Glutathione S-transferase M1 and T1 gene polymorphisms in Brazilian women with endometriosis

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Received: 17 March 2015 / Accepted: 31 July 2015 / Published online: 9 September 2015
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Abstract

Purpose The glutathione family (GST) genes appear to play a role in the genesis of endometriosis. This case–control study aimed to compare the frequencies of GSTM1 and GSTT1 polymorphisms in women with endometriosis and women without endometriosis.

Methods Polymerase chain reaction was performed to analyze the GSTM1 and GSTT1 genotypes among women with surgically and histologically confirmed endometriosis (case group $n=121$) and in women without evidence of endometriosis confirmed by laparoscopy for investigation the infertility or for laparoscopic tubal sterilization (control group $n=97$).

Result(s) No differences in the frequencies of GSTM1 polymorphism (null genotype) were observed between the cases and controls: odds ratio (OR)=1.13; 95 % CI 0.656–1.93 ($p=0.659$). The GSTT1 polymorphism (null genotype) was more prevalent in the endometriosis group than in the control group (OR=0.53; 95 % CI 0.94–0.29 ($p=0.039$). No relationship

between menstrual cycle interval and GSTM1 null genotype frequency was observed in either cases or controls ($p=0.370$ and $p=0.664$, respectively). In addition, no relationship between menstrual cycle interval and GSTT1 null genotype was observed in cases ($p=0.797$) or controls ($p=0.052$).

Conclusions GSTM1 null genotype frequency was similar between cases and controls. The GSTT1 null genotype was more frequent in the control group.

Keywords Endometriosis · DNA polymorphisms · Glutathione S-transferase · Molecular biology · Null genotype

Capsule GSTT1 null polymorphism may protect against endometriosis development in mixed-race Brazilian women.

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Abbreviations

CEP-	Research Ethics Committee—Julio Muller
HUJM	Hospital University
DNA	Deoxyribonucleic acid
GSH	Glutathione
GSTM1	Gene mu (μ) 1 the glutathione S-transferase system
GSTT1	Gene theta (θ) 1 the glutathione S-transferase system
BMI	Body mass index
bp	Base pairs
PCR	Polymerase chain reaction
UFMT	Federal University of Mato Grosso
USA	United States of America

Introduction

Endometriosis affects 10 to 15 % of women of reproductive age and is characterized by the invasion and proliferation of

endometrial tissue outside the uterine cavity [1, 2]. Affected women may have an unfavorable quality of life, and the major signs and symptoms include chronic pelvic pain, dysmenorrhea, and infertility [1, 2]. Although a number of etiopathogenic theories have been proposed to explain the development of endometriosis, the most widely accepted theories are celomic metaplasia and retrograde menstruation [3, 4]. With respect to the diagnosis of endometriosis, a physical examination may reveal violaceous lesions in the vagina wall, painful uterine mobilization, palpable nodules in the posterior vaginal fornix, or rectovaginal septum and thickening of the uterosacral ligaments [5, 6]. Imaging examinations, such as transvaginal ultrasound and magnetic resonance, should be performed after bowel preparation to allow better identification of focal lesions and deep endometriosis. The gold standard tool for the diagnosis of endometriosis is laparoscopy, but the use of this technique as a routine procedure is debatable [5, 6].

The role of genetic polymorphisms in the development of endometriosis was examined in several studies [7–14]. Some of these studies focused on genes related to the cell cycle and found that endometriotic lesions may be genetically controlled and regulated [8, 9]. Specifically, polymorphisms in the genes that encode estrogen and progesterone receptors appear to increase susceptibility to the development of endometriosis [8, 9]. Genetic polymorphisms in NRIP1, PGR, and PROGINS estrogen receptors (ESR1 and ESR2) have been associated with endometriosis affliction [8–14]. Regarding the GSTM1 and GSTT1 variants, two recent meta-analyses demonstrated a consistent association between GSTT1 polymorphism and endometriosis [11, 15].

