Total antioxidant capacity and oxidative stress in Polycystic ovary syndrome, a case-control study

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Abstract

Introduction: Polycystic ovary syndrome (PCOS) is the most common endocrinological syndrome among reproductive-age women. Oxidative stress (OS) elaborates on PCOS pathological process. OS is a state where oxidative powers exceed the antioxidant systems, serum's ability to reduce the free radical's formation and protect the cell from oxidative stress. TAC protects the cell from the harmful effects of radicals. Examples are superoxide and radical hydroxyl ions. Any change in the level of plasma's antioxidants or oxidative stress can disturb TAC.

Aim of the study: This study evaluated serum Total Antioxidant Capacity (TAC) levels in PCOS patients compared to the healthy control group.

Methods: The women with PCOS were considered cases and TAC levels compared to healthy women. All were recruited from outpatient clinics in the Duhok governorate in Iraqi Kurdistan between November 2021 and February 2022. One hundred twenty women (60 PCOS patients and 60 healthy subjects). PCOS was diagnosed according to the Rotterdam criteria (2003). All patients underwent clinical assessment.

Results: The PCOS patients and controls were similar in age and BMI. The PCOS showed highly significant differences in the clinical parameter as hirsutisms. Patients with PCOS are likely to have a morphology of>12 follicles per ovary (95.0%) in contrast to <12 follicles per ovary (93.33% in the controls (P<0.0001). The PCOS patients had substantially higher volumes of the right and left ovary than the controls (P=0.0008). A similar pattern was found for the morphology of the left ovary. This study showed highly significant differences in the mean concentration of testosterone in PCOS patients, which was significantly higher (0.31) compared to its attention in the controls (0.19; P<0.0001). PCOS patients had a substantially lower level of TAC (2.24) compared to the controls (2.51; P<0.0001).

These parameters showed in PCOS patients on the level of TAC; all parameters have no significant effect except for age.

Conclusions: This study revealed that the serum TAC levels

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are significantly lower in PCOS patients than in control patients.

Keywords: Total antioxidant capacity, Polycystic ovary syndrome, oxidative stress

Introduction

Polycystic ovarian syndrome (PCOS) is the most typical endocrine disorder that causes reproductive complaints. This syndrome is characterized by the presence of two or more criteria, which are both clinical and biochemical. It includes excessive androgen, chronic anovulation (CA) or oligo-ovulation, and polycystic ovaries morphology (PCOM) [1-6].

PCOS is associated with a metabolic disorder [7], a heterogeneous disease with individualized predisposition detected by genetic and environmental causes between 9% and 18% in reproduction ages. About 80% of women have anovulatory infertility [8]. PCOS has been mainly related to low-grade chronic inflammation and oxidative stress (OS) [9, 10].

OS is identified as a situation where oxidative powers exceed the antioxidant systems leading to imbalance. The Reactive Oxygen Species (ROS) rise in response to oxidative stress [11]. The main actions of ROS in the cell functions are activating redox-sensitive transcription factors; it is an essential issue in human reproductive medicine [12].I t elaborates on the pathological processes that go with insulin resistance (IR), hyperandrogenism, and obesity [13]Metabolic syndromes accelerate the OS progression in patients with PCOS and minuscule the antioxidative capacity [14]. OS is closely related to PCOS pathogenesis as it suggests OS in PCOS patients is more severe than in non-PCOS patients [15]. The molecules in the cells that avert these OS reactions are named antioxidants, organized by a complex antioxidant system.[16].

Total Antioxidant Capacity (TAC) is the ability of serum to reduce the free radical's formation and protect the cell construction from the harmful effects of radicals. It is one of the antioxidant fortifications present in the body. Any modification in the plasma level of antioxidants or oxidative stress can disrupt TAC [17]. TAC may be used in the Workup of PCOS as a test of diagnosis and progress [18]. TAC measures the total antioxidants in the patient serum [17]. Also, TAC in Follicular fluid (FF) creates a microenvironment for the emerging oocyte and has a straight effect on the quality of the oocyte; it would increase during the growth of follicles because of the TAC developmental capability [19]. Insufficient antioxidants in FF may oppose these roles [20].

