ACE I/D Polymorphism in Brazilian Women with Endometriosis

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ABSTRACT

Endometriosis is a chronic gynecological disease that displays some features similar to malignancy, such as local invasion, aggressive spread to distant organs and angiogenesis. Polymorphisms of the ACE gene have been linked with some vascular disease. To determine the frequency of the ACE I/D polymorphism in Brazilian patients with endometriosis compared to controls. This case-control study included a total of 134 women (49 endometriosis patients and 85 controls) who had undergone a laparoscopy or laparotomy. Molecular analysis was performed by polymerase chain reaction (PCR). For the statistical analysis, the chi-square and multiple logistic regression tests were used. The I/D ACE genotype frequencies in cases and controls were, respectively: II 16.3% and 16.5%; ID 24.5% and 20%; DD 59.2% and 63.5%. There was no statistically significant difference between cases and controls, either in the genotype frequencies (χ² = 0.385; p = 0.825) or in the allele frequencies (χ² = 0.098; p = 0.75) of the ACE I/D polymorphism. However, the genotype distribution was not consistent with the Hardy-Weinberg equilibrium, either in patients (χ² = 7.84; p = 0.005) or in controls (χ² = 20.09; p <0.0001). Multiple logistic regression analysis has not shown any differences amongst groups for the polymorphism studied [(OR 1.51; CI 95% 0.52-4.41); p=0.4523]. Despite of the small sample size, the present study suggests that I/D ACE polymorphism is not related with endometriosis in brazilian patients.

Keywords: Angiotensin I-converting enzyme. Endometriosis. Polymorphisms. Brazilian population.

INTRODUCTION

Endometriosis is a common gynecological disease, characterized by the growth of endometrial tissue in sites outside the uterine cavity. The main clinical features are chronic pelvic pain, dysmenorrhea, dyspareunia and infertility (Bulun, 2009). Endometriosis has a negative impact on different aspects of women’s lives, including marital/sexual relations, social life, besides physical and psychological aspects (Moradi et al., 2014). The gold standard for the diagnosis is videolaparoscopic surgery, an invasive procedure that allows visualizing the typical lesions and obtaining samples for biopsy, to classify the stage of the disease (Hsu et al., 2010).

Its etiology is still unknown, but several studies have revealed an association between endometriosis and polymorphisms in genes which are related with inflammation, immune response, oxidative stress, detoxification, hormonal activity, matrix remodeling, growth factors, adhesion molecules, cell cycle regulation, among others (Trovó de Marqui, 2012; Kobayashi et al., 2014). Endometriosis displays some features which are similar to malignancy, such as local invasion, aggressive spread to distant organs and angiogenesis. Increasing evidence has demonstrated that, for endometrial fragments to survive and develop into an endometriotic lesion, new vessel formation is essential (Taylor et al., 2009; Djokovic & Calhaz-Jorge, 2014). It has further been proven that the renin-angiotensin system (RAS) plays a role in the formation of new blood vessels (Khakoo et al., 2008), and a major component of RAS is the angiotensin I-converting enzyme (ACE) that catalyzes the conversion of angiotensin I to angiotensin II. Angiotensin II modulates the angiogenic reaction and could be involved in both normal and pathological angiogenesis (Pupilli et al., 1999; Tamarat et al., 2002). In vitro studies have shown that angiotensin II stimulates cell growth and invasion, and increases vascular endothelial growth factor (VEGF) expression in endothelial cells via the angiotensin II type 1 receptor (Fujiyama et al., 2001). ACE is present in the endometrial glandular...
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Coombs et al. (2010) in Estonian women. In addition, no association was referred by Lamp et al. (2014). In addition, no association was referred by Lamp et al. (2014). Researchers have investigated the association between endometriosis and ACE I/D, -240 A/T, and 2350 A/G polymorphisms in Chinese (Hsieh et al., 2005; Hsieh et al., 2007), Estonian (Lamp et al., 2010) and Polish (Kowalczyńska et al., 2011; Kowalczyńska et al., 2014). In addition, no association was referred by Lamp et al., (2010) in Estonian women.

The aim of this study was to determine the frequency of the ACE I/D polymorphism in Brazilian patients with endometriosis compared to controls, in order to verify if there is any association between this polymorphism and the susceptibility to endometriosis in this population.

