

Correlation of Markers of Inflammation with Hormonal, Metabolic Parameters, Insulin Resistance and Adiposity Indices in First-Degree Relatives of Patient with Polycystic Ovary Syndrome

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is a state of chronic low-grade inflammation. Low-grade inflammation has been linked to the development of cardiovascular disease (CVD). There is evidence of clustering for metabolic syndrome, hypertension, dyslipidaemia in type 2 diabetes mellitus and insulin resistance (IR) in mothers, fathers, sisters and brothers of women with PCOS.

Aims: The aim is to study the levels of inflammatory markers and IR in first-degree relatives of patients with PCOS and find any correlation with hormonal parameters, metabolic parameters and adiposity indices in them. **Settings and Design:** A total of 66 first-degree relatives of a patient with PCOS were included in this cross-sectional study. **Materials and Methods:** All participants underwent detailed clinical evaluation and biochemical investigations, including high-sensitivity C-reactive protein (hsCRP), interleukin 6 (IL-6), luteinising hormone (LH), follicle-stimulating hormone (FSH) and total testosterone (only in females). Homeostasis model assessment of IR (HOMA-IR), lipid accumulation product and visceral adiposity index were calculated using standard equations. Visceral adipose tissue thickness and subcutaneous adipose tissue thickness were assessed using ultrasonography. **Statistical Analysis Used:** Spearman's and Pearson's correlation coefficients were used to analyse the correlation between different non-parametric and parametric data, respectively. Multiple linear regression was used to correlate multiple dependent factors. **Results:** The mean hs-CRP level was 2.4 ± 1.1 mg/L, which is greater than the cut-off of 2 mg/L and hs-CRP >2 mg/L was found in 62% ($n = 41$) participants. The mean IL-6 (3.5 ± 1.1 pg/ml) and total white blood cell count ($7244 \pm 2190/\text{mm}^3$) were in the normal range. The mean HOMA-IR was 2.35 ± 0.76 , which is elevated, considering HOMA IR >2 as a predictor of IR and metabolic syndrome. HOMA IR >2 was found in 64% ($n = 42$) of the participants. Inflammatory markers were significantly correlated with LH and HOMA IR, even after multiple linear regression was fitted for each marker individually.

Conclusion: Apparently, healthy first-degree relatives of PCOS patients had evidence of chronic low-grade inflammation. The chronic inflammation in them

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correlated well with HOMA-IR and LH but was independent of body mass index. This low-grade inflammation may predispose the first-degree relatives of PCOS to CVD.

KEYWORDS: *First-degree relatives, Homeostasis model assessment of insulin resistance, high-sensitivity C-reactive protein, interleukin 6, polycystic ovary syndrome*

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a heterogeneous disorder with a prevalence varying between 6% and 20%, possibly making this syndrome the most common endocrine and metabolic disorder in women of reproductive age.^[1-3] Various criteria have been laid to diagnose PCOS, out of which the most commonly used is the Rotterdam criteria, according to which, after excluding disorders which mimic PCOS, the diagnosis of PCOS is made if any 2 out of three following criteria are met: androgen excess, ovulatory dysfunction, or ultrasonographic appearance of polycystic ovarian morphology.^[4] The incidence of PCOS is on the rise and this is probably going to be the most common disorder leading to chronic anovulation.

PCOS not only includes disorders related to anovulation and reproduction but, association of various metabolic disorders, such as hyperinsulinaemia, insulin resistance (IR), glucose intolerance, dyslipidaemia, obesity and non-alcoholic fatty liver disease along with it.^[5,6] Recently, it has been observed that women with PCOS have raised levels of inflammatory markers. In these women, C-reactive protein (CRP), and various cytokines such as interleukin 6 (IL-6), interleukin 18, tumour necrosis factor- α , etc., were found to be elevated.^[5,7] Chronic low-grade inflammation, associated with obesity and IR has been implicated as a risk factor for endothelial dysfunction, atherosclerosis and coronary heart disease.^[5,8] Visceral obesity is associated with IR, glucose intolerance, diabetes mellitus, increase in androgen production rate and decreased levels of sex hormone-binding globulin that leads to increased levels of free testosterone.^[9] Visceral obesity, in contrast to general obesity, is a more specific risk factor for PCOS. However, IR and androgen excess are not confined to obese anovulatory women but also occur in nonobese anovulatory women.^[10] It has been observed that in comparison to women with isolated obesity and IR, women with PCOS along with obesity and IR have higher levels of inflammatory markers.^[11]

