Introduction

Polycystic ovarian syndrome (PCOS) is a common endocrinological condition marked by oligo-anovulation, clinical or biochemical hyperandrogenism, and polycystic ovary syndrome, with prevalence rates varying across different populations. It is characterized by the presence of multiple ovarian cysts and menstrual irregularities, and it affects approximately 5-10% of women of reproductive age. Despite being a common condition, the underlying mechanisms and genetic predisposition are still largely unknown. Recent studies have suggested a genetic component in PCOS pathogenesis, with single nucleotide polymorphisms (SNPs) in genes associated with immune response and inflammation being implicated.

The study by Yousuf et al. aimed to investigate the association of PAI-1 -675 4G/5G and MCP-1 -2518 A/G genetic polymorphisms with the occurrence of PCOS in Kashmiri women. The authors conducted a case-control study involving 220 PCOS participants and 220 healthy controls.

The study employed allele-specific polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods to investigate the PAI-1 -675 4G/5G and MCP-1 -2518 A/G SNPs, respectively. The results showed a strong link between the PAI-1 -675 4G/5G SNP and PCOS, with an odds ratio (OR) of 3.2 (P = 0.001) for the 4G/4G genotype and 2.39 (P = 0.001) for the 4G/5G genotype. The carriers with the 4G/4G and 4G/5G genotypes showed significantly increasing trends in triglyceride levels (P < 0.05).

Similarly, the MCP-1 -2518 A/G SNP differed significantly between the PCOS patients and healthy controls, with the GG genotype remaining a strong predictor of PCOS (OR = 8.7, P = 0.01) and the AG genotype (OR = 2.40, P = 0.01), indicating an elevated risk of predisposing women to PCOS. There was a significant variation in the glucose 2-h levels between -2518A/G MCP-1 genotypes with AG heterozygous and GG mutant genotype showing increasing trends of glucose 2-h levels (P < 0.05).

The conclusion of the study is that both PAI-1 -675 4G/5G and MCP-1 -2518 A/G polymorphisms are associated with predisposition to PCOS and its complications in Kashmiri women.

Keywords: Coagulation, inflammation, monocyte chemoattractant protein-1, plasminogen activator inhibitor-1, polycystic ovary syndrome, polymorphism
ovaries. It is usually encountered in 4–18% of the women in the reproductive age group.1,2 PCOS is very common among Kashmiri women and it is perhaps the highest in the world.3 Weight gain, elevated blood pressure, abnormal lipid levels, and insulin resistance-induced diabetes are all common metabolic and cardiovascular concerns in PCOS women.4 Patients with PCOS may visit their primary care physician for hirsutism, acne, menstrual irregularities, infertility, obesity, and psychiatric issues like anxiety and depression.

Screening for type 2 diabetes, dyslipidemia, hypertension, as well as inflammatory and coagulatory states, should all be part of the treatment plan for these patients. The treatment should be tailored to each patient's phenotype as well as personal goals, such as the desire to become pregnant. Hence, primary care physicians should be aware of the clinical characteristics and have a well-grounded knowledge about its pathogenesis. Chronic low-grade inflammation, endothelial dysfunction, and a prothrombotic state have all been identified as major derangements in the etiology of this condition in recent years.5,6 Though the exact origin of this condition is unknown, it appears to be complex, with environmental risk factors combining with sensitive genes to create a wide range of reproductive and metabolic issues.7 Single nucleotide polymorphisms (SNPs) in the genes participating in coagulatory and inflammatory pathways have recently been associated with increased vulnerability to PCOS and its metabolic and reproductive repercussions8–10 which is relevant to their role in its etiology. Thus, the inflammatory hypothesis of PCOS is supported by the association of SNPs in the inflammatory genes with the development of this disorder. This may have therapeutic implications by targeting the inflammatory pathways for the treatment of this disorder.

Plasminogen activator inhibitor-1 (PAI-1), a serine protease inhibitor (SERPIN) family member, inhibits the fibrinolytic action of the tissue-type plasminogen activator (tPA) and is a critical regulator of endogenous fibrinolysis.11 Due to a disruption in the hemostatic or fibrinolytic system, PCOS patients have a prothrombotic propensity and have higher levels of PAI-1, which is linked to higher BMI, visceral obesity, thrombosis, and cardiovascular disease (CVD).12,13 The SERPINE1 gene, which has nine exons and is located on chromosome 7 (q21.3–q22), encodes PAI-1. The SERPINE1 gene harbors many SNPs, the most common of which is the -6754G/5G (rs1799889) SNP in the promoter region, which affects the SERPINE1 expression and has been associated with a variety of inflammatory pathologies.14–17

