**RESEARCH ARTICLE**

**The ICAM-1 Gly241Arg Polymorphism is Not Associated With Polycystic Ovary Syndrome - Results from a Case Control study in Kashmir, India**

Syed Douhath yousuf¹, Mohammad Ashraf Ganie², Mohammad Afzal Zargar¹, Tabasum Parvez³, Fouzia Rashid¹*  

Abstract

**Background:** Polycystic ovary syndrome (PCOS) is considered to be a multifactorial disorder resulting from the interaction of several predisposing and protective genetic variants. PCOS is associated with low-grade chronic inflammation. Elevated levels of inflammatory markers including intercellular adhesion molecule-1 (ICAM-1) are demonstrated in women with PCOS. Recent evidence indicates a significant linkage between a locus on chromosome 19p13 and multifactorial diseases that have an inflammatory component. The aim of the study was to assess the possible association of Gly241Arg polymorphism of ICAM-1 gene located on chromosome 19p13 in determining risk of PCOS in Kashmiri women. **Materials and Methods:** Gly241Arg SNP in DNA from peripheral blood leukocytes of 220 PCOS cases and 220 age matched non-PCOS healthy controls was analysed using allel specific PCR. **Results:** The genotype and allele frequency distributions of Gly241Arg SNP showed insignificant difference between the PCOS cases and control women, indicating no role of this SNP in PCOS susceptibility. The odds ratio for Arg/Arg genotype was 0.87 (95% CI=0.32-2.3) [P=0.79], for Gly/Arg genotype was 0.98 (95% CI=0.66-1.47) [P=1] and for Arg/Arg+Gly/Arg genotype was 0.97 (95% CI=0.65-1.45) [P=0.92]. The genotypic frequencies of ICAM-1 codon 241 showed statistically insignificant difference between cases and controls (χ²=0.07; p=0.96) Nor the studied polymorphism was found to affect clinical and laboratory parameters significantly. **Conclusions:** Although Gly241Arg polymorphism have not shown significant association with PCOS. Further, specifically designed studies on large cohorts are required to conclusively establish any role of ICAM-1 gene polymorphisms in PCOS in our study.  

Keywords: Gly241Arg - ICAM-1 - polycystic ovary syndrome - polymorphism - SNP

**Introduction**

The polycystic ovary syndrome (PCOS) is a common and complex endocrinopathy affecting 3-10% of women of reproductive age (Azziz et al., 2004; Nidhi et al., 2011) and is characterized by hyperandrogenism, chronic anovulation and polycystic ovarian morphology on ultrasonography (Rosenfield, 2015). It may be associated with increased risk of endometrial cancer (Shen et al., 2015). Insulin resistance and its compensatory hyperinsulinemia is a central feature of PCOS (Asante et al., 2015). Insulin resistance has been increasingly recognised as having a key role in chronic inflammation and endothelial dysfunction, which represent an early sign of atherosclerosis (Prieto et al, 2014). Adhesion molecules are one of the main markers of low-grade inflammation and endothelial dysfunction (Blankenberg et al., 2003). Intercellular adhesion molecule-1 (ICAM-1, CD54) is a member of immunoglobulin superfamily of adhesion molecules. It is expressed on the surface of the endothelium cells, smooth muscle cells, macrophages and activated lymphocytes. ICAM-1 plays an important role in the adhesion of circulating leukocytes to the blood vessel wall and transendothelial migration to vascular intima (Hayflick et al., 1998). ICAM-1 binds to β2 integrins of leukocytes, leukocyte function associated antigen-1 (LFA-1, Integrin αLβ2) and macrophage antigen -1 (MAC-1, Integrin αMβ2) (Springer, 1990). Fibrinogen could also be a ligand for ICAM-1 (Languino et al., 1993). There are soluble forms of adhesion molecules in circulation that allows assessment of the protein’s concentration. During the last decade, several studies have analyzed the serum ICAM-1 levels in women with PCOS in an attempt to link PCOS with risk of cardiovascular diseases (CVD) and it has been reported that women with PCOS have significantly increased ICAM-1 concentrations (Nasiek

¹Biochemistry, Biological Sciences, Kashmir University; ²Endocrinology, ³Gynecology, Sheri Kashmir Institute of Medical Sciences, Srinagar, India  *For correspondence: rashid.fouzia@gmail.com
et al., 2004; Vrbikova, 2005; Diamanti-Kandarakis et al., 2006; Gonzalez et al., 2009) suggesting ICAM-1 as a marker of low-grade inflammation, and a predictor of disease related to PCOS.