There is the clustering of PCOS in families, which suggests that it has an underlying genetic basis.^[12,13] Family studies of PCOS investigated mainly ovarian morphology, menstrual irregularities, symptoms of hyperandrogenism and hyperandrogenaemia.^[13-20] In a Dutch twin-family study, the tetrachoric correlation

between monozygotic twins for PCOS was 0.71, and for dizygotic twin or non-twin sister pairs, the correlation was 0.38.^[21] In the Han Chinese population, genome-wide PCOS association signals showed evidence of enrichment for candidate genes related to insulin signalling (INSR), gonadotropin receptors (FSHR, LHCGR), and type 2 diabetes (HMGGA2, THADA, DENND1A).^[22] Few meta-analyses do show evidence of clustering for metabolic syndrome, hypertension, dyslipidaemia, type 2 diabetes mellitus (T2DM) and IR in mothers, fathers, sisters and brothers of women with PCOS.^[23,24]

A recent study in India showed the presence of metabolic syndrome or related metabolic derangements to be high in the family members of women with PCOS.^[25] Although limited, the available evidence raises the hypothesis that first-degree relatives of women with PCOS are at risk of having increased markers of inflammation along with IR and altered hormonal profile. Thus, we propose this study to be the first of its kind to study the correlation of markers of inflammation and IR with hormonal, metabolic parameters and adiposity indices in first-degree relatives of a patient with PCOS.

MATERIALS AND METHODS

Study design and population

This cross-sectional study was conducted at an academic institute. The study was approved by the institutional ethics committee (IEC Appln no. 248/26 August 2020). Ethical principles of the World Medical Association Declaration of Helsinki (2013) were adhered to while conducting the study.

Inclusion criteria

Father, mother, brothers and sisters of patients with a confirmed diagnosis of PCOS by Rotterdam criteria, either newly diagnosed or on follow-up, who were accompanying the patient or were available for evaluation were screened for inclusion in the study.

Exclusion criteria

Relatives with a diagnosis of diabetes mellitus, coronary artery disease, cerebrovascular disease, chronic kidney disease and chronic liver disease, presence of PCOS in sisters and mothers, any acute or chronic infection, any history of connective tissue disorders or other inflammatory disorder, relatives with any type of

malignancy (ca. breast, ca. endometrium, ca. ovary, ca. prostate, ca. lungs, etc.), pregnant and lactating women, alcoholics and smokers were excluded from the study. Furthermore, relatives with current or previous use (within 6 months) of glucocorticoids, non-steroidal anti-inflammatory drugs, oral contraceptives, anti-androgens, anti-diabetics, statins or other hormonal drugs were excluded from the study.

After applying the above inclusion and exclusion criteria, 66 first-degree family members of PCOS patients could be enrolled in the current study. The number of participants in this study fulfilled the minimum number of samples needed for research in a health study of 30 samples. Hence, the data generated are expected to be normally distributed in calculating statistics. The study participants were explained in detail about the disorder of the patient, the implication of the same in first-degree relatives, purpose of the study and the work up plan. A valid consent was taken from the patients and relatives included, followed by a detailed history, physical examination and basic biochemical evaluation. After applying the exclusion criteria, the participants were qualified for further evaluation in inflammatory markers, hormonal parameters and USG to determine subcutaneous and visceral adiposity.

Clinical assessment

A detailed history was taken, including history suggestive of hyperandrogenism in female relatives and a history of early onset androgenetic alopecia in male relatives. History of all relevant diseases and medication use was obtained.

Physical examination included vitals, anthropometry, general examination and systemic examination. Height, weight, waist circumference (WC) and hip circumference were measured with participants in light clothes and without shoes using standard protocols. Body mass index (BMI) was calculated as weight in kg divided by the square of height in metres. Weight classification by BMI for Asians was used in the study; BMI <17.5 kg/m² (underweight), 17.5–22.9 kg/m² (normal weight), 23–27.4 kg/m² (overweight) and ≥27.5 kg/m² (obesity). Further obesity was classified as BMI 27.5–32.4 kg/m² (Obesity Class I), 32.5–37.5 kg/m² (Obesity Class II) and ≥ 37.5 kg/m² (Obesity Class II).^[26]

