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CYP2C19*3 polymorphism in patients with endometriosis

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ABSTRACT. Endometriosis is a pathology that affects women in Brazil and around the world. It is characterized by the presence of endometrial cells outside the uterine cavity, leading to chronic pain, infertility and hormonal cycle deregulations. Ectopic endometrial foci may be responsible for changes in anatomy, in hormonal metabolism, immune and inflammatory systems and the pelvic peritoneum, causing pain and infertility. CYP2C19 is an important gene of the cytochrome p450 family that encodes an aromatase associated with estrogen metabolism. The *CYP2C19*3* polymorphism is related to a higher susceptibility for endometriosis in women. This gene is overexpressed in chronic inflammation. The CYP2C19 gene is associated with drug metabolism, detoxification of potential carcinogenic agents and steroid metabolism . We analyzed the prevalence of the *CYP2C19*3* polymorphism in women with endometriosis. The control group comprised 14 health women and the case group 19 patients with endometriosis. We found that the *CYP2C19*3* polymorphism at a significantly higher rate in endometriosis patients. No individual in the control group presented

had this polymorphism and we found no association between this polymorphism and any social habits in patients with endometriosis.

Key words: Endometriosis; Polymorphism; *CYP2C19*3*; Steroid

INTRODUCTION

Endometriosis is a condition that affects 70 million women worldwide. It is one of the main causes of hospitalization within the gynecology area. The prevalence of the disease reaches 5-15% of women during the reproductive age and up to 3-5% in the postmenopausal age (Vercellini et al., 2007). Endometriosis is defined as the presence of endometrial cells and stroma outside the endometrial cavity, mediated by hormonal stimuli. Diagnosis is performed by histopathological analysis of tissue removed through laparoscopy, which is an invasive procedure (Figueiredo et al., 2011). The disease has a neoplastic feature, with the ability to induce neovascularization, growing and invading tissues (Baldi *et al*., 2008; Abrão et al., 2009). Ectopic endometrial foci may be responsible for changes in anatomy in the pelvic peritoneum, as well as changes in hormonal metabolism, immune and inflammatory systems, causing pain and infertility. The release of prostaglandins promotes the adhesion process, contributing to the anatomical variations presented by endometriotic patients. The production of ovarian hormones is responsible for the maintenance of anatomical variations (Giudice and Kao, 2004).

Although a small number of patients with endometriosis are asymptomatic, most women with the disease present clinical manifestations, such as dysmenorrhea, pelvic pain, infertility, deep dyspareunia, intestinal and urinary symptoms (Marqui, 2012). Several causes are associated with endometriosis onset, such as genetic, endocrine, immunological and environmental factors. In a study made by Bellelis et al. (2011), 5.3% of the patients reported having first-degree relatives with the pathology. The relation of endometriosis and genetic factors has been investigated (Bozdag et al., 2010). A single mutation in the CYP2C19 gene may result in inability to metabolize chemicals and cytotoxic agents. CYP2C19 also takes part in estrogen metabolism, which may be linked to the development of endometriosis. Because of its metabolizing phenotype, CYP2C19 is capable of stimulating chronic inflammation. The presence of the polymorphism CYP2C19*3 has been associated with endometriosis (Bozdag et al., 2010).

CYP2C19 is an important gene of the cytochrome p450 family that encodes an aromatase associated with estrogen metabolism. It converts estradiol into estrone and 2α- and 16α-hydroxylated metabolites of estradiol and estrone (Christofolini et al., 2015). The gene is located on the long arm of chromosome 10. The CYP2C19*3 polymorphism is featured by an exchange of a guanine residue for an adenine residue in position 636, and in the coded protein an exchange of the amino acid isoleucine for leucine at position 359 (Yilmaz et al., 2001; Bozdag et al., 2010; Mrazek, 2010).

