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ASSOCIATION OF FREE ANDROGEN INDEX WITH LOW-GRADE INFLAMMATION IN DIFFERENT PHENOTYPES OF POLYCYSTIC OVARY SYNDROME

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is a common endocrine disorder associated with hyperandrogenism, insulin resistance, and chronic low-grade inflammation. This study evaluated the relationship between free androgen index (FAI) and inflammation across different PCOS phenotypes.

Methods: In this hospital-based cross-sectional study, 330 women with PCOS diagnosed according to the modified Rotterdam criteria were classified into four phenotypes (A–D). Serum C-reactive protein (CRP), FAI, and HOMA-IR were measured and compared among phenotypes using appropriate statistical analyses.

Results: Phenotype A was the most common (51.2%) and showed the highest CRP, FAI, and HOMA-IR values. CRP levels differed significantly among phenotypes (P=0.014), with elevated CRP most frequently observed in phenotype A (50.3%). FAI was positively correlated with HOMA-IR (r=0.255, P<0.001), but neither FAI nor HOMA-IR showed a significant association with CRP. Multivariable analysis confirmed that FAI, HOMA-IR, BMI, and age were not independent predictors of CRP.

Conclusion: Hyperandrogenic PCOS phenotypes exhibit greater inflammatory and metabolic disturbances; however, FAI is not independently associated with systemic inflammation. These findings suggest that inflammation in PCOS is multifactorial and varies across phenotypes.

KEYWORDS: Polycystic ovary syndrome; Free androgen index; C-reactive protein; Hyperandrogenism; Insulin resistance; PCOS phenotypes.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common endocrine disorder among women of reproductive age and is associated with adverse reproductive, metabolic, and cardiovascular outcomes. Traditionally, PCOS has been defined by menstrual dysfunction, hyperandrogenism, and polycystic ovarian morphology. However, growing evidence suggests that PCOS is a heterogeneous syndrome with distinct phenotypic presentations and varying clinical manifestations. [1,2] The Rotterdam consensus broadened the diagnostic framework and enabled the classification of phenotypes with differing clinical severity and long-term risk profiles.[3] Hyperandrogenism is considered a central pathophysiological feature of PCOS and contributes not only to clinical manifestations such as hirsutism, acne, and anovulation, but also to adverse metabolic outcomes.[4] Excess androgen exposure has been implicated in altered adipose tissue function, impaired insulin signalling, and ovarian dysfunction.[5] Insulin resistance is highly prevalent in women with PCOS, even in those without overt obesity.[6] The resulting hyperinsulinemia may increase ovarian androgen production through stimulation of theca cells and suppression of sex hormone-binding globulin (SHBG) synthesis. This reciprocal interaction creates a vicious cycle between excess androgen levels and metabolic dysregulation.[7]

Low-grade chronic inflammation is an important feature of PCOS.[8] Elevated inflammatory markers, including C-reactive protein (CRP), are common in PCOS and may be linked to obesity, insulin resistance, endothelial dysfunction, and ovarian inflammation. [8,9] However, phenotypic differences in inflammatory burden remain unclear.

Given the heterogeneity of PCOS, phenotype-based assessment of hormonal, metabolic, and inflammatory markers may provide clinically meaningful insights. This study evaluated the interrelationship between free androgen index (FAI), insulin resistance, and CRP levels across PCOS phenotypes.

MATERIALS AND METHODS

This hospital-based cross-sectional study was conducted in the department of Biochemistry with collaboration of Obstetrics and Gynaecology at a tertiary care teaching hospital from August 2024 to May 2026. Women of reproductive age presenting with suspected PCOS were evaluated clinically and biochemically. Eligible

