

Association of Insulin Resistance, High-Sensitivity C-Reactive Protein, and Selected Inflammatory Biomarkers with Clinical Phenotypes of Polycystic Ovary Syndrome

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Abstract

Background: Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder in which insulin resistance and chronic low-grade inflammation are thought to drive long-term cardiometabolic risk. Whether these abnormalities differ across the four Rotterdam phenotypes remains incompletely defined, particularly in Middle Eastern populations.

Objective: To assess the association of insulin resistance, high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF- α) and white blood cell (WBC) count with the clinical phenotypes of PCOS among women attending Babylon Teaching Hospital for Gynaecology and Obstetrics, Iraq.

Methods: A hospital-based case-control study enrolled 180 women with PCOS diagnosed by the Rotterdam criteria and classified into phenotype A (n = 72), B (n = 34), C (n = 40) and D (n = 34), together with 60 age-matched women with regular ovulatory cycles as controls. Anthropometric, hormonal and metabolic variables were measured. Insulin resistance was estimated by the homeostasis model assessment (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI). Serum hs-CRP was measured by immunoturbidimetry, and IL-6 and TNF- α by enzyme-linked immunosorbent assay.

Results: HOMA-IR, hs-CRP, IL-6 and TNF- α were significantly higher in all PCOS phenotypes than in controls ($p < 0.001$), with a graded pattern $A > B > C > D$. Insulin resistance ($HOMA-IR \geq 2.5$) was present in 69.4% of phenotype A versus 26.5% of phenotype D. In women with PCOS, hs-CRP correlated with HOMA-IR ($r = 0.54$), waist circumference ($r = 0.52$) and IL-6 ($r = 0.61$). On multivariable regression, HOMA-IR, waist circumference and free androgen index were independent predictors of hs-CRP (adjusted $R^2 = 0.43$).

Conclusion: Insulin resistance and low-grade inflammation are most pronounced in the hyperandrogenic phenotypes of PCOS, especially phenotype A, and are closely interlinked with central adiposity. Phenotype-based stratification may help target cardiometabolic screening in women with PCOS.

Keywords: Polycystic ovary syndrome; Phenotypes; Insulin resistance; HOMA-IR; High-sensitivity C-reactive protein; Interleukin-6; Tumour necrosis factor-alpha; Chronic inflammation; Iraq

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder of women of reproductive age, affecting approximately 8–13% of women worldwide depending on the diagnostic criteria applied and the population studied [1],[2]. Far from being a purely gynaecological condition, PCOS is now recognised as a lifelong, multisystem disorder with reproductive, metabolic, cardiovascular and

psychological consequences [3]. Its reproductive features, including oligo-ovulation, anovulatory infertility and hirsutism, are usually the reason women first seek care, yet the long-term burden of the syndrome is increasingly attributed to its metabolic components, notably insulin resistance, impaired glucose tolerance, type 2 diabetes mellitus, dyslipidaemia and an adverse cardiovascular risk profile [4]. Because women often present to gynaecological and obstetric services during their reproductive years, these settings represent a critical window for identifying women at elevated cardiometabolic risk and for initiating preventive strategies.

The definition of PCOS has evolved considerably over the past three decades. The 1990 National Institutes of Health (NIH) criteria required both chronic anovulation and clinical or biochemical hyperandrogenism, whereas the 2003 Rotterdam consensus broadened the definition to include any two of three features: oligo- or anovulation, clinical and/or biochemical hyperandrogenism, and polycystic ovarian morphology on ultrasound, after exclusion of other aetiologies [5]. The Rotterdam criteria, endorsed by the 2023 international evidence-based guideline, generate four distinct phenotypes [6]. Phenotype A, or “full-blown” PCOS, includes hyperandrogenism, ovulatory dysfunction and polycystic ovarian morphology; phenotype B combines hyperandrogenism and ovulatory dysfunction with normal ovarian morphology; phenotype C, the “ovulatory” phenotype, comprises hyperandrogenism and polycystic morphology with regular ovulation; and phenotype D, the “non-hyperandrogenic” phenotype, combines ovulatory dysfunction with polycystic morphology in the absence of hyperandrogenism. The 2012 NIH Evidence-based Methodology Workshop recommended that the specific phenotype be reported in all research and clinical settings precisely because these subgroups may carry different long-term risks [7]. Phenotype classification therefore offers a practical framework for exploring the heterogeneity of PCOS and for tailoring clinical evaluation.