Genome-wide scans have predicted that PCOS susceptibility genes may reside over a broad region of chromosome 19p13.2 (Urbanek et al., 2005). ICAM-1 gene is located in 19p13.3-p13.2 chromosomal region. A common genetic polymorphism (rs1799969) at codon 241 in exon 4 of ICAM-1 gene resulting in substitution of an G with an A nucleotide and replaces Glycine (G) with a Arginine (R) in ICAM-1 gene has been described (Vora DK et al., 1994). Limited data are available on Gly241Arg polymorphism of ICAM-1 gene and its relationship with development of PCOS especially among Indian women. Since polymorphism of ICAM-1 are common genetic variation, several polymorphisms of ICAM-1 gene have been studied in number of inflammatory diseases like peripheral occlusive arterial disease (Flex et al., 2007), inflammatory bowel disease (Song et al., 2015), diabetes type 1 (Nejentsev et al., 2003), diabetes mellitus (Petrovic et al., 2008) and coronary artery disease (Chou et al., 2015). Therefore, the exon 4 of ICAM-1 gene represents a strong positional and biological candidate for the susceptibility to the development of inflammatory and metabolic diseases which may include PCOS. In the present study the genetic association between Gly241 Arg polymorphism on exon 4 of ICAM-1 gene and incidences of PCOS in kashmiri women was examined.

Materials and Methods

Study population

220 women with established PCOS and 220 healthy women were studied. The Rotterdam criteria (2003) was used for the diagnosis of PCOS which states 2 of the 3 features needs to be present to make the diagnosis of PCOS. These features includes (1) Oligo- or anovulation (< eight menstrual cycles in the presenting year) (2) Clinical and/or biochemical signs of hyperandrogenism and (3) Polycystic ovaries (either 12 or more follicles measuring 2-9 mm in diameter, or an ovarian volume of >10 cm³), with the exclusion of other etiologies (Non classic congenital adrenal hyperplasia, androgen-secreting tumors, cushing’s syndrome). Non classic congenital adrenal hyperplasia (NCAH), cushing’s syndrome, thyroid dysfunction, hyperprolactinemia, and androgen-producing tumors were ruled out by doing relevant investigation. All the PCOS patients belonged to Department of Endocrinology Sher-i-kashmir Institute of Medical Sciences (SKIMS) Srinagar, Kashmir. The non-PCOS group represented 220 apparently normal women having regular menstrual cycles (21-35d), displaying no evidence of clinical/biochemical hyperandrogenism, and having normal ovarian morphology on trans-abdominal ultrasonography. Controls were collected from various medical camps organised at colleges and at university of Kashmir. Women consuming any hormonal preparations or drug(s) known or suspected to affect reproductive or metabolic functions within 6 months of the study entry, or those having known diabetes mellitus instead, renal, hepatic, or cardiac dysfunction were also excluded from the study. PCOS cases and non PCOS healthy controls of same age group were preferentially selected and enrolled in the study. The study protocol was approved by the Institutional Ethics Committee and written informed consent was obtained from all the participants.

Anthropometric and systemic examination

All women underwent anthropometric assessment like measurement of height, weight, waist-hip circumference ratio and detailed systemic examination. Hirsutism assessment was done using modified Ferriman-Gallway score by counting nine specified body areas. A score of > 8 out of a total of 36 was taken as significant.

Biochemical Analysis

Biochemical analysis includes oral glucose tolerance test(OGTT), insulin, triglyceride(TG), low density lipoprotein(LDL), high density lipoprotein(HDL) and total cholesterol.

Calculations

Insulin resistance was assessed by means of the fasting glucose to insulin ratio (FGIR), homeostasis model assessment insulin resistance index (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI). The FGIR values were calculated as fasting glucose (mg/dL)/ fasting insulin (μIU/mL). The HOMA index was calculated as [fasting insulin (μIU/mL) x fasting glucose (mg/dL)]/405. The QUICKI was calculated as 1/ [log fasting insulin (μIU/mL) + log fasting glucose (mg/dL)]. High HOMA-IR, low QUICKI and low FGIR scores denote insulin resistance (low insulin sensitivity). Body mass index (BMI) was calculated as body weight (kg) divided by body height squared (m²).

Hormonal analysis

Hormonal analysis included; LH, FSH, Testosterone, 17-OHP and cortisol. 17 OHP to rule out non classical congenital adrenal hyperplasia and cortisol to rule out cushing’s syndrome. T4 - to rule out hyperthyroidism, TSH - to rule out hypothyroidism, PRL- to rule out prolactinoma. The sampling was arranged in such a way so that the sample for LH, FSH, 17-OHP and testosterone was collected on 3rd to 7th day of the follicular phase of either spontaneous or progesterone induced menstrual cycle.

