Original Article

The 763C>G Polymorphism of The Secretary PLA2IIa Gene is Associated with Endometriosis in Iranian Women

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Abstract:

**Background:** Endometriosis is a chronic gynecological disease characterized by complex interactions between genetic, the stress oxidative and intrinsic inflammatory, hormonal and environmental components. The aim of this study was to investigate the potential association of the 763C>G polymorphism in the secretary phospholipase A2 group IIa gene (PLA2G2A) with the risk of endometriosis in Iranian women.

**Materials and Methods:** Ninety seven patients with endometriosis and 107 women who were negative for endometriosis after laparoscopy and laparotomy served as control groups and were enrolled for this cross-sectional study. Samples were analyzed for polymorphism of the PLA2G2A using polymerase chain reaction –restriction fragment length polymorphism based methods.

**Results:** Multivariate analysis was used to examine the association between the risk of endometriosis and 763C>G polymorphism of PLA2G2A. Genotype distributions of PLA2G2A show significant difference between patients and the controls (P<0.001, OR =0.22, 95%CI=0.21-0.39). Correlation analysis showed that there was a significant difference between the normal homozygous genotype and susceptibility to endometriosis (P<0.001).

**Conclusion:** The present study suggested that the 763C>G polymorphism of PLA2G2A plays an important role as independent factor in the risk of endometriosis in Iranian women.

**Keywords:** Endometriosis, 763 C>G Polymorphism, PLA2G2A, Iranian Women

Introduction

Endometriosis is one of the most frequent disorders in gynecology that is characterized by development of the endometrial tissue outside the uterus (1). Endometriosis may affect > 10% of all women of reproductive age and > 30% of all infertile women (2,3). The common symptoms of endometriosis are pelvic pain, dysmenorrhoea, dyspareunia and infertility (4).
The disease is diagnosed by laparoscopy with or without biopsy for histologic diagnosis (4,5). According to extent, endometriosis is classified as stage I (minimal), stage II (mild), stage III (moderate) and stage IV (severe)(6). Previous studies have demonstrated that oxidative stress and inflammatory activity as well as genetic abnormalities and mutations may be associated with the development and progression of endometriosis (2,7,8).

A similar process exists in cardiovascular disease, an abnormal lipid profile including increase low-density lipoprotein (LDL), decrease high density lipoprotein (HDL) and subsequently decreased high density lipoprotein (HDL) and production of oxidized LDL (oxLDL) particles in peritoneal fluid, that could be involved in the development of endometriosis (9,10). Melo et al. study showed that the abnormal lipid profile with elevated LDL and Non-HDL may increase oxidative stress and inflammation in the peritoneal fluid of women and subsequently the risk of endometriosis (11). Secretary phospholipase A2 group IIa (sPLA2IIa) is a superfamily of enzyme that hydrolyse glycerophospholipids through the sn-2 ester bond to produce non-esterified free fatty acids (NEFAs) and lysophospholipids (12). Release of the NEFA arachidonic acid (AA) is a key step as a precursor in the production of eicosanoids such as leukotrienes, thromboxanes and prostaglandin E2 (13). It therefore promotes these pro-inflammatory lipid mediators which aid the imitation and maintenance of prolonged inflammatory responses in the body (14,15). Also studies have shown that overexpression of human sPLA2IIa resulted in increased lesion size and oxidative stress (16,17). The human sPLA2IIa gene (PLA2G2A) located on chromosome locus 1p34-36. Several single nucleotide polymorphism (SNP) previously identified, capturing 92% of the variation of PLA2G2A (18). The 763C>G polymorphism (rs11573156), in the 5’UTR promoter lies in the exon 2 region of PLA2G2A, showed strong association with sPLA2IIa levels and coronary artery disease (CAD) risk (19-21). sPLA2II enzyme contribute to atherogenesis by hydrolysing the outer phospholipid layer of low density lipoprotein (LDL) particles in the circulation, generating small-dense LDL (sd-LDL) particles which can then transverse the endothelial cell layer of the artery wall into the intima, where they are further modified (22). An aggregation of particles leads to form foam cells, resulting in an increase atherosclerotic plaque size (22). Santanam et al. study suggests that oxLDL particles can also be involved in the development of atherosclerosis and endometriosis (23). Existence a similar lipid profile of atherosclerosis in women with endometriosis in plasma and peritoneal fluid (PF), suggests that during PF formation, lipid components of the plasma is seeping into the PF (24). This hypothesis may associate dyslipidemia with endometriosis. This dyslipidemia could be, in turn, a suitable pathological substrates for inflammatory process.
and oxidative stress in endometrial tissue (24,25). Several studies have found an association between sPLA2IIa gene polymorphisms and CAD risk (18,26,27) but no study has been conducted on endometriosis. The ultimate aim of this study was to determine the prevalence of the 763C>G polymorphism of PLA2G2A in women with endometriosis respect to control group and its relationship with the risk of endometriosis in the Iranian population.