participants were diagnosed with PCOS according to the modified 2003 Rotterdam criteria after exclusion of related disorders. A total of 330 women aged 18–45 years who were diagnosed with PCOS according to the modified 2003 Rotterdam criteria were included in the study. The sample size was calculated using a prevalence of 31% reported in a previous study,[10] with a 95% confidence level and an absolute precision of 5%, yielding a minimum required sample size of 330 participants. Diagnosis required the presence of at least two of the following three criteria (i) oligo-ovulation or anovulation (cycle length <21 or >35 days, or fewer than eight menstrual cycles per year); (ii) clinical or biochemical hyperandrogenism, defined as a modified Ferriman–Gallwey (mFG) score ≥8 or elevated serum total testosterone above the institutional laboratory reference range; and (iii) polycystic ovarian morphology (PCOM) on transvaginal ultrasonography, defined as ≥20 follicles measuring 2–9 mm in diameter per ovary or ovarian volume >10 mL in either ovary, according to the 2018 International Evidence-Based Guideline for PCOS.[11] Participants were included after providing written informed consent and completion of clinical, biochemical, and ultrasonographic evaluation. Women with diabetes mellitus, thyroid dysfunction, hyperprolactinaemia, congenital adrenal hyperplasia, Cushing syndrome, or androgen-secreting adrenal or ovarian tumours were excluded. Additional exclusion criteria included use of insulin sensitisers and anti-inflammatory medications within the preceding three months. Participants were classified into four PCOS phenotypes based on the modified 2003 Rotterdam criteria: (1) Phenotype A: Oligo/anovulation (OA) + hyperandrogenism (HA) + PCOM (2) Phenotype B: OA + HA (3) Phenotype C: HA + PCOM (4) Phenotype D: OA + PCOM.[3] Phenotypes A and B were considered classic hyperandrogenic phenotypes, whereas phenotype D represented the non-hyperandrogenic phenotype. Anthropometric measurements were obtained by using calibrated instruments under standardised conditions. Body weight was measured using a digital weighing scale, and height was recorded using a wall-mounted stadiometer. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Waist circumference was measured at the midpoint between the lower rib margin and iliac crest, while hip circumference was measured at the level of the greater trochanters using a non-stretchable measuring tape. Waist-to-hip ratio (WHR) was subsequently calculated. Clinical hyperandrogenism was assessed using the modified Ferriman–Gallwey (mFG) scoring system across nine androgen-sensitive body areas, with a threshold of ≥8 defining clinically significant hirsutism.[12]

Venous blood samples were collected after an overnight fast of at least 8–12 hours during days 2–3 of the menstrual cycle. Fasting blood glucose, fasting serum insulin, total testosterone, follicle-stimulating hormone (FSH), luteinising hormone (LH), free triiodothyronine (FT3), free thyroxine (FT4), thyroid-stimulating hormone (TSH), and prolactin were measured by chemiluminescent immunoassay on the VITROS 5600/VITROS XT 7600 Integrated Systems (QuidelOrtho™ Corporation, San Diego, CA, USA). Serum SHBG was measured by electrochemiluminescence immunoassay (ECLIA) using the Cobas e 411 analyzer (Roche Diagnostics, Germany). C-reactive protein (CRP) was measured by particle-enhanced immunoturbidimetric assay on the VITROS platform. All assays were performed in the hospital biochemistry laboratory under internal and external quality control protocols. The free androgen index (FAI) was calculated according to the standard formula: FAI (%) = Total Testosterone (nmol/L) × 100 / SHBG (nmol/L).[13] HOMA-IR was calculated as: fasting insulin (pmol/L) × fasting glucose (mg/dl) / 2430, following the original formulation by Matthews et al.[14] The threshold for elevated FAI was set at ≥2.56%, based on evidence from a systematic review and diagnostic meta-analysis informing the 2023 International Evidence-based Guideline for PCOS, which reported a median FAI cutoff of 2.56 for identifying biochemical hyperandrogenism.[11]

Statistical analysis was performed using [IBM SPSS STATISTICS version 31]. Baseline characteristics were presented as mean ± standard deviation (SD) for continuous variables. Categorical variables were expressed as frequency and percentage. Normality was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Comparison of continuous variables across the four phenotype groups was performed using the Kruskal–Wallis test, followed by post-hoc pairwise comparisons using Bonferroni correction when significant. The Mann–Whitney U test was used to compare two groups (high vs normal FAI). Categorical variables are compared using the Pearson chi-square test or Fisher's exact test, as appropriate. Correlations between FAI, HOMA-IR, and CRP were assessed using Spearman's rank correlation. Multivariable linear regression with robust standard errors was performed to identify independent predictors of CRP. A two-tailed P-value <0.05 was considered statistically significant.