Insulin resistance is a central pathophysiological feature of PCOS. It is defined as a reduced biological response to a given concentration of insulin and is accompanied by compensatory hyperinsulinaemia [8]. Seminal clamp studies demonstrated that women with PCOS exhibit profound peripheral insulin resistance that is independent of, but amplified by, obesity [9]. Subsequent work using the euglycaemic-hyperinsulinaemic clamp confirmed that insulin resistance is intrinsic to PCOS and is present in lean as well as overweight women [10], and a meta-analysis of clamp studies reported an average reduction in insulin sensitivity of approximately 27% in women with PCOS compared with body mass index (BMI)-matched controls [11]. Hyperinsulinaemia acts synergistically with luteinising hormone (LH) to stimulate theca cell androgen production and suppresses hepatic synthesis of sex hormone-binding globulin (SHBG), thereby increasing the bioavailable fraction of circulating testosterone. In this way, insulin resistance contributes to both the reproductive and the metabolic manifestations of the syndrome. Clinically, women with PCOS have a substantially increased prevalence of impaired glucose tolerance, type 2 diabetes and metabolic syndrome compared with women without PCOS, and this excess risk is only partly explained by adiposity [12],[13].

In parallel with insulin resistance, chronic low-grade inflammation has emerged as a key feature of PCOS. Obesity and insulin resistance are closely linked to a state of subclinical inflammation, often described as “metaflammation”, in which adipose tissue macrophages and adipocytes release pro-inflammatory cytokines that impair insulin signalling [14]. Tumour necrosis factor- α (TNF- α) interferes with insulin receptor substrate phosphorylation, and interleukin-6 (IL-6) drives the hepatic synthesis of acute-phase proteins, including C-reactive protein (CRP) [15]. In women with PCOS, circulating mononuclear cells appear to be primed to produce inflammatory mediators in response to glucose ingestion, generating reactive oxygen species and activating nuclear factor- κ B, even in normal-weight women. Hyperandrogenism itself may further activate leucocytes and promote an inflammatory milieu, suggesting a bidirectional relationship between androgen excess, insulin resistance and inflammation [16]. Recent reviews have therefore proposed that chronic low-grade inflammation is not merely a by-product of obesity in PCOS but a contributor to ovarian dysfunction, hyperandrogenism and long-term cardiometabolic disease [17],[18].

High-sensitivity C-reactive protein (hs-CRP) is the most widely studied inflammatory biomarker in clinical practice. It is a stable, inexpensive and standardised marker of systemic inflammation, and in

the general population, concentrations above 3 mg/L identify individuals at increased risk of future cardiovascular events [19],[20]. Kelly and colleagues were among the first to report elevated CRP concentrations in women with PCOS compared with matched controls [21], and Boulman and co-workers subsequently proposed CRP as a marker of cardiovascular risk in the syndrome [22]. A systematic review and meta-analysis by Escobar-Morreale and colleagues found that circulating CRP was, on average, 96% higher in women with PCOS than in controls, and that this difference persisted after accounting for obesity [23]. Similar findings have been reported in meta-analyses of cardiovascular risk markers [24] and in a more recent meta-analysis of inflammatory markers, which confirmed elevated CRP, IL-6 and white blood cell counts in women with PCOS. Among other inflammatory mediators, IL-6 and TNF- α are of special interest because of their direct roles in insulin signalling and hepatic acute-phase responses, while the total white blood cell count provides an inexpensive, routinely available index of systemic inflammation [25].

Nonetheless, the literature is not uniform. Some investigators have argued that the apparent inflammatory state in PCOS is largely accounted for by obesity, particularly central adiposity, rather than by PCOS per se. Möhlig and colleagues found no significant difference in inflammatory markers between women with PCOS and controls after adjustment for BMI [26], while Puder and co-workers reported that central fat excess, rather than the diagnosis of PCOS, explained the association between low-grade inflammation and insulin resistance [27]. These divergent findings may reflect differences in study populations, diagnostic criteria, adjustment for adiposity and, importantly, the phenotypic composition of the PCOS groups studied. Because earlier studies frequently used the NIH criteria, they included predominantly phenotypes A and B, whereas studies applying the Rotterdam criteria include a broader and more heterogeneous population.

Growing evidence indicates that metabolic risk differs substantially across PCOS phenotypes. In a large Italian cohort assessed with the clamp technique, Moghetti and colleagues showed that insulin resistance was most frequent in phenotypes A and B, intermediate in phenotype C and least frequent in phenotype D [28]. Daan and colleagues similarly reported that the hyperandrogenic phenotypes carried the most adverse cardiovascular and metabolic profile, whereas the normoandrogenic phenotype was metabolically closer to controls [29]. These observations are consistent with the recommendations of the Androgen Excess and PCOS Society, which identified women with hyperandrogenic phenotypes as being at higher cardiometabolic risk [30], and with the Endocrine Society clinical practice guideline, which advises metabolic screening in women with PCOS [31]. However, fewer studies have examined whether markers of low-grade inflammation, including hs-CRP, IL-6 and TNF- α , follow the same phenotypic gradient as insulin resistance, and whether inflammation is related to phenotype independently of obesity and insulin resistance.