In PCOS patients, central obesity appears to activate inflammatory pathways as well as enhance lipogenesis and adipogenesis.18–20 The macrophages are directed from the circulatory system to the adipose tissue, where they release cytokines or chemokines such as intercellular cell adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM), monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-α (TNF-α), interleukin (IL-β), and IL-6, which are powerful mediators of pro-inflammatory and insulin-resistant states. Furthermore, this cytokine environment has been shown to aggravate atherosclerosis.21–24 Monocyte chemoattractant protein-1 (MCP-1) is the most important chemokine in macrophage recruitment.25,26 In humans, the MCP-1 gene is located on chromosome 17 (q11.2).27 The genetic changes in the MCP-1 gene's promoter region (rs1024611) impact the gene's distal regulatory region and transcriptional activity, affecting the MCP-1 expression and increasing susceptibility to a variety of inflammatory diseases.28–30 Even though these variables are important in multifactorial ailments, the results obtained thus far have been inconsistent.

Unhealthy eating habits, reduced physical exercise, and stress contribute to a gain in body weight. These lead to pathological conditions such as thrombosis, obesity-related adipose inflammation, and insulin resistance. In today's world, the scenario of people getting into sedentary lives has multiplied, especially owing to the present situation of the pandemic where everyone has been confined to restricted zones. In the future, the implications of deranged metabolism will evolve and the job of primary care physician will become more important and challenging. So, the overall profiles of the patients need to be considered while deciding on the treatment and management plans.

Hence, this study was envisaged to validate the relationship between MCP-1 and PAI-1 SNPs and PCOS in the Kashmiri population, specifically, the link between PAI-1 -6754G/5G and MCP-1-2518A/G genetic variations and the probability of the development of PCOS in our indigenous ethnic community. The knowledge gained through such translational research may have far-reaching implications of public health importance and help the primary care physician better appreciate the pathogenesis of PCOS and better inform his/her approach toward this disease.

**Methods**

**Subjects**

The patients with PCOS were screened for the condition at the Department of Endocrinology, Sher-i-Kashmir Institute of Medical Sciences (SKIMS). This study included 220 healthy women and 220 women with well-established PCOS. The Rotterdam criteria (2003)19 were used to diagnose PCOS, which stipulate that two of the three symptoms must be present to diagnose PCOS: (1) Oligomenorrhea or anovulation (less than eight cycles in the preceding year or missing periods for 6 months), (2) Clinical and/or biochemical evidence of hyperandrogenism, and (3) Polycystic ovaries (having 12 or more follicles of 2–9 mm diameter or ovarian volume >10 cm³), after ruling out other manifestations like non-classical congenital adrenal hyperplasia, androgen-secreting tumor, Cushing’s syndrome, thyroid dysfunction, and hyperprolactinemia using relevant investigations. The women who had recently taken any hormones or medications questionable to influence hormonal or biochemical functions, or who had diabetic, renal, hepatic, or cardiac abnormalities, were not included in the study. The control group consisted of 220 women with normal monthly cycles (21–35 days), no evidence of clinical or biochemical hyperandrogenism, normal ovarian volume, and
morphology on pelvic ultrasonography. The control group was gathered during medical camps conducted at several colleges in Kashmir including the University of Kashmir. The PCOS females and females in the control group of a similar age were considered in the study in a preferred manner.

The Sher-i-Kashmir Institute of Medical Sciences, Soura, Institutional Ethics Committee recommended/approved the study under IEC No: SIMS 131/IEC-SKIMS/2013-6479, and all the subjects gave written informed permission.

**Anthropometric and clinical assessment**

All women were measured for height, weight, and waist-hip circumference ratio. The details of their menstrual history as well as blood pressure were recorded. Hirsutism was evaluated using the Ferriman–Gallwey score by numbering nine specific body regions. A count of more than 8 out of 36 was considered significant.

**Biochemical analysis**

After an overnight fast of 10–12 h, an oral glucose tolerance test (OGTT) was done; 75 g anhydrous glucose dissolved in 200–300 mL of water was given orally to the patient. Blood samples were taken at 0, 60, and 120 min. Other investigations in the biochemical analysis included lipid profile, liver and renal function tests.

**Calculations**

The homeostasis model assessment insulin resistance index (HOMA-IR), the fasting glucose/insulin ratio (GIR), and the quantitative insulin sensitivity check index (QUICKI) were used to assess insulin resistance (Fasting insulin [IU/mL] × fasting glucose [mg/dL]/405 was used to calculate the HOMA-IR index. Fasting glucose (mg/dL)/fasting insulin (IU/mL) was used to compute the GIR values. 1/[log fasting insulin (IU/mL) + log fasting glucose (mg/dL)] was used to determine QUICKI. Insulin resistance is defined by low QUICKI, low GIR scores (low insulin sensitivity), and high HOMA-IR. The body mass index (BMI) was derived by dividing the body weight (kg) by body height squared (m²).

