The *Ser128Arg* polymorphism of *E-selectin* gene is not associated with polycystic ovary syndrome

**ABSTRACT**

**Objective:** Low-grade chronic inflammation and endothelial dysfunction have important role in the pathogenesis of polycystic ovary syndrome (PCOS). E-selectin is a cell adhesion molecule involved in first attachment and transmigration of leukocytes to activated endothelium in conditions with chronic inflammation. This study examined for the first time the possible association of *Ser128Arg* (c.561 A>C) single nucleotide polymorphism (SNP) of *E-selectin* gene with the occurrence of PCOS, and evaluates the relationship between genotypes and clinical/biochemical characteristics of PCOS.

**Methods:** The *Ser128Arg* polymorphism of *E-selectin* gene in DNA from peripheral blood leukocytes of 169 PCOS patients and 259 healthy control women were investigated by real-time PCR combined with melting curve analysis using fluorescence-labeled hybridization probes.

**Results:** It was found that insulin resistance (IR) was present in 85 (50%) of women with PCOS. Additionally, insulin (72.8%) levels and HOMA (82.3%) value were higher, and QUICKI (13.2%) was lower in PCOS women when compared with controls. No significant association between PCOS and the variant allele of *E-selectin Ser128Arg* (OR: 1.17, 95% CI= 0.75-1.82) was observed. This polymorphism was found not to affect insulin resistance (IR) indices and lipid profile parameters significantly.

**Conclusion:** These preliminary results suggest that the *E-selectin Ser128Arg* polymorphism is not a significant risk factor for PCOS alone. However, further studies on the same topic are necessary to support our observation before any statement can be made about the relationship between PCOS and *E-selectin* polymorphism.

**Key Words:** E-selectin, insulin resistance, polycystic ovary syndrome, polymorphism.

**Conflict of Interest:** The authors declare no conflict of interest.

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**ÖZET**

**Amaç:** Polikistik over sendromun (PCOS) patojenezinde düşük derece kronik inflamasyon ve endotel disfonksiyonu önemli rol oynamaktadır. E-selektin, kronik inflamasyon durumlarında aktifleşen endotele lökositlerin tutunması ve transmigrasyonunu sağlayan bir hücre adhesiyon molekülüdür. *E-selektin Ser128Arg* polimorfizminin PCOS oluşumu ve PCOS’un klinik ve biyokimyasal özellikleri ilişkisi bu çalışma ile ilk kez incelenmektedir.

**Metod:** 169 PCOS’lu ve 259 sağlıklı kadının DNA örnekleri, real-time PCR yöntemiyle incelendi. Bu analiz, PCOS’lu ve kontrol kadınlarda insülin rezistansı (IR) ve HOMA, insulin direnci ve QUICKI değerlerine göre değerlendirildi.

**Bulgular:** PCOS’lu kadınlarda 85 (50%) insülin rezistansı (IR) bulunuyordu. Ayrıca, IR (72.8%) ve HOMA (82.3%) değerleri artmış, QUICKI (13.2%) değerleri ise düşük bulunuyordu. *E-selectin Ser128Arg* genindeki polymorfizm PCOS gelişimine ilişkin değildir (OR: 1.17, 95% CI= 0.75-1.82). Bu polymorfizm, insülin direnci ve lipid parametreleri açısından bir etkisi bulunmadi.

**Sonuç:** Bu sonuc, *E-selectin Ser128Arg* polymorfizminin PCOS için tek başına bir risk faktörü olmadığını düşündürüyor. Ancak, aynı konuda daha fazla çalışma yapılması gereklidir.

**Anahtar Kelimeler:** E-selektin, insülin rezistansı, polikistik over sendromu, polymorfizm.

**Çıkış Çatışması:** Yazarlar çıksış çatışması yoktur.
Introduction

The polycystic ovary syndrome (PCOS) is a common reproductive endocrinopathy characterized by chronic anovulation, oligoamenorrhea and hyperandrogenism [1] and is associated with increased risk of obesity, insulin resistance and type 2 diabetes mellitus [2]. Although the exact pathophysiological mechanisms of PCOS remains elusive, there is a growing evidence that the disease is a consequence of interaction between genetic and environmental factors [3,4]. A positive family history appears to be the most informative risk factor for the development of PCOS [3]. The heritability of PCOS is probably complex, similar to that type 2 diabetes. Indeed, many genes involved insulin signaling, steroid hormone synthesis and type 2 diabetes have been interrogated as susceptibility genes for PCOS [3,4]. Available data suggest that low-grade chronic inflammation and endothelial dysfunction have important roles in the pathogenesis of PCOS [1,2].