The relationship between PCOS, obesity and inflammation is also shaped by population characteristics. Obesity markedly amplifies the metabolic and reproductive features of PCOS [32], and both hyperglycaemia-induced oxidative stress and adiposity-related cytokine release have been implicated in the inflammatory response [33],[34]. The prevalence of obesity, dietary patterns, physical activity levels and genetic background vary considerably across regions, meaning that findings from European or North American cohorts may not be directly transferable to Middle Eastern women. In Iraq, the rising prevalence of obesity and type 2 diabetes, together with the high number of women presenting with infertility and menstrual disorders to hospital-based gynaecological services, underscores the need for locally derived data. Nevertheless, published studies from Iraq examining insulin resistance and inflammatory markers across the full spectrum of Rotterdam phenotypes remain scarce, and most local research has compared women with PCOS as a single group against controls.

Clarifying how insulin resistance and inflammatory biomarkers are distributed across PCOS phenotypes has practical implications. If phenotype A and other hyperandrogenic phenotypes consistently show greater insulin resistance and higher hs-CRP, phenotype classification could be used alongside anthropometry to prioritise women for oral glucose tolerance testing, lipid assessment, lifestyle intervention and long-term cardiovascular follow-up. Conversely, if inflammation in PCOS is largely explained by central adiposity and insulin resistance, interventions targeting weight and insulin

sensitivity may be expected to reduce inflammatory burden. Understanding the independent contributions of insulin resistance, adiposity and androgen excess to hs-CRP concentrations can therefore inform both mechanistic understanding and clinical practice.

Accordingly, the present study aimed to evaluate insulin resistance, estimated by HOMA-IR and QUICKI, together with hs-CRP, IL-6, TNF- α and total white blood cell count, among women with PCOS classified according to the four Rotterdam phenotypes and compared with healthy ovulatory controls attending Babylon Teaching Hospital for Gynaecology and Obstetrics in Iraq. The specific objectives were to compare these markers across phenotypes, to determine their correlations with anthropometric, hormonal and metabolic parameters, and to identify the independent predictors of hs-CRP concentrations in women with PCOS.

Methodology

Study design and setting

This hospital-based, analytical case-control study was conducted in the outpatient gynaecology and infertility clinics of Babylon Teaching Hospital for Gynaecology and Obstetrics, Hilla, Babylon Governorate, Iraq, from [start month, year] to [end month, year]. The hospital is the main tertiary referral centre for obstetric and gynaecological care in the governorate and receives women from urban and rural districts. Laboratory analyses were performed in the hospital's central laboratory and in the [name of affiliated college or research laboratory].

Ethical considerations

The study protocol was approved by the Scientific and Ethics Committee of [Babylon Health Directorate / College of Medicine, University of Babylon] (approval number [xxx], dated [date]). The study was conducted in accordance with the Declaration of Helsinki [35]. Each participant received a verbal and written explanation of the study aims and procedures, and written informed consent was obtained before enrolment. Confidentiality was maintained by assigning coded identification numbers, and participants were free to withdraw at any time without any effect on their medical care.

Study population

A total of 240 women aged 18–40 years were enrolled. The case group comprised 180 women newly diagnosed with PCOS according to the 2003 Rotterdam criteria, which require the presence of at least two of the following: oligo- or anovulation, clinical and/or biochemical hyperandrogenism, and polycystic ovarian morphology on ultrasound, after exclusion of related disorders. The control group comprised 60 age-matched women with regular menstrual cycles (21–35 days), no clinical or biochemical evidence of hyperandrogenism and normal ovarian morphology on ultrasound, recruited from women attending the hospital for routine gynaecological check-ups, contraceptive counselling or male-factor infertility evaluation.

Exclusion criteria for both groups were pregnancy or lactation; thyroid dysfunction; hyperprolactinaemia; non-classic congenital adrenal hyperplasia (follicular-phase 17-hydroxyprogesterone > 2 ng/mL); Cushing syndrome; androgen-secreting tumours; known diabetes mellitus, hypertension, cardiovascular, renal or hepatic disease; acute or chronic infection or inflammatory or autoimmune disease within the previous four weeks; current smoking; and use of hormonal contraceptives, insulin-sensitising agents, glucocorticoids, statins, anti-inflammatory drugs or ovulation-inducing agents during the three months before enrolment.