Using the American Diabetic Association (ADA) recommendations, impaired fasting glucose (IFG) was defined as fasting plasma glucose (FPG) 100 mg/dL to 125 mg/dL and impaired glucose tolerance was defined as 2 h PGPG during 75-g OGTT as 140 mg/dL to 199 mg/dL.^[27] Metabolic syndrome was defined

by using revised NCEP ATP III criteria^[28] which require at least three of the following components: (1) abdominal obesity (WC ≥90 cm for men, or ≥80 cm for women); (2) triglycerides ≥150 mg/dL, and/or drug treatment for elevated triglycerides; (3) high-density lipoprotein (HDL)-cholesterol <40 mg/dL for men, or <50 mg/dL for women; (4) systolic blood pressure (BP) ≥130 mmHg or diastolic BP ≥85 mmHg or antihypertensive medication treatment, and/or a history of hypertension and (5) FPG ≥100 mg/dL. The cut-off point for homeostasis model assessment of IR (HOMA-IR) in non-diabetic individuals was considered two taking occurrence of metabolic syndrome in various studies.^[29-31] High-sensitivity CRP (hs-CRP) <2.0 mg/L was considered low risk for cardiovascular disease (CVD) based on recent guidelines.^[32,33]

Laboratory tests

In all study participants, blood samples were collected for fasting insulin, fasting glucose, 2 h PGPG, fasting lipid profile, liver function test, renal function test, thyroid function test and complete blood count. After review of history, examination and basic biochemical evaluation those patients who qualified for further evaluation, for them blood samples were collected for hsCRP, IL-6, luteinising hormone (LH), follicle-stimulating hormone (FSH) and total testosterone (only in females). Hormonal evaluation was done on 2nd or 3rd days of menstrual cycle. Serum insulin, LH, FSH, total testosterone and IL-6 assay were measured using automated electrochemiluminescence immunoassay method (cobas e 411 analyser, Roche Diagnostics International Ltd). hs-CRP was measured by particle enhanced immunoturbidimetric test (AU480 Chemistry Analyzer, Beckman Coulter).

Ultrasonography

Trans-abdominal ultrasound was performed using a high-resolution B-mode Ultrasound system (SAMSUNG HS70A) by a single experienced investigator. On the same sitting adipose tissue depots were estimated. Both subcutaneous adipose tissue and visceral adipose tissue (VAT) were assessed twice and were calculated as the average of the two measurements.

Calculations

- HOMA IR as a marker of IR was calculated as (FPG in mg/dl X fasting insulin in mU/L)/405.^[34]
- Lipid accumulation product (LAP) Index was calculated as:^[35]
 - LAP for women = (WC [cm]-58) × (TG concentration [mmol/L])
 - LAP for men = (WC [cm]-65) × (TG concentration [mmol/L])
- Visceral adiposity index (VAI) was calculated as:^[36]

- Females:

$$VAI = \left[\frac{WC}{\{36.58 + (1.89 \times BMI)\}} \right] \times \frac{TG}{0.81} \times \frac{1.52}{HDL}$$

- Males:

$$VAI = \left[\frac{WC}{\{36.68 + (1.88 \times BMI)\}} \right] \times \frac{TG}{1.03} \times \frac{1.31}{HDL}$$

where WC is in cm, BMI in Kg/m², TG in mmol/L and HDL in mmol/L.

Statistical analysis

Descriptive statistical methods such as mean and standard deviation were applied to summarise continuous variables. Categorical data were summarised as percentages or proportion. Normality distribution of all parameters was checked using Shapiro–Wilk test. Parametric tests (independent *t*-test) and non-parametric test (Mann–Whitney *U*-test) were performed as required. Spearman's and Pearson's correlation coefficient were used to analyse correlation between different nonparametric and parametric data, respectively. Multiple linear regression was used to correlate multiple dependent factors. The data were analysed using IBM SPSS 26 statistical software. Graphs and charts were generated using IBM SPSS 26 software and Microsoft Excel 2019 (SPSS Inc., IBM Corporation, Armonk, New York, United States).

RESULTS

A total of 66 participants were included in the study, of which 38% (*n* = 25) were male and 62% (*n* = 41) were female. The mean BMI of the study group was 25.9 ± 3.7 kg/m². 23% (*n* = 15) of participants were found to have normal weight, 48% (*n* = 32) were overweight and 29% (*n* = 19) were obese [Table 1]. The mean SBP was 126.6 ± 10.77 mm of Hg and mean diastolic blood pressure (DBP) is 78.5 ± 6.9 mm of Hg. Systolic hypertension was present in 36% (*n* = 24) participants and diastolic hypertension was present in 20% (*n* = 13) of participants [Table 1]. Mean FPG and 2 h PGPG were 94.8 ± 11.3 mg/dl and 113.3 ± 21.7 mg/dl, respectively. 32% (*n* = 21) of participants had IFG and 15% (*n* = 10) of participants had impaired glucose tolerance [Table 1]. The mean lipid profile parameters were within normal range. In our study, 42% (*n* = 28) participants had metabolic syndrome. The mean HOMA-IR was 2.35 ± 0.76 which is elevated and HOMA-IR >2 was found in 64% (*n* = 42) of the participants, showing mild degree of IR [Table 1]. The mean LH and FSH were 7.5 ± 2.3 mIU/ml and 7.8 ± 1.9 mIU/ml, respectively, which are within normal range for female of reproductive age and males [Table 1]. The mean Total Testosterone levels in female participants was