RESULT

Of the 330 women with PCOS, 169 (51.2%) were classified as phenotype A, 103 (31.2%) as phenotype B, 37 (11.2%) as phenotype C, and 21 (6.4%) as phenotype D. No significant inter-phenotype differences were observed for age ($P = 0.083$), BMI ($P = 0.558$), or BMI category distribution ($\chi^2 = 5.01$, $P = 0.172$), indicating that differences in study outcomes across phenotypes were not confounded by overall adiposity. Baseline demographic, anthropometric, hormonal, and metabolic characteristics are presented in Table 1.

Table 1- Baseline characteristics of demographic, anthropometric, hormonal, and metabolic profile of PCOS phenotypes

Variable	A (n=169)	B (n=103)	C (n=37)	D (n=21)	p-value
Age	27.49 ± 6.55	27.79 ± 6.34	29.95 ± 6.32	28.38 ± 7.69	0.083
BMI	25.67 ± 4.30	25.24 ± 3.94	24.94 ± 4.14	24.83 ± 3.81	0.558

Waist Circumference	34.91 ± 3.94	33.14 ± 4.44	31.50 ± 4.11	33.05 ± 4.13	<0.001
Waist-Hip Ratio	0.89 ± 0.03	0.87 ± 0.04	0.86 ± 0.04	0.88 ± 0.04	<0.001
m-FG score	11.20 ± 4.38	10.88 ± 2.93	9.62 ± 3.19	3.62 ± 2.22	<0.001
TT (nmol/L)	2.75 (2.25–3.47)	2.12 (1.76–2.71)	2.08 (1.42–2.91)	2.15 (1.39–2.32)	<0.001*
SHBG (nmol/L)	47.25 (32.00–74.60)	54.74 (34.00–87.26)	61.20 (37.50–86.70)	111.50 (94.10–128.70)	<0.001*
FAI (%)	5.82 (3.80–9.73)	3.82 (2.57–6.17)	3.54 (1.90–6.92)	1.75 (1.26–1.99)	<0.001*
FBS (mg/dL)	91 (83–101)	87 (76–99)	89 (80–99)	85 (80–97)	0.078*
Fasting insulin (pmol/L)	98.06 (71.20–129.00)	90.00 (66.00–112.00)	78.90 (58.00–103.20)	64.00 (31.00–102.00)	0.002*
HOMA-IR	3.62 (2.57–4.81)	3.11 (2.27–4.07)	2.89 (1.90–3.78)	2.47 (1.16–3.44)	<0.001*
LH (mIU/L)	10.19 (8.39–12.88)	11.71 (7.92–16.30)	11.46 (8.32–13.72)	6.68 (4.86–9.68)	<0.001*
FSH (mIU/L)	5.10 (4.12–6.21)	6.79 (5.59–7.54)	5.10 (4.26–6.20)	3.33 (2.62–5.18)	<0.001*
LH:FSH ratio	2.08 (1.39–2.86)	1.71 (1.20–2.38)	2.38 (1.62–2.86)	1.87 (1.28–2.15)	0.008*
FT3 (pmol/L)	6.18 (5.32–6.91)	6.14 (5.42–7.04)	6.62 (5.68–7.19)	6.43 (4.92–7.00)	0.447*
FT4 (pmol/L)	18.10 (14.10–22.40)	19.40 (13.70–22.50)	18.30 (15.30–21.70)	17.60 (15.40–23.80)	0.870*
TSH (mIU/L)	2.00 (1.13–2.97)	2.22 (1.13–3.02)	2.32 (1.34–3.00)	1.91 (1.48–2.88)	0.809*
PRL (ng/mL)	237.00 (167.10–307.00)	244.70 (174.50–319.70)	223.50 (122.10–310.80)	203.20 (158.50–262.20)	0.301*