**Hormonal analysis**

To rule out non-classical congenital adrenal hyperplasia, hypothyroidism, prolatinoma, hypergonadotropism, Cushing’s syndrome, hyperandrogenism, and androgen-secreting tumors, 17-hydroxypregosterone (17-OHP), thyroxine (T4) and thyroid stimulating hormone (TSH), prolactin (PRL), luteinizing hormone (LH) and follicle-stimulating hormone (FSH), cortisol, and testosterone were used. The LH, FSH, 17-OHP, and testosterone were collected on the 3rd to 7th day of the follicular phase of either a spontaneous or a progesterone-induced menstrual cycle, respectively.

**Laboratory analysis**

The glucose and other biochemical parameters were determined on an autoanalyzer (Roche Hitachi 912, Minatoku, Tokyo, Japan). Hormonal assays were performed in duplicate by radioimmunoassay/immunoradiometric assay (RIA/IRMA) using commercial kits and following the supplier procedure (Diagnostic Products Corporation, Los Angeles, CA & Immunotech & Medicorp Inc., Montreal, Canada). Electrochemiluminescence was used to measure the plasma insulin levels (Cobas e411, Roche Diagnostics Limited, Charles Avenue, West Sussex).

**DNA extraction**

The phenol-chloroform technique was used to extract deoxyribonucleic acid (DNA) from peripheral blood leukocytes. Gel electrophoresis and optical density measurements at 260 and 280 nm with a double-beam spectrophotometer were used to examine the quality and quantity of the DNA extracted. The 260/280 nm ratio was computed, and only DNA samples with a ratio between 1.7 and 1.9 were considered for the experiment.

**Genotyping of -6754G/5G PAI-1 gene polymorphism by allele-specific PCR**

A 139-bp fragment of the PAI-1 gene was amplified using allele-specific PCR and the appropriate primers. The gene’s promoter region contains a single allele deletion (4G)/insertion (5G) at position -675. All the patient’s genotypes were determined using allele-specific primers and PCR amplification of genomic DNA. The primers used were: (a) Insertion 5G allele: 5’-GTC TGGACACGTGGGGG-3’ (b) Deletion 4G allele: 5’-GTCTGGACACGTGGGGG-3’ Each was used in conjunction with the 5’-TGCAGCCACGGCATGATTGCTAG-3’ downstream primer, which resulted in a 139 bp DNA fragment. A control upstream primer, 5'-AAGCTTTTACCATGTTACCCCTGTT-3' was used, as a positive control in the PCR. Two PCR reactions were run per sample (one for the 4G allele and one for the 5G allele), that is, each reaction contained a downstream primer, an upstream primer, and one primer for 4G or 5G. The amplification reaction was carried out in a 20 µL reaction volume in a 0.2 mL PCR tube. The reaction components were 2 µL of 10 × PCR buffer, 1.2 µL of MgCl₂, 0.4 µL of 10 mM deoxyribonucleotide triphosphate (dNTPs), 1 µL of 20 nm upstream control primer, 1 µL of 20 nm common downstream (reverse) primer, 1 µL of allele-specific primers 4G or 5G, 0.3 µL (1.5 U) Taq DNA polymerase, 0.5 µL genomic DNA 12.6 µL deionized water. The PCR was carried out with an initial denaturation at 94°C for 5 min and 30 cycles of 1 min of denaturation at 94°C, 1 min of annealing at 60°C, 1 min of extension at 72°C, and final extension of 5 min at 72°C. The amplified PCR products were electrophoresed on a 2% agarose gel and visualized using a UV transilluminator. The upstream and downstream primers produced a 257 bp control band. The downstream primer and the 4G or 5G allele-specific primer yielded 139 bp fragments. The participants with just 4G primers were 4G/4G-variant homozygote, those with only 5G primers were 5G/5G homozygote wild, and those with both primers depicted 4G/5G heterozygous condition [Figure 1].
Genotyping of -2518 A/G polymorphism of MCP-1 gene
PCR-RFLP was used to amplify a 321 bp fragment of the MCP-1 gene. Two primers were used. Forward primer F: 5'-CCGAGATGTTCCAGCACAG-3' and reverse primer R: 5'-ATCTCTGGAAAGTGACTTGGC-3'. A 300-ng genomic DNA was amplified in a total volume of 20 µL of the reaction mixture, which included 2 µL of 10× PCR buffer, 1.2 µL of MgCl₂, 0.4 µL of 10 mM dNTPs, 1 µL of 20 nm primer (F), 1 µL of 20 nm primer (R), 0.3 µL of Taq DNA polymerase, 0.5 µL of genomic DNA, and 13.6 µL of deionized water. The initial denaturation at 94°C for 5 min was followed by 30 cycles of 30 s denaturation at 94°C, 30 s annealing at 60°C, 30 s extension at 72°C, and final extension at 5 min at 72°C. Following that, 10 µL of 321 bp PCR product was treated with 0.3 µL of proteus vulgaris (Pvu) II in a total volume of 20 µL of 10× buffer (G + 18 µL of nuclease-free water) and was kept for incubation at 37°C for 16 h for optimal digestion. When restriction digestion is performed, the AA homozygous wild-type yields a single band of 321 bp, while the GG homozygous mutant produces two bands of 222 and 99 bp. Electrophoresis on a 3% ethidium bromide-stained agarose gel was used to examine the fragments [Figure 2].