Molecular Analysis of Gly241Arg Polymorphism of ICAM-1 Gene

DNA was extracted from peripheral leukocytes according to Phenol-Chloroform method. The quality of the resulting genomic DNA was stringently assessed by low percentage agarose gel electrophoresis. The concentration of the DNA obtained was measured in a Evolution 201 UV-Vis Spectrophotometer (Thermo Fisher Scientific) at 260nm wavelength and the purity of DNA was checked by using A260/ A280 ratio.

ICAM-1 Codon 241 Genotyping

An allele specific PCR method was used to amplify a 137 bp fragment of exon 4 of ICAM-1 gene using
the appropriate primers. The primer sets consisted of Primer G: 5GTGGTCTGTTCCCTGGACG3; Primer R: 5GTGGTCTGTTCCCTGGACA3 Common primer: 5GCCGTCACACTGACTGAGGCCCT3. For each individual two distinct PCR reactions were performed, one with primers G and the common primer and another with primers R and the common primer. Approximately 300 ng genomic DNA was amplified in a total volume of 25 μl of the reaction mixture containing 2.5μl of 10X PCR buffer, 2 μl of MgCl2, 0.5μl of 10mM dNTP mix, 0.5μl of forward Primer (G) (20nm), 0.5μl of forward Primer (R) (20nm), 0.5 μl of common (reverse) Primer (20nm), 0.3μl of Taq DNA polymerase (1U), 3.0μl of Genomic DNA, 15.2μl of deionized water to make total volume equal to 25.0μl. PCR amplification was done using touch-down method including an initial denaturation at 94°C for 5 min and two loops of amplification. Loop one included: 10 cycles of 94°C for 20s, 65°C for 20 s, 72°C for 20 s. Loop two included: 20 cycles of 94°C for 20 s, 61.5°C for 20 s, 72°C for 20 s. Final extension was performed at 72°C for 5 min. The amplified PCR products of 137 bp were run on 2% ethidium bromide stained agarose gel and visualized under UV transilluminator. (Figure 1)

**Statistical analysis**

Data was statistically analysed for mean values and standard deviations (SD) in Microsoft Office Excel. ANOVA and unpaired Student t-tests were used to compare the means of variables. Allel and genotype frequencies in the case and control groups were compared using Chi-square and Fischer exact tests. Statistical significance was set at P <0.05. Statistical analyses were

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>CASES</th>
<th>CONTROLS</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23.0 ± 4.82</td>
<td>22.9 ± 3.58</td>
<td>0.86 (NS)</td>
</tr>
<tr>
<td>FG score</td>
<td>13.0 ± 3.23</td>
<td>7.0 ± 2.33</td>
<td>0.0011</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>23.8 ± 3.03</td>
<td>23.4 ± 3.31</td>
<td>0.14 (NS)</td>
</tr>
<tr>
<td>Waist Hip Ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>8.8 ± 6.23</td>
<td>6.94 ± 1.99</td>
<td>0.07 (NS)</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>5.0 ± 5.22</td>
<td>6.66 ± 1.23</td>
<td>0.0001</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>8.8 ± 15.8</td>
<td>32.8 ± 11.2</td>
<td>0.14 (NS)</td>
</tr>
<tr>
<td>Blood glucose Fasting (mg/dl)</td>
<td>8.8 ± 12</td>
<td>87.7 ± 11.2</td>
<td>0.75 (NS)</td>
</tr>
<tr>
<td>Blood glucose 1 hr (mg/dl)</td>
<td>136 ± 17.8</td>
<td>131.2 ± 15.5</td>
<td>0.0002</td>
</tr>
<tr>
<td>Blood glucose 2 hr (mg/dl)</td>
<td>102.3 ± 19.4</td>
<td>94.1 ± 20.2</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td>Insulin Fasting (µIU/ml)</td>
<td>20.8 ± 10.8</td>
<td>10.8 ± 7.72</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td>FGIR</td>
<td>4.44 ± 7.85</td>
<td>11.2 ± 3.06</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.3 ± 0.33</td>
<td>0.09 ± 0.09</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>4.55 ± 2.14</td>
<td>2.14 ± 1.23</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td>Cholesterol(mg/dl)</td>
<td>181.2 ± 20.2</td>
<td>158 ± 17.6</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td>Triglycerides(mg/dl)</td>
<td>151.2 ± 22.6</td>
<td>104 ± 20.7</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td>HDL(mg/dl)</td>
<td>43 ± 6.21</td>
<td>48 ± 7.81</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td>LDL(mg/dl)</td>
<td>119 ± 16.1</td>
<td>93 ± 18.3</td>
<td>&lt;0.00</td>
</tr>
</tbody>
</table>