The glutathione family (GST) genes appear to play a role in the genesis of endometriosis [11, 12, 29, 30]. Two genes, mu (μ) and theta (θ) of the glutathione S-transferase system 1 (GSTM1 and GSTT1), express the phase II multifunctional enzymes that play a key role in cellular detoxification and bioactivation reactions [11, 12]. Therefore, GSTM1 and GSTT1 are considered important antioxidant enzymes that participate in the oxidation and isomerization reactions involved in steroidogenesis [11, 14, 16]. The GSTM1 gene, which is polymorphic in the human population, may have a pair of functional alleles with the same metabolic efficiency or a null activity allele [8, 14]. Due to the extensive deletion in its structure, the latter allele does not promote the synthesis of the specific protein product. Individuals who are homozygous for the missing GSTM1 allele are considered to be at risk for endometriosis, because the implantation of ectopic endometrial tissues occurs in these patients as a result of the enzymatic defect in the detoxification system [8–11]. The GSTT1 gene also has an inactivating homozygous deletion polymorphism, which may be related to an increased risk of endometriosis [10–12, 16, 17].

In the clinical setting, it is essential to identify molecular markers that facilitate the early identification of women with a higher risk of developing endometriosis, particularly the most serious forms of the disease. Studies of the prevalence of these genetic polymorphisms in Brazilian women are scarce. However, no ethnic differences in the prevalence of GSTM1 and GSTT1 null genotypes among women with or without endometriosis have so far been observed [11]. The aim of the current study was to compare the prevalence of these genetic polymorphisms in Brazilian women with and without endometriosis.

Material and methods

Study population

The study enrolled 218 outpatients at the Tropical Institute of Reproductive Medicine and Menopause in Cuiabá, Mato Grosso, Brazil—from November 2010 to January 2013. The mean age of the entire group was 31.5 years. The laparoscopy procedure was performed in 218 women. A total of 121 infertile women with visual laparoscopic and histologic evidence supporting a diagnosis of endometriosis were enrolled as cases, and 97 women who were either infertile or candidates for laparoscopic tubal sterilization without a diagnosis of endometriosis were enrolled as controls. All women underwent a standardized interview to collect information concerning the variables of interest. The sample size was calculated using the allele frequencies of the respective polymorphisms, which are approximately 15–20 % [18, 19]. The Research Ethics Committee of the Federal University of Mato Grosso (UFMT) approved the study (954/CEP-HUJM/2010), and all participants were informed of the study objective and signed an informed consent form.

Definitions

The body mass index (BMI) was calculated as body weight (kg)/height (meters) squared. Abnormal menstrual cycle was defined using a range of 26–34 days, with a menstrual duration of 2 to 7 days. The revised American Society for Reproductive Medicine (ASRM 2008) criteria were used for endometriosis staging [32].

Blood collection

Peripheral blood samples (8 ml) were collected via venipuncture and immediately transferred to ethylenediamine tetraacetic acid (EDTA) tubes (Becton Dickinson, Franklin Lakes, NJ, USA). The samples were frozen and stored at -20°C until DNA extraction. All experiments were

performed in the Genetics Laboratory in the Medical School, Federal University of Mato Grosso.

DNA extraction and genotyping

Genomic DNA was extracted using the salting out method [21]. Genotyping was performed using multiplex PCR with the following primers: to detect GSTM1 with fragments of 480 bp, primers 5'-GAA CTC CCT AAG GAA CTA AAG C-3' and 5'-GTT CTC AAA GGG TAT ACG GTG G-3' were used; to detect GSTT1 with fragments of 215 bp, primers 5'-TTC CTT ACT GGT CCT CAC ATC TC-3' and 5'-TCA CCG GAT CAT GGC CAG CA-3' were used; β -globin with fragments of 268 bp, primers 5'-CAA CTT CAT CCA CGT TCA CC-3' and 5'-GAA GAG CCA AGG ACA GGT AC-3' were used as a positive control. Ultrapure Milli-Q water (PURELAB®, Indianapolis, IN, USA) was used as a negative DNA control. A DNA fragment with a molecular weight of 100 bp was used as marker. PCR reactions contained 5 μ l of 10 $\times$ reaction buffer (100 mM/l Tris, pH 8.3, 500 mM/l KCl 500, 20 mM/l MgCl₂), 0.5 μ l of 50 mM MgCl, 0.2 μ l of 10 mM/ μ l dNTPs, 1.2 μ l GSTM1, 1.2 μ l GSTT1, 1.2 μ l of β -globin primers (10 mM/ μ l), 0.5 μ l of Taq DNA polymerase (5 U/ml) (Invitrogen, Carlsbad, CA), 1 μ l of DNA sample (100 ng/ μ l), and 5.4 μ l H₂O to generate a total volume of 18 μ l. The amplification was performed with an initial denaturation at 95 °C for 3 min and 35 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min. The final extension occurred at 72 °C for 10 min. The amplified samples were visualized in a 1.5 % agarose gel. Electrophoresis was performed at 90 V, 81 A, and 7 W, using a specific power source (ENDURO-LABNET®, Edison, NJ, USA).