Table 1: Clinical, anthropometric, biochemical and hormonal parameters in study subjects

	Subjects (<i>n</i> =66), mean±SD	Range (minimum- maximum)
Age (years)	39.4±9.5	20-60
BMI (kg/m ²)	25.9±3.7	18.6-34.2
WC (cm)	88.6±10.4	68-110
WHR	0.90±0.05	0.70-0.99
SBP (mm of Hg)	126.6±10.77	106-152
DBP (mm of Hg)	78.5±6.9	64-94
FPG (mg/dl)	94.8±11.3	73-120
2 h PGPG (mg/dl)	113.3±21.7	82-166
TC (mg/dl)	158.1±26.0	115-210
TG (mg/dl)	111.5±24.4	74-172
HDL (mg/dl)	40.62±8.6	21-58
LDL (mg/dl)	94.5±28.7	35-166
VLDL (mg/dl)	22.9±5.9	12-36
Fasting Insulin (mIU/ml)	9.9±2.8	5.4-18
HOMA IR	2.32±0.74	1.16-4.98
LH (mIU/ml)	7.5±2.1	4.1-12.9
FSH (mIU/ml)	7.8±1.9	4.6-12.1
Total testosterone (female) (ng/dl)	30.5±9.4	14-52
WBC/ml	7244±2190	3600-11,730
hs-CRP (mg/l)	2.4±1.1	0.5-5.3
IL-6 (pg/ml)	3.5±1.1	1.8-6.2

BMI: Body mass index, WC: Waist circumference, WHR: Waist-to-hip ratio, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, FPG: Fasting plasma glucose, TC: Total cholesterol, TG: Triglyceride, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very LDL, HOMA-IR: Homeostasis Model Assessment of Insulin Resistance, LH: Luteinising hormone, FSH: Follicle-stimulating hormone, WBC: White blood cell, hs-CRP: High-sensitivity C-reactive protein, IL: Interleukin, SD: Standard deviation, PGPG: Post Glucose Plasma Glucose

30.5 ± 9.4 ng/dl [Table 1]. The mean hs-CRP level was 2.4 ± 1.1 mg/L which is greater than the cut off of 2 mg/L. The mean IL-6 was 3.5 ± 1.1 pg/ml and total white blood cell (TWBC) counts were 7244 ± 2190/ml which were in normal range though in the upper half of normal limits [Table 1].

The study group was stratified according to BMI into normal weight, overweight and obese categories [Table 2]. The VAT thickness was higher in higher BMI categories and difference between the groups was statistically significant (*P* = 0.049). The LAP index was also higher in higher BMI categories and difference between the groups was statistically significant (*P* < 0.001). When the participants were grouped according to the hs-CRP cut off of 2 mg/L [Table 3], the HDL levels were significantly lower among those with hs-CRP levels ≥2 mg/L (*P* = 0.02). Fasting insulin levels and HOMA IR were significantly higher in the participants

Table 2: Comparison of Clinical, anthropometric, biochemical, hormonal parameters and adiposity indices between normal weight (body mass index 17.5-22.9), overweight (body mass index 23.0-27.4) and obese (body mass index ≥ 27.5) study subjects