Regarding female infertility, particularly PCOS, evidence has shown decreased antioxidant status in the insulin resistance of PCOS patients [21]. Some studies are conducted to identify the correlation between PCOS and oxidative stress using other microelements rather than TAC [1, 17, 18, 22-26]. One only depended on biochemical criteria [22]. However, results would be more reliable when reassessed by more than one parameter. TAC may be used in the Workup of PCOS as a test of diagnosis and progress [18]

Patients and methods

In this case-control study, the women with PCOS were considered the cases, and levels of TAC were compared to healthy women. One hundred twenty women (60 PCOS patients and 60 healthy subjects). PCOS was diagnosed according to the Rotterdam criteria (2003).

The cases and controls were recruited from the outpatient clinics in Sumel and Sennuny, Duhok governorate in Iraqi Kurdistan, between November 2021 and February 2022. The patients who attended the outpatient clinics were clinically screened for the eligibility criteria. The data were collected from the patients and controls following approval by the Duhok General Health Directorate and

ethical committee of Duhok University and the ministry of health of Kurdistan. The letter of consent was obtained from the patients before inclusion in the study. The procedure study procedure explained the patients before inclusion in the study.

The patients included in this study were 18 and 38 years old. The patients were screened endovaginally by ultrasound. A blood sample was taken from each patient. The diagnosis of PCOS was made according to the Rotterdam European Society of Human Reproduction, ion and Embryology (ESHRE) revised consensus criteria 2003 [2-5]. Chronic anovulation (CA), hyperandrogenism, clinical (including signs such as hirsutism) or biological increase level of testosterone, and polycystic ovaries visible on ultrasound as the presence of at least one of ovaries with >10 cm3 or comprise at least 12 follicles between 2 to 9 mm in diameter.

Clinical examination

Regarding the three criteria, CA (chronic anovulation) is defined as fewer than eight menstrual cycles per year or more than 35 days between cycles. Hyperandrogenism is characterized by clinical features (acne, hirsutism, and androgenic alopecia) or raised testosterone levels. Ultrasound features are classified as extra than 12 antral follicles (measuring 2–9mm in diameter) or an ovarian volume more significant than 10 cm3 in either ovary.

Physical examination

All women underwent measurement for weight and body mass index (BMI). Systemic examination and assessment of the hair according to the Ferriman-Gallwey score were done [27].

Exclusion Criteria

They are hypo and hyperthyroidism, congenital adrenal hyperplasia, androgen tumors, hyperprolactinemia, chronic medical diseases such as insulin-dependent diabetes mellitus, hypertension, heart and blood vessels disease, and uterine cancer.

Measurements

All patients underwent assessment for main risk factors such as age, previous pregnancy, parity, marital status, education, irregular cycle, alcohol consumption, family history of PCOS, family history of diabetes, infertility problem, and mother's history of menstrual abnormality [28].

Transvaginal ultrasound

All have a transvaginal ultrasound, DW C60 made by DAWEI medical brand from China with a CE-supported certificate. The frequency othe f vaginal probe was between 7.5-10 MHz to identify PCOS, which means the presence of at least one of the ovaries with >10 cm3 or containing at least 12 follicles 2 to 9 mm in diameter.

Blood Samples

Hormonal tests, testosterone, and TAC testosterone were measured from plasma by an automated instrument. Plasma total antioxidant capacity (TAC) was assessed using the Enzyme-Linked Immunosorbent Assay (Human Total antioxidant status (TAOS) ELISA Kit

And Human Total antioxidant capacity-AOC ELISA Kit) [29, 30]. Serum sample serum was selected and frozen until the complete number was ordered. The testosterone test was performed by Cobas e411 (ROCH diagnostics), an automated instrument, principle, ELICIA.

