The Effect of PAI-1 Gene Variants and PAI-1 Plasma Levels on Development of Thrombophilia in Patients with Klinefelter Syndrome

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**Abstract:** Klinefelter Syndrome (KS) is a common sex chromosome related abnormality seen among men. KS negatively affects spermatogenesis and testosterone production. It also increases the risk of thrombosis but its molecular mechanism has not been well described yet. Elevated PAI-1 is a risk factor for thrombosis. The rs1799889 polymorphism located in the promotor region of the PAI-1 gene was detected in patients with deep venous thrombosis. In this study, PAI-1 gene variant and its plasma levels in KS patients were examined. Forty-one KS patients (47, XXY) and 50 age-matched healthy controls participated. DNA was isolated from peripheral blood and Real-Time PCR method was used to detect known SNPs in PAI-1 gene. In addition, PAI-1 plasma levels were measured by using ELISA method. There was no significant difference between PAI-1 gene polymorphisms of KS patients and controls (p>0.05). However, the significant difference was observed in PAI-1 plasma levels between two groups (high PAI-1 plasma level in KS patients compared to controls). Patient group's mean was 55.13 and control group's mean in PAI-1 level was 29.89 ng/ml (p=0.020). Moreover, clinical features related to thromboembolism especially varicose veins were detected in KS patients frequently (p= 0.04). These results suggest that thromboembolism related to clinical features are seen more frequently in cases with KS, but it may not be dependent only on PAI-1 gene polymorphism structure.

**Key words:** Klinefelter Syndrome, PAI-1 polymorphism, thrombosis, varicose vein
1. Introduction

Klinefelter Syndrome (KS), is a disease with chromosomal abnormality detected only in men. KS as a sex chromosomal abnormality is a common genetic disease and can be seen in every 1/500-1000 births throughout all ethnic groups. According to karyotype analyses, these patients have an extra one or more X chromosomes. Eighty percent of these patients have 47, XXY karyotype, others have 46,XY/XXY mosaicism, chromosomal aneuploidies and structurally damaged X chromosome (1,2).

Clinodactyly and other malformations like undescended testes and cleft plate may manifest at higher frequencies in patients with extra X chromosomes. Besides, KS patients have risks of several complications such as epilepsy, abdominal obesity, taurodontism, osteoporosis, cancer, systemic lupus erythematosus, heart disease. Also, they suffer from vascular diseases, exclusively varicose veins, thromboembolism and pulmonary embolism during their lifespan compared to healthy people (3,4,5).

Many studies have demonstrated that PAI-1 gene polymorphisms may have an important role in the formation of thrombosis. PAI-1 gene has a role in the fibrinolytic system and is in a balance with t-PA (plasminogen activator) in the coagulation process. PAI-1 gene is a member of serpin family (6). The increased PAI-1 level is a risk factor for thrombosis. 4G/4G, 4G/5G, 5G/5G polymorphisms (rs1799889), which are located in the promotor region of PAI-1 gene. Moreover, several studies demonstrated that low testosterone increases the PAI-1 level and elevated PAI-1 activity is associated with the pathogenesis of ulceration, thromboembolism etc.(7). In addition, increased PAI-1 activity affects fibrinolysis negatively in KS and this inverse relationship supports the beneficial role of androgen therapy in KS patients with venous leg ulcers (8). Based on
this information we hypothesized that PAI-1 gene polymorphism and PAI-1 plasma level may affect clinical findings of KS patients related to the formation of thrombosis.

2. Materials and methods

2.1. Study cohort and protocols

The study groups were composed of 41 KS patients (age range: 19-42; mean age: 30.80±5.69 years) and 50 unrelated healthy males as control group (age range: 25-38; mean age: 32.68±3.26 years). These KS patients were admitted to Istanbul University Cerrahpasa Medical Faculty Department of Medical Genetics and Istanbul University Medical Faculty Department of Medical Genetics between 2009-2016 to infertility outpatient clinic. This study was approved by Cerrahpasa Faculty of Medicine ethics committee. These patients were asked for their history of unbalanced hormonal level and any other medical treatments. Patients were examined whether they had stroke, circulatory disease, vasculitis, varicose, leg ulcer, diabetes, high tension, thrombosis, thromboembolism, hormone treatment, autoimmune disease, hepatitis B and C infections. All patients had 47XXY genotype. Those patients who had 46 XY/47XXY mosaicism and other chromosomal abnormalities were excluded.