	Mean \pm SD			P
	Normal weight subjects (n=15)	Overweight subjects (n=32)	Obese subjects (n=19)	
Age (years)	40.7 \pm 11.2	38.9 \pm 8.4	39.0 \pm 10.4	0.825
WC (cm)	81.8 \pm 11.0	87.21 \pm 8.85	96.4 \pm 7.2	<0.001
WHR	0.87 \pm 0.06	0.90 \pm 0.05	0.92 \pm 0.02	0.006
SBP (mm of Hg)	126 \pm 11	127 \pm 10	126 \pm 12	0.896
DBP (mm of Hg)	78 \pm 7	78 \pm 7	79 \pm 7	0.793
FPG (mg/dl)	92.7 \pm 13.5	95.0 \pm 10.4	96.3 \pm 11.2	0.654
2 h PGPG (mg/dl)	110.2 \pm 23.9	112.5 \pm 18.2	116.9 \pm 25.8	0.657
TG (mg/dl)	112.2 \pm 19.4	108.1 \pm 20.5	116.6 \pm 33.1	0.493
HDL (mg/dl)	42 \pm 9.4	40.9 \pm 8.2	39.0 \pm 8.8	0.587
Fasting insulin (mIU/ml)	9.51 \pm 2.19	10.00 \pm 3.23	10.04 \pm 2.78	0.839
HOMA IR	2.15 \pm 0.49	2.34 \pm 0.79	2.42 \pm 0.81	0.576
LH (mIU/ml)	7.7 \pm 1.9	7.2 \pm 2.1	7.9 \pm 2.2	0.503
FSH (mIU/ml)	7.9 \pm 2.2	7.6 \pm 1.9	8.1 \pm 1.6	0.726
Total testosterone (female) (ng/dl)	27.0 \pm 10.0	29.5 \pm 8.4	34.7 \pm 9.4	0.121
WBC/ml	7212 \pm 2591	7123 \pm 2021	7476 \pm 2231	0.858
hs-CRP (mg/l)	2.5 \pm 0.9	2.3 \pm 1.0	2.7 \pm 1.1	0.371
IL-6 (pg/ml)	3.6 \pm 1.1	3.4 \pm 1.1	3.5 \pm 1.0	0.936
SAT (cm)	2.1 \pm 0.6	2.1 \pm 0.6	2.3 \pm 0.6	0.487
VAT (cm)	7.3 \pm 1.3	7.8 \pm 1.6	8.8 \pm 2.3	0.049
LAP	27.5 \pm 14.5	31.4 \pm 13.4	47.0 \pm 17.9	<0.001
VAI	2.2 \pm 0.9	1.9 \pm 0.6	2.5 \pm 1.5	0.217

WC: Waist circumference, WHR: Waist-to-hip ratio, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, FPG: Fasting plasma glucose, TG: Triglyceride, HDL: High-density lipoprotein, HOMA-IR: Homeostasis Model Assessment of Insulin Resistance, LH: Luteinising hormone, FSH: Follicle-stimulating hormone, WBC: White blood cell, hs-CRP: High-sensitivity C-reactive protein, IL: Interleukin, SD: Standard deviation, VAT: Visceral adipose tissue, SAT: Subcutaneous adipose tissue, LAP: Lipid accumulation product, VAI: Visceral adiposity index, PGPG: Post Glucose Plasma Glucose

with hs-CRP levels ≥ 2 mg/L ($P < 0.001$) and ($P = 0.001$) respectively. We also found that mean serum LH was significantly higher in the participants with hs-CRP levels ≥ 2 mg/L ($P = 0.001$).

In our study, hs-CRP correlated negatively and significantly with HDL ($r = -0.34$, $P = 0.005$). There was significant positive correlation of hs-CRP with fasting Insulin ($r = 0.59$, $P < 0.001$), HOMA IR ($r = 0.57$, $P < 0.001$), LH ($r = 0.58$, $P < 0.001$) and VAI ($r = 0.27$, $P = 0.028$) [Table 4]. In multiple linear regression analysis with hs-CRP as dependent variable LH and HOMA-IR contributed significantly to the model ($\beta=0.192$, $P = 0.004$) and ($\beta =0.485$, $P = 0.007$), respectively, but HDL and VAI did not ($\beta = -0.015$, $P = 0.377$) and ($\beta =0.061$, $P = 0.674$), respectively [Table 5]. IL-6 correlated negatively and significantly with HDL ($r = -0.24$, $P = 0.045$). There was significant positive correlation of IL-6 with fasting insulin ($r = 0.55$, $P < 0.001$), HOMA IR ($r = 0.55$, $P < 0.001$) and LH ($r = 0.49$, $P < 0.001$) [Table 4]. In multiple linear regression analysis with IL-6 as dependent variable LH and HOMA IR contributed

significantly to the model ($\beta = 0.130$, $P = 0.043$) and ($\beta =0.601$, $P = 0.002$) m respectively, but HDL did not ($\beta = 0.001$, $P = 0.919$) [Table 5]. There was significant positive correlation of TWBC count with fasting insulin ($r = 0.48$, $P < 0.001$), HOMA IR ($r = 0.46$, $P < 0.001$) and LH ($r = 0.52$, $P < 0.001$) [Table 4]. In multiple linear regression analysis with TWBC count as dependent variable LH contributed significantly to the model ($\beta = 383$, $P = 0.004$), but HOMA IR did not ($\beta = 734$, $P = 0.055$) [Table 5].