Materials and methods

Subjects

Women with chronic pelvic pain or infertility referred to the Kosar Hospital Qazvin-Iran for diagnostic laparoscopy between April 2011 and April 2012 were selected for this cross-sectional study. Among 310 women, 204 were eligible and consented to participation in the study. Endometriosis patients and control group were between 18 and 42 years old. To obtain more homogeneous population and verifying that no endometriosis is present, controls were also selected from women undergoing laparoscopy. A total of 97 patients had surgical and histological evidence of endometriosis, while 107 patients without the disease served as controls (women with uterine myoma, dermoid cyst, paraovarian cyst, serous cyst and healthy women). Endometriosis condition was confirmed by diagnostic laparoscopy or laparotomy in both groups. In the endometriosis group, stage of the disease was diagnosed according to the revised American Fertility Society Classification (6). Among the endometriosis patients, 10 patients were diagnosed with stage I, 13 patients with stage II, 35 patients with stage III and 39 patients with stage IV. None of the patients had received hormone therapy during the previous one year. In addition, women who had received anti inflammatory drug and contraceptives in the past three months, or if they had urological disease, endocrine disorders, familial dyslipidemia and chronic inflammatory were not included to the study. The study was approved by the ethics committee of Qazvin University of Medical Sciences.

Assay of plasma lipid

Total plasma cholesterol (TC), HDL-C and triglyceride (TG) were assessed using enzymatic method. (Selectra XL-VITA lab. Holland). LDL-C was calculated using Friedewald equation:
(LDL-C=total cholesterol (TC) - (HDL-C)+TG/5) (28). All samples were stored at -70°C for later simultaneous measurement.

Genomic DNA analysis

Genomic DNA was extracted from the leukocytes of blood samples using the DNA purification kit (Qiagen-USA). A 86 bp sequence of the sPLA2IIa gene was amplified by polymerase chain reaction (PCR) in a DNA thermal cycle (ABI,Veriti,USA) by using oligonucleotide primers F: 5' - CAGCCTTGTGCCTCACCTA -3' and R: 5' CAGGCCGTCTTTTGTTCTTG -3'.

The PCR condition were 94 °C for 5 min, 35 °C cycles of 94 °C for 45s, 55 °C for 1min,72 °C for 1min, followed by 72 °C for 7 min (18). TseI enzyme (New England Biolabs, Inc., Beverly, MA) was used as Restriction Enzyme. Samples were electrophoresed in polyacrylamide gel, and then the gel was visualized by standard staining method (Fig.1).

Based on different restriction fragment size, results showed 86 bp band (rare homozygotes), 86/46/40 bp ( heterozygotes) and 46/40 bp (common homozygotes).

Statistical analysis

Values were presented as the mean± SD, and statistical significance was defined as P values less than 0.05(p≤0.05). Statistically significant differences in mean results between genotypes were assessed by t-test. Multivariate analysis was used to compare variable parameters between groups. Logistic regression analyses were performed for evaluating genotypes distribution with respect to the presence of endometriosis as a dependent variable. All analyses were carried out using statistical package for social sciences for windows version 11.0(SPSS,Chicago,IL,USA).