Statistical analysis
The mean and standard deviations were calculated using Microsoft Office Excel. To compare the means of the variables, analysis of variance (ANOVA) and unpaired Student's t-tests were performed. The Chi-square and Fischer's exact tests were used for the comparison of allelic and genotypic frequencies between case and controls. A P < 0.05 was considered as the level of statistical significance. The Chi-square (χ²) test was used to compare the genotype distribution in the controls to that predicted by the Hardy–Weinberg equilibrium (HWE). A P > 0.05 was in line with HWE. SPSS version 22 and VassarStats online software were used for analyzing the data.

Results
Table 1 depicts the comparison of the baseline characteristics of the subjects with and without PCOS. The mean age and BMI of the PCOS and control subjects were not significantly different (23.04 ± 4.82 years vs. 22.97 ± 3.58 years) (P = 0.86); (23.87 ± 3.03 kg/m² vs. 23.43 ± 3.31 kg/m²) (P = 0.14), respectively. The PCOS group revealed significant differences in the ferriman-gallwey (FG) score, waist-hip ratio, cycles per year, lipid profile, and total testosterone when compared to the control group (P < 0.0001). However, the difference between the cases and controls in terms of fasting glucose and FSH levels could not reach statistical significance. The PCOS women presented deranged baseline clinical and metabolic milieu compared to normal healthy women.

Genotypic distributions of -6754G/5G SNP of PAI-1 gene
The genotypic and allelic patterns of PAI-1-4G/5G polymorphism in PCOS and control women are presented in Figure 1 and Table 2. In some instances, the genotypic frequency of the cases was 20.45% (N = 45) for 4G/4G (mutant), 56.81% (N = 125) for 4G/5G (heterozygote), and 22.72% (N = 50) for 5G/5G (wild-type) compared to 11.81% (N = 26) for 4G/4G, 45% (N = 99) for 4G/5G, and...
43.18% (N = 95) for 5G/5G genotype in healthy controls. The odds ratio (OR) for the 4G/4G genotype was 3.2 (95% CI = 1.8–5.9) (P = 0.001), while the OR for the 4G/5G genotype was 2.39 (95% CI = 1.55–3.69) (P = 0.001). There was a significant difference in the genotypic frequencies of the PAI-1 gene between PCOS women and controls (χ² = 22.07, P = 0.001). The Hardy–Weinberg equilibrium (HWE) was found to be in agreement with the genotype frequencies among the controls for the -675 4G/5G SNP of the PAI-1 gene (i.e. P > 0.05). When comparing the PCOS women to controls, the allelic frequency revealed a statistically significant and uneven distribution of the 4G allele, with OR = 1.8 (95% CI = 1.39–2.39); (P = 0.001). Upon thoroughly investigating the relationship between the genotypes of the selected polymorphism and their corresponding clinical and laboratory markers among the cases, it was found that the triglyceride levels were significantly higher in the homozygous mutants (4G/4G) and heterozygous (4G/5G) states [Figure 3, Table 3].

### Table 1: Clinical and laboratory profile of cases and controls

| Variables                        | Cases n=220 | Controls n=220 | P      |
|----------------------------------|-------------|----------------|--------|
| Age (years)                      | Mean 23.04  | Mean 22.97     | 0.86 (NS) |
| FG score                         | SD 4.82     | SD 3.58        |        |
| No. of cycles per year           | 7.2         | 11.55          | <0.0001 |
| BMI (kg/m²)                      | 23.87       | 23.43          | 0.31 (NS) |
| Waist-Hip ratio                  | 0.87        | 0.84           | 0.05 (NS) |
| LH (IU/l)                        | 8.06        | 6.13           | <0.0001 |
| FSH (IU/l)                       | 6.66        | 6.94           | 0.07 (NS) |
| Testosterone (ng/dL)             | 85.22       | 32.85          | <0.0001 |
| Blood glucose fasting (mg/dL)    | 88.07       | 87.70          | 0.75 (NS) |
| Blood glucose 1 h (mg/dL)        | 136         | 131.22         | 0.002   |
| Blood glucose 2 h (mg/dL)        | 102.36      | 94.14          | <0.0001 |
| Insulin Fasting (µU/mL)          | 20.8        | 10.81          | <0.0001 |
| FGIR                             | 4.44        | 7.85           | <0.0001 |
| QUICKI                           | 0.30        | 0.33           | 0.09 (NS) |
| HOMA-IR                          | 4.55        | 2.14           | <0.0001 |
| Cholesterol (mg/dL)              | 181.23      | 158.02         | <0.0001 |
| Triglycerides (mg/dL)            | 151.27      | 104.09         | <0.0001 |
| HDL (mg/dL)                      | 43.01       | 48.05          | 0.001   |
| LDL (mg/dL)                      | 119.02      | 93.03          | <0.0001 |

