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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 ($\chi 2 = 0.385$; $p = 0.825$) or in the allele frequencies ($\chi 2 = 0.098$; $p = 0.75$) of the ACE I/D polymorphism. However, the genotype distribution was not consistent with the Hardy-Weinberg equilibrium, either in patients ($\chi 2 = 7.84$; $p = 0.005$) or in controls ($\chi 2 = 20.09$; $p < 0.0001$). 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.

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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

epithelium throughout the menstrual cycle, so an imbalance in ACE activity may affect menstruation (Li & Ahmed, 1997; Jeunemaitre, 2008), which in turn may predispose certain women to endometriosis. The ACE gene, located on chromosome 17q23.3, has more than 160 polymorphisms listed in the National Center for Biotechnology Information (NCBI) records, most of which are single nucleotide polymorphisms (SNPs) (Sayed-Tabatabaei et al., 2006), however only three SNPs has been studied relating with endometriosis: (1) an insertion/deletion (I/D) of the Alu sequence (287bp) within intron 16; (2) -240 A/T; and (3) 2350 A/G. Some reports have supported the role of the ACE gene polymorphism in endometriosis risk, but the results are controversial. The three polymorphisms of ACE gene were associated with increased risk for endometriosis in Chinese women (Hsieh et al., 2005; Hsieh et al., 2007). Studies published by Kowalczyńska et al. related the polymorphism of 2350 A/G to endometriosis, but no the ACE I/D (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 GTC 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 $MgCl_2$, 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 477 bp product corresponds to the insertion allele (I) and the 190 bp product to the deletion allele (D). 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 ($\chi2 = 0.385$; $p = 0.825$) and allele frequencies ($\chi2 = 0.098$; $p = 0.75$) of the ACE I/D polymorphism.

However, the genotype distribution was not consistent with the Hardy-Weinberg equilibrium in patients ($\chi2 = 7.84$; $p = 0.005$) and controls ($\chi2 = 20.09$; $p < 0.0001$).

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., 2007; 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

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

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

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.

Study	Country	Samples	Association with Endometriosis
Hsieh et al. (2007)	China	125 cases 120 leiomyoma patients 128 controls	Positive ACE Jeunemaitre I-related genotypes and allele
Kowalczyńska et al. (2011)	Poland	121 cases 122 controls	Negative
Kowalczyńska et al. (2014)	Poland	241 cases 127 controls	Negative
Present study	Brazil	49 cases 85 controls	Negative

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 Júnior 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).

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 ($\chi2 = 0,385$; $p = 0,825$) ou nas frequências alélicas ($\chi2 = 0,098$; $p = 0,75$) do polimorfismo ACE I/D. Entretanto, a distribuição genotípica não foi consistente com o equilíbrio de Hardy-Weinberg, tanto nos pacientes ($\chi2 = 7,84$; $p = 0,005$) ou nos controles ($\chi2 = 20,09$; $p < 0,0001$). 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.

Palavras-chave: Enzima conversora da angiotensina. População brasileira. Endometriose. Polimorfismos.

REFERENCES

ASRM. Revised American Society for Reproductive Medicine classification of endometriosis: 1996. Fertil Steril. 1997;67(5):817-21.

Attar R, Agachan B, Kuran SB, Toptas B, Eraltan IY, Attar E, Isbir T. Genetic variants of vascular endothelial growth factor and risk for the development of endometriosis. In Vivo. 2010;24(3):297-301.

Bulun SE. Endometriosis. N Engl J Med 2009;360(3):268-79.

Costa IR, Silva RC, Frare AB, Silva CT, Bordin BM, Souza SR, Ribeiro Júnior CL, Moura KK. Polymorphism of the progesterone receptor gene associated with endometriosis in patients from Goiás, Brazil. Genet Mol Res. 2011;10(3):1364-70.

Djokovic D, Calhaz-Jorge C. Angiogenesis as a therapeutic target in endometriosis. Acta Med Port 2014;27(4):489-97.

Frare AB, Barbosa AM, Costa IR, Souza SR, Silva RC, Bordin BM, Ribeiro Júnior CL, Moura KK. GSTM1 and GSTT1 polymorphisms in endometriosis in women from Goiás, Brazil. Genet Mol Res. 2013;12(3):2764-70.

Fujiyama S, Matsubara H, Nozawa Y, Maruyama K, Mori Y, Tsutsumi Y, Masaki H,

Uchiyama Y, Koyama Y, Nose A, Iba O, Tateishi E, Ogata N, Jyo N, Higashiyama S,

Iwasaka T. Angiotensin AT(1) and AT(2) receptors differentially regulate angiopoietin-2 and vascular endothelial growth factor expression and angiogenesis by modulating heparin binding-epidermal growth factor (EGF)-mediated EGF receptor transactivation. Circ Res. 2001 Jan 19;88(1):22-9.