MATERIAL AND METHODS

This case-control study was approved by the Ethics Committee of Universidade Federal do Triângulo Mineiro/UFTM (CEP/UFTM protocol nº1628). Written informed consent was obtained from all the participants. Data were obtained from medical records at one hospital from the Brazilian public health system, carried out from 2013 through 2014. A total of 134 women (20–70 years of age), 49 endometriosis patients (mean age ± SD: 33.14 ± 7.66 years) and 85 controls (mean age ± SD: 40.48 ± 10.03), who had undergone a laparoscopy (n=88) or laparotomy (n=41) were included in the study. According to the revised American Fertility Society classification (ASRM, 1997), 7 (22.6%) patients had minimal or mild endometriosis (stages I–II), 24 (77.4%) had moderate or severe endometriosis (stages III–IV) and 18 (36.7%) had these information unavailable. The mains symptoms of the 47 women with endometriosis were: chronic pelvic pain, problems to conceive, dysmenorrhea and dyspareunia, with frequencies varying amongst 25% to 50%.

Genomic DNA was extracted from blood samples using the salting out method (Miller et al., 1988). A polymerase chain reaction (PCR) assay was performed to detect the ACE I/D polymorphism. The primer sequence used was: F: 5’ CTG GAG ACC ACT CCC ATC CTT TCT 3’, R: 5’ GAT GTG GCC ATC ACA TTC GTG AGA T 3’. The PCR reactions were carried out in a final volume of 30μL, containing 20pMols/μL of each primer, 2μM of dNTP, 1X PCR buffer, 1.5mM of MgCl₂, 1U of Taq polymerase, and approximately 100ng of genomic DNA. Amplification conditions were: 10 min at 95°C, followed by 35 cycles of 45 s at 95°C, 30 s at 63°C and 45 s at 72°C, with a final extension at 72°C for 10min. The PCR products were separated on 2% agarose gel and stained with GelRed.

The chi-square test was used to compare allele and genotype frequencies between groups and to estimate the Hardy-Weinberg equilibrium. Multiple logistic regression model was used to evaluate the effects of demographic and clinical variables and genotype distribution between groups. The result was presented in odds ratio (OR) and confidence interval of 95% (CI 95%). A P-value of less than 0.05 was considered statistically significant. The statistical power of the samples in this study, estimated using GPower 3.1, was 88.4%.

RESULTS

The genotype and allele frequencies for the ACE I/D polymorphism are summarized in Table 1. There was no statistically significant difference between the patient and control groups regarding both genotype frequencies (χ² = 0.385; p = 0.825) and allele frequencies (χ² = 0.098; p = 0.75) of the ACE I/D polymorphism.

However, the genotype distribution was not consistent with the Hardy-Weinberg equilibrium in patients (χ² = 7.84; p = 0.005) and controls (χ² = 20.09; p <0.0001).

Multiple logistic regression analysis has not shown any differences amongst groups for the polymorphism studied [(OR 1.51; CI 95% 0.52-4.41); p=0.4523].

DISCUSSION

The role of angiogenesis in the development and persistence of endometriosis foci, which are surrounded by blood vessels, has been increasingly emphasized, in particular the RAS components that operate in the formation of new blood vessels (Khakoo et al., 2008; Djokovic & Calhaz-Jorge, 2014). Researchers have investigated the association between endometriosis and ACE I/D, -240 A/T and 2350 A/G polymorphisms in Chinese (Hsieh et al., 2005; Hsieh et al., 2007), Estonian (Lamp et al., 2010) and Polish (Kowalczyńska et al., 2011; Kowalczyńska et al., 2014) women, and a positive association was reported in the Chinese population. Only three studies were found in the literature concerning the association of ACE I/D polymorphism with the development of endometriosis (Hsieh et al., 2005; Kowalczyńska et al., 2011; Kowalczyńska et al., 2014) (Table 2). In the present study, no association was observed between the analyzed polymorphism and endometriosis, a result that is in line with those published by Kowalczyńska et al., (2011; 2014). The distribution of genotypes, however, was not in Hardy-Weinberg equilibrium. One possible explanation
A limitation of our study is the small size and thus it is difficult to determine whether ACE I/D polymorphism is correlated with endometriosis. In spite of this, our results suggest that in Brazilian women endometriosis is not associated with the ACE I/D polymorphism.

DISCLOSURE: The authors declare that they have no conflict of interest.