The control group was selected randomly from age-matched healthy males. In the selection process, the following imperative criteria were considered: marriage and fertility. Individuals with symptoms as deep vein thrombosis, autoimmune disease, hepatitis B and C infections and individuals under hormonal treatment etc. were excluded from the study as well. At the study onset, as a total of 2 EDTA (anticoagulated tubes) tubes, two milliliters of whole blood sample was collected with the standard venipuncture technique. One of these tubes was taken for the isolation of DNA by using genomic DNA purification mini kit (E.Z.N.A.® Omega Kit, Georgia), the other one was used to obtain
plasma, which is used to determine PAI-1 levels. Real-time PCR technique (FLUORINE; PAI-1 4G-5G QLP 3.0 Real-Time PCR Kit) was applied to assess PAI-1 gene polymorphisms (4G/4G, 4G/5G, 5G/5G) in patients with KS and control group. Moreover, ELISA method was carried out to determine PAI-1 plasma levels. All of these data were analyzed using SPSS software package (version 22 IBM SPSS; parametric, non-parametric, chi-square tests etc.).

3. Results

Patients and control group were matched in terms of their ages and the mean and standard deviation of ages was given above. Related to the aim of our study Table 1 shows all parameters. The PAI-1 gene polymorphism (4G/4G; mutant polymorphism, 4G/5G; heterozygote polymorphism, 5G/5G; wild-type polymorphism) of patients with KS and control group were shown. The distribution of PAI-1 gene polymorphisms was in accordance with the Hardy-Weinberg equilibrium (patients p= 0.1, controls p=0.157).

There were no statistically significant differences between two types of polymorphisms (4G/4G; mutant, 5G/5G; wild type) and PAI-1 plasma levels. But, PAI-1 plasma levels of heterozygote polymorphism carriers (4G/5G) was found to be statistically significant compared to heterozygote 4G/5G polymorphism carriers among controls (Patient mean= 67.01 - control mean= 28.60 / p= 0.033). In general, without considering polymorphic groups, the PAI-1 plasma levels of patients were detected higher than the control group (p= 0.020). Moreover, clinical symptoms related to thromboembolism especially the presence of varicose veins were found more frequent in patients compared to control group (p= 0.040).
Table 1: All observed parameters and p values of patients with KS and controls

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients (±SD)</th>
<th>Controls (±SD)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>(N= 41)</td>
<td>(N= 50)</td>
<td>0.052</td>
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<tr>
<td></td>
<td>30.80 ± 5.69</td>
<td>32.68 ± 3.2</td>
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<tr>
<td>Polymorphism</td>
<td></td>
<td></td>
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<tr>
<td>4G/4G (mut)</td>
<td>10 (24.3%)</td>
<td>15 (30%)</td>
<td>0.46</td>
</tr>
<tr>
<td>4G/5G (het)</td>
<td>16 (39%)</td>
<td>20 (40%)</td>
<td>0.8</td>
</tr>
<tr>
<td>5G/5G (wild)</td>
<td>15 (36.5%)</td>
<td>15 (30%)</td>
<td>0.32</td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4G</td>
<td>36</td>
<td>50</td>
<td>0.41</td>
</tr>
<tr>
<td>5G</td>
<td>46</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>N=40</td>
<td>N=50</td>
<td>0.020*</td>
</tr>
<tr>
<td></td>
<td>(55.13 ± 60.25)</td>
<td>(29.89 ± 32.19)</td>
<td></td>
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<tr>
<td>PAI-1 plasma level</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>4G/4G (mut)</td>
<td>10 (30.43 ± 10.9)</td>
<td>15 (28.11 ± 21.83)</td>
<td>0.73</td>
</tr>
<tr>
<td>4G/5G (het)</td>
<td>16 (67.01 ± 63)</td>
<td>20 (28.60 ± 23.74)</td>
<td>0.033*</td>
</tr>
<tr>
<td>5G/5G (wild)</td>
<td>14 (59.2 ± 74.6)</td>
<td>15 (33.41 ± 48.6)</td>
<td>0.28</td>
</tr>
<tr>
<td>Varicose veins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>17/38</td>
<td>6/29</td>
<td>0.040*</td>
</tr>
<tr>
<td>Absent</td>
<td>21/38</td>
<td>23/29</td>
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</tr>
</tbody>
</table>
4. Discussion

Klinefelter Syndrome has a tendency towards hypercoagulability because of hormonal imbalance and one or more inherited thrombophilic factors (9,15). In several studies, venous thrombosis, thromboembolism (especially pulmonary thromboembolism) and leg ulcers' prevalence were seen to be increased in patients with KS because of androgen deficiency. According to Campbell and Price's study (10) where 412 KS patients were examined the prevalence of deep vein thrombosis and pulmonary embolism was observed to be increased in adults who are 1-20 years. Winkler (11) reported the reason for this disease is androgen imbalance which in turn affects hemostasis negatively.