NS=Statistically Non significant (P>0.05); SD=Standard Deviation; BMI, Body Mass Index; FGIR, fasting glucose insulin ratio; FG Score, Ferrimen 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>
<thead>
<tr>
<th>ICAM-1 Gene Codon 241</th>
<th>Variants</th>
<th>Cases (N=220)</th>
<th>Controls (N=220)</th>
<th>OR (95% CI); P* Value</th>
<th>( \chi^2 ); P Value (Overall)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypic Frequencies (N)</td>
<td>Gly/Gly (Wild)</td>
<td>73 (33.1%)</td>
<td>72 (32.7%)</td>
<td>1</td>
<td>0.07; 0.96</td>
</tr>
<tr>
<td></td>
<td>Gly/Arg (Hetereozygous)</td>
<td>139 (63.1%)</td>
<td>139 (63.1%)</td>
<td>0.98 (0.66-1.47)</td>
<td>[1]</td>
</tr>
<tr>
<td></td>
<td>Arg/Arg (variant)</td>
<td>8 (3.63%)</td>
<td>9 (4.09%)</td>
<td>0.87 (0.32-2.3)</td>
<td>[0.79]</td>
</tr>
<tr>
<td></td>
<td>Gly/Arg+ Arg/Arg</td>
<td>147 (66.8%)</td>
<td>148 (67.2%)</td>
<td>0.97 (0.65-1.45)</td>
<td>[0.92]</td>
</tr>
<tr>
<td>Allelic Frequency (2N)</td>
<td>Gly (G allele)</td>
<td>285 (64.8%)</td>
<td>283 (64.3%)</td>
<td>1.0*</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Arg (R allele)</td>
<td>155 (35.2%)</td>
<td>157 (35.7%)</td>
<td>0.98 (0.74-1.29); 0.88</td>
<td>-</td>
</tr>
</tbody>
</table>

P*= Pearsons P value
Syed Douhath Yousuf et al


Results

Clinical and laborator characteristics of women with PCOS and control women are summarized in Table 1. The mean age of case and control women was comparable (23.04± 4.82 years in women with PCOS versus 22.97± 3.58 years in women without PCOS; p=0.862) and was BMI (23.87± 3.03 in PCOS group 23.43±3.31 in control group; p=0.146). FG score, cholesterol, triglycerides, LDL, HDL, total testosterone, fasting Insulin, FGIR, HOMA IR, and QUICKI showed significant differences between PCOS group compared to control group (p<0.0001). There was no significant difference in mean values of age, BMI, fasting glucose, and FSH among women with PCOS as compared to controls

All PCOS cases and controls were genotyped for the ICAM-1 codon 241 SNP. ICAM1 GG (Gly/Gly) represents homozygous wild-type, RR (Arg/Arg) represents homozygous variant type, GR (Gly/Arg) represents heterozygous genotype (Figure 1). All the studied genotypes were almost equally distributed among cases and controls and the distribution was statistically insignificant.

The genotypic distributions of ICAM-1 Gly241Arg polymorphism for cases and controls are shown in Table 2. The odds ratio (OR) for Arg/Arg genotype was 0.87 (95% CI=0.32-2.3) [P=0.79], for Gly/Arg genotype OR was 0.98 (95% CI=0.66-1.47) [P=1] and for Arg/Arg +Gly/Arg genotype OR was 0.97 (95% CI=0.65-1.45) [P=0.92]. The allelic frequency revealed statistically insignificant and almost equal distribution of R allel among cases and controls with OR=0.98 (95% CI=0.74-1.29); P=0.88. The genotypic frequencies of ICAM-1 codon 241 showed a insignificant difference between cases and controls (χ²=0.07, p=0.96). The genotypic and allelic distributions of ICAM-1 Gly241Arg polymorphism for cases and controls are shown in Table 2. Furthermore the association between polymorphism with that of the clinical and laboratory parameters was also carefully analysed. However, we did not find any significant association (P>0.05) with any of the PCOS characteristic. With respect to ICAM-1 Gly241Arg polymorphism our negative findings are performed using SPSS and vassarstats online software

Discussion

In our study the objective was to find out the possible association between Gly241Arg polymorphism of ICAM-1 gene with risk of developing PCOS in Kashmiri women. Our results revealed that there was no difference in the distribution of genotypic and allelic frequencies of Gly241Arg genotypes between cases and controls, thereby, suggesting that the polymorphism in this codon may not be associated with the risk of PCOS in our population. Furthermore the association between polymorphism with that of the clinical and laboratory parameters was also carefully analysed. However, we did not find any significant association (P>0.05) with any of the PCOS characteristics shown in Table 3.
ICAM-1 Gly241Arg Polymorphism is Not Associated with Polycystic Ovary Syndrome in Kashmir, India.

DOI:http://dx.doi.org/10.7314/APJCP.2016.17.3.1583

ICAM-1 Gly241Arg Polymorphism is Not Associated with Polycystic Ovary Syndrome in Kashmir, India.

References