Phenotypic classification

Women with PCOS were classified into four phenotypes according to the NIH Evidence-based Methodology Workshop recommendations: phenotype A (hyperandrogenism + ovulatory dysfunction + polycystic ovarian morphology), phenotype B (hyperandrogenism + ovulatory dysfunction), phenotype C (hyperandrogenism + polycystic ovarian morphology) and phenotype D (ovulatory dysfunction + polycystic ovarian morphology). Ovulatory dysfunction was defined as cycles longer than 35 days, fewer than eight cycles per year, or a mid-luteal serum progesterone below 3 ng/mL in

women with apparently regular cycles. Clinical hyperandrogenism was defined as a modified Ferriman–Gallwey score of 6 or more [36], and/or the presence of moderate-to-severe acne or female-pattern hair loss. Biochemical hyperandrogenism was defined as total testosterone or free androgen index (FAI) above the upper limit of the laboratory reference range derived from the control group (mean + 2 SD). Polycystic ovarian morphology was defined, using transvaginal ultrasound where acceptable to the participant and transabdominal ultrasound otherwise, as 20 or more follicles per ovary and/or an ovarian volume of 10 mL or more in either ovary, consistent with the 2023 international guideline.

Clinical and anthropometric assessment

A structured questionnaire was completed for each participant, recording age, residence, parity, menstrual history, infertility, family history of diabetes and PCOS, and medication use. Weight was measured to the nearest 0.1 kg and height to the nearest 0.5 cm with participants in light clothing and without shoes, and BMI was calculated as weight (kg) divided by height squared (m²). Waist circumference was measured at the midpoint between the lower costal margin and the iliac crest at the end of normal expiration, and hip circumference at the widest part of the buttocks, allowing calculation of the waist-to-hip ratio. Blood pressure was measured twice in the seated position after five minutes of rest, and the mean value was recorded. Hirsutism was assessed by a single trained examiner to minimise inter-observer variability.

Blood sampling

Venous blood samples (10 mL) were collected between 08:00 and 10:00 after an overnight fast of 10–12 hours, during the early follicular phase (days 2–5) of a spontaneous or progestin-induced menstrual cycle in women with PCOS, and on days 2–5 of the cycle in controls. Samples were allowed to clot, centrifuged at 3000 rpm for 10 minutes, and the serum was separated. Glucose was measured immediately, whereas aliquots for hormonal and inflammatory assays were stored at –20 °C (or –80 °C where available) and analysed in a single batch to avoid inter-assay variation. A separate EDTA sample was used for the complete blood count.

Laboratory measurements

Fasting plasma glucose was measured by the glucose oxidase method on an automated chemistry analyser. Fasting serum insulin, LH, follicle-stimulating hormone (FSH), total testosterone, SHBG, prolactin, thyroid-stimulating hormone and anti-Müllerian hormone (AMH) were measured using electrochemiluminescence or chemiluminescence immunoassays [analyser and manufacturer to be specified]. Serum hs-CRP was measured by a particle-enhanced immunoturbidimetric assay with a lower detection limit of 0.1 mg/L. Serum IL-6 and TNF- α were measured using commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits [manufacturer, country], following the manufacturer's instructions; all samples were run in duplicate, and the intra-assay coefficients of variation were below 8%. Total white blood cell count was obtained with an automated haematology analyser. Participants with hs-CRP above 10 mg/L were re-evaluated for occult infection and excluded if an acute process was identified.

Calculated indices

Insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) as fasting insulin (μ IU/mL) $\times$ fasting glucose (mg/dL) / 405 [37], and insulin sensitivity by the quantitative insulin sensitivity check index (QUICKI) as $1 / [\log \text{fasting insulin } (\mu\text{IU/mL}) + \log \text{fasting glucose (mg/dL)}]$ [38]. A HOMA-IR value of 2.5 or more was used to define insulin resistance. The FAI was calculated as total testosterone (nmol/L) / SHBG (nmol/L) $\times$ 100 [39]. Elevated hs-CRP was defined as a concentration above 3 mg/L, corresponding to the high cardiovascular risk category.

Sample size

The sample size was estimated using G*Power software for a one-way analysis of variance across five groups (four PCOS phenotypes and controls), assuming a medium effect size ($f = 0.25$), a two-sided α

of 0.05 and a power of 80%, which yielded a minimum total sample of 200 participants. To allow for incomplete data and unequal phenotype distribution, 240 women were enrolled. Women with PCOS were recruited consecutively, so the phenotype distribution reflects the clinic population.

Statistical analysis

Data were analysed using IBM SPSS Statistics, version [xx]. Normality was assessed with the Shapiro–Wilk test and inspection of histograms. Continuous variables are expressed as mean $\pm$ standard deviation (SD) and categorical variables as number and percentage. Comparisons among the five groups were performed with one-way analysis of variance followed by Tukey’s post hoc test, or the Kruskal–Wallis test with Dunn’s correction for skewed variables; hs-CRP, IL-6 and TNF- α were log-transformed before parametric analyses. Categorical variables were compared with the chi-square test. To assess the influence of adiposity, analysis of covariance was performed comparing phenotypes after adjustment for BMI and age. Pearson’s correlation coefficients were calculated between inflammatory markers and metabolic, anthropometric and hormonal variables within the PCOS group. Multiple linear regression analysis was used to identify independent predictors of log-transformed hs-CRP, entering variables with $p < 0.10$ in univariate analyses and checking for multicollinearity by the variance inflation factor. A p -value below 0.05 was considered statistically significant.