Hsieh YY, Chang CC, Tsai FJ, Hsu CM, Lin CC, Tsai CH. Angiotensin I-converting enzyme ACE 2350*G and ACE-240*T-related genotypes and alleles are associated with higher susceptibility to endometriosis. Mol Hum Reprod 2005;11(1):11-4.

Hsieh YY, Lee CC, Chang CC, Wang YK, Yeh LS, Lin CS. Angiotensin I-converting enzyme insertion-related genotypes and allele are associated with higher susceptibility of endometriosis and leiomyoma. Mol Reprod Dev 2007;74(7):808-14.

Hsu AL, Khachikyan I, Stratton P. Invasive and noninvasive methods for the diagnosis of endometriosis. Clin Obstet Gynecol 2010;53(2):413-19.

Jeunemaitre X. Genetics of the human renin angiotensin system. J Mol Med (Berl). 2008;86(6):637-41.

Khakoo AY, Sidman RL, Pasqualini R, Arap W. Does the renin-angiotensin system participate in regulation of human vasculogenesis and angiogenesis? Cancer Res 2008;68(22):9112-5.

Kobayashi H, Imanaka S, Nakamura H, Tsuji A. Understanding the role of epigenomic, genomic and genetic alterations in the development of endometriosis (review). Mol Med Rep. 2014;9(5):1483-505.

Kowalczyńska LJ, Ferenc T, Wojciechowski M, Mordalska A, Pogoda K, Malinowski A. Endometriosis and RAS system gene polymorphisms: the association of ACE A2350G polymorphism with endometriosis in Polish individuals. DNA Cell Biol. 2014;33(5):328-35.

Kowalczyńska LJ, Tomasz F, Wojciechowski M, Mordalska A, Pogoda K, Malinowski A. ACE I/D polymorphism in Polish patients with endometriosis. Ginekol Pol. 2011;82(2):102-7.

Lamp M, Saare M, Laisk T, Karro H, Kadastik U, Metspalu A, Peters M, Salumets A. Genetic variations in vascular endothelial growth factor but not in angiotensin I-converting enzyme genes are associated with endometriosis in Estonian women. Eur J Obstet Gynecol Reprod Biol. 2010;153(1):85-9.

Li XF, Ahmed A. Compartmentalization and cyclic variation of immunoreactivity of renin and angiotensin converting enzyme in human endometrium throughout the menstrual cycle. Hum Reprod. 1997;12(12):2804-9.

Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16(3):1215.

Moradi M, Parker M, Sneddon A, Lopez V, Ellwood D. Impact of endometriosis on women's lives: a qualitative study. BMC Womens Health. 2014;14:123.

Pena SD, Di Pietro G, Fuchshuber-Moraes M, Genro JP, Hutz MH, Kehdy FS, Kohlrausch F, Magno LA, Montenegro RC, Moraes MO, Moraes ME, Moraes MR, Ojopi EB, Perini JA, Racciopi C, Ribeiro-Dos-Santos AK, Rios-Santos F, Romano-Silva MA, Sortica VA, Suarez-Kurtz G. The genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected. PLoS One. 2011;6(2):e17063.

Pupilli C, Lasagni L, Romagnani P, Bellini F, Mannelli M, Misciglia N, Mavilia C, Vellei U, Villari D, Serio M. Angiotensin II stimulates the synthesis and secretion of vascular permeability factor/vascular endothelial growth factor in human mesangial cells. J Am Soc Nephrol. 1999;10(2):245-55.

Ribeiro Júnior CL, Arruda JT, Silva CT, Moura KK. Analysis of p53 codon 72 gene polymorphism in Brazilian patients with endometriosis. Genet Mol Res. 2009;8(2):494-9.

Sayed-Tabatabaei FA, Oostra BA, Isaacs A, van Duijn CM, Witteman JC. ACE polymorphisms. Circ Res. 2006;98(9):1123-33.

Silva KSF, Moura KKVO. Genetic polymorphisms in patients with endometriosis: An analytical study in Goiânia (central west of Brazil). Genet Mol Res. 2016;15(2): gmr.15028135. DOI http://dx.doi.org/10.4238/gmr.15028135

Song K, Elston RC. A powerful method of combining measures of association and Hardy-Weinberg disequilibrium for fine-mapping in case-control studies. Stat Med. 2006;25(1):105-26.

Tamarat R, Silvestre JS, Durie M, Levy BI. Angiotensin II angiogenic effect in vivo involves vascular endothelial growth factor- and inflammation-related pathways. Lab Invest. 2002;82(6):747-56.

Taylor RN, Yu J, Torres PB, Schickedanz AC, Park JK, Mueller MD, Sidell N. Mechanistic and therapeutic implications of angiogenesis in endometriosis. Reprod Sci. 2009;16(2):140-6.

Trovó de Marqui AB. Genetic polymorphisms and endometriosis: contribution of genes that regulate vascular function and tissue remodeling. Rev Assoc Med Bras. 2012;58(5):620-32.

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