Serum CRP levels differed significantly across PCOS phenotypes ($P = 0.014$). The highest median CRP level was observed in Phenotype A [5.14 (IQR: 1.64–8.12) mg/dL], followed by Phenotype B [3.21 (0.87–6.46) mg/dL], Phenotype C [2.34 (0.82–5.41) mg/dL], and Phenotype D [2.14 (0.87–6.21) mg/dL]. Pairwise comparisons showed higher CRP levels in Phenotype A than in Phenotypes B and C (both $P = 0.019$); however, these differences were not significant after Bonferroni correction. No significant differences were observed among the remaining phenotype comparisons (B vs. C: $P = 0.392$; B vs. D: $P = 0.514$; C vs. D: $P = 0.955$).

Table 2 - Prevalence of elevated CRP (>5 mg/dL) across PCOS Phenotypes

Phenotype	Elevated CRP (>5 mg/dL) *N* (%)	Normal CRP (≤5 mg/dL) *N* (%)
A	85 (50.3)	84 (49.7)
B	39(37.9)	64 (62.1)
C	11(29.7)	26 (70.3)
D	6 (28.6)	15 (71.4)
Total	141 (42.7)	189 (57.3)

A significant difference in the prevalence of elevated CRP (>5 mg/dL) was observed across the PCOS phenotypes ($\chi^2 = 9.23$, $P = 0.026$). The highest prevalence was noted in Phenotype A (50.3%), followed by Phenotype B (37.9%), Phenotype C (29.7%), and Phenotype D (28.6%). Overall, 50.3% of the study participants had high CRP levels (>5 mg/dL). These data are summarised in Table 2.

CRP levels did not differ significantly between women with BMI ≥25 kg/m² and those with normal BMI <25 kg/m² [3.63 (IQR: 1.00–7.04) vs. 3.58 (IQR: 1.04–7.41) mg/dL, P =0.655]. Biochemical hyperandrogenism was most prevalent in Phenotype A (91.1%), followed by Phenotype B (75.7%), Phenotype C (73.0%), and was absent in Phenotype D. In line with these findings, FAI levels differed significantly across PCOS phenotypes ($P < 0.001$), with the highest median values observed in Phenotype A [5.82 (3.80–9.73)], followed by Phenotype B [3.82 (2.61–6.08)], Phenotype C [3.54 (1.90–6.92)], and lowest in Phenotype D [1.75 (1.26–1.99)]. Post hoc analysis showed that FAI was significantly higher in Phenotype A than in Phenotypes B, C, and D. Phenotype D had significantly lower FAI than Phenotypes B and C, whereas no significant difference was observed between Phenotypes B and C.

No significant correlation was observed between CRP and FAI, total testosterone, or SHBG in the overall cohort (all $P > 0.05$), Similarly, no significant association between FAI and CRP was observed within any PCOS phenotype. The mFG score also showed no significant correlation with CRP ($r = -0.073$, $P = 0.186$). Women with

elevated FAI (≥2.56%) had a higher mean CRP level than those with normal FAI (4.95 ± 4.10 vs. 3.88 ± 3.54 mg/dL), although this difference was not statistically significant ($P = 0.053$).

Table 3. Multiple Linear Regression Analysis for Predictors of CRP

Predictor	β – Coefficient	P - value
log-FAI (%)	0.076	0.188
log-HOMA-IR	0.032	0.577
BMI (kg/m²)	0.047	0.397
Age (years)	0.053	0.342

In multiple linear regression analysis with log-CRP as the dependent variable, the overall model was not statistically significant [F (4,325) = 1.19, $P = 0.313$, $R^2 = 0.015$, adjusted $R^2 = 0.002$]. None of the evaluated predictors, including log-FAI, log-HOMA-IR, BMI, and age demonstrated an independent association with log-CRP (Table 3).