DISCUSSION

In our study, we have correlated the markers of inflammation with hormonal, metabolic parameters and adiposity indices in first-degree relatives of patient with PCOS. We found that inflammatory markers hs-CRP, IL-6 and TWBC all are elevated in first-degree relatives of PCOS patient. HOMA-IR was also elevated, showing the presence of IR. Inflammatory markers were significantly correlated with LH and HOMA-IR even after multiple linear regression was fitted for each marker individually which indicates that each of them individually contributes to the increased inflammation.

Table 3: Clinical, anthropometric, biochemical, hormonal parameters and adiposity indices with high sensitivity C-reactive protein cut-off of 2 mg/L

	Mean±SD		P
	hs-CRP <2 mg/L subjects (n=25)	hs-CRP ≥2 mg/L subjects (n=41)	
Age (years)	39.7±9.5	39.1±9.7	0.795
BMI (kg/m ²)	25.3±3.4	26.3±3.9	0.267
WC (cm)	87.7±11.9	89.2±9.3	0.573
WHR	0.89±0.07	0.90±0.03	0.641
SBP (mm of Hg)	126±9	126±11	0.837
DBP (mm of Hg)	77±7	79±6	0.222
FPG (mg/dl)	95.1±13.0	94.7±10.3	0.911
2 h PGPG (mg/dl)	115.2±21.5	112.1±22.1	0.585
TC (mg/dl)	166.1±25.9	153.2±25.2	0.052
TG (mg/dl)	108.3±23.1	113.4±25.3	0.418
HDL (mg/dl)	43.7±9.0	38.7±7.9	0.020
LDL (mg/dl)	100.5±27.0	90.9±29.4	0.192
VLDL (mg/dl)	21.8±6.1	23.6±5.8	0.237
Fasting insulin (mIU/ml)	8.20±1.92	10.9±2.8	<0.001
HOMA IR	1.93±0.55	2.56±0.74	0.001
LH (mIU/ml)	6.4±1.3	8.2±2.3	0.001
FSH (mIU/ml)	7.7±1.6	7.9±2.1	0.736
Total testosterone (female) (ng/dl)	31.3±9.9	30.0±9.3	0.690
WBC/ml	5713±1615	8178±1967	<0.001
IL-6 (pg/ml)	2.7±0.6	4.0±1.0	<0.001
SAT (cm)	2.1±0.6	2.1±0.6	0.569
VAT (cm)	7.8±1.7	8.1±1.8	0.591
LAP	33.1±17.1	36.2±16.7	0.471
VAI	1.9±0.8	2.3±1.1	0.162

BMI: Body mass index, WC: Waist circumference, WHR: Waist-to-hip ratio, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, FPG: Fasting plasma glucose, TC: Total cholesterol, TG: Triglyceride, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very LDL, HOMA-IR: Homeostasis Model Assessment of Insulin Resistance, LH: Luteinising hormone, FSH: Follicle-stimulating hormone, WBC: White blood cell, hs-CRP: High-sensitivity C-reactive protein, IL: Interleukin, SD: Standard deviation, VAT: Visceral adipose tissue, SAT: Subcutaneous adipose tissue, LAP: Lipid accumulation product, VAI: Visceral adiposity index, PGPG: Post Glucose Plasma Glucose

In our study, 52% of participants were either overweight or obese, similar findings were reported in studies by Sasidevi *et al.*, Yildiz *et al.* and Shabir, *et al.*^[25,37,38] Systolic hypertension was present in 36% participants and diastolic hypertension was present in 20% of participants in our study population. In a meta-analysis by Yilmaz *et al.* mothers, sisters and brothers of PCOS patient had significantly higher SBP than control, whereas fathers had similar SBP to that of controls, whereas diastolic BP was comparable between all the first-degree relatives and controls.^[23] In the study by Shabir *et al.* SBP (>130 mmHg) was found in 27% and

DBP (>85 mmHg) was found in 27% of first-degree relatives of PCOS patients.^[25] These findings suggest that first-degree relatives of PCOS patient are at predisposed to hypertension which increases the risk of CVD. In our study, the mean glycaemic parameters of FPG and 2 h PGPG were 94.8 ± 11.3 mg/dl and 113.3 ± 21.7 mg/dl, respectively. We found 32% of participants had IFG and 15% of participants had impaired glucose tolerance. As the study was designed to evaluate the low-grade chronic inflammation and other metabolic parameters in apparently healthy first-degree relatives of PCOS, all presenting diabetes mellitus patients were excluded. In a similar study by Yildiz *et al.* glucose intolerance in 33% of Motherpcos group, 31% of Father_{PCOS} group, 5% in SistersPCOS group, and 4% in the BrothersPCOS group^[37] were found. In another study by Yilmaz *et al.*, the prevalence of any degree of glucose intolerance was 40% in Mothers_{PCOS} and 52% in Fathers_{PCOS} group.^[38] The above findings suggest that first-degree relatives of PCOS patient are having increased prevalence of IFG/IGT. According to ADA history of PCOS is considered criteria for screening of T2DM.^[27] Therefore, further large-scale studies are required to find out whether first-degree relatives having a family history of PCOS can be considered a screening criterion or not.