Statistical Analysis

The general information of the patients and cases were presented in mean and standard

deviation or number and percentage. The homogeneity of the PCOS and control groups in terms of age and BMI was examined in an independent t-test and Pearson Chi-squared test. The transvaginal ultrasound (U/S) comparisons between patients with PCOS and controls were analyzed in an independent t-test and Pearson Chi-squared test. Comparisons of Testosterone and TAC test results between patients with PCOS and controls were examined in an independent t-test. The role of general characteristics, Testosterone, and transvaginal ultrasound outcomes on the level of TAC in PCOS patients was examined in standard least squares with affect leverage. The normality of the data was checked by drawing a histogram, and the outliers were checked by drawing the box plots of two study groups. The extreme outliers were not included in the mean and Standard Deviation (SD) measurement of the outcomes and other information in this study. A p-value of less than 0.05 determined the significant level of difference. The statistical calculations were performed in JMP Pro 14.3.0 tool.

Results

The study found that the PCOS patients and controls were similar in age (26.34 vs. 27.91 yrs., P=0.0739) and BMI (26.55 vs. 26.60 kg/m2; P=0.9532). The majority of the patients and controls were obese (45.0% vs. 38.33%), followed by average weight (33.33% vs. 38.33%; P=0.9034), respectively (Table 1).

The study showed that the PCOS patients had moderate (85.0%) and severe (15.0%) hirsutism in contrast with minimal (45.0%) and mild hirsutism (55.0%; P<0.0001). In terms of the morphology of the right ovary, the study showed that PCOS patients were more likely to have >12 follicles per ovary (95.0%), while the controls were more likely to have <12 follicles per ovary (93.33%; P<0.0001). A similar pattern was found for the morphology of the left ovary. In addition, the study showed that PCOS patients were more likely to have an irregular menstrual cycle (68.33%) compared to a regular menstrual cycle (58.33%; P=0.0033), see Table 2, Figs 1-2).

The study showed that the mean concentration of testosterone was significantly higher in PCOS patients (0.31) compared to its concentration in the controls (0.19; P<0.0001). But, the PCOS patients had a substantially lower level of TAC (2.24) compared to the controls (2.51; P<0.0001), see Table 3 and Fig 3.

In terms of the role of general characteristics and biomedical measurements on the level of TAC in PCOS patients, the study showed that TAC is highly associated with increasing age. The study showed that the level of TAC significantly decreased with age older than 26.0 years. Other medical and clinical factors were not shown to associate with the level of TAC in PCOS patients (Table 4 and Fig 4).

DISCUSSION

This case-control study showed that the PCOS patients had a significantly lower TAC level than matched control women. The literature has stated that oxidative stress (in contrast to TAC) is higher in PCOS patients [14, 15, 21, 31-33]. TAC is the facility of serum to overpower the oxidative stress to retain the cell structure healthy from the adverse effects of oxidative materials. TAC was lower in PCOS patients in our study, in agreement with other studies, as all documented a decrease in TAC level. Patient with PCOS. TAC could be used to prevent the growth or development of insulin resistance and oxidative stress by routine assessments. Also it may be working as an indicator in early diagnosis of PCS .[17, 22, 26].

Regarding the general characteristics, the mean age was 26.34 and 27.91 for both PCOS and the control group. BMI with a mean value of 26.55 for the study group and 26.60for

the control group. No significant difference among both groups was noticed for age and BMI. This could be one of the strong points of this study. So, the general characteristics were found to have no significant difference between both groups. Some studies documented that age negative correlates with TAC[34]. Some studies also reported that weight had decreased the TAC level [6, 35]. Neither age nor weight affected the TAC in this study, as the differences in these characteristics were insignificant between both groups.

The menstrual cycle assessment was considered one essential parameter of PCOS confirmation diagnosis. The confirmatory biochemical testosterone was found to be agreed with TAC which is found to be highly significant between PCOS and the control group. It was found that the mean value of testosterone level is higher in the study group, 0.31, than in control 0.11. Such an outcome level of testosterone means a correct choice for our study group of patients.

On physical examination between both groups of patients, hirsutism was assessed according to the Ferriman-Gallwey score [27]. Patients with moderate and severe hirsutism were mainly distributed among the PCOS group, with a highly significant outcome.