Statistical analysis

Continuous variables were summarized as the mean and standard deviation. Non-Gaussian data were reported as the median and range. Data with a parametric distribution were compared using the *t* test. Associations between polymorphisms of GSTM1 and GSTT1 null genotypes were examined using the proportion χ^2 test. Comparisons of GSTM1 and GSTT1 null genotypes between groups were performed using odds ratios

(ORs) with 95 % confidence intervals (95 % CI). Differences were considered statistically significant with *p* value <0.05. All data were examined using EpiData Software, version 2.2.2 build 177 (June 2004, Foringen, Denmark).

Results

The mean ages of the women with and without endometriosis were 32.07 $\pm$ 5.62 and 31.01 $\pm$ 6.2 years, respectively (*p*=0.208). The age of menarche was 12.68 $\pm$ 2.35 and 12.49 $\pm$ 3.1 years in the cases and controls, respectively (*p*=0.116). No difference in BMI was observed between the two groups (23.55 $\pm$ 3.14 and 25.40 $\pm$ 4.76, *p*=0.260). The frequencies of GSTM1 and GSTT1 null genotypes observed in the cases and controls are presented in Table 1. No differences in GSTM1 gene polymorphisms were observed between the two groups (OR=1.128; 95 % CI 0.660–1.927; *p*=0.659). On the other hand, GSTT1 null genotype were more frequent in women without endometriosis (OR=0.529; 95 % CI 0.285–0.983; *p*=0.039).

As shown in Table 2, no relationship was observed between menstrual cycle interval and frequency of GSTM1 null genotype in either the cases or controls (*p*=0.370 and *p*=0.664, respectively). Similarly, no association between menstrual interval and GSTT1 null genotype was observed in either the cases or controls (*p*=0.797 and *p*=0.052, respectively). It was observed associations between menstrual bleeding duration and GSTM1 or GSTT1 null genotypes observed in cases or controls (*p*=0.645 and *p*=0.198, respectively, for GSTM1; *p*=0.853 and *p*=0.950, respectively, for GSTT1). In addition, no association between GSTM1 and GSTT1 null genotypes and stage of endometriosis was demonstrated (*p*=0.580).

Discussion

The present study demonstrated that GSTM1 and GSTT1 genetic polymorphisms (null genotypes) were present in 54.5 and 20.7 % of women with endometriosis, respectively, using laparoscopy and biopsy to confirm the diagnosis. In contrast to, these genetic polymorphisms were present in 51.5 and 33 % of women without endometriosis, respectively. A frequency of the

Table 1 Frequencies of polymorphisms in *GSTM1* and *GSTT1* genes in women with endometriosis

	Genotype	Cases (n%)	Controls (n%)	OR 95 % CI	<i>p</i> Value
<i>GSTM1</i>	Null	66 (54.50)	50 (51.50)	1.128 (0.660–1.927)	0.659
	Present	55 (45.50)	47 (48.50)		
<i>GSTT1</i>	Null	25 (20.70)	32 (33.0)	0.529 (0.285–0.983)	0.039
	Present	96 (79.30)	65 (67.0)		
Total (<i>n</i>)		121 (55.5)	97 (44.5)	218 (100)	