In our study, 42% of participants had metabolic syndrome. In a meta-analysis by Yilmaz *et al.*, mothers, sisters and fathers of PCOS patients had a significantly higher prevalence of Metabolic syndrome than controls. Although brothers of PCOS had a higher prevalence of MetS yet, it did not reach statistical significance.^[23] In another Indian study by Shabir *et al.* at 46% of first-degree relatives of PCOS had Metabolic syndrome.^[25] In the study by Shabir *et al.*, maximum participants (PCOS women as well as family members) had dyslipidaemia in the form of low HDL and high triglycerides.^[25] Increased levels of LDL cholesterol along with MetS in affected sisters of women with PCOS were reported by Sam *et al.*^[5,39] We found the mean HOMA IR was 2.35 ± 0.76, showing a mild presence of IR. In our study, HOMA IR >2 was found in 64% (n = 42) of the participants. Various studies have shown the presence of higher HOMA IR among first-degree relatives of PCOS patients in comparison to controls.^[37,38] We found among the study participants, 32% (n = 21) had acanthosis nigricans in neck and 44% (n = 29) had acanthosis nigricans in axilla. Thus, there was a higher prevalence of metabolic syndrome in first-degree relatives of PCOS patients.

In our study, the mean LH and FSH were 7.5 ± 2.3 mIU/ml and 7.8 ± 1.9 mIU/ml, respectively, which are within the normal range for a female of

Table 4: Correlation of Inflammatory markers with clinical, anthropometric, biochemical, hormonal parameters and adiposity indices

	hsCRP correlation coefficient (r)	P	IL-6 correlation coefficient (r)	P	TWBC correlation coefficient (r)	P
Age (years)	-0.04	0.725	-0.07	0.052	0.08	0.488
BMI (kg/m ²)	0.12	0.329	0.02	0.889	0.10	0.424
WC (cm)	0.143	0.253	-0.02	0.874	0.11	0.373
WHR	-0.02	0.872	-0.17	0.162	0.03	0.777
SBP (mm of Hg)	0.03	0.814	-0.09	0.433	-0.02	0.869
DBP (mm of Hg)	0.06	0.602	-0.07	0.556	0.01	0.987
FPG (mg/dl)	0.08	0.496	0.17	0.178	0.05	0.656
2 h PGPG (mg/dl)	0.01	0.997	0.06	0.602	-0.01	0.936
TG (mg/dl)	0.20	0.098	-0.12	0.306	-0.04	0.710
HDL (mg/dl)	-0.34	0.005	-0.24	0.045	-0.22	0.076
Fasting insulin (mIU/ml)	0.59	<0.001	0.55	<0.001	0.48	<0.001
HOMA IR	0.57	<0.001	0.55	<0.001	0.46	<0.001
LH (mIU/ml)	0.58	<0.001	0.49	<0.001	0.52	<0.001
FSH (mIU/ml)	-0.007	0.957	-0.05	0.678	0.02	0.814
Total testosterone (female) (ng/dl)	-0.05	0.743	-0.20	0.193	-0.04	0.778
hsCRP (mg/L)	1	-	0.71	<0.001	0.74	<0.001
WBC/ml	0.74	<0.001	0.63	<0.001	1	-
IL-6 (pg/ml)	0.71	<0.001	1	-	0.63	<0.001
SAT (cm)	0.09	0.468	-0.05	0.686	-0.01	0.968
VAT (cm)	0.14	0.245	-0.13	0.265	0.01	0.970
LAP	0.110	0.379	-0.089	0.476	0.10	0.405
VAI	0.27	0.028	0.03	0.758	0.15	0.226

BMI: Body mass index, WC: Waist circumference, WHR: Waist-to-hip ratio, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, FPG: Fasting plasma glucose, TG: Triglyceride, HDL: High-density lipoprotein, HOMA-IR: Homeostasis Model Assessment of Insulin Resistance, LH: Luteinising hormone, FSH: Follicle-stimulating hormone, WBC: White blood cell, hs-CRP: High-sensitivity C-reactive protein, IL: Interleukin, SD: Standard deviation, VAT: Visceral adipose tissue, SAT: Subcutaneous adipose tissue, LAP: Lipid accumulation product, VAI: Visceral adiposity index, TWBC: Total white blood cell, PGPG: Post Glucose Plasma Glucose