Table 4 - Key correlation findings

Variable pair	Spearman's rho (r)	P - value
FAI (%) vs CRP (mg/dL)	0.090	0.102
HOMA-IR vs CRP (mg/dL)	0.017	0.765
Total testosterone vs CRP (mg/dL)	0.091	0.098
mFG score vs CRP (mg/dL)	−0.073	0.186
SHBG vs CRP (mg/dL)	−0.048	0.387
BMI vs CRP (mg/dL)	0.040	0.472
FAI (%) vs HOMA-IR	0.255	<0.001
HOMA-IR vs BMI	0.118	0.032
FAI (%) vs TT (nmol/L)	0.592	<0.001
FAI (%) vs SHBG (nmol/L)	−0.698	<0.001

HOMA-IR differed significantly across PCOS phenotypes ($P < 0.001$), with the highest values in phenotype A [3.62 (2.57–4.81)] and the lowest in phenotype D [2.47 (1.16–3.44)]. Post-hoc analysis showed significantly higher HOMA-IR in phenotype A than in phenotypes C and D. The prevalence of insulin resistance differed significantly across phenotypes ($\chi^2 = 10.09$, $P = 0.018$), decreasing progressively from phenotype A (75.7%) to phenotype D (47.6%). HOMA-IR was not significantly correlated with CRP, either in the overall cohort ($r = 0.017$, $P = 0.765$) or within individual phenotypes (all $P > 0.05$). FAI and HOMA-IR were significantly positively correlated ($r = 0.255$, $P < 0.001$), and HOMA-IR showed a weak but significant positive correlation with BMI ($r = 0.118$, $P = 0.032$). All key correlation findings are presented in Table-4.

DISCUSSION

This cross-sectional study was conducted on 330 women diagnosed with PCOS in tertiary care teaching hospital. The primary objective was to examined systemic inflammation assessed by serum CRP across various PCOS phenotypes and investigate its correlation with FAI and IR among PCOS patients. Serum CRP levels differed significantly across PCOS phenotypes, with the highest values observed in phenotype A and the lowest in phenotype D. Similarly, the prevalence of elevated CRP (>5 mg/dL) was greatest in Phenotype A and least in Phenotype D. Although studies directly comparing CRP across PCOS phenotypes are limited, previous studies showing that the classic hyperandrogenic phenotype has higher hs-CRP concentrations than the normo-androgenic PCOS phenotype. [15]

Hyperandrogenism may promote chronic low-grade inflammatory state of PCOS through its effects on visceral adiposity, oxidative stress, immune cell activation, and pro-inflammatory cytokine production.[16] However, the absence of a significant correlation between FAI and CRP in the present study suggests that androgen excess alone may not independently determine systemic inflammation. The higher CRP levels observed in the classic PCOS phenotypes likely reflect the combined effects of hyperandrogenism, insulin resistance, central adiposity, and ovulatory dysfunction. These findings support the multifactorial nature of inflammation in PCOS, with

hyperandrogenism contributing to the overall endocrine-metabolic disturbance rather than directly influencing CRP.

Insulin resistance was more prevalent in the hyperandrogenic PCOS phenotypes. HOMA-IR was not significantly associated with CRP, in the overall cohort or within individual phenotypes and was not an independent predictor of CRP. This suggest that insulin resistance alone may not be the primary cause of systemic inflammation in PCOS.

The positive association between FAI and HOMA-IR supports the well-recognized bidirectional relationship between hyperandrogenism and insulin resistance, whereby androgen excess aggravates insulin resistance, while hyperinsulinaemia further stimulates androgen production.[17] Collectively, these findings suggest that insulin resistance is an important metabolic feature of the hyperandrogenic phenotype but does not independently explain the increased inflammatory burden, which is likely driven by the combined effects of androgen excess, adiposity, and other metabolic disturbances.[18]

CONCLUSION

The higher CRP levels observed in the phenotype A support previous evidence that PCOS is a chronic low-grade inflammatory state and further suggest that inflammatory burden varies across PCOS phenotypes. The lack of a significant association between CRP and HOMA-IR suggests that inflammation in PCOS cannot be attributed to insulin resistance alone but likely reflects the combined effects of hyperandrogenism and other metabolic factors.[19] Differences from previous studies may be due to variations in study populations and phenotype distribution.

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