Results

Demographic and metabolic parameter of patients and controls are shown in Table 1. The mean of age was (endometriosis: 29.8 ± 5.4 years vs. control: 29.5 ± 5.5 years, P=0.66), BMI (endometriosis: 25.1 ± 3.3 kg/m2 vs. control:26.9 ±3.9 kg/m2, P=0.001), waist circumference (endometriosis: 80.6 ± 9.1 cm vs. control: 81.2 ± 9.7 cm, P=0.69). The TC level of the endometriosis group was higher than the control group (216 ± 38 mg/dL vs. 175± 30
mg/dL, P<.0001) and also the LDL level of the endometriosis group was higher (130 ± 22 mg/dL vs. 101 ± 20 mg/dL, P<.0001). Similarly HDL levels were higher in the endometriosis group (46±10 mg/dL vs. 40 ± 9 mg/dL, P<0.001). TG level was higher in the endometriosis group but was not significantly different between the groups (128±48 mg/dL vs. 127 ± 47 mg/dL, P=0.86). The genotype distributions of both groups were in the Hardy-Weinberg equilibrium (both P>0.05). Chi-square analysis between genetic groups identified that normal genotypes have more susceptibility than those carrying rare alleles (P<0.001). The distribution of genotypes was different between the endometriosis group and the control group (68.4% CC, 29.5% CG, 2.1% GG versus 31.8%, 51.4%, 16.8% respectively, P< 0.001) (Table 2). By logistic regression analysis the risk of endometriosis in different genetic groups was calculated (Table 3). The analysis showed that in normal individuals with homozygous genotype, the risk of endometriosis was more than other groups. Moreover, the analysis, after adjustment factors such as BMI, HDL-C and LDL-C, the risk of endometriosis in patients with normal homozygous genotype were more than rare allele (P<0.001, OR =0.30, 95%CI=0.14-0.63). Analysis of variance showed that in women with endometriosis there is increase in total cholesterol (TC) and LDL-C compared to the control (P<0.001) (Table 4).

**Discussion**

The atherogenic lipids have a potent activity to induce endothelium dysfunction and lesion formation (29,30,31). Secretary PLA2IIa enhances LDL oxidation and promotes the formation of bioactive phospholipids via the release of polyunsaturated free fatty acids, can be cause an enhanced to stimulate monocyte-endothelium interaction (32). Secretary PLA2IIa and oXLDL are involved in inflammation and oxidative stress and are accepted as oxidative and inflammatory markers(33). Immunohistochemistry (IHC) with anti-human
PLA2IIa demonstrated that sPLA2 is present in peritoneal environment and is secreted by the endometrial gland (34).

The present study examines the association between sPLA2IIa gene polymorphism in patients with endometriosis and in control subjects in Iran. We observed that genotype distributions for PLA2G2A polymorphism were significantly different between the individuals with and without endometriosis. In previous showed the strong relationship between PLA2G2A polymorphism with serum sPLA2IIa levels (P<0.0001) (18). Moreover, by the haplotype analysis reported that of the potential 64 haplotypes defined by SNPs of PLA2G2A, only six haplotypes occurred at frequency >5% and these haplotypes associated with sPLA2IIa levels (18). Secretary PLA2IIa could modify LDLs particle and it has been shown that this modified LDL has enhanced affinity for proteoglycans and glycosaminoglycan binding (35-37). The interaction between LDL and proteoglycans to induce LDL aggregation and fusion is a factor that contributes to the pathogenesis. Moreover the macrophage-specific over expression of group IIa sPLA2 increases atherosclerosis and enhances collagen deposition (38,39). Ploak et al showed an increased oxLDL in the peritoneal fluid of women with endometriosis, in advanced stages (40).

The data from our experimental study demonstrate that women with endometriosis have a dyslipidemia compared to control subjects. These findings were consistent with previous studies (11).

A study of the Caucasian men and women with Type II diabetes mellitus (T2D) reported that the frequency of the minor allele was (23% , 95%CI=0.20-0.25), that confirmed nearly similar to the frequency this allele in control group in our study (18). Furthermore, Wooton et al reported no effect of 763C>G polymorphism PLA2G2A in HDL-C levels and triglyceride (TG) levels in the Caucasian population with T2D (18) that these findings were consistent with the present study. Our results showed no significant difference in HDL-C levels in individuals with GG compared with CC genotype. As, HDL-C levels depended on different factors including diets, exercise, environment and genetics. Probably, the G allele alone is not the determinant serum HDL-C levels. We observed significant interactions between PLA2G2A and endometriosis risk.

Our research has shown that in subjects with 763C>G polymorphism of PLA2G2A, the risk of endometriosis is lower than normal homozygous individuals.