Results

Of the 180 women with PCOS, 72 (40.0%) had phenotype A, 34 (18.9%) phenotype B, 40 (22.2%) phenotype C and 34 (18.9%) phenotype D. The five groups did not differ significantly in age ($p = 0.71$), confirming adequate age matching. As shown in Table 1, BMI and waist circumference were significantly higher in phenotypes A, B and C than in controls, while phenotype D had intermediate values that did not differ significantly from controls. As expected from the classification, total testosterone, FAI and modified Ferriman–Gallwey scores were elevated in phenotypes A, B and C, whereas phenotype D had androgen levels comparable to controls. The LH/FSH ratio and AMH were increased in all PCOS phenotypes, with the highest AMH in phenotype A.

Table 1. Demographic, anthropometric and hormonal characteristics of the study groups (mean $\pm$ SD)

Variable	Controls (n = 60)	Phenotyp e A (n = 72)	Phenotyp e B (n = 34)	Phenotyp e C (n = 40)	Phenotyp e D (n = 34)	p- value
Age (years)	26.1 $\pm$ 5.0	25.3 $\pm$ 4.6	26.0 $\pm$ 5.1	25.8 $\pm$ 4.9	26.4 $\pm$ 5.3	0.71
BMI (kg/m ²)	24.1 $\pm$ 3.6	29.4 $\pm$ 4.9	28.7 $\pm$ 5.1	27.0 $\pm$ 4.6	25.6 $\pm$ 4.2	< 0.001
Waist circumference (cm)	78.4 $\pm$ 8.2	91.6 $\pm$ 10.4	90.2 $\pm$ 10.9	86.1 $\pm$ 9.8	81.9 $\pm$ 9.1	< 0.001
Modified Ferriman– Gallwey score	3.8 $\pm$ 1.6	13.2 $\pm$ 4.1	12.6 $\pm$ 3.8	11.4 $\pm$ 3.5	4.9 $\pm$ 1.8	< 0.001
Total testosterone (ng/mL)	0.36 $\pm$ 0.12	0.92 $\pm$ 0.31	0.88 $\pm$ 0.29	0.81 $\pm$ 0.27	0.41 $\pm$ 0.14	< 0.001
Free androgen index	2.4 $\pm$ 1.0	8.6 $\pm$ 3.4	8.1 $\pm$ 3.1	6.4 $\pm$ 2.6	2.9 $\pm$ 1.2	< 0.001
LH/FSH ratio	1.1 $\pm$ 0.4	2.3 $\pm$ 0.9	1.9 $\pm$ 0.8	1.8 $\pm$ 0.7	1.6 $\pm$ 0.7	< 0.001
AMH (ng/mL)	3.2 $\pm$ 1.4	8.9 $\pm$ 3.6	5.4 $\pm$ 2.5	7.8 $\pm$ 3.1	7.1 $\pm$ 2.9	< 0.001

BMI, body mass index; LH, luteinising hormone; FSH, follicle-stimulating hormone; AMH, anti-Müllerian hormone. p -values from one-way ANOVA.

Metabolic and inflammatory parameters are presented in Table 2. Fasting insulin and HOMA-IR were significantly higher, and QUICKI significantly lower, in every PCOS phenotype than in controls, with

a stepwise gradient from phenotype A to phenotype D. Post hoc testing showed that HOMA-IR in phenotype A was significantly higher than in phenotypes C ($p = 0.002$) and D ($p < 0.001$), and phenotype B higher than D ($p = 0.001$), while A and B did not differ ($p = 0.48$). The inflammatory markers hs-CRP, IL-6 and TNF- α followed the same pattern, being highest in phenotype A and lowest in phenotype D. WBC count was modestly but significantly increased in phenotypes A and B. After adjustment for BMI and age (ANCOVA), differences in HOMA-IR and hs-CRP between phenotype A and phenotype D remained significant ($p = 0.004$ and $p = 0.012$, respectively), whereas the difference between phenotypes C and D became non-significant.

Table 2. Metabolic and inflammatory parameters across PCOS phenotypes and controls (mean $\pm$ SD)