RESUMO
Polimorfismo ACE I/D em Mulheres Brasileiras com Endometriose

A endometriose é uma doença ginecológica crônica que apresenta algumas características semelhantes à malignidade, tais como invasão local, disseminação para órgãos distantes e angiogênese. Polimorfismos no gene ACE têm sido relacionados com algumas doenças vasculares. Determinar a frequência do polimorfismo ACE I/D em pacientes brasileiros com endometriose em comparação aos controles. Estudo caso-controle que incluiu um total de 134 mulheres (49 pacientes com endometriose e 85 controles) que se submeteram a uma laparoscopia ou laparotomia. A análise molecular foi realizada por Reação em Cadeia da Polimerase (PCR). A análise estatística utilizou os testes de qui-quadrado e regressão logística. As frequências genotípicas ACE I/D em casos e controles foram, respectivamente: II 16,3% e 16,5%; ID 24,5% e 20%; DD 59,2% e 63,5%. Não houve diferença estatisticamente significativa entre os casos e controles, tanto nas frequências genotípicas ($\chi^2 = 0,385; p = 0,825$) ou nas frequências alélicas ($\chi^2 = 0,098; p = 0,75$) do polimorfismo ACE I/D. Entretanto, a distribuição genotípica não foi consistente com o equilíbrio de Hardy-Weinberg, tanto nos pacientes ($\chi^2 = 7,84; p = 0,005$) ou nos controles ($\chi^2 = 20,09; p <0,0001$). A análise de regressão logística não mostrou qualquer diferença entre os grupos para o polimorfismo estudado [(OR 1,51; CI 95% 0,52-4,41); $p=0,4523$]. Apesar do pequeno número de amostras, o presente estudo mostra que em pacientes brasileiras o polimorfismo ACE I/D não está relacionado com endometriose.


REFERENCES

Table 1 – Genotype and allele frequencies of the ACE I/D polymorphism in endometriosis patients and controls.

<table>
<thead>
<tr>
<th>ACE polymorphism</th>
<th>Endometriosis (n=49)</th>
<th>Controls (n=85)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes</td>
<td>n (%)</td>
<td>n (%)</td>
<td>p = 0.825</td>
</tr>
<tr>
<td>II</td>
<td>8 (16.3)</td>
<td>14 (16.5)</td>
<td></td>
</tr>
<tr>
<td>ID</td>
<td>12 (24.5)</td>
<td>17 (20)</td>
<td></td>
</tr>
<tr>
<td>DD</td>
<td>29 (59.2)</td>
<td>54 (63.5)</td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td>p = 0.75</td>
</tr>
<tr>
<td>I</td>
<td>0.29</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>0.71</td>
<td>0.74</td>
<td></td>
</tr>
</tbody>
</table>

ACE: angiotensin I converting enzyme; *p-value: value of p for significance; I/D: insertion/deletion.

Table 2 – Findings of different studies regarding the I/D ACE polymorphism in endometriosis.

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Samples</th>
<th>Association with Endometriosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hsieh et al. (2007)</td>
<td>China</td>
<td>125 cases</td>
<td>120 leiomyoma patients 128 controls</td>
</tr>
<tr>
<td>Kowalczyńska et al. (2011)</td>
<td>Poland</td>
<td>121 cases</td>
<td>122 controls</td>
</tr>
<tr>
<td>Kowalczyńska et al. (2014)</td>
<td>Poland</td>
<td>241 cases</td>
<td>127 controls</td>
</tr>
<tr>
<td>Present study</td>
<td>Brazil</td>
<td>49 cases</td>
<td>85 controls</td>
</tr>
</tbody>
</table>

is genetic drift, in which random fluctuations can cause the disappearance of an allele in small samples (Song & Elston, 2006).

Some authors found a positive association between ACE polymorphism and endometriosis in subjects with the same ethnicity (Hsieh et al., 2005; Hsieh et al., 2007). The present study did not collect information on ethnicity or skin color and all individuals were from the same region of Brazil. A Brazilian study estimated the Amerindian, European and African genomic ancestry of 934 individuals from the four most populous geographical regions of the country (North, Northeast, South and Southeast) self-categorized as White, Brown and Black. In all regions studied, the European ancestry was predominant, with proportions ranging from 60.6% in the Northeast to 77.7% in the South. In conclusion, genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected (Pena et al., 2011).

Our study was limited by the sample size, once confirmation of the endometriosis diagnosis depends on an invasive procedure, performed only with a specific indication. However, there are published studies with a small sample size - approximately 100 samples (50 cases and 50 controls) (Ribeiro Junior et al., 2009; Attar et al., 2010; Costa et al., 2011; Frare et al., 2013; Silva & Moura, 2016). In some studies, the control group was defined as healthy women without clinical symptoms of the disease (Costa et al., 2011; Frare et al., 2013).


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