The role of general characteristics on the level of TAC. It is found that the level of TAC is decreased with increasing age [36]. Fortunately, the mean value of age in both control and PCOS were close, which doesn't affect our study's results. BMI in the profiler showed that as weight increased, the TAC level decreased, which agreed with a number of studies because these studies also reported that TAC level decreases with increased BMI and age [34, 36], but in underweight patients, that showed a decrease in TAC. A systematic review by Solmi also proved that the underweight has high oxidative stress [37]. Risi argued in a narrative review that obesity and underweight are two sides of one coin [38].

Other parameters include ovarian morphology and volume of both right and left ovaries, testosterone hormone level, menstrual cycle, and degree of hirsutism; all were found to be no significant change at the level of TAC in PCOS patients. Instead, the morphology assessment by endovaginally ultrasound and testosterone test profiler showed a negative correlation but not to a significant level.

Limitations

In this study, we only used total testosterone as a biochemical test far better if we used more than one test. We only used two-dimensional ultrasound to assess the ovarian volume and follicular number per ovary. Three-dimensional (3-D) ultrasound may be more promising regarding image quality, storage, data, and image interpretation. It may provide more understanding pathophysiology of PCOS severity assessment, regardless of the accuracy [39, 40].

Conclusions and Recommendations

This study showed that serum TAC levels are significantly lower in PCOS patients than in non-affected individuals. A prospective case-control and randomized control study are advisable to know about the fluctuation of the level of TAC with the change of weight and age and the severity of PCOS. Also, to know if the administration of antioxidants will affect the level of TAC. It is essential to consider TAC in routine monitoring of PCOS patients as a regular assessment if the above-recommended points are considered. Systematic review and metanalysis may be suggestable in this area of research.

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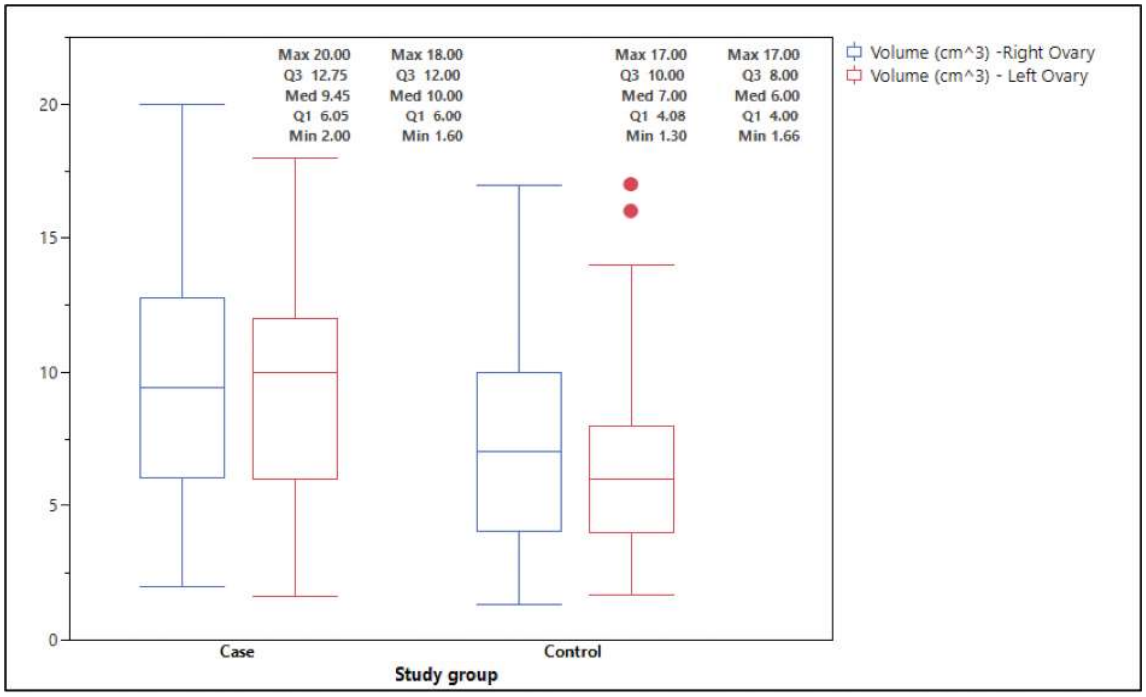