Hypogonadism increases PAI-1 plasma level in this way high PAI-1 plasma level causes to decrease fibrinolytic activity. The formation of thrombosis or thromboembolism does not depend on only hormonal imbalance but also depends on hereditary thromboembolic factors (10,11). It is thought that the polymorphic structure formed in the 675. pair in the PAI-1 gene promoter region may play an important role in thrombophilia formation. The polymorphic structure seen in the PAI-1 gene; 4G / 4G, 4G / 5G, and 5G / 5G. The 5G allele has been found to have an additional protein binding site. In the 4G allele, this region is not present. It is understood that the elevated level of PAI-1 is positively correlated with the 4G allelic polymorphism structure. Thus, the idea that the additional protein binding site in the 5G allele is involved in a repressor protein is gaining in importance (13,16).

In this study, we investigated that the polymorphism in the promoter region at 675 base pair of PAI-1 gene could have an important role in the formation of thrombophilia. 5G allele of PAI-1 gene was shown to have an additional linkage protein region in contrast to the 4G allele. Increased PAI-1 levels and 4G allele polymorphisms were seen to be
positively correlated. In this regard, the 5G allele can bind E2F transcription repressor of PAI-1 and 4G allele does not. 4G allele polymorphism may explain for increased PAI-1 plasma levels in patients with Klinefelter Syndrome\(\text{\textregistered}\)\(\text{\textregistered}\)(12). In addition, we compared both PAI-1 gene polymorphisms 4G/4G, 4G/5G, 5G/5G and PAI-1 plasma level in KS and control group. The risk of thromboembolism increases with the age, which was the reason for us to compose a cohort which would include young individuals within a patients' group and control group. The clinical symptoms related to thromboembolism (especially varicose vein) were searched in the patients with KS and control group who were included in our study.

PAI-1 polymorphisms and the increased plasma levels of PAI-1 may trigger the formation of thromboembolism in patients with KS due to hypoandrogenism (7,14). In our study, heterozygote polymorphism (4G/5G) PAI-1 gene was found to be statistically significant as compared to PAI-1 plasma levels \(p=0.033\). The appearance of high PAI-1 plasma levels in patients with KS who have 4G / 5G polymorphism, suggests that thromboembolism formation may be more common in these individuals. It was determined that individuals with 4G / 5G polymorphism in the patient group had a significant effect on clinical features related to thromboembolism rather than the control group.

PAI-1 plasma levels of patients and control group were determined in this study and according to obtained results, the mean of PAI-1 plasma level in KS was found to be about 2 times higher than the control group (mean of patients: 55.13 / controls: 29.89) when plasma levels were compared between patients and control group and the \(p\)-value of respective test was 0.02. When we compared PAI-1 gene polymorphism with PAI-1 plasma levels in both patients and control group, the PAI-1 plasma level was found to be
statistically significant in patients with 4G/5G polymorphism (p= 0.033). These results
demonstrate that there is a correlation between PAI-1 plasma levels and heterozygote
(4G/5G) polymorphism. In addition, we examined patients and the control group for the
clinical features related to thromboembolism. Patients and control group were compared
for PAI-1 polymorphism structures and clinical findings related to the formation of
thromboembolism and no statistically significant difference was seen (patients p = 0.55,
controls p= 0.99). When patients and control group were compared, clinical findings
related to thromboembolism (especially varicose vein) was found to be more frequent in
cases individuals KS. These results suggest that thromboembolism related to clinical
features are seen more frequently in cases with KS, but it may not be dependent only on
PAI-1 gene polymorphism. Also, the PAI-1 gene polymorphism suggests that it may also
be associated with other mechanisms that are effective in the fibrinolysis and coagulation
system as inhibitors or as catalysts.

In conclusion, mutant type 4G/4G and heterozygote type 4G/5G were not found
frequent in observed patients with KS compared to wild-type 5G/5G. However, increased
number of formation of varicose veins and PAI-1 plasma levels were found to be
statistically significant, suggesting that the PAI-1 plasma level was effective in patients
with KS. In further studies, other factors that are associated with PAI-1 gene in both
fibrinolysis and coagulation mechanism should be investigated separately by increasing
the number of patients with KS and controls.

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References


1 Theses