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No association between glutathione S-transferase M1 and T1 gene polymorphisms and susceptibility to endometriosis

Ausência de associação entre polimorfismos nos genes da glutationa-S transferase M1 e T1 e suscetibilidade à endometriose

Letícia C. Batista; Mariangela T. Ruiz-Cintra; Marco Fábio P. Lima; Alessandra Bernadete Trovó de Marqui

Universidade Federal do Triângulo Mineiro (UFTM), Minas Gerais, Brazil.

ABSTRACT

Introduction: Endometriosis is a common gynecologic disorder influenced by genetic and environmental factors. The glutathione S-transferase family is associated with endometriosis because its main function is cellular detoxification, so the absence of those enzymes may be a factor for the development of the disorder. **Objective:** Investigate the relationship between polymorphisms of *GSTM1* and *GSTT1* genes and endometriosis, in order to gain a better understanding of the association between detoxification genes and the susceptibility to endometriosis. **Material and methods:** Case-control study in 132 women (49 with endometriosis and 83 of the control group). The genotype was determined using multiplex polymerase chain reaction (PCR), observed in 10% polyacrylamide gel electrophoresis stained with silver nitrate, and statistical analysis was performed. **Results:** There was not a significant difference between the *GSTM1* and *GSTT1* null genotype in the endometriosis group and the control group ($p = 0.9956$). The same result was observed with the combined genotype ($p = 0.8129$). **Conclusion:** In the present study, the *GSTM1* and *GSTT1* polymorphisms are not associated with a higher risk of endometriosis.

Key words: endometriosis; polymorphism; genetic; multiplex polymerase chain reaction.

INTRODUCTION

Endometriosis is characterized by the presence of endometrial glands and stroma outside the uterine cavity. It is a common gynecologic disease that affects 5%-10% of women of reproductive age, but its incidence has been rising in recent years. The most common symptoms of endometriosis are chronic pelvic pain, dysmenorrhea and infertility[1, 2]. Because of the symptoms, the condition has a negative impact on quality of life[3, 4], causing anxiety, depression[5], and even reduced work productivity[6].

The etiology and the pathogenesis of this disease are still unclear, however the most accepted theory is the retrograde menstruation described by Sampson in 1927[7]. Endometriosis is considered a complex trait caused by the interaction between genetic[8-10] and environmental factors[11, 12].

The genetic factors can be related to polymorphism in some genes, like the ones that happen in the glutathione S-transferase family (GST). The role of GST polymorphisms as a risk factor for endometriosis has been researched in many studies, especially in meta-analyses[13-16]. The GST family comprises enzymes of phase II conjugation, and they are involved in the detoxification of estrogen, reactive oxygen species (ROS), dioxin and products of oxidative stress, all of them associated with the development of endometriosis[16]. Glutathione S-transferase M1 (*GSTM1*) and T1 (*GSTT1*) are two candidate genes of this family. The *GSTM1* gene is located on chromosome 1p13.3[17], and the *GSTT1* gene is located on chromosome 22q11.2[18]. In both genes there is a deletion polymorphism that the null alleles provoke, which results in the reduced activity of a functional gene product[16]. A recent study concludes that an interaction between GST genes and environmental factors plays an important role in human disease[19].

The aim of this study was to investigate the relationship between polymorphisms of *GSTM1* and *GSTT1* genes and endometriosis, in

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order to develop a better understanding of the association between detoxification genes and susceptibility to endometriosis.

MATERIAL AND METHODS

Subjects

In this case-control study, the investigated women were those treated at the Clinic of Gynecology and Obstetrics, in Universidade Federal do Triângulo Mineiro (UFTM), Brazil. The sample consisted of 49 patients with endometriosis and 83 controls that underwent laparoscopy or laparotomy. The inclusion factors were the surgical procedure, which confirmed the presence (patients) or absence (controls) of endometriosis, and the written informed consent for participation in this research. Patients were classified into two groups according to the criteria of the American Society for Reproductive Medicine (ASRM)[20]: seven patients at early-stage endometriosis (stages I-II), and 23 patients at advanced-stage endometriosis (stages III-IV). For the other 19 patients, the data were not available. The control group consisted of women who had undergone this surgical procedure, with no observed evidence of endometriosis. Ages ranged from 24 to 58 (mean: 36.14 ± 7.66) years in the endometriosis group, and from 20 to 70 (mean: 40.36 ± 10.08) years in the control group. The most common symptoms were: chronic pelvic pain, dysmenorrhea and infertility. This research was approved by the UFTM Ethics Committee (1628 protocol).

Deoxyribonucleic acid (DNA) analysis

The DNA was extracted from peripheral blood according to the technique described by Miller *et al*. (1988)[21]. Quality and quantity of extracted DNA were checked by agarose gel electrophoresis 1% and spectrophotometer, respectively.