Figure 1: Comparisons of ovarian volume between patients with PCOS and controls

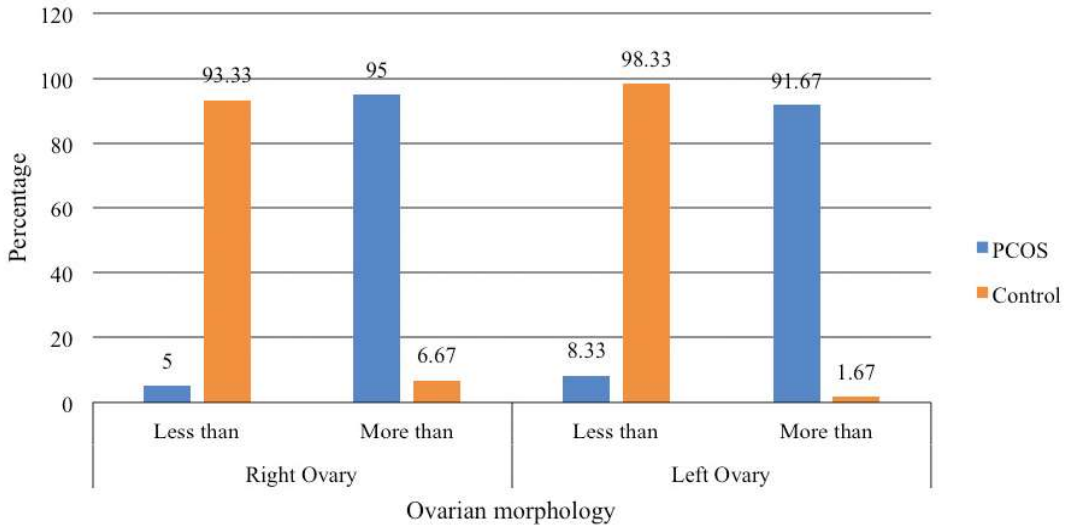

Figure 2: Comparisons of ovarian morphology between patients with PCOS and controls