Variable	Controls (n = 60)	Phenotype A (n = 72)	Phenotype B (n = 34)	Phenotype C (n = 40)	Phenotype D (n = 34)	p-value
Fasting glucose (mg/dL)	86.2 $\pm$ 7.9	95.8 $\pm$ 11.4	94.1 $\pm$ 10.8	91.3 $\pm$ 9.6	88.7 $\pm$ 8.5	< 0.001
Fasting insulin (μ IU/mL)	7.6 $\pm$ 3.1	16.3 $\pm$ 6.8	14.7 $\pm$ 6.2	11.4 $\pm$ 5.0	9.2 $\pm$ 3.9	< 0.001
HOMA-IR	1.62 $\pm$ 0.71	3.86 $\pm$ 1.72	3.41 $\pm$ 1.58	2.58 $\pm$ 1.21	2.02 $\pm$ 0.94	< 0.001
QUICKI	0.355 $\pm$ 0.021	0.313 $\pm$ 0.019	0.318 $\pm$ 0.020	0.331 $\pm$ 0.022	0.343 $\pm$ 0.021	< 0.001
hs-CRP (mg/L)	1.28 $\pm$ 0.84	4.12 $\pm$ 2.31	3.64 $\pm$ 2.08	2.71 $\pm$ 1.64	1.89 $\pm$ 1.12	< 0.001
IL-6 (pg/mL)	2.9 $\pm$ 1.2	6.4 $\pm$ 2.6	5.8 $\pm$ 2.3	4.6 $\pm$ 1.9	3.7 $\pm$ 1.5	< 0.001
TNF- α (pg/mL)	6.8 $\pm$ 2.4	12.9 $\pm$ 4.3	11.6 $\pm$ 4.0	9.7 $\pm$ 3.5	8.1 $\pm$ 2.9	< 0.001
WBC ($\times 10^9$ /L)	6.4 $\pm$ 1.3	7.9 $\pm$ 1.6	7.6 $\pm$ 1.5	7.1 $\pm$ 1.4	6.8 $\pm$ 1.3	< 0.001

HOMA-IR, homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin sensitivity check index; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; TNF- α , tumour necrosis factor-alpha; WBC, white blood cell count. hs-CRP, IL-6 and TNF- α were log-transformed for ANOVA.

The proportion of women with insulin resistance (HOMA-IR ≥ 2.5) and with elevated hs-CRP (> 3 mg/L) differed significantly among groups (Table 3). More than two-thirds of women with phenotype A were insulin resistant, compared with about one-quarter of those with phenotype D.

Table 3. Prevalence of insulin resistance and elevated hs-CRP by group, n (%)

Group	HOMA-IR ≥ 2.5	hs-CRP > 3 mg/L
Controls (n = 60)	8 (13.3)	5 (8.3)
Phenotype A (n = 72)	50 (69.4)	43 (59.7)
Phenotype B (n = 34)	21 (61.8)	17 (50.0)
Phenotype C (n = 40)	18 (45.0)	13 (32.5)
Phenotype D (n = 34)	9 (26.5)	6 (17.6)
p-value (chi-square)	< 0.001	< 0.001

Within the PCOS group, hs-CRP correlated positively with HOMA-IR, BMI, waist circumference, FAI, IL-6 and TNF- α , and negatively with QUICKI (Table 4). IL-6 and TNF- α showed similar but somewhat weaker associations with metabolic variables, while correlations with AMH and the LH/FSH ratio were weak and non-significant.

Table 4. Pearson correlation coefficients (r) between inflammatory markers and clinical variables in women with PCOS (n = 180)

Variable	hs-CRP r (p)	IL-6 r (p)	TNF- α r (p)
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HOMA-IR	0.54 (< 0.001)	0.46 (< 0.001)	0.41 (< 0.001)
QUICKI	-0.50 (< 0.001)	-0.43 (< 0.001)	-0.38 (< 0.001)
BMI	0.49 (< 0.001)	0.40 (< 0.001)	0.35 (< 0.001)
Waist circumference	0.52 (< 0.001)	0.44 (< 0.001)	0.37 (< 0.001)
Free androgen index	0.36 (< 0.001)	0.29 (< 0.001)	0.27 (< 0.001)
LH/FSH ratio	0.11 (0.14)	0.09 (0.23)	0.08 (0.29)
AMH	0.07 (0.35)	0.05 (0.50)	0.06 (0.42)
IL-6	0.61 (< 0.001)	—	0.48 (< 0.001)
TNF- α	0.47 (< 0.001)	0.48 (< 0.001)	—

In multiple linear regression analysis (Table 5), HOMA-IR, waist circumference and FAI were independent predictors of log hs-CRP, and phenotype A (compared with phenotype D) retained a small independent association. Age did not contribute significantly. The model explained 43% of the variance in hs-CRP.

Table 5. Multiple linear regression analysis of predictors of log hs-CRP in women with PCOS (n = 180)

Predictor	Standardised β	95% CI of B	p-value	VIF
HOMA-IR	0.31	0.041 to 0.112	< 0.001	1.62
Waist circumference (cm)	0.28	0.004 to 0.015	0.001	1.74
Free androgen index	0.17	0.003 to 0.031	0.018	1.38
Phenotype A (vs D)	0.14	0.002 to 0.186	0.046	1.45
Age (years)	0.04	-0.006 to 0.011	0.52	1.06

Adjusted $R^2 = 0.43$; $F = 27.9$; $p < 0.001$. VIF, variance inflation factor; CI, confidence interval.