Polymorphisms of *GSTM1* and *GSTT1* genes were evaluated by multiplex polymerase chain reaction (PCR). The *GSTM1* primers were forward (F): 5' GAA CTC CCT GAA AAG CTA AAG C 3' and reverse (R): 5'GTT GGG CTC AAA TAT ACG GTG G3' (219 bp); the *GSTT1* primers were F: 5' TTC CTT ACT GGT CCT CAC ATC TC 3' and R: 5' TCA CCG GAT CAT GGC CAG CA 3' (480 bp). The *CYP1A1* primers were F: 5 'CTG GAA CTG TCT CCA AGC CTT 3' and R: 5 'CAG CTG TTG GAA CAT GTC CTC 3' (312 bp); and this gene was used as an internal positive control reaction. Multiplex PCR was performed in 25 µl reaction volume containing 100 ng of genomic DNA, 0.1 µm of *GSTM1*, *GSTT1* and *CYP1A1* primers, 2.5 mM MgCl2, 0.2 µm deoxy-nucleotide triphosphate, 2.5 µl of 1× PCR buffer, and 1U Taq DNA polymerase. Amplification was performed with initial denaturation at 95°C, followed by 35 cycles at 94°C for 2 min, 59°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 7 min. The PCR products were analyzed in polyacrylamide gel electrophoresis and stained with silver nitrate. The null genotype indicated that both alleles were deleted. The *GSTT1* and *GSTM1* genes were identified by the absence of the fragments of 480 bp and 219 bp, respectively. The presence of the 312 bp fragment corresponding to the *CYP1A1* gene showed a positive amplification in all samples (**Figure**).

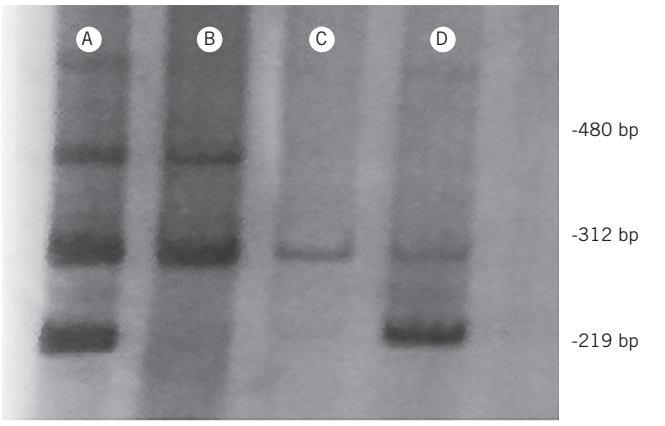

FIGURE – *Patterns of multiplex PCR for* GSTT1 *(480 bp),* CYP1A1 *(312 bp) and* GSTM1 *(219 bp) in a 10% polyacrylamide gel stained with silver nitrate. The four sample genotypes were: A)* GSTT1+ *and* GSTM1+*; B)* GSTT1+ *and* GSTM1-*; C)* GSTT1- *and* GSTM1-*; D)* GSTT1- *and* GSTM1+

PCR: polymerase chain reaction.

Statistical analysis

The statistical analysis used the chi-square test, considering the significance of $p < 0.05$. The Hardy-Weinberg equilibrium analyses were performed to compare the observed and expected genotype frequencies.

RESULTS

In order to investigate whether *GSTM1* and *GSTT1* polymorphisms were associated with endometriosis in the Brazilian female population, a comparison was drawn among 132 Brazilian women (49 with endometriosis and 83 of the control group). **Table 1** shows the distribution of *GSTT1* and *GSTM1* genotypes in both cases and controls.

Analysis of *GSTM1* and *GSTT1* polymorphisms showed no significant difference between the study and control groups ($\chi^2 = 0.0001$, $p = 0.9956$, Yates correction $p = 0.8611$; $\chi^2 = 0.082$, $p = 0.7740$, Yates correction $p = 0.9164$, respectively) (Table 1). Moreover, the analysis of the combined genotypes of polymorphisms showed no statistical difference between patients and controls ($\chi^2 = 0.952, p = 0.8129$) (**Table 2**).

TABLE 1 – Frequencies of *GSTM1* and *GSTT1* genotypes in the study and control groups

Groups	Genotypes			
	GSTM1+ *n* (%)	*GSTM1-* *n* (%)	*GSTT1+* *n* (%)	*GSTT1-* *n* (%)
Endometriosis (*n* = 49)	26 (53.1)	23 (46.9)	20 (40.8)	29 (59.2)
Control (*n* = 83)	44 (53)	39 (47)	36 (43.4)	47 (56.6)

TABLE 2 – Frequency of *GSTM1* and *GSTT1* combined genotypes in the study and control groups

Genotypes	Endometriosis *n* (%)	Control *n* (%)
GSTM1+/GSTT1+	12 (24.5)	18 (21.7)
GSTM1+/GSTT1-	14 (28.6)	26 (31.3)
GSTM1-/GSTT1-	15 (30.6)	21 (25.3)
GSTM1-/GSTT1+	8 (16.3)	18 (21.7)
Total	49 (100)	83 (100)

DISCUSSION

The aim of this study was to evaluate whether the polymorphisms in the glutathione S-transferase genes, especially *GSTM1* and *GSTT1*, could be associated with susceptibility to endometriosis. This study showed that *GSTM1* and *GSTT1* null genotypes were not associated with an increased risk for endometriosis. In addition, no significant difference was observed for the combined genotype, again showing the lack of association between endometriosis and the investigated polymorphisms.