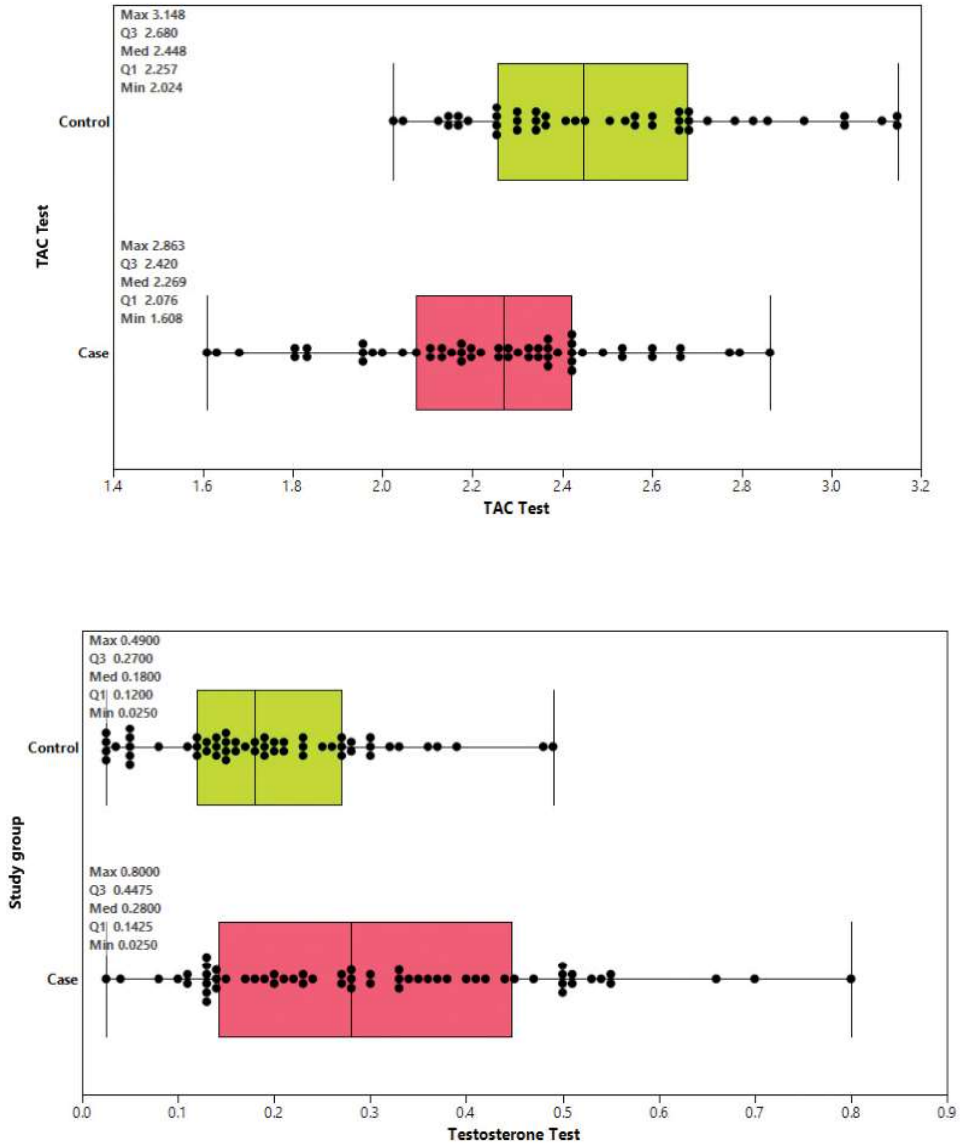

Figure 3: Comparisons of Testosterone and TAC test results between patients with PCOS and controls