The adherence of circulating leukocytes to the activated endothelium is one of the earliest events of the inflammatory process [5-7].

E-selectin (CD62E) is a cell surface glycoprotein expressed in peripheral lymphocytes, monocytes, neutrophils and endothelial cells, and plays a key role in the initiation of leukocyte migration from circulation to subendothelial tissues [7]. Recent studies demonstrated increased E-selectin levels in patients with type 2 diabetes mellitus [8] and PCOS [9], or insulin resistance [10], and association of elevated E-selectin with diabetic microvascular and macrovascular complications as well [11]. Increased expression of E-selectin on endothelium in insulin resistant states is related to increased insulin secretion using an MAPK-dependent pathway [12,13].

P-selectin, similar to E-selectin, is another adhesion molecule (CD62P), found on the surface of activated platelets and endothelial cells [14]. P-selectin mediates initial leukocyte “rolling” to the subendothelial region, plays a major role in the cascade of inflammation, thrombosis, and coagulation at the site of vascular injury [15]. Levels of P-selectin are reported to be increased in patients with hypertension [16], dyslipidemia [17], atherosclerosis [18], and PCOS as well [19]. Although the P-selectin and E-selectin shares many properties, E-selectin is has been accepted to be a marker of endothelial dysfunction, whereas P-selectin usually reflects the procoagulant state [20]. The fact that there are different ligands for P-selectin and E-selectin suggest disparate roles for P-selectin and E-selectin during leukocyte recruitment in inflammatory responses [21].

More importantly, the association between circulating P-selectin and fibrinogen levels in cardiovascular disease (CVD) patients supports the thrombotic functions of P-selectin [22]. Therefore, as E-selectin reflects predominantly the endothelial dysfunction, we chased it as study parameter.

The E-selectin gene polymorphisms have been shown as independent risk factors for the development of endothelial dysfunction in cardiovascular disease [23-25]. The most studied polymorphism of E-selectin gene is Ser128Arg (rs5361, c.561A>C) which causes a substitution of serine (Ser) to arginine (Arg) in the epidermal growth factor domain at position 128 of protein, affecting importantly the binding of E-selectin to the endothelium. The 128Arg allele exhibits a decreasing binding specificity and an increased affinity for additional ligand such as sialyl-lactosamine [26], and has been associated with increased risk for cardiovascular disease [24]. The Ser128Arg polymorphism has been found to be associated with the increased risk for type 2 diabetes mellitus in general [27,28], whereas Meigs et al.[29] did not found any association in diabetic women. Considering the recognized roles of increased E-selectin levels in diabetes mellitus and PCOS, and lack of information about the relationship between PCOS and E-selectin polymorphisms, we aimed to investigate whether the Ser128Arg polymorphism of the E-selectin gene could predispose women to PCOS, and to evaluate is this polymorphism related to indices of IR and lipid profile parameters in PCOS.

Materials and Methods

The study was approved by the Institutional Ethical Committee at Şişli Etfal Research and Training Hospital. A total number of 428 women (169 women with established PCOS and 259 healthy controls) were studied. PCOS was defined when at least two of the following three features were present: oligo-/amenorrhea (<8 menstrual cycles in the presenting year); hyperandrogenism (and/or hirsutism); and polycystic ovaries [30]. Cushing’s syndrome, late-onset 21-hydroxylase deficiency, thyroid dysfunction, hyperprolactinemia, diabetes mellitus or androgen secreting tumors were excluded. Among the exclusion criteria, also, were current or previous use (within 6 months) of oral contraceptives, antiandrogens, statins, glucocorticoids, or infertility medications. The control group consisted of 259 unrelated healthy fertile women matched for age. None of the controls had symptoms of hyperandrogenism, a history of menstrual dysfunction, infertility, or sonographic sings of PCOS. All women were nonpregnant, non-smokers, and normotensive. Also, all of women were studied within the first 10 days after onset of menstruation in the case of mild oligomenorrhea or at random in those suffering from severe oligo-/amenorrhea. The characteristics of PCOS women and controls are shown in Table 1.