NS = Statistically non-significant (P>0.05); SD = Standard deviation; BMI, body mass index; FGIR, fasting glucose/insulin ratio; FG, Score, Ferriman-Gallwey score; FSH, follicular stimulating hormone; HDL, high-density lipoprotein; HOMA-IR, Homeostasis Model Assessment Insulin resistance index; LDL, Low-density lipoprotein; LH, luteinizing hormone; QUICKI, quantitative insulin sensitivity index

### Table 2: Genotypic and allelic frequencies of -675 4G/5G PAI-1 SNP among cases and controls and their association with the risk of PCOS

| PAI-1 Gene | Variants | Cases (n=220) | Controls (n=220) | OR (95% CI); P* | χ²; P (Overall) |
|------------|----------|--------------|-----------------|-----------------|-----------------|
| Genotypic  | 5G/5G (Wild) | 50 (22.72%) | 95 (43.18%) | 22.07; 0.001 |                |
|            | 4G/4G (Mutant) | 45 (20.45%) | 26 (11.81%) | 3.2 (1.8-5.9); P=0.001 |                |
| Allelic Frequency (2N) | 5G allele (Wild) | 225 (51.13%) | 289 (65.68%) | 2.39 (1.55-3.69); P=0.001 |                |
|            | 4G allele (Mutant) | 215 (48.86%) | 151 (34.31%) | 1.8 (1.39-2.39); P=0.001 |                |

P* = Pearson's P; OR = Odds ratio

### Genotypic distributions of -2518A/G SNP of the MCP-1 gene

The genotypic and allelic patterns of the MCP-1 -2518 A/G polymorphisms in PCOS patients and controls are presented in [Figure 2 and Table 4]. The cases had a genotypic frequency of 16.81% (N = 37) for GG, 60.5% (N = 121) for AG, 28.18% (N = 62) for AA, and 71.8% (N = 158) for GG + AG genotype, compared to healthy controls who had a genotypic frequency of 3.63% (N = 8) for GG, 43.18% (N = 95) for AG, 53.18% (N = 117) for AA, and 46.81% (N = 103) for GG + AG genotype. The OR for the GG, AG, and GG + AG genotype was 8.7 (95% CI = 3.8–19.8) (P = 0.01), 2.40 (95% CI = 1.59–3.61) (P = 0.01), and 2.89 (95% CI = 1.94–4.29) (P = 0.01), respectively. The allelic frequency demonstrated a statistically significant and unequal distribution of the G allele between the patients and controls, with the cases having a considerably higher frequency of G allele with OR = 2.35 (95% CI = 1.77–3.13) (P = 0.01). The genotype frequencies of the MCP-1 gene codon -2518 indicated a statistically significant variation in the distribution between the genotypes for the GG, AG, and GG + AG genotypes compared to the healthy controls [Figure 4, Table 5].

### Discussion

PCOS is a broad and varied condition caused by a complex interaction of environmental and genetic variables. Genetics plays an important part in the pathophysiology of PCOS because it is a heritable disease that runs in families. Chronic...
low-grade inflammation, a prothrombotic condition, and endothelial dysfunction have all been implicated in the disease development.\[5-8\]

Genetic variations play a role in modifying the disease’s pathogenesis. SNPs indicate the functional alterations caused by amino acid differences or gene expression regulation. SNPs may be responsible for individual disparities in the treatment response as well as the development of complicated and common diseases like diabetes, obesity, hypertension, and psychiatric disorders. SNPs can affect the gene expression, mRNA shape, and mRNA and protein subcellular localization, which can lead to a diseased state. As a result, identifying the various gene variants and analyzing their consequences may lead to a greater understanding of their impact on gene function and individual health. This new insight could pave the way for the

