–17] Progesterone maintains the secretion of GnRH during the menstrual cycle. In PCOS, progesterone level decreases and cannot suppress GnRH/LH levels. Therefore, increased estrogen levels lead to the production of auto-antibodies, such as anti-nuclear antibodies (ANA), anti-thyroid peroxidase (anti-TPO), anti-thyroglobulin (anti-TG).[18]

ANA is the hallmark of several autoimmune disorders, including SLE, Sjogren's syndrome, and polymyositis.[19] During reproductive age, autoimmune thyroiditis is three times more common in PCOS women than in normal women.[20] Anti-thyroid antibodies, such as anti-TPO antibodies, thyrotrophic receptor antibodies, and anti-TG thyroglobulin antibodies, are associated with infertility, miscarriage, preterm birth, postpartum thyroiditis, Graves's disease, and Hashitomoto's thyroditis.[21–24]

Production of ANA, anti-thyroid, and anti-islet cell antibodies (anti-ICAb) are hallmarks of autoimmunity. Thus, the present study was designed to determine

levels of ANA, anti-thyroid, and anti-ICAb in women with PCOS.

## Methods

The present comparative cross-sectional study was carried out at the Department of Immunology University of Health Sciences, Lahore, Pakistan, after obtaining approval from the Ethical Review Committee of UHS. A total of 82 subjects were recruited for this study following the inclusion and exclusion criteria, and these subjects were divided into two groups with 42 individuals in each group. Group I comprised healthy individuals as controls, and Group II comprised patients diagnosed with PCOS.

**Inclusion criteria.** Group I (control group): healthy individuals (non-PCOS); female; reproductive age, 12–45 years. Group II (patient group): clinically suspected PCOS confirmed on ultrasonography; female; reproductive age, 12–45 years.

Females of reproductive age with clinical features of PCOS confirmed on ultrasound were included in the patient group, while patients with autoimmune disorders, pregnancy, and chronic disorders such as tuberculosis, Cushing syndrome, thyroid diseases, and malignancy were excluded. A total of 82 subjects were recruited to this study by purposive sampling after obtaining written informed consent. Determination of ANA, anti-TPO, anti-TG, and anti-ICAb was performed by using ELISA.

The data were analyzed by using IBM SPSS-20. Quantitative variables were expressed as mean±SD, while qualitative variables were expressed as frequencies and percentages. During comparison of auto-antibodies, the Mann–Whitney U test was applied for non-normally distributed data, and the chi-squared test was used to determine the association between clinical history and PCOS. A $p \leq 0.05$ was considered statistically significant.

## Results

In Group II, two (4.8%) subjects had ANA, 5 (12.1%) had anti-TPO, and 3 (7.31%) had anti-TG antibodies. In Group I, none of the subjects had ANA (0%), 4 (9.7%) had anti-TPO, and 1 (2.43%) had anti-TG antibodies. Comparisons showed no statistically significant difference between groups ($p$ = 0.49, 1.00, 0.61, respectively; Table 1). Levels of anti-TPO antibody were higher in Group II (6.01 IU/ml) than in Group I (5.98 IU/ml), and the difference observed was not statistically significant ($p$ = 0.65). Levels of anti-TG and anti-ICAb were higher in Group I (19.86 and 32.49 IU/ml, respectively) than in Group II (19.78 and 26.04 IU/ml, respectively), and comparisons revealed no statistically significant difference between groups ($p$ = 1.00, 0.56, respectively; Table 2).

**Table 1.** Frequency, percentage, and comparison of auto-antibodies between the two groups

| Antibodies | Group I N (%) | Group II N (%) | $p$ |
|---|---|---|---|
| ANA | 0 (0%) | 2 (4.8%) | 0.494 |
| Anti-TPO | 4 (9.7%) | 5 (12.1%) | 1.000 |
| Anti-TG | 1 (2.43%) | 3 (7.31%) | 0.616 |

ANA: anti-nuclear antibodies, Anti-TPO: anti-thyroid peroxidase, Anti-TG: anti-thyroglobulin.

**Table 2.** Level and comparison of different auto-antibodies between two groups

| Antibodies | Group I Median (IU/ml) | Group II Median (IU/ml) | $p$ |
|---|---|---|---|
| Anti-TPO | 5.98 | 6.01 | 0.65 |
| Anti-TG | 19.86 | 19.78 | 1.00 |
| Anti-ICAb | 32.49 | 26.04 | 0.56 |

Anti-TPO: anti-thyroid peroxidase, Anti-TG: anti-thyroglobulin, Anti-ICAb: anti-islet cell antibodies.