Fasting peripheral venous blood samples were collected in plain tubes for routine biochemical analysis, and in EDTA.K1 for genotype analysis. Serum glucose, triglyceride, cholesterol, HDL-and LDL-cholesterol measurements were performed on 1800 DPP Roche autoanalyzer; LH, FSH, prolactin, TSH, free T4, free T3, total testosterone, progesterone, DHEAS and insulin were measured on
Insulin resistance was assessed by means of the homeostasis model (HOMA), fasting glucose/insulin ratio (GIR) and quantitative insulin sensitivity check index (QUICKI). High HOMA, low QUICKI and low GIR scores denote IR (low insulin sensitivity). HOMA, GIR and QUICKI were calculated using the following formulas [31].

\[
\text{HOMA} = \frac{\text{Fasting glucose (mg/dL)} \times \text{Fasting insulin (µU/ml)}}{405}
\]

\[
\text{GIR} = \frac{\text{Fasting glucose (mg/dL)}}{\text{Fasting insulin (µU/ml)}}
\]

\[
\text{QUICKI} = \frac{1}{\log \text{fasting insulin concentration (µU/mL)} + \log \text{fasting glucose concentration (mg/dL)}}
\]

Body mass index (BMI) was calculated as body weight (kg) divided by body height squared (m²).

Genomic DNA was isolated from peripheral blood leukocytes by using High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany). We established a real-time PCR method combined with melting curve analyses using primer and probe system on LightCycler instrument (Roche Diagnostics, Mannheim, Germany) for the detection of E-selectin Ser128Arg (c.561 A>C). The sequences of primers and probes were as follows: primers (5'-TGCTGATGTCTCTGTTGC-3' and 5'-GGTCTCTACATGGTCACG-3'), and probes (5'-TTTGTATTTTCCGTAGCTGCTGTACC-FL and 5'-LC640-ATACATCCTGCGTGCATTGC-3'). Analysis was done in 20 μl volumes using glass capillaries. The PCR mix contained 2 μl of the genomic DNA, 2 μl of LC™ FastStart DNA Master HybProbe kit (Roche Diagnostics), 0.5 μM of each primer, 0.2 μM of each probe and 2.5 mM total MgCl₂. The initial 10 min denaturation at 95 °C was followed by 45 cycles - denaturation (95 °C; 10 s), annealing (49 °C; 10 s), and elongation (72 °C; 12 s). Melting curve analysis was done with an initial denaturation step at 95 °C for 5 s and 20 s at 45 °C, slow heating to 70 °C, with a ramping rate of 0.15 °C/s and continuous fluorescence detection. Melting curves were converted to melting peaks by plotting the negative derivative of fluorescence against temperature (-dF/dT) (Fig.1). A negative control containing all reagents but water instead of the DNA template was included to each amplification set. Melting curves were evaluated by two independent observers who were blinded to the analy-

![Figure 1](image_url)
sis of the clinical data. In addition, 10% of randomly selected samples were repeated independently to verify genotyping results and 100% concordance was found. The nomenclature of studied polymorphism was achieved from “Recommendation for the description of sequence variants” (http://www.hgvs.org/mutnomen/).

The differences in the distribution of alleles and genotypes between studied groups were estimated by chi-square ($\chi^2$) test. Deviation from Hardy-Weinberg Equilibrium (HWE) was tested using the Pearson $\chi^2$-test. Odds ratios (ORs) were calculated and given with 95% confidence intervals (CIs). The wild-type genotype/allele served as a reference category. Comparisons of individual clinical variables between genotypes were assessed with $\chi^2$-test. Mann-Whitney U and Spearman correlation tests were used for the evaluation of clinical and biochemical parameters. All statistical analyses were performed with SPSS 15.0 (Chicago, IL, USA) for Windows. In addition, the NCSS 2000 statistical package (Kaysville, Utah, USA) was used to evaluate the power analysis.