Table 3: Clinical and laboratory parameters of cases in accordance with their genotypes of the PAI-1-675 4G/5G gene polymorphism

| Variables          | 5G/5G (Wild) | 4G/5G (Heterozygous) | 4G/4G (Mutant) | P     |
|--------------------|--------------|----------------------|----------------|-------|
|                    | Mean         | SD                   | Mean           | SD    | Mean        | SD    |       |       |
| BMI (kg/m²)        | 23.48        | 3.03                 | 23.83          | 2.62  | 24.27       | 3.63  | 0.42  |
| Waist-Hip ratio    | 0.866        | 0.02                 | 0.869          | 0.03  | 0.874       | 0.02  | 0.32  |
| Testosterone (ng/dL) | 83.31      | 10.53                | 85.04          | 11.66 | 87.32       | 11.32 | 0.22  |
| Blood glucose fasting (mg/dL) | 85.97 | 10.52               | 88.88          | 11.2  | 89.76       | 9.97  | 0.17  |
| Glucose 2 (mg/dL)  | 100.01       | 13.43                | 102.52         | 12.76 | 104.55      | 15.53 |       |       |
| Fasting insulin (µIU/mL) | 19.77 | 6.43                 | 20.75          | 4.91  | 21.98       | 3.82  | 0.11  |
| FGIR               | 4.48         | 1.30                 | 4.40           | 1.06  | 4.60        | 1.11  | 0.58  |
| HOMA-IR            | 4.85         | 1.56                 | 4.55           | 1.76  | 4.25        | 1.28  | 0.20  |
| QUICKI             | 0.307        | 0.01                 | 0.308          | 0.01  | 0.309       | 0.01  | 0.62  |
| Cholesterol (mg/dL) | 176.33      | 20.77                | 182.23         | 19.87 | 185.13      | 17.74 | 0.07  |
| TG (mg/dL)         | 138.75       | 14.97                | 151.23         | 12.23 | 163.84      | 15.83 | 0.00* |
| HDL (mg/dL)        | 43.33        | 7.81                 | 43.31          | 5.42  | 42.41       | 6.77  | 0.69  |
| LDL (mg/dL)        | 115.27       | 15.45                | 120.32         | 13.09 | 121.48      | 16.86 | 0.06  |

NS=Statistically non-significant (P>0.05); SD=Standard deviation; *Statistically significant

Table 4: Genotypic and allelic frequencies of -2518 A/G MCP-1 SNP among cases and controls and their association with the risk of PCOS

| MCP-1 Gene Codon -2518 | Variants          | Cases (n=220) | Controls (n=220) | OR (95% CI); P* | χ²; P (Overall) |
|------------------------|-------------------|--------------|------------------|---------------- |----------------|
| Genotypic Frequencies (N) | AA-Wild          | 62 (28.18%)  | 117 (53.18%)     | 1              | 38.72;0.00     |
|                        | GG ‑Variant       | 37 (16.81%)  | 8 (3.63%)        | 8.7 (3.8-19.8); P=0.01 |
|                        | AG ‑Heterozygous  | 121 (60.5%)  | 95 (43.18%)      | 2.40 (1.59-3.61); P=0.01 |
|                        | GG + AG           | 158 (71.8%)  | 103 (46.81)      | 2.89 (1.94-4.29); P=0.01 |
| Allelic Frequency (2N) | (A allele)        | 245 (55.6%)  | 329 (74.77%)     | 1              | 2.35 (1.77-3.13); P=0.01 |
|                        | (G allele)        | 195 (44.3%)  | 111 (25.22%)     |                |                |

P*=Pearson’s P

Table 5: Clinical and Laboratory parameters of cases in accordance with their genotypes of the MCP-1-2518 A/G gene polymorphism

| Variables          | AA (Wild) | AG (Heterozygous) | GG (Mutant) | P     |
|--------------------|-----------|-------------------|-------------|-------|
|                    | Mean      | SD                | Mean        | SD    | Mean        | SD    |       |       |
| BMI (kg/m²)        | 23.11     | 3.43              | 24.03       | 3.00  | 24.47       | 3.43  | 0.08  |
| Waist-Hip ratio    | 0.876     | 0.03              | 0.879       | 0.02  | 0.880       | 0.02  | 0.67  |
| Testosterone (ng/dL) | 82.25     | 13.17             | 85.64       | 12.16 | 87.78       | 11.32 | 0.07  |
| Blood glucose fasting (mg/dL) | 87.77 | 10.62              | 88.88       | 9.83  | 89.96       | 10.77 | 0.57  |
| Glucose 2 (mg/dL)  | 97        | 15.63             | 103.09      | 10.96 | 107         | 10.53 | 0.00* |
| Fasting insulin (µIU/mL) | 19.87 | 5.93              | 20.15       | 4.61  | 21.08       | 4.52  | 0.48  |
| FGIR               | 4.41      | 1.50              | 4.41        | 1.86  | 4.26        | 1.81  | 0.89  |
| HOMA-IR            | 4.50      | 1.36              | 4.65        | 1.34  | 4.32        | 1.02  | 0.37  |
| QUICKI             | 0.308     | 0.01              | 0.307       | 0.01  | 0.308       | 0.01  | 0.80  |
| Cholesterol (mg/dL) | 177.33    | 20.77             | 182.23      | 19.87 | 184.13      | 17.74 | 0.17  |
| TG (mg/dL)         | 149.03    | 17.77             | 150.08      | 14.23 | 154.72      | 20.83 | 0.22  |
| HDL (mg/dL)        | 44.33     | 7.81              | 43.31       | 5.42  | 41.41       | 6.77  | 0.09  |
| LDL (mg/dL)        | 116.27    | 15.45             | 118.32      | 13.09 | 122.48      | 16.86 | 0.11  |