The GST family plays an important role in the detoxification of environmentally toxic compounds and products of oxidative stress, and these are related to an increased susceptibility to endometriosis. The presence of unbalanced ROS can cause cellular damage and change cellular functions because they regulate the protein activity and gene expression, which can lead to severe effects[22].

The *GSTM1* and *GSTT1* gene polymorphisms have been searched in the association with endometriosis in different populations, most of them from Asia[13-16, 23-31]. In that population, five studies[15, 26-29] investigated only *GSTM1*, and three of them found a positive association[15, 27, 29]. The other eight studies searched both genes, *GSTM1* and *GSTT1*. Similarly to the results found in this research, a study conducted in Japan, by Matsuzaka *et al*. (2012)[24], found a negative association. A study from Korea[31] found a negative association with *GSTM1* and a positive association with *GSTT1*; and three studies, one from Turkey, one from Iran and one from Taiwan showed a positive association with *GSTM1* gene and a negative association with *GSTT1*. Three meta-analyses from China reported positive association with both genes[13, 14, 16].

These gene polymorphisms were also searched in Italy and Tunisia. In Italy, the study conducted by Vichi *et al*. (2012)[32] found a negative association with both genes as our study, while the one from Tunisia, searched by Henidi *et al*. (2015)[33], showed a positive association with both genes.

Three studies were conducted in Brazil[34-36]. The first one was carried out in Goiás, by Frare *et al*. (2013)[35], and found a positive association with *GSTM1* and a negative association with *GSTT1* in 50 cases and 46 controls. Another research also conducted in Goiás reported positive association with both genes[36]. The last study conducted in Brazil was carried out in Cuiabá, in the state of Mato Grosso, by Kubiszeski *et al*. (2015)[34], who found a negative association with *GSTM1* and a positive one with *GSTT1* in 121 cases and 97 controls.

Results found in those studies are very different, with a few of them showing the same results. Similarly to the results in our research, we observed that only two studies which investigated both genes also reported no association[24, 32]. So, the difference between the populations' eating habits and environmental pollution can be one of the reasons influencing different results found in the studies. The environmental pollution and food intake are related to endometriosis because some compounds can affect endometrial function; such compounds are difficult to break down, have long half-lives and can be accumulated in various tissues[11, 12]. Therefore, diet and environmental pollution have an influence on the pathogenesis and progression of endometriosis.

Another reason influencing the difference of the found results is the genetic background among ethnic groups in these populations.

So, a limitation of our study is that our sample was small, what happened because the diagnostic method was invasive. Another limitation is that because of the lack of information about some patients we were unable to classify them according to the ASRM[20] criteria, and due to this we were also unable to compare the stages with the genotypes.

CONCLUSION

In conclusion, the present study shows that the *GSTM1* and *GSTT1* null polymorphisms are not associated with an increased risk of endometriosis in our population.

CONFLICT OF INTEREST

None of the authors has any conflict of interest to disclose.

RESUMO

Introdução: *A endometriose é uma doença ginecológica comum influenciada por fatores genéticos e ambientais. A família de glutationa-S-transferase está associada à endometriose porque tem como principal função a detoxificação celular, de modo que a ausência dessas enzimas pode ser um fator no desenvolvimento da doença.* ***Objetivo:*** *Investigar a relação entre polimorfismos nos genes* GSTM1 *e* GSTT1 *e endometriose, a fim de melhor compreender a associação entre os genes de detoxificação e a suscetibilidade à endometriose.* ***Material e métodos:*** *Estudo caso-controle em 132 mulheres (49 com endometriose e 83 do grupo- controle). O genótipo foi determinado utilizando reação em cadeia da polimerase (PCR) multiplex, observado em eletroforese em gel de poliacrilamida 10% corado com nitrato de prata, e a análise estatística foi empregada.* ***Resultados:*** *Não houve diferença significativa entre o genótipo nulo* GSTM1 *e* GSTT1 *no grupo endometriose e no controle (*p = *0,9956). O mesmo resultado foi observado para o genótipo combinado (*p = *0,8129).* ***Conclusão:*** *No presente estudo, os polimorfismos* GSTM1 *e* GSTT1 *não estão associados a maior risco de endometriose.*

Unitermos: *endometriose; polimorfismo genético; reação em cadeia da polimerase multiplex.*

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CORRESPONDING AUTHOR

Alessandra Bernadete Trovó de Marqui

Universidade Federal do Triângulo Mineiro (UFTM); Instituto de Ciências Biológicas e Naturais/ICBN; Departamento de Patologia, Genética e Evolução; Disciplina de Genética; Campus I; Praça Manoel Terra, 330; CEP: 38015-050; Uberaba-MG, Brasil; Phone: +55 (34) 3700-6434; Fax: +55 (34) 3700-6466; e-mail: alessandratrovo@hotmail.com.