Table 5: Multiple linear regression analysis

	R ²	variables	β-coefficient	P
hs-CRP	0.441	HDL (mg/dl)	-0.015	0.377
		LH (mIU/ml)	0.192	0.004
		HOMA-IR	0.485	0.007
		VAI	-0.061	0.674
IL-6	0.357	HDL (mg/dl)	0.001	0.919
		LH (mIU/ml)	0.130	0.043
		HOMA-IR	0.601	0.002
TWBC count	0.312	LH (mIU/ml)	383.706	0.004
		HOMA-IR	734.320	0.055

hs-CRP: High-sensitivity C-reactive protein, IL: Interleukin, HDL: High-density lipoprotein, HOMA-IR: Homeostasis Model Assessment of Insulin Resistance, VAI: Visceral adiposity index, LH: Luteinising hormone, TWBC: Total white blood cell

reproductive age and males. The mean total testosterone levels in female participants, i.e., 30.5 ± 9.4 ng/dl. Our study was designed to exclude first-degree relatives who had PCOS in order to evaluate the metabolic parameters and inflammatory markers in the apparently healthy individual. Therefore, probably the gonadotropins and total testosterone were within the normal range in our study participants. Few of similar studies have shown

higher LH and total testosterone in first-degree relatives of PCOS in comparison to controls, but those studies included all the participants irrespective of the presence of clinical features of PCOS in relatives, which could explain the variation from our results.^[40,41]

Atherosclerosis is an inflammatory disease, and hs-CRP has been endorsed by multiple guidelines as a biomarker of atherosclerotic CVD risk.^[32,42] A large prospective clinical trial demonstrated significantly less cardiovascular risk for patients with hs-CRP <2.0 mg/L.^[33] In the present study, the mean hs-CRP level was 2.4 ± 1.1 mg/L, which is greater than the cut-off of 2 mg/L, showing the presence of a mild degree of inflammation. The mean IL-6 and TWBC counts in our study were in the normal range though in the upper half of normal limits. In a similar study by Vipin *et al.* from India, the mean hs-CRP levels were >2 mg/L in first-degree relatives of PCOS, but it was not significantly higher than in controls.^[43] These results show that the chronic inflammatory state of PCOS is heritable, and the factors responsible for this in PCOS patient are also present in the first-degree relatives of the same.

To the best of our knowledge, probably, this is the first Indian study to analyse the correlation of inflammatory markers with hormonal, metabolic parameters and adiposity indices in first-degree relatives of PCOS patients. Our findings show that inflammatory markers were significantly correlated with LH and HOMA IR even after multiple linear regression was fitted for each marker individually. This suggests that the higher LH levels (though within normal range) and IR are significant contributors to the state of chronic inflammation, though there is a possibility that these findings are due to intrinsic multigenic abnormality associated with PCOS.

The study had some limitations, one being the absence of a control group to compare and show that there is a significant difference in the findings. However, we were able to compare many key parameters with the established cut-off values available for the standard population. Second, was the small sample size of the study, but our sample size was similar to many of the published studies of similar nature. Third, was despite a proper history, examination and basic investigations done to rule out all causes of acute and chronic inflammatory state few rarer causes may have been missed, which may have affected the results. Fourth, the free androgen index could have been used to better represent the testosterone levels in female participants. Fifth, was the gold standard test for insulin sensitivity, i.e., hyperinsulinaemic euglycaemic clamp was not used in the study; instead, we relied on HOMA IR. However, many published studies have correlated well between the HOMA IR cut-off used in our study with metabolic syndrome, dysglycaemia and CVD. Despite the above limitations, our study was the first of its kind to correlate the various clinical, anthropometric, biochemical, hormonal parameters and adiposity indices in first-degree relatives of PCOS patients.

CONCLUSIONS

We demonstrated that first-degree relatives of PCOS patients had evidence of chronic low-grade inflammation. The chronic inflammation in them correlated well with HOMA IR and LH but were independent of BMI, suggesting the source of inflammation in these participants is not due to altered adipose tissue dysfunctions alone. This low-grade inflammation may predispose the first-degree relatives of PCOS to CVD and future well-controlled, larger studies are required to prove this association and find the mechanisms behind the above findings.

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Conflicts of interest

There are no conflicts of interest.

Data availability statement

The data that support the findings of the study are available from the author, upon reasonable request.

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