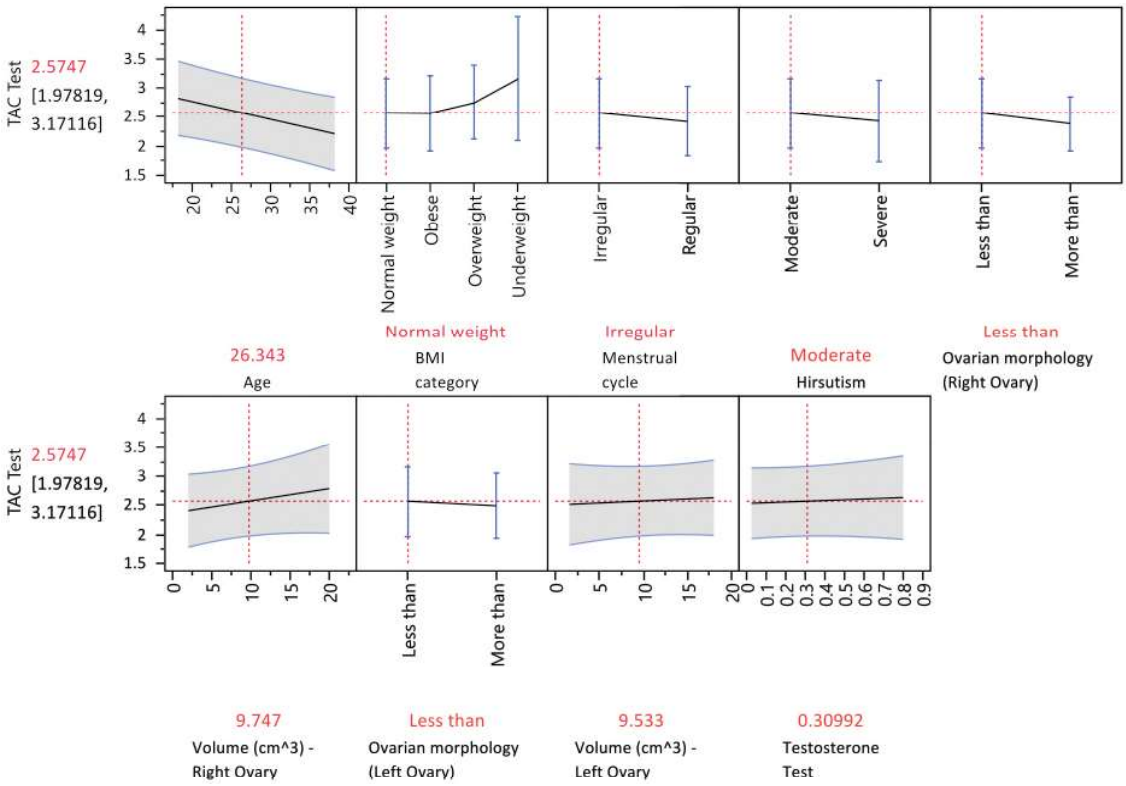

Figure 3: Profiler of the role of general characteristic, Testosterone, and endovaginally U/S outcomes on level of TAC in PCOS patients

Table 1: Comparisons of general characteristics between PCOS and control groups

Age and BMI	Study groups		p-value (two-sided
	PCOS (n=60)	Control (n=60)	
Age	26.34 (4.92	27.91 (4.61	0.0739[a]
BMI	26.55 (4.40	26.60 (4.15	
Underweight	1 (1.67	1 (1.67	0.9532[a]
Normal weight	20 (33.33	23 (38.33	
Overweight	27 (45.00	23 (38.33	0.9034[b]
Obese	12 (20.00	13 (21.67	
[a]An independent t-test and [b] Pearson Chi-squared tests were performed for statistical analyses.			

Table 2: Comparisons of physical examination and Endovaginally U/S between patients with PCOS and controls

Physical examination and Endovaginal U/S outcomes	Study groups		p-value (two-sided
	PCOS (n=60)	Control (n=60)	
Hirsutism Minimal Mild Moderate Severe	 0 (0.00 0 (0.00 51 (85.00 9 (15.00	 27 (45.00 33 (55.00 0 (0.00 0 (0.00	**<0.0001[b]**
Right Ovary **Ovarian morphology** Less than 12 follicles More than 12 follicles Volume (cm^3)	 3 (5.00 57 (95.00 9.75 (4.08	 56 (93.33 4 (6.67 7.30 (3.72	**<0.0001[b]** **0.0008[a]**
Left Ovary **Ovarian morphology** Less than 12 follicles More than 12 follicles Volume (cm^3)	 5 (8.33 55 (91.67 9.53 (3.79	 59 (98.33 1 (1.67 6.76 (3.44	**<0.0001[b]** **<0.0001[a]**
Menstrual cycle Irregular Regular	 41 (68.33 19 (31.67	 25 (41.67 35 (58.33	**0.0033**
[a]An independent t-test and [b] Pearson Chi-squared tests were performed for statistical analyses.			

Table 3: Comparisons of Testosterone and TAC test results between patients with PCOS and controls

Outcomes	Study groups		p-value (two-sided
	PCOS (n=60)	Control (n=60)	
Testosterone test **Range (Min-max)**	0.31 (0.18 0.025-0.8	0.19 (0.11 0.025-0.57	**<0.0001**
TAC Test **Range (Min-Max)**	2.24 (0.29 1.61-4.10	2.51 (0.31 2.02-17.23	**<0.0001**
[a]An independent t-test was performed for statistical analyses. The red bold numbers show significant differences.			

Table 4: Role of general characteristics, Testosterone, and endovaginally U/S outcomes on level of TAC in PCOS patients

Factors (n=60)	Outcome: TAC test Presentations	P-value
Age		**0.00933**
Menstrual cycle		0.25754
Volume (cm^3) -Right Ovary		0.27451
BMI category		0.30380
Hirsutism		0.38885
Ovarian morphology (Right Ovary)		0.51705
Testosterone Test		0.70679
Volume (cm^3) - Left Ovary		0.71295
Ovarian morphology (Left Ovary)		0.71545
Standard least squares with effect leverage were performed for statistical analyses. The red bold numbers show the predictors of TAC in PCOS group.		