### Results

One hundred sixty nine women with established PCOS and 259 unrelated nonpregnant healthy women) were enrolled in the study. We had a 97% power to detect an effect size (W) of 0.20 using a 2 degrees of freedom ($\alpha=0.05$). Table 1 summarizes the clinical and biochemical characteristics of controls and PCOS women. The BMI in PCOS women was increased according to healthy controls. IR was found to be present in 85 (50%) of PCOS patients. Insulin (72.8%) levels and HOMA (82.3%) were higher, and QUIKCI (13.2%) was lower in PCOS women when compared to controls. Spearman correlation test revealed that there were positive correlations between BMI/insulin ($r=0.322$, $p=0.0001$), BMI/HOMA ($r=-0.702$, $p=0.0001$), BMI/LDL ($r=0.217$, $p=0.038$), BMI/cholesterol ($r=0.278$, $p=0.008$); and negative correlations between BMI/GIR ($r=-0.325$, $p=0.0001$), and BMI/QUICKI ($r=-0.319$, $p=0.0001$). In addition, strong correlations between the indices of IR were observed: GIR/QUICKI ($r=0.804$, $p=0.0001$), GIR/HOMA ($r=-0.702$, $p=0.0001$), HOMA/

### Table 2. Distribution of genotypes and allele frequencies of the E-selectin Ser128Arg (rs5361) gene for Polycystic Ovary Syndrome (PCOS) and control group

<table>
<thead>
<tr>
<th>Genotype/Allele</th>
<th>Controls n (%)</th>
<th>PCOS n (%)</th>
<th>OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ser/Ser</td>
<td>209 (80.7)</td>
<td>131 (77.5)</td>
<td>1.0*</td>
<td>–</td>
</tr>
<tr>
<td>Ser/Arg 48 (18.5)</td>
<td>37 (21.9)</td>
<td>1.23 (0.76-1.99)</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>2 (0.8)</td>
<td>1 (0.6)</td>
<td>0.80 (0.07-8.89)</td>
<td>0.85</td>
</tr>
<tr>
<td>Ser/Arg + Arg/Arg</td>
<td>50</td>
<td>38</td>
<td>1.21 (0.75-1.95)</td>
<td>0.43</td>
</tr>
<tr>
<td>Ser allele frequency</td>
<td>0.90</td>
<td>0.88</td>
<td>1.0*</td>
<td></td>
</tr>
<tr>
<td>Arg allele frequency</td>
<td>0.10</td>
<td>0.12</td>
<td>1.17 (0.75-1.82)</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Each p-value was based on Chi-square($\chi^2$) analysis

*Reference values for

### Table 3. Insulin resistance and lipid profile parameters of PCOS women in accordance with their genotypes of the E-selectin Ser128Arg gene polymorphism [mean (range)]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ser/Ser</th>
<th>Ser/Arg + Arg/Arg</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>24 (18-44)</td>
<td>25 (19-40)</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>88 (68-117)</td>
<td>92 (67-135)</td>
</tr>
<tr>
<td>Insulin (µIU/mL)</td>
<td>14.1 (2.5-59.0)</td>
<td>13.7 (3.7-36.5)</td>
</tr>
<tr>
<td>GIR</td>
<td>9.9 (1.6-34.4)</td>
<td>9.4 (2.8-24.5)</td>
</tr>
<tr>
<td>HOMA</td>
<td>3.1 (0.5-13.9)</td>
<td>3.1 (0.8-9.4)</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.34 (0.26-0.67)</td>
<td>0.33 (0.28-0.40)</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>170.0 (106-255)</td>
<td>160.5 (108-229)</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>100.0 (33-348)</td>
<td>90.0 (33-171)</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>48.0 (27-73)</td>
<td>49.0 (35-65)</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>95 (50-161)</td>
<td>97 (56-159)</td>
</tr>
</tbody>
</table>

Mann-Whitney U test (when Ser/Arg + Arg/Arg genotype carriers were compared to SS). BMI: Body mass index; GIR: Glucose/insulin ratio; HOMA: Homeostasis model assessment; QUICKI: Quantitative insulin sensitivity check index; HDL-C: High density lipoprotein-cholesterol; LDL-C: Low density lipoprotein-cholesterol
The genotypic and the allelic distributions of S128R polymorphism of E-selectin gene for cases and controls are shown in Table 2. E-selectin Ser128Arg genotype distributions among the cases and controls were in accordance with the HWE. The allelic frequencies of Ser (0.90) and Arg (0.10) found in our control population were similar to those reported for the American [25], English [19] and Austrian [32] populations. We did not find any association between PCOS and E-selectin Ser128Arg (Table 2).