NS=Statistically non-significant (P>0.05); SD=Standard deviation; *Statistically significant
development of new and more useful SNP markers for medical testing as well as safer and more customized medication to treat the most common and devastating diseases. In the future, this will transform the healthcare profession. To adequately identify the genetic foundations of a multigenic complex disorder like PCOS, the selection of suitable candidate genes should continue. This would open the way for the creation of genetic risk profiles that may be used to develop therapeutic management methods in the future.\[^{32}\]

This study aimed to investigate the relationship between the 4G/5G polymorphism at position -675 in the PAI-1 gene's 3'-UTR region and the prevalence of PCOS in Kashmiri women. For the first time, a study focusing on the association between PAI-1-675 4G/5G and the prevalence of PCOS in Kashmiri women was conducted, which adds to our understanding of the genetic vulnerability of this disease. According to our findings, the homozygous mutant state (4G/4G) and heterozygous mutant state (4G/5G) were frequently prevalent in PCOS women as compared to the normal women of the same ethnicity. These genotypes dramatically elevated the chance of PCOS development. The allelic frequency revealed that the 4G allele is more prevalent in the PCOS cases. Additionally, the patients who were homozygous mutant (4G/4G) or heterozygous (4G/5G) for the variant allele had considerably higher triglyceride levels. The genotype frequencies among the controls for -675 4G/5G SNPs in the PAI-1 gene were found to be in concordance with HWE (i.e. \( P > 0.05 \)).

Our findings resembled those of Diamanti-Kandarakis et al.\[^{33}\] who found that the genotypic subtypes 4G/4G and 4G/5G were present at a statistically higher frequency in Greek Caucasian women with PCOS than controls. A meta-analysis backs up our findings, revealing that the PAI-1 4G/5G polymorphism enhanced susceptibility to PCOS, with 4G allele carriers having a greater probability of developing the syndrome than 5G allele carriers.\[^{34}\] In the PCOS instances, a relationship was found between recurrent pregnancy loss and implantation failures and the PAI-1 4G/5G polymorphism.\[^{35}\] Early on, PCOS women with 4G/4G and 4G/5G situations were sensitive to pregnancy risks due to elevated PAI-1 levels, which produced clotting issues.\[^{36}\] Because there has been little investigation into the association between the PAI-1 mutations and PCOS, our findings can only be compared to a few other studies. However, several scholars have looked at the relationship between PAI-1 plasma levels and PAI-1 gene genotypes, which may give indirect support to our findings. According to a meta-analysis, the 4G carriers had significantly higher PAI-1 plasma levels.\[^{37}\] The increased incidence of 4G/4G and 4G/5G states, as well as their link to greater PAI-1 levels, raises cardiovascular risks in women\[^{38,39}\] and increases the chance of first-trimester miscarriage in PCOS women.\[^{40}\]

We also examined the correlation between the MCP-1-2518A/G polymorphism and PCOS in Kashmiri women. MCP-1 is one of the most significant secretory factors in adipocytes. When compared to normal women of the same ethnicity, the homozygous mutant state (GG) and heterozygous state (AG) were frequently observed in PCOS women. These genes dramatically elevated the chance of PCOS development. When compared to normal women, the PCOS patients with the variant allele in homozygous (GG) and heterozygous (AG) conditions had significantly higher 2-h glucose levels. The G allele (mutant allele) frequency was greater in the PCOS individuals than in the controls. MCP-1-2518A/G SNP genotype frequencies in controls differ from HWE (i.e. \( P < 0.05 \)). Our findings are consistent with a previous study in the Korean population, which found that the MCP-1-2518A/G polymorphism is connected to PCOS risk through altering transcriptional activity resulting in an increased MCP-1 expression.\[^{41}\] Because there has been limited investigation into the association between MCP-1 mutations and PCOS, our work can only be compared to a few other studies of this type. However, several studies have looked at the genetic link between the -2518 A/G polymorphism in the MCP-1 gene and chronic inflammatory conditions, which can provide indirect support to our findings. The MCP-1 variations may be exploited to predict long-term kidney graft failure in the future.\[^{42}\] It has been established that the MCP-1 AA genotype and A allele play a role in determining diabetic nephropathy (DN) susceptibility.\[^{43}\] The A-2518G polymorphism in the MCP-1 gene may play a role in the development of ischemic heart disease and stroke according to an increasing body of evidence.\[^{44,45}\] These findings are similar to those of Wang et al.\[^{46}\] who did a meta-analysis to check into the correlation between the MCP-1 gene -2518A/G polymorphism and coronary artery disease (CAD), but found no link in the Asian subgroup population.