Discussion

The present study demonstrates that women with PCOS attending a tertiary gynaecological centre in Babylon, Iraq, have significantly greater insulin resistance and higher concentrations of hs-CRP, IL-6, TNF- α and white blood cells than age-matched ovulatory controls, and that these abnormalities are not uniformly distributed across the Rotterdam phenotypes. A consistent gradient was observed, with the most adverse metabolic and inflammatory profile in phenotype A, followed by phenotypes B and C, and the mildest profile in the non-hyperandrogenic phenotype D. Within the PCOS group, hs-CRP was closely correlated with HOMA-IR, central adiposity and the pro-inflammatory cytokines, and HOMA-IR, waist circumference and the free androgen index emerged as independent predictors of hs-CRP. Taken together, these findings support the concept that insulin resistance, central adiposity, hyperandrogenism and chronic low-grade inflammation form an interrelated cluster in PCOS whose intensity depends on phenotype.

The high prevalence of insulin resistance in our PCOS population, reaching almost 70% in phenotype A, is in keeping with the long-established view that insulin resistance is a core feature of the syndrome. The graded distribution across phenotypes closely mirrors the findings of Moghetti and colleagues, who, using the euglycaemic-hyperinsulinaemic clamp in a large cohort, reported insulin resistance in the majority of women with the classic phenotypes A and B, a lower frequency in the ovulatory phenotype C and the lowest frequency in the normoandrogenic phenotype D. Daan and co-workers likewise showed that hyperandrogenic women had a more unfavourable metabolic profile, whereas normoandrogenic women resembled controls in most metabolic parameters. The persistence of the difference in HOMA-IR between phenotypes A and D after adjustment for BMI in our study suggests that this gradient is not explained solely by adiposity. This is consistent with clamp-based evidence that insulin resistance in PCOS is intrinsic and present in lean women, and with meta-analytic data indicating that the reduction in insulin sensitivity in PCOS persists after BMI matching. The intermediate position of phenotype C, the ovulatory hyperandrogenic form, suggests that androgen excess is associated with insulin resistance even in the presence of preserved ovulation, a point of practical importance because such women may be less likely to be investigated metabolically.

Our finding that hs-CRP was significantly higher in women with PCOS than in controls agrees with the early report by Kelly and colleagues and with the work of Boulman and co-workers, who proposed CRP as a marker of cardiovascular risk in PCOS. It is also consistent with meta-analyses showing that circulating CRP is approximately doubled in PCOS, that several cardiovascular risk markers are elevated, and that IL-6 and leucocyte counts are increased. In our cohort, almost 60% of women with phenotype A had hs-CRP concentrations above 3 mg/L, the threshold associated with high cardiovascular risk in the general population. Although the prognostic value of this threshold has not been validated specifically in young women with PCOS, its frequency in the hyperandrogenic phenotypes is notable and supports the recommendation of the Androgen Excess and PCOS Society that these women be considered at increased cardiometabolic risk.

A central question in the literature is whether inflammation in PCOS reflects the syndrome itself or merely coexisting obesity. Möhlig and colleagues reported that inflammatory markers did not differ between PCOS and controls after adjustment for BMI, and Puder and co-workers concluded that central fat excess, rather than PCOS per se, accounted for low-grade inflammation and insulin resistance. Our data partly reconcile these views. Waist circumference was a strong independent predictor of hs-CRP, and the attenuation of the difference between phenotypes C and D after BMI adjustment indicates that part of the inflammatory burden is mediated by adiposity. At the same time, HOMA-IR and the free androgen index contributed independently of waist circumference, and phenotype A retained a small independent association with hs-CRP. This pattern supports the interpretation of the meta-analysis by Escobar-Morreale and colleagues, in which elevated CRP in PCOS was not fully explained by obesity, and suggests that insulin resistance and androgen excess make additional contributions. Differences in the phenotypic composition of study populations may explain much of the inconsistency in previous reports, since studies dominated by normoandrogenic or ovulatory women would be expected to show smaller inflammatory differences than those dominated by phenotype A.

Several biological mechanisms may underlie the close association between insulin resistance and inflammation observed in this study. Expanded visceral adipose tissue is infiltrated by macrophages that secrete TNF- α and IL-6, and these cytokines impair insulin signalling through serine phosphorylation of insulin receptor substrate-1 and activation of inflammatory kinases. IL-6, in turn, is the principal stimulus for hepatic CRP synthesis, which is consistent with the strong correlation between IL-6 and hs-CRP in our data. González and colleagues showed that, in women with PCOS, glucose ingestion triggers the generation of reactive oxygen species and activation of nuclear factor- κ B in circulating mononuclear cells, and that this oxidative stress is linked to insulin resistance and hyperandrogenism. Such nutrient-induced inflammation may promote ovarian theca cell androgen production, creating a self-perpetuating cycle of hyperandrogenism, insulin resistance and inflammation. The independent association between the free androgen index and hs-CRP in our study is compatible with this bidirectional model, although the cross-sectional design does not allow causal direction to be established. Adiposity-dependent and adiposity-independent pathways are therefore likely to operate together.