Due to the low frequency of Arg/Arg homozygotes in both PCOS and control groups, these subjects were combined with the Ser/Arg heterozygotes for further analysis. The alterations of the indices of IR (glucose, insulin, GIR, HOMA and QUICKI) and lipid profile parameters between genotypes of Ser128Arg polymorphism of E-selectin gene were not observed to be statistically significant (Table 3).

Discussion

Insulin resistance and hyperinsulinemia are widely acknowledged to be common biochemical features of PCOS. It is present in both obese and nonobese PCOS women, and probably have a pivotal role in the pathogenesis of the syndrome [33]. Moreover, it was estimated that women with PCOS are more insulin resistant than age- and BMI-matched control women [2]. In addition, women with PCOS are three to seven times more likely than weight-matched controls to develop non-insulin-dependent diabetes mellitus in later life [34]. It is seen from the results that IR was present in 85 (%50) of PCOS patients. In addition, our results showing increased insulin (72.8%), HOMA (82.3%), decreased QUICKI (13.2%) in PCOS women, and significant correlations between indices of insulin resistance and BMI in PCOS, agree with these observations.

It is well recognized that low-grade chronic inflammation and endothelial dysfunction reflected in increased plasma levels of adhesion molecules and C-reactive protein [2,9], are present in type 2 diabetes mellitus [8], diabetic microvascular and macrovascular complications [10,11] and PCOS [9]. Cell adhesion molecules, mediating neutrophil, monocyte and memory T-cell adhesion and transmigration through the activated endothelium, play important role in early stages of vascular disease in conditions with chronic inflammation such as insulin resistance and PCOS [2,7]. Mononuclear cells isolated from insulin resistant subjects have been reported to bind to endothelial cells with enhanced affinity [35,36]. Although the exact mechanisms are still poorly understood, this process seems to be modulated by various cell adhesion molecules including E-selectin, and might explain the increased risk of atherosclerosis in insulin resistant state [35].

It has been revealed that Ser128Arg polymorphism of E-selectin gene is a risk factor for development of endothelial dysfunction [23] and has profound effects on ligand recognition and binding to activated endothelium. The substitution of serine to arginine has been shown to decrease importantly binding specificity with increased affinity for additional ligands, leading to an increase in cellular adhesion two- to threefold. The 128Arg allele may thus increase leukocyte adherence to endothelium contributing to the progression of endothelial dysfunction. The data about the impact of Ser128Arg polymorphism on type 2 diabetes mellitus is controversial. While a number of studies reported the association between Ser128Arg polymorphism and increased risk for diabetes mellitus in general [27,28], Meiggs and coworkers [29] suggested that E-selectin variant is not important risk factor for diabetes in women. Moreover, it was demonstrated that the presence of 128Arg allele is associated with acquired risk for coronary heart disease in patients with diabetes mellitus [37]. Although increased E-selectin levels in patients with PCOS were reported [9], there is no information in the literature about the relationship between E-selectin gene polymorphism and PCOS. Therefore, we investigated Ser128Arg polymorphism in PCOS women. We did not find an association between Ser128Arg polymorphisms and the risk of developing PCOS in the present study, indicating that these polymorphisms alone may not play a major role in the PCOS etiopathogenesis. In addition, we reported recently the lack of association between intercellular adhesion molecule 1 (ICAM1) and vascular adhesion molecule 1 (VCAM1) polymorphisms with PCOS [38,39]. However, the VCAM1 -1591 polymorphism was found to be related with increased triglyceride and decreased HDL-C in PCOS, supporting increased risk for atherosclerosis and cardiovascular disease in PCOS women [39].

Another objective of our study was to investigate the probable relationship between studied polymorphism and indices of IR and lipid profile parameters. When parameters of PCOS patients were classified in accordance with their Ser128Arg genotype, no significant differences among genotypes were found.

As a conclusion, there is no any association between E-selectin Ser128Arg polymorphism and susceptibility to PCOS. Additionally, there isn’t any significant difference among genotypes in mean of IR and lipid profile parameters. Further studies on the same topic are warranted to confirm our observations before any statement can be made about the relationship between E-selectin polymorphism and risk for PCOS.

Acknowledgement

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Conflict of Interest

There are no conflicts of interest among the authors.
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