The exposure of patients to dioxins in the environment leads to a greater susceptibility for the development of endometriosis, ovarian issues, estrogen cycle suppression, fertility reduction and mammary gland disorders. Moreover, it can affect immunocompetence, leading to suppression of antibody responses of T- dependent cells, decreased anti-tumor cytolytic activity of lymphocytes and increased production of inflammatory cytokines, such as tumor growth factor (TNF-α) and interleukin-6 (IL-6) in

leukocytes present in the peripheral blood and peritoneum (Rier, 2008). Women carrying the CYP2C19*3 polymorphism and exposed to dioxins supposedly have a greater predisposition to develop endometriosis. This fact is due to the presence of the environmental factor associated with the inefficacy of the gene responsible for metabolizing this chemical and cytotoxic xenobiotic, which stimulates chronic inflammation (Bozdag et al., 2010).

The study of genetic polymorphisms is of extreme importance in the elucidation of several diseases, among them endometriosis. Since the CYP2C19 gene is associated with drug metabolism, detoxification of potential carcinogenic agents and the metabolism of steroids, the we investigated the prevalence of CYP2C19*3 polymorphism in patients with endometriosis.

MATERIAL AND METHODS

We collected peripheral blood samples from 33 women with age between 19 and 57 years old. Patients were divided into the case group (diagnosed with endometriosis) and control (women without the pathology). All patients participated voluntarily in the research, signed a questionnaire on social habits and the informed consent form. The project was approved by the PUC Goiás Ethics Committee (number 35321614.3.0000.0037).

DNA samples were extracted using the Kaswi kit® protocol (Genomic DNA Purification Kit). DNA amplification was performed by the ARMS-PCR technique with a final volume of 25 μL. For the thermal cycling steps, we used a 10 min cycle for denaturation at 94°C, 44 cycles for fragment amplification (30 s at 94°C, 30 s at 50°C and 60 s at 72°C); and 7 min at 72ºC for a final extension. After amplification, the DNA segments were identified by 2% agarose gel electrophoresis and stained with ethidium bromide. The images were captured by the BIORAD photodocumentation system (Bio-Rad, Hercules, California, USA). Table 1 shows the primer sequences used. The statistical analysis of the results was performed using Bioestat 5.3 software.

Table 1. Sequences of the CYP2C19*3 gene primers.

Primer	Sequence	Molecular Size
2C19Ex4Ue	TATGAAGTGTTTTATATCTAATGTTTACTCA	309 bp
2C19Ex4Lf	ACTTCAGGGCTTGGTCAATATAGA	309 bp
2C19*3mutU	GTAAGCACCCCCTGA	110 bp
2C19*3wtL	GGCCTTACCTGGATC	228 bp

RESULTS

Among the 33 patients analyzed, 19 were patients with endometriosis and 14 comprised the control group. The mean age of the patients in the case group was 31.8 and in the control group it was 38.3 years old. In the case group, 5/19 patients presented the

polymorphism and 14/19 did not. Conversely, in the control group, no patient presented the CYP2C19*3 gene polymorphism. The result was significant (P = 0.0130, Table 2).

Table 2. Comparison of CYP2C19*3 gene genotypes in patients with endometriosis and controls.

	wtl/wtl	wtl/*3 ou *3/*3	Total	P^{α}
	n	n	n	
Case	14	5	19	0.0130
Control	14	0	14	

$^{\alpha}$ G test; wtl – wild type allele

Table 3 correlates the case group with social habits and the CYP2C19*3 polymorphism. Most of the patients who presented the polymorphism were Caucasian and did not use contraception (3). All patients carrying the polymorphism practiced some kind of physical activity (4), did not smoke (4) and did not drink alcohol (4).

Table 3. Correlation between social habits of patients with and without endometriosis and the presence of the CYP2C19*3 polymorphism.

Groups	wtl/wtl	wtl/*3 or *3/*3				
	n	n	p^{α}	OR	Min	Max
* Ethnicity						
Caucasians	13	3				
Black	3	1	0.6750	1.444	--	--
Total	16	4				
Physical activity						
Yes	8	4				
No	5	0	0.0704	0.000	--	--
Total	13	4				
Smoking						
Yes	1	0				
No	12	4	0.5201	0.000	--	--
Total	13	4				
Alcohol consumption						
Yes	2	0				
No	11	4	0.9584	0.000	--	--
Total	13	4				
Contraception						
Yes	4	1				
No	9	3	0.6848	1.333	--	--
Total	13	4				

*Self-declared.

$^{\alpha}$ G Test and Odds Ratio

DISCUSSION

Endometriosis reduces the quality of life of patients. It features several stages of clinical manifestation, and in some cases it is asymptomatic (Nácul and Spritzer, 2010). Genetic and environmental factors contribute to the development of the pathology (Dun et al., 2010). The exposure to environmental factors and the oxidative processes on the metabolism of estrogens lead to the production of substances that trigger endometriosis (Cribb et al., 2006). At the genetic level, the presence of polymorphisms increases the

susceptibility to endometriosis. Studies of various populations would bring a better understanding of the disease (Dun et al., 2010).

In our study, only five women with endometriosis presented the CYP2C19*3 polymorphism and in the control group this mutation was not identified. Although the prevalence of this polymorphism is low in the general population, it seems to be related to the development of endometriosis (Cayan et al,. 2009). The presence of the CYP2C19*3 polymorphism was confirmed in women with endometriosis through genomic sequencing (Painter et al., 2014). However, Bozdag et al. (2010) conducted a study on CYP2C19*3 polymorphism in women with endometriosis and found no significant difference between case and control groups; nevertheless, they concluded that the the CYP2C19*3 polymorphism influences etiopathogenic aspects of endometriosis. The lack of consensus regarding the relationship of the CYP2C19*3 polymorphism and endometriosis is due to main factors. Firstly, the size of samples under study varies considerably and secondly, ethnicity influences the distribution of genetic polymorphisms.

We assessed the relation between the CYP2C19*3 polymorphism with some social habits, such as skin color, physical activity, smoking, alcohol consumption and contraceptive usage. The study conducted by Jacoby et al. (2010) showed that Asian women have a higher prevalence of endometriosis, and black women have a higher prevalence of fibrous process development at younger ages when compared to white women (Jacoby et al., 2010). Another study found that black women have a lower incidence of endometriosis than Asian women, and Asian women have a higher incidence than white women (Cramer and Missmer, 2002). We found no significant association between skin color and the disease.

The practice of physical activity plays a protective role in the inflammatory processes of endometriosis, inducing cytokines and reducing menstrual flow during ovarian stimulation and estrogen activity (Bonocher et al., 2014). Regarding the smoking habit, a lower level of estrogen has been detected because tobacco has properties capable of decreasing the efficacy of the estrogen cycle, and stimulates variation in progesterone levels (Zhou et al., 2011). Finally, alcohol changes hepatic metabolism, alters redox processes and interferes in steroid degradation, leading to an increased level of this lipid in the blood circulation (Eagon, 2010).

Estrogen-free contraceptive drugs can be used as therapeutic agents against endometriosis (Nácul and Spritzer, 2010). We found no association between the use of contraception and the *CYP2C19*3* polymorphism. Two independent studies did not find any association between CYP polymorphisms and the use of contraception drugs (Dieudonné et al., 2014; Cong et al., 2018).

It is important to understand endometriosis at the genetic level and take into account the social habits because the phenotype depends on both parameters. Here, we found that the presence of CYP2C19*3 gene polymorphism is associated with the development of endometriosis. Studies with different population groups and larger size samples should be performed. Specific characteristics of patients may trigger factors that increase endometriosis susceptibility and those characteristics should also be considered for a specific kind of treatment.

CONFLICTS OF INTEREST

Authors declare no conflict of interest for the present work.

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