In our investigation, the patients with mutant and heterozygous genotypes had higher triglyceride levels in the case of PAI-1 and higher glucose 2-h levels in the case of MCP-1, indicating that the genetic variants had an impact on the patients' clinical and metabolic outcomes. In non-diabetic people, increased circulating triglyceride levels can increase the risk of type 2 diabetes or boost fasting glucose or fasting insulin levels. One explanation for our findings is that insulin resistance, dyslipidemia, and insulin production by -cells are all complicated interactions between fat, muscle, and liver. Excess triglycerides in the liver, for example, produce fatty liver disease and are thought to disrupt hepatic insulin signaling, leading to insulin resistance, whereas free fatty
acids (FFAs) are known to decrease insulin production in the cell. Furthermore, a positive correlation between mutant and heterozygous MCP-1 genotypes and 2-h glucose levels obtained by OGTT should be a reasonable indicator to assess insulin sensitivity in PCOS patients.  

Greater knowledge of cytokine impacts will provide insight into normal tissue regulation and may lead to therapeutic breakthroughs. In women with PCOS, a dysregulated inflammatory state has a wide-ranging influence. Insulin resistance, inflammation, hypertension, cardiovascular risk, coagulation, oocyte differentiation and maturation, and embryonic development are just a few of the biological pathways that cytokines interact with. As a result, other reproductive processes in these women are impaired leading to difficulties such as delayed conceptions, increased miscarriage rates, and poor outcomes in aided conceiving treatments.  

During fetal development, functional polymorphisms in the coagulation factor gene can be employed as a predictive factor in the prevention of abortion. Several coagulation factor polymorphisms disrupt the interchange of food and other materials between the fetus and the mother, impede placental formation throughout the embryonic stages, increase clot formation and lead to abortion, and have a role in regulating pregnancy duration and hemostasis. Procoagulant abnormalities cause thrombosis and infarction of the placental arteries resulting in recurrent miscarriages. Before starting a procoagulant examination, the patients should be examined for hormonal and anatomical problems by a primary care physician. If this is done, the right therapy could result in a great outcome.

Since the pathophysiology of PCOS is multifactorial, the possibility of genetic variants influencing the clinical outcomes should be duly considered, especially while choosing the right treatment regimen. In our earlier publications also, we have observed that inflammation and coagulation markers are disrupted in PCOS women, especially those taking OCP as treatment, thus, anti-inflammatory and insulin-sensitizing therapeutics are recommended in addition to lifestyle interventions. Our study’s limitation is that we were unable to conduct expression studies at the mRNA level that would have assisted in identifying the molecular processes by which these SNPs exert their effect.

**Conclusion/Key points**

The PAI-1 -675 4G/5G and MCP-1 -2518 A/G genetic variants are linked to PCOS risk in Kashmiri women. Further, these SNPs were found to be linked with the biochemical and metabolic aspects of the syndrome in our study implying that they may increase the PCOS women’s vulnerability to metabolic syndrome and insulin resistance.

The association of PCOS with genetic variations in the inflammatory and coagulatory genes should be a clue for the primary care physician to manage PCOS women in accordance with such scenarios so that further complications, especially reproduction-related, can be managed.

Advanced pharmacological therapy, as well as dietary and lifestyle changes, may be beneficial to women with PCOS and may operate as effective preventive treatments against the syndrome’s major consequences.

More research into the pathophysiology of PCOS, as well as the underlying inflammation and coagulation-mediated mechanisms, are required.

**Key take-home message**

People being sedentary has become more common in today’s world, particularly because of the current pandemic crisis, which has confined everyone to restricted zones. An unhealthy diet and insufficient physical activity result in obesity which can lead to pathological disorders like thrombosis, inflammation, and insulin resistance. The consequences of a malfunctioning metabolism will become more apparent in the future. So, a take-home message is a healthy diet, regular exercise, and routine screening.

**Novelty/New knowledge emerging from the manuscript**

We previously reported that inflammation and coagulation markers are disrupted in PCOS women and that this was exacerbated in PCOS women taking OCP treatment. The current study discovered a link between the MCP-1 and PAI-1 gene polymorphisms and PCOS. Thus, anti-inflammatory and insulin-sensitizing therapies are recommended.

Because the pathophysiology of PCOS is complicated, the likelihood of genetic variations influencing the clinical and metabolic results should be taken into account while selecting the best treatment plan.

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**Conflicts of interest**

There are no conflicts of interest.

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