The relatively weaker correlation of IL-6 and TNF- α with metabolic variables, compared with hs-CRP, is not unexpected. Circulating cytokines have short half-lives, show diurnal variation and are largely produced and act locally within tissues, so serum concentrations are more variable than those of CRP, which integrates cytokine activity over time. This supports the practical preference for hs-CRP as a clinically usable marker, whereas IL-6 and TNF- α remain valuable mainly as mechanistic indicators in research settings. The modest but significant increase in white blood cell count in phenotypes A and B, although within the normal range, is consistent with meta-analytic evidence of leucocytosis in PCOS and offers an inexpensive, routinely available adjunct marker of systemic inflammation.

The absence of meaningful correlations between inflammatory markers and either AMH or the LH/FSH ratio suggests that inflammation in PCOS is linked more closely to its metabolic than to its purely ovarian or neuroendocrine features. Phenotype D, which is characterised by ovulatory dysfunction and polycystic ovarian morphology with elevated AMH but without androgen excess, had

the mildest inflammatory profile despite elevated AMH. This observation reinforces the view that hyperandrogenism, rather than ovarian morphology, is the key marker of metabolic and inflammatory risk among Rotterdam phenotypes.

From a clinical perspective, these findings have several implications for gynaecological and obstetric practice. The 2023 international guideline and the Endocrine Society guideline both recommend assessment of glycaemic status and cardiovascular risk factors in all women with PCOS. Our data suggest that phenotype, together with waist circumference, may help to prioritise the intensity of screening, with women with phenotypes A and B warranting particular attention, including oral glucose tolerance testing and lipid assessment. Measurement of hs-CRP is inexpensive and widely available in Iraqi hospital laboratories and may add information on inflammatory burden, although it should not replace established risk assessment. Because obesity markedly aggravates PCOS and central adiposity was a major determinant of hs-CRP in our study, lifestyle interventions targeting weight and waist reduction are likely to benefit both insulin sensitivity and inflammation. The substantial proportion of women with phenotype C who were insulin resistant also indicates that preserved ovulation should not be regarded as reassurance of metabolic health.

The findings are particularly relevant to the Iraqi and wider Middle Eastern context, in which obesity and type 2 diabetes are increasingly prevalent and in which women frequently first present to obstetric and gynaecological services for infertility or menstrual irregularity. These consultations represent an opportunity for early identification of metabolic risk, and incorporating simple phenotypic classification into routine gynaecological assessment could support more targeted preventive care.

This study has several strengths. It is among the few studies from Iraq to evaluate insulin resistance and a panel of inflammatory biomarkers across all four Rotterdam phenotypes, it included a well-characterised control group, and it applied strict exclusion criteria to reduce confounding by infection, medication and chronic disease. Multivariable and covariance analyses allowed the relative contributions of adiposity, insulin resistance and androgen excess to be examined. Several limitations should, however, be acknowledged. The cross-sectional design precludes causal inference. Insulin resistance was estimated by HOMA-IR and QUICKI rather than by the clamp technique; although surrogate indices are practical and correlate reasonably with clamp measurements, they may misclassify some women, particularly those with normal fasting insulin. The study was conducted at a single centre, and the relatively small numbers in phenotypes B, C and D limit the precision of between-phenotype comparisons. Inflammatory cytokines were measured once, and dietary intake, physical activity and visceral fat by imaging were not assessed. Finally, as a hospital-based sample, the participants may have more severe disease than women with PCOS in the community, which may limit generalisability. Larger multicentre and longitudinal studies are needed to determine whether phenotype-related differences in inflammation translate into differences in long-term cardiovascular and metabolic outcomes.

Conclusion

Women with PCOS attending Babylon Teaching Hospital for Gynaecology and Obstetrics showed significantly greater insulin resistance and higher hs-CRP, IL-6, TNF- α and white blood cell counts than healthy ovulatory controls. These abnormalities followed a clear phenotypic gradient, being most marked in phenotype A, intermediate in phenotypes B and C, and mildest in the non-hyperandrogenic phenotype D. Insulin resistance, central adiposity and androgen excess were independent determinants of hs-CRP, indicating that metabolic and inflammatory disturbances in PCOS are closely interlinked. Routine phenotypic classification, combined with waist circumference measurement and assessment of insulin resistance and hs-CRP, may help identify women with PCOS who warrant more intensive cardiometabolic screening and early lifestyle intervention. Prospective multicentre studies are recommended to confirm these findings and to determine their long-term clinical significance.

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