**Table 3.** OHIP-14 scores according to oral hygiene practices (N= 31)

| Variables | Group I | Group II | $p$ |
|---|---|---|---|
| Age (years) | 26±6.39 | 27±6.53 | 0.54 |
| Menarche age (years) | 14±1.31 | 13±1.46 | 0.23 |
| Weight (kg) | 51.46±9.24 | 60.39±9.57 | <0.001[*] |
| Height (meters) | 4.79±0.44 | 5.11±0.47 | 0.07 |
| Married | 22 (53.6%) | 19 (46.3%) | 0.050 |
| Menstrual irregularity | 5 (12.1%) | 35 (85.3%) | <0.001[*] |
| Acne | 6 (14.6%) | 30 (73.1%) | <0.001[*] |
| Hirsutism | 3 (7.3%) | 33 (80.4%) | <0.001[*] |

Data presented as mean ± SD or frequency and percentage; *$p \leq 0.05$

The percentage of females with menstrual irregularity, acne, and hirsutism was higher in Group II (85.3%, 73.1%, and 80.4%, respectively) than in Group I (12.1%, 14.6%, and 7.3%, respectively); upon comparison, differences between these two groups were statistically significant (all $p < 0.001$). The mean±SD of weight was higher in Group II (60.39 ± 9.57) than in Group I (51.46±9.24). Comparisons revealed statistically significant differences between the two groups ($p < 0.00$; Table 3).

## Discussion

The percentage of subjects who had ANA was higher in Group II than in Group I, which agrees with a previous study[25] that found ANA in 8.6% of PCOS and 0% of healthy controls. This result, however, disagrees with a study[26] that reported ANA in 36% of PCOS and 6% of healthy controls and another study[27] that revealed ANA in 38% of PCOS and 8% of healthy controls. This difference in results may be due to differences in sample size and environmental factors.

In this study, levels of anti-TPO antibodies were slightly higher in PCOS than in controls. This finding is in concordance with other researchers[28] who suggested anti-TPO levels of 28±9.1 in PCOS and 26±8.2 in controls. The present study, however, disagrees with a previous study[29] that documented anti-TPO antibody levels of 321±190 IU/mL in PCOS and 22±7.2 IU/mL in healthy controls. The disagreement between results could be due to the inclusion of young euthyroid girls with a complaint of PCOS and the larger sample size (175 subjects) in the previous study.[29]

In the current study, anti-TG antibody levels were nearly identical between PCOS and the controls, with agrees with a previous study[30] that reported the presence of these antibodies in 47% of PCOS and 41% of healthy females. The current study also agrees with another study[31] performed by Oun and his coworkers that found anti-TG antibody in 36% of PCOS and 30% of healthy controls. However, this result contrasts other researchers[20] who found anti-TG contents of 113±312 IU/mL in PCOS and 4±17 IU/mL in healthy controls. Differences in lifestyle, obesity, smoking, alcohol consumption, and stress, as well as a larger sample size, may explain this contradiction.

The anti-ICAb levels of the controls and PCOS were below the reference range; specifically, the subjects were negative for anti-ICAb. This result is in accordance with a previous study[27] that suggested that none of its PCOS and healthy subjects had ICAb. Although data on levels of anti-ICAb in PCOS are limited, a previous study[32] reported that 18.8% of diabetic females have PCOS whereas 6.5% of non-diabetic subjects have PCOS. The difference between these groups was statistically significant.

The mean±SD of age in Group II was higher than that in Group 1, in line with a previous study[33] that suggested similar findings among PCOS and controls. However, this result contrasts another study.[31] Although the mean age of PCOS patients in this previous study is relatively similar to that in the present study, environmental factors, such as diet and stress level, could contribute to the differences observed.

The mean of age of menarche was higher in the controls than in PCOS, which agrees with a published study[34] that also suggested a high age of controls. This result, however, contradicts another study[29] in which the mean age of menarche of PCOS patients was higher compared with that of controls. The lower mean age of menarche in such studies may be due to racial differences in study populations.

The percentage of menstrual irregularity was higher in PCOS than in controls, in accordance with a previous study[28] that observed a high number of menstrual irregularities in PCOS patients. The present study, however, contrasts a previous study[35] that observed more menstrual irregularities in controls. Differences observed may be due to the small sample size of the current study. Trends related to such epidemiological factors can only be predicted accurately by using larger study samples.

The percentage of acne was higher in PCOS than in controls, in accordance with a study[35] that suggested a higher frequency and percentage of acne in PCOS as compared with controls. The percentage of hirsutism was higher in PCOS compared with controls, in line with previous study[28] that also found a higher frequency and percentage of hirsutism in PCOS.

The mean of weight of PCOS was higher than that of the controls. This result agrees with a previous study[26] that documented high weight in PCOS patients but contradicts the findings of a study.[34] in which the mean weight of the controls was high. Differences in the body weight of the subjects of a study may be due to inclusion the of diabetic females as controls.[34] The mean of height of PCOS was higher than that of controls, which contrasts a previous study[26] that found a greater mean height in controls.

## Conclusion

ANA and anti-TPO antibodies were expressed more extensively in patients with PCOS than in controls. By contrast, anti-TG and anti-ICAb antibodies were expressed more extensively in the control group than in

patients with PCOS. However, differences in expression between groups were not statistically significant. Therefore, autoimmunity may not play a significant role in PCOS.

## Funding

## Conflict of Interest Statement

The authors declare(s) that there is no conflict of interest regarding the publication of this paper.

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