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POLYMORPHISMS OF THE GSTT1 AND GSTM1 GENES IN WOMEN OF CENTRAL SERBIA – ABSENCE OF ASSOCIATION WITH UTERINE MYOMA

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Abstract - Since *glutathione S-transferase* (GST) enzymes are involved in cellular protection, we aimed to determine the distribution of GSTT1 and GSTM1 null genotypes in women in central Serbia in order to assess the risk of development of uterine myoma. The study consisted of 34 clinically diagnosed uterine myoma patients and 35 healthy control women. Analyses of GST polymorphism were carried out by multiplex PCR. Our results showed no significant differences in the GSTT1 and GSTM1 null genotypes between the patients and controls. Using the GSTT1 positive/GSTM1 positive combination as reference, there was no statistically significant risk of uterine myoma with the combination of GSTT1 null and GSTM1 null genotypes. We conclude that polymorphism of both GSTT1 and GSTM1 genes, alone or in combination, did not present the main risk for uterine myoma in women from central Serbia.

Key words: Uterine myoma, GSTT1, GSTM1, polymorphism, central Serbia

INTRODUCTION

Uterine myoma is one of the most common benign tumors, occurring in 20-40% of women in their reproductive years (Duhan, 2011). The precise etiology of this tumor is not clear and some of the possible factors that promote the development and the growth of myoma, as well possible risk factors, have been reviewed by Parker (2007).

In order to better understand the molecular pathway of diseases, polymorphisms of the enzymes involved in cellular protection have long been evaluated. So far, the greatest interest has been focused on GST enzymes, a large family of xenobiotic-detoxifying phase II enzymes, which take part in the conjugation of endogenous and exogenous electrophiles and play an important role in the detoxification of several toxic and carcinogenic substances (Clapper, 2000). Deletion variants or null alleles exist for both GSTT1 and GSTM1 genes which are presented biochemically as a failure to express protein (McIlvan et al., 2006). Numerous studies have investigated the role of GST polymorphisms in the increased risk for predisposition to various cancers, including bladder (Salagovic et al., 1999), oral (Zhang et al., 2011), uterine cervical (Wang et al., 2011), gastric (Chen et al., 2010), prostate (Srivastava et al., 2005), endometrial (Karageorgi et al., 2011) and other diseases such as type 2 diabetes mellitus (Bid et al., 2010), neurological diseases (Stroombergen and Waring, 1999) and male infertility (Safarinejad et al., 2010).

The aim of the present study was to determine the distribution of GSTT1 and GSTM1 null polymorphisms in women in central Serbia with and without uterine myoma in order to assess the risks presented by individual and combined genotypes in the development of uterine myoma. To the best of our knowledge, this is the first study of the incidence of GSTT1 and GSTM1 polymorphisms among patients from central Serbia with clinically diagnosed uterine myoma.

MATERIALS AND METHODS

Subjects

The case-control study population consisted of 34 uterine myoma patients (aged from 32 to 71) with both ultrasound and histological diagnosis of uterine myoma, and 35 healthy control women without any kind of gynecological abnormality or malignancy (aged from 24 to 58). Patients were recruited during 2010 and 2011 at the Clinic of Obstetrics and Gynecology in the Clinical Center Kragujevac (Kragujevac, Serbia). Blood samples were collected for DNA extraction.

This study was approved by the Ethics Committee of the Clinical Center Kragujevac (N° 01 - 100).

GSTT1 and GSTM1 genotyping

Genomic DNA from patients and healthy controls was extracted from whole peripheral blood using an EZ1 DNA Blood 350 µl Kit (Qiagen, Hilden, Germany) and BioRobot EZ1(Qiagen, Hilden, Germany).

GSTT1 and GSTM1 genotyping for gene deletions was carried out by the multiplex polymerase chain reaction described by Abdel-Rahman et al. (1996), with some modification. DNA samples were amplified with the primers: 5' - TTCCTTACTGGTCCTCACATCTC- 3' and 5' - TCACCGGATCATGGCCAGCA – 3' for GSTT1, which produced a 480 bp product; 5' - GAACTCCCTGAAAAGCTAAAGC – 3' and 5'- GTTGGGCTCAAATATACGGTGG- 3' for GSTM1, which produced a 215 bp product. Amplification of exon 7 of CYP1A1 with the primers 5'- GAACTGCCACTTCAGCTGTCT– 3' and 5'- CAGCTGCATTTGGAA GTGCTC - 3' was used as an internal control and produced a 312 bp product. PCR was performed in a final volume of 50 µl consisting of 30 pmol of primers for GSTT1, GSTM1 and CYP1A1 primers (Invitrogen, California, USA), 200 µM of dNTP (Invitrogen, California, USA), 1.5mM of $MgCl_2$, 1X PCR buffer and 2U of Taq polymerase (Invitrogen, California, USA), glycerol and 5% dimethyl sulfoxide (DMSO). PCR was performed with an initial denaturation at 94°C for 5 min, followed by 35 cycles of amplification at 94°C for 2 min, annealing at 58°C for 1 min and extension at 72°C for 1 min. The final extension was performed at 72°C for 10 min.

The amplified products were visualized in 2% agarose gel using SYBR Safe DNA gel stain (Invitrogen, California, USA). Bands of 480 bp and 215 bp correspond to GSTT1 and GSTM1 genes, respectively.

Statistical analysis

The data were analyzed using the statistical package for social sciences (SPSS) for Windows, version 11.5. The chi-square (χ^2) and Fischer's (F) exact test were used to compare variables between groups. The level of significance was taken as $p < 0.05$. The odds ratios (OR) with 95% confidence interval (CI) were calculated simultaneously as an estimate of the risk for uterine myoma in individuals with GSTT1 and GSTM1 genotype polymorphisms.

RESULTS

The distribution of persons and OR for GSTT1 and GSTM1 genotypes as well as the GSTT1 and GSTM1 genotypes in patients and healthy controls, are shown in Tables 1 and 2.

The proportion of persons in the patient sample with GSTT1 null genotype was 17.6%. No statistically significant difference was found between the uterine myoma patients and the healthy control (17.6% vs. 34.3%) for the GSTT1 null genotype (Table 1).