Moreover, our findings suggested that in woman with the rare G homozygotes, total cholesterol and LDL-C levels are less than normal homozygous genotype. Cholesterol and LDL-C, as inflammatory risk factors, can be involved in the occurrence and severity of
disease, which may indicate beneficial effects of mutation in this gene. The 763C>G polymorphism of PLA2G2A has also in linkage disequilibrium with an identified functional polymorphism in PLA2G2A that may influences the risk of endometriosis. The main limitation of the present study was the relatively small sample size and the absence of data on sd-LDL and oxLDL particles. Another limitation of the present study was no standardization of the intensity of patients’ physical activity, a factor that may also influence the levels of serum lipids. Another limitation was that sPLA2IIa activity did not obtain.

Conclusion

This study reported for the first time a significant positive correlation between PLA2G2A gene polymorphism with the risk of endometriosis. Despite the 763 C>G polymorphism showed a tendency to decrease endometriosis risk, larger series are warranted to confirm these observations and to further study the interaction between PLA2G2A genotype and endometriosis development.

Acknowledgments

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Table 1: Demographic and Metabolic parameters of patients with endometriosis versus control

<table>
<thead>
<tr>
<th></th>
<th>Control (n=107)</th>
<th>Endometriosis (n=95)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>29.5±5.5</td>
<td>29.8±5.4</td>
<td>0.66</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.9±3.9</td>
<td>25.1±3.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>81.2±9.7</td>
<td>80.6±9.1</td>
<td>0.69</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>175±30</td>
<td>216±38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>127±47</td>
<td>128±48</td>
<td>0.86</td>
</tr>
<tr>
<td>HDL-C, mg/dl</td>
<td>40±9</td>
<td>46±10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C, mg/dl</td>
<td>101±20</td>
<td>130±22</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean±standard deviation.
Table 2: Genotype distributions in patients with endometriosis versus control

<table>
<thead>
<tr>
<th>Gene</th>
<th>control (n=107)</th>
<th>Endometriosis (n=95)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA2G2A (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>34 (31.8%)</td>
<td>65 (68.4%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CG</td>
<td>55 (51.4%)</td>
<td>28 (29.5%)</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>18 (16.8%)</td>
<td>2 (2.1%)</td>
<td></td>
</tr>
</tbody>
</table>

P-values: Chi-square tests.
Table 3: Logistic regression analysis of individual alleles with respect to the presence of endometriosis

<table>
<thead>
<tr>
<th></th>
<th>Univariate</th>
<th></th>
<th>Multivariate*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
<td>P</td>
</tr>
<tr>
<td>PLA2G2A</td>
<td>0.22</td>
<td>0.12-0.39</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are 95% confidence interval (95% CI), the odds ratio (OR).

*Adjusted for body mass index (BMI), HDL-C and LDL-C.
Table 4: Metabolic parameter according to genotypes

<table>
<thead>
<tr>
<th>PLA2G2A</th>
<th>CC</th>
<th>CG</th>
<th>GG</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>99</td>
<td>83</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>29.8±5.5</td>
<td>29.3±4.6</td>
<td>30.7±5.5</td>
<td>0.55</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.5±3.2</td>
<td>26.5±4.0</td>
<td>26.4±4.8</td>
<td>0.19</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>81.9±8.5</td>
<td>80.5±10.2</td>
<td>78.0±9.8</td>
<td>0.19</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>206±40</td>
<td>186±38</td>
<td>173±29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>131±52</td>
<td>124±42</td>
<td>125±45</td>
<td>0.52</td>
</tr>
<tr>
<td>HDL-C, mg/dl</td>
<td>44±9</td>
<td>42±10</td>
<td>42±9</td>
<td>0.19</td>
</tr>
<tr>
<td>LDL-C, mg/dl</td>
<td>121±25</td>
<td>109±25</td>
<td>101±21</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± SD, ANOVA.
The ischemic picture of the sPLA2IIa gene including: the sequence of the amplicone, the position of nucleotide polymorphism, the place of the restriction enzyme with its recognition site as well as location of primers.
Fig. 1 Gel picture analysis with the restriction enzyme TseI of the PLA2G2A 763C>G genotypes in genomic DNAs of the study subjects. Lane M: molecular weight marker 50 bp; lanes 10 and 13: homozygous GG; lanes 3, 4, 8, 9, 12: heterozygous CG; lanes 1, 2, 5, 6, 7, 11: homozygous wild-type CC.