OR odds ratio, CI (95 %) confidential interval

Table 2 Association between menstrual cycle interval and frequencies of *GSTM1* or *GSTT1* polymorphisms in patients with or without endometriosis

Menstrual cycle interval								
	Cases (<i>n</i> =121)				Controls (<i>n</i> =97)			
	Abnormal	Normal	95 % CI	<i>p</i> Value	Abnormal	Normal	95 % CI	<i>p</i> Value
<i>GSTM1</i>								
Null	20	46	0.3281–1.513	0.370	17	33	0.5107–2.902	0.664
Present	21	34			14	33		
<i>GSTT1</i>								
Null	9	16	0.4308–2.823	0.797	6	26	0.1243–1.009	0.052
Present	32	64			25	40		

OR odds ratio, CI (95 %) confidential interval

GSTM1 null genotype of 53 % was previously reported in both Caucasian and Asian women with different clinical conditions. Currently, no available evidence proves that women with this *GSTM1* null genotype have an increased risk of developing endometriosis [11]. The equal frequencies of the *GSTM1* polymorphism (null genotype) in cases and controls observed in the current study is supported by other studies in other populations [9, 12, 13]. However, the OR for endometriosis with the *GSTM1* null genotype compared to other genotypes ranges from 0.75 to 32.6 [21], with a pooled summary OR of 1.96 [11, 31]. The frequency of *GSTT1* null genotype was reported to be lower in women with endometriosis, within a range of 11–20 % in Caucasian women and of approximately 47 % in Asian women [22, 23]. According to results of some studies, women with this *GSTT1* null genotype may have a higher risk of developing endometriosis [11, 21].

The first case–control study reporting possible involvement of a *GSTT1* deletion polymorphism (null genotype) in the development of endometriosis demonstrated that endometriosis was two to three times more frequent in women with this polymorphism. Worldwide, the frequencies of *GSTT1* polymorphism range from 9 to 74 % in women with endometriosis and from 8 to 9 % in women without endometriosis [9, 12]. With respect to the higher frequency of the *GSTT1* null genotype found in Brazilian women without endometriosis in the present study, several reports published worldwide have been inconsistent in different populations [9, 12, 14, 17, 24, 25]. Whether this *GSTT1* null genotype confers a protective effect against endometriosis remains uncertain [25]. However, by comparing women with and without endometriosis, this polymorphism has a pooled OR of 1.77 and is approximately one to seven times more common in women without endometriosis [11, 25].

Menarche age, BMI, menstrual cycle interval, and menstrual flow volume were not different between the cases and controls in the current study. These findings are in agreement with other studies performed in women of different ethnicities [18, 26]. As GST enzymes have been found in ovarian steroidogenic cells and may participate in 3 β -hydroxysteroid

dehydrogenase enzyme activity during the conversion of pregnenolone to progesterone and dehydroepiandrosterone to androstenedione, it is possible that GST enzymes may play a role in normal steroidogenesis and participate in endometriosis pathogenesis [20, 27, 28]. The current study examined the possible association between *GSTM1* and *GSTT1* null genotypes and menstrual cycle interval or bleeding volume. The observed absence of associations between these variables suggests that any modifications in menstrual cycle that occur in women with endometriosis are not impacted by these polymorphisms.

The presence or absence of *GSTT1* null genotypes in a small group of women with the disease allows at best to speculate on whether it protects women against endometriosis or facilitates disease emergence. This is not enough to reach any definitive conclusion, but it should be considered that the population studied is formed by a mixed-race Brazilian population. In summary, the current results suggest that the higher prevalence of *GSTT1* null genotype in women without endometriosis may protect them against endometriosis development. It is possible that the absence of activity of this gene may facilitate and increase the production of reactive oxygen species (ROS) which promote DNA damage and apoptosis, impeding the proliferation and invasion of ectopic endometrial cells. In addition, the contribution of polymorphisms in other genes involved in the synthesis and metabolism of sex steroids that are potentially implicated in the development of endometriosis should be considered in future studies.

Acknowledgments This study was supported by the National Council of Research and Development (CNPq) and the Mato Grosso Foundation for Research (FAPEMAT).

Conflict of interest There is no conflict of interest in connection with this article for any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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