ORIGINAL ARTICLE

NUREXUS

Journal of Medverse Research & Practice

ISSN: 3107-4278

Study of Free Testosterone in Polycystic Ovarian Syndrome

Dr. A Reshma[1]
Professor
Department of Obstetrics & Gynaecology, ACSR Government Medical College, Nellore.
Email ID: reshmaa45@gmail.com,

Submission Date: 15.11.2025	Accepted Date: 16.12.2025	Published Date: 31.12.2025

Abstract

Background: Polycystic ovarian syndrome (PCOS) is a prevalent endocrine disorder in women of reproductive age, characterized by hyperandrogenism, menstrual irregularities, and metabolic disturbances. Free testosterone is the biologically active androgen, yet its levels may vary among different PCOS phenotypes.

Objective: To evaluate free testosterone levels in women with PCOS and compare them with those of age-matched healthy controls.

Methods: A case-control study was conducted at the Department of Obstetrics and Gynecology, ACSR Government Medical College, Nellore, from August 2022 to November 2023. A total of 74 women (37 diagnosed with PCOS and 37 healthy controls) were enrolled after informed consent. Serum-free testosterone was measured using ELISA. Statistical analysis included descriptive statistics, independent t-tests, and Pearson correlation, with significance set at $p < 0.05$.

Results: The mean age of participants was 24.24 ± 5.60 years in the PCOS group and 23.49 ± 3.85 years in controls. Mean free testosterone levels were 9.66 ± 4.44 pg/mL in PCOS and 10.70 ± 5.57 pg/mL in controls, showing a statistically significant difference ($p < 0.05$). Weak positive correlations between age and free testosterone were observed in both groups (PCOS: $r = 0.129$; controls: $r = 0.031$).

Conclusion: Free testosterone levels in women with PCOS were slightly lower than those of controls, with minimal correlation to age. These results underscore the heterogeneity of PCOS and suggest that assessing free testosterone alone may not adequately reflect androgen excess. Comprehensive evaluation, including total testosterone, SHBG, free androgen index, and phenotypic assessment, is recommended for accurate diagnosis and management.

Keywords: Polycystic ovarian syndrome; free testosterone; hyperandrogenism; case-control study; reproductive-age women

Introduction

Polycystic ovarian syndrome (PCOS) is a multifaceted endocrine disorder affecting women of reproductive age and is a leading cause of anovulatory infertility [1]. Its prevalence globally ranges from 5% to 20%, depending on the diagnostic criteria applied, including Rotterdam, NIH, and AE-PCOS Society guidelines [2]. In India, community-based studies report prevalence rates between 9% and 36%, highlighting an increasing burden of reproductive and metabolic disorders among young women [3].

Clinically, PCOS manifests as menstrual irregularities, chronic anovulation, infertility, obesity, acne, hirsutism, and metabolic abnormalities such as insulin resistance and dyslipidemia [4]. Hyperandrogenism is a defining biochemical feature and may present clinically or be confirmed through elevated androgen levels [5]. Among androgens, free testosterone represents the biologically active

fraction, while total testosterone may be misleading due to variations in sex hormone-binding globulin (SHBG), which is often reduced in PCOS secondary to hyperinsulinemia and obesity [6]. Therefore, free testosterone measurement is considered a more reliable marker of androgen excess.

Accurate assessment of hyperandrogenemia remains challenging, as clinical practice frequently relies on total testosterone, which may not reflect true androgen activity. Free testosterone correlates more closely with clinical manifestations such as hirsutism, acne, and menstrual irregularities; however, measurement accuracy depends on the assay employed [7]. Misestimation can lead to underdiagnosis or misclassification of disease severity.

Moreover, free testosterone levels are associated with metabolic complications, including insulin resistance, visceral adiposity, and increased cardiovascular risk [8]. Variability in assay methods further complicates interpretation, as direct analog assays can be inaccurate while equilibrium dialysis, though more precise, is expensive and not widely available [9]. Understanding the patterns of free testosterone in PCOS is therefore essential for accurate diagnosis and optimal management.

Materials and Methods

Study Design

This hospital-based case-control study was conducted to evaluate serum free testosterone levels among women with Polycystic Ovary Syndrome (PCOS) and compare them with healthy age-matched controls.

Study Setting and Duration

The study was carried out in the Department of Obstetrics and Gynecology, ACSR Government Medical College, Nellore, over a period of 16 months from August 2022 to November 2023.

Study Population

The study population comprised women diagnosed with Polycystic Ovary Syndrome (PCOS) attending the outpatient department and healthy age-matched women without PCOS who served as controls.

Sample Size

A total of 74 participants were included in the study, comprising 37 women with PCOS and 37 healthy controls. The sample size was calculated based on the study by Hurjahan Banu et al. using the formula $n = 2(Z\alpha + Z1\text{-}\beta)^2\sigma^2 / d^2$, considering a standard deviation of 17.39, an effect size of 11.26, 95% confidence level, and 80% study power.

Inclusion Criteria

Women aged 18 years and above diagnosed with Polycystic Ovary Syndrome according to the Rotterdam criteria who had not received metformin, clomiphene citrate, oral contraceptive pills, or other hormonal medications for more than one month prior to enrolment were included in the case group. Healthy age-matched women without clinical or ultrasonographic evidence of PCOS were included as controls.

Exclusion Criteria

Women with hyperprolactinemia, ovarian tumors, Cushing syndrome, current pregnancy, or those unwilling to participate were excluded from the study.

Data Collection Tool

Following written informed consent, detailed demographic information, menstrual history, obstetric history, medical history, and relevant clinical findings were recorded using a structured case record form. General and systemic examinations were performed for all participants. Approximately 5 mL of venous blood was collected under aseptic precautions, centrifuged to separate serum, and stored at –20°C until biochemical analysis. Serum free testosterone levels were measured using a standardized Enzyme-Linked Immunosorbent Assay (ELISA) technique following the manufacturer's instructions and quality control procedures.

Ethical Considerations

The study protocol was reviewed and approved by the Institutional Ethics Committee of ACSR Government Medical College, Nellore, prior to commencement of the study. Written informed consent was obtained from all participants before enrolment. Participant confidentiality was maintained by assigning unique identification numbers, and all collected data were used exclusively for research purposes.

Statistical Analysis

Data were entered into Microsoft Excel for coding and analyzed using the Statistical Package for the Social Sciences (SPSS) software version 26.0. Continuous variables were expressed as mean ± standard deviation, while categorical variables were presented as frequencies and percentages. Comparisons between cases and controls were performed using the independent sample *t*-test for continuous variables and the Chi-square test for categorical variables where appropriate. Pearson's correlation coefficient was used to evaluate the relationship between serum free testosterone levels and clinical variables. A *p*-value of less than 0.05 was considered statistically significant.

Results

This case-control study was conducted in the Department of OBG, Vanivilas Hospital, BMCRI, from August 2022 to November 2023. Following Institutional Ethics Committee approval, 37 women with PCOS and 37 controls meeting the inclusion criteria were enrolled after providing informed consent.

Table 1: Distribution of participants by group

Group	Frequency (n)	Percentage (%)
PCOS	37	50
Control	37	50
Total	74	100

This table shows that the study included 74 participants, evenly divided between the PCOS group (37, 50%) and the control group (37, 50%).

Table 2: Age distribution between PCOS and Control groups

Group	Mean ± SD	Median (IQR)	Range	P value
PCOS	24.24 ± 5.595	23.00 (16-34)	18	0.000
Control	23.49 ± 3.849	(18–33)	15	0.001

The mean age of participants was slightly higher in the PCOS group (24.24 ± 5.60 years) compared to the control group (23.49 ± 3.85 years). Median ages were 23 years (IQR 16–34) for PCOS and 23 years (IQR 18–33) for controls. The age difference between groups was statistically significant ($P < 0.05$).

Table 3: Free testosterone levels in PCOS vs Control groups

Group	Mean ± SD	Median (IQR)	Range	P value
PCOS	9.66 ± 4.443	10.85 (2–18)	16	0.001
Control	10.70 ± 5.568	12.67 (2–27)	25	0.000

The mean free testosterone level was slightly lower in the PCOS group (9.66 ± 4.44 pg/mL) compared to controls (10.70 ± 5.57 pg/mL). Median levels were 10.85 pg/mL (IQR 2–18) for PCOS and 12.67 pg/mL (IQR 2–27) for controls. The difference between groups was statistically significant ($P < 0.05$).

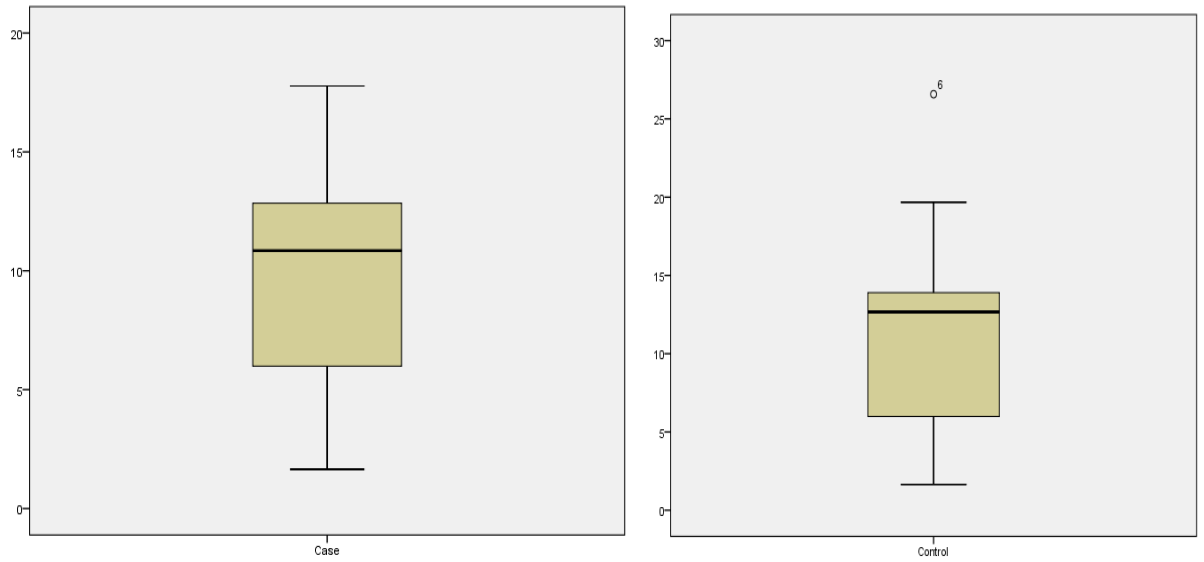

Figure 1: Free testosterone levels in the case PCOS groups vs the Control groups

Table 4: Comparison of free testosterone between groups (Independent t test)

Parameter	Mean difference	t statistic	Df	P value
Free testosterone in the case	9.66.	13.225	36	0.000
Free testosterone in control	10.698	11.686	36	0.000

The independent t-test comparing free testosterone levels between groups showed a mean of 9.66 pg/mL in the PCOS group and 10.70 pg/mL in controls. Both comparisons were statistically significant (PCOS: $t = 13.23$, $df = 36$, $p < 0.001$; Control: $t = 11.69$, $df = 36$, $p < 0.001$), indicating a significant difference between the groups.

Table 5: Correlation between age and free testosterone

Parameter	Pearson correlation (r)	P value
Age vs Free Testosterone case	0.129	0.00
Age vs Free Testosterone control	0.031	0.00

There was a weak positive correlation between age and free testosterone in both groups (PCOS: $r = 0.129$; Control: $r = 0.031$). Both correlations were statistically significant ($P < 0.05$), suggesting a minimal but significant association between age and free testosterone levels.

Discussion

In the present case-control study involving 74 women (37 with polycystic ovary syndrome [PCOS] and 37 age-matched healthy controls), the mean serum free testosterone (FT) levels were 9.66 ± 4.44 pg/mL in the PCOS group and 10.70 ± 5.57 pg/mL in the control group, with a statistically significant difference. Weak positive correlations between age and free testosterone were observed in both groups. These findings suggest that, in this cohort, biochemical hyperandrogenism as assessed by free testosterone was not markedly elevated in women with PCOS, which differs from the classical expectation of consistently increased androgen levels in this condition. Earlier studies have documented higher free testosterone concentrations in women with PCOS. Penttilä reported significantly elevated serum free testosterone levels in women with PCOS compared to healthy controls, supporting the role of biochemical hyperandrogenism as a diagnostic feature of the syndrome [12]. Similarly, Legro et al. demonstrated that androgen excess, including elevated testosterone levels, was a prominent feature in several PCOS phenotypes and influenced both diagnosis and treatment approaches [13]. Grassi et al., using liquid chromatography tandem mass spectrometry (LC-MS/MS), further confirmed significantly higher

testosterone and androstenedione levels in women with PCOS, highlighting the sensitivity of advanced analytical techniques in detecting androgen excess [14].

The apparent discrepancy between our findings and previous reports may be explained by the well-recognized heterogeneity of PCOS. Legro et al. described substantial phenotypic variation within PCOS, noting that certain phenotypes, particularly those without overt hyperandrogenism, may exhibit androgen levels comparable to healthy controls [13]. Grassi et al. also emphasized that androgen profiles differ significantly depending on the PCOS phenotype and the analytical method used, suggesting that cohorts enriched with less androgenic phenotypes may demonstrate lower free testosterone levels [14]. Methodological differences in androgen assessment may also contribute to variability across studies. Lerchbaum and Obermayer-Pietsch highlighted that free testosterone levels are strongly influenced by assay methodology, timing of sample collection, and circulating sex hormone-binding globulin concentrations, which can lead to under- or overestimation of biochemical hyperandrogenism [15]. Patil et al. similarly emphasized that reliance on a single marker, such as free testosterone, may be insufficient, advocating for the use of the free androgen index and complementary androgen measurements for accurate evaluation [16].

Recent evidence suggests that androgens beyond testosterone play a significant role in the hyperandrogenic milieu of PCOS. O'Reilly et al. demonstrated that 11-oxygenated androgens, particularly 11-ketotestosterone, are markedly elevated in women with PCOS and may better reflect androgen excess than conventional testosterone measurements [17]. Wang et al. reinforced this concept, identifying androgen excess as a hallmark of PCOS and highlighting the contribution of non-classical androgens to its pathophysiology [18]. Studies focusing on specific populations, including Indian women, have further shown that LC-MS/MS–measured androgen profiles can reveal patterns not captured by routine immunoassays, underscoring the importance of population-specific assessment [19,20]. With respect to age, the weak correlations observed in the present study are consistent with recent findings indicating minimal influence of age on free testosterone levels within reproductive-age women with PCOS. Kugelman et al. reported that basal total and free testosterone levels did not vary significantly with age and did not impact reproductive outcomes in women with PCOS undergoing assisted reproduction [22]. Similarly, Zhang et al. found that androgen levels were not strong age-dependent predictors in reproductive outcome analyses among women with PCOS [23].

From a clinical perspective, these findings highlight that biochemical hyperandrogenism, as assessed solely by free testosterone, may not be universally present in all women with PCOS. Exclusive reliance on free testosterone may therefore lead to underdiagnosis or misclassification of androgen excess. A comprehensive diagnostic approach incorporating total testosterone, sex hormone-binding globulin, free androgen index, androstenedione, 11-oxygenated androgens, and detailed phenotypic assessment is essential for accurate diagnosis, appropriate risk stratification, and individualized management of women with PCOS.

Limitations

The study had several limitations. The sample size was modest, which may limit the generalizability and statistical power of the findings. We did not measure SHBG, total testosterone, or other androgen metabolites, limiting the ability to fully characterize androgen status. Blood samples were not controlled for menstrual cycle phase, which could influence hormone levels. Furthermore, the cross-sectional design precludes evaluation of longitudinal changes or causal relationships between androgen levels and clinical outcomes. Finally, the study cohort was drawn from a single tertiary care center, which may limit applicability to broader populations.

Conclusion

In this case-control study, women with PCOS exhibited slightly lower free testosterone levels compared to healthy controls, and only weak positive correlations were observed between age and free testosterone. These findings emphasize the heterogeneity of PCOS and suggest that not all patients exhibit biochemical hyperandrogenism. Reliance on free testosterone alone may underestimate androgen excess, potentially leading to an incomplete diagnosis. A comprehensive approach incorporating total testosterone, SHBG, free androgen index, other androgen metabolites, and phenotypic evaluation is recommended for accurate diagnosis and optimal management of PCOS.

Conflict of interest: Nil
Source Of Fund: Nil
Acknowledgement: I sincerely thank my department for their invaluable support throughout the study, and I am grateful to the management of the institution for providing the resources and environment necessary for the successful completion of this work.

Reference

1. Teede H, Misso M, Costello M, et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Clin Endocrinol (Oxf). 2018;89(3):251-268.
2. Azziz R, Woods KS, Reyna R, et al. The prevalence and features of the polycystic ovary syndrome in an unselected population. J Clin Endocrinol Metab. 2004;89(6):2745-2749.
3. Dunaif A. Insulin resistance and the polycystic ovary syndrome. Endocr Rev. 1997;18(6):774-800.
4. Rosner W, Auchus RJ, Azziz R, et al. Utility, limitations, and pitfalls in measuring testosterone. J Clin Endocrinol Metab. 2007;92(2):405-413.
5. Escobar Morreale HF. Polycystic ovary syndrome. Nat Rev Endocrinol. 2018;14(5):270-284.
6. Carmina E, Azziz R, Bergfeld W, et al. Female pattern hair loss and androgen excess. J Clin Endocrinol Metab. 2019;104(7):2875-2891.
7. Goldman AL, Bhasin S, Wu FC, et al. A reappraisal of testosterone binding in circulation. Endocr Rev. 2017;38(4):302-324.
8. Sam S. Obesity and polycystic ovary syndrome. Obes Manag. 2007;3(2):69-73.
9. Lim SS, Davies MJ, Norman RJ, Moran LJ. Overweight, obesity and central obesity in women with polycystic ovary syndrome. Hum Reprod Update. 2012;18(6):618-637.
10. Hussein S, et al. Reference intervals and biologic variation of testosterone in women: comparison between PCOS and controls. Fertil Steril. 2014;101(6):1695 1701.
11. Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO. The prevalence and features of the polycystic ovary syndrome in an unselected population. J Clin Endocrinol Metab. 2004;89(6):2745 2749.
12. Penttilä TA. Serum free testosterone in polycystic ovary syndrome. Fertil Steril. 1996;65(3):693 698.
13. Legro RS, Kunselman AR, Dodson WC, Dunaif A. Phenotypic variation in polycystic ovary syndrome: impact on diagnosis and treatment. Fertil Steril. 1998;69(6):1051 1058.
14. Grassi G, Polledri E, Fustinoni S, Chiodini I, Ceriotti F, D'Agostino S, et al. Hyperandrogenism by Liquid Chromatography Tandem Mass Spectrometry in PCOS: Focus on Testosterone and Androstenedione. J Clin Med. 2021;10(1):119.
15. Lerchbaum E, Obermayer-Pietsch B. Hyperandrogenemia in Polycystic Ovary Syndrome. PLoS One. 2014;9(5):e97530.
16. Patil P, et al. Free-androgen index in women with polycystic ovarian syndrome. J Hum Reprod Sci. 2015;8(4):222 228.
17. O'Reilly MW, Kempegowda P, Jenkinson C, et al. 11-oxygenated androgens in PCOS: implications for diagnosis and therapy. J Clin Endocrinol Metab. 2017;102(9):3330 3339.
18. Wang K, et al. Androgen excess: a hallmark of polycystic ovary syndrome. Front Endocrinol (Lausanne). 2023;14:1273542.

19. Subramaniam K, et al. Insights from liquid chromatography-mass spectrometry-measured androgens in Indian women with PCOS. Gynecol Endocrinol. 2024;40(5):375 382.
20. Chen F, Chen M, Zhang W, Yin H, Chen G, Huang Q, Yang X, Chen L, Lin C, Yin G. Comparison of the efficacy of different androgens measured by LC MS/MS in representing hyperandrogenemia and an evaluation of adrenal origin androgens with a dexamethasone suppression test in patients with polycystic ovary syndrome. J Ovarian Res. 2021;14:32.
21. Mansour A, Noori M, Hakemi MS, Haghgooyan Z, Mohajeri Tehrani MR, Mirahmad M, Sajjadi Jazi SM. Hyperandrogenism and anthropometric parameters in women with polycystic ovary syndrome. BMC Endocr Disord. 2024;24:201.
22. Kugelman N, Digby A, Rotshenker Olshinka K, Bellemare V, Pooni A, Son W Y, Dahan MH. Basal serum luteinizing hormone, total testosterone, and free testosterone levels do not impact in vitro fertilization outcomes in patients with polycystic ovary syndrome. J Turk Ger Gynecol Assoc. 2024;25(4):192–199.
23. Zhang Y, Liu X, Li Z, et al. The discriminatory capability of anthropometric measures in predicting reproductive outcomes in Chinese women with polycystic ovary syndrome. J Ovarian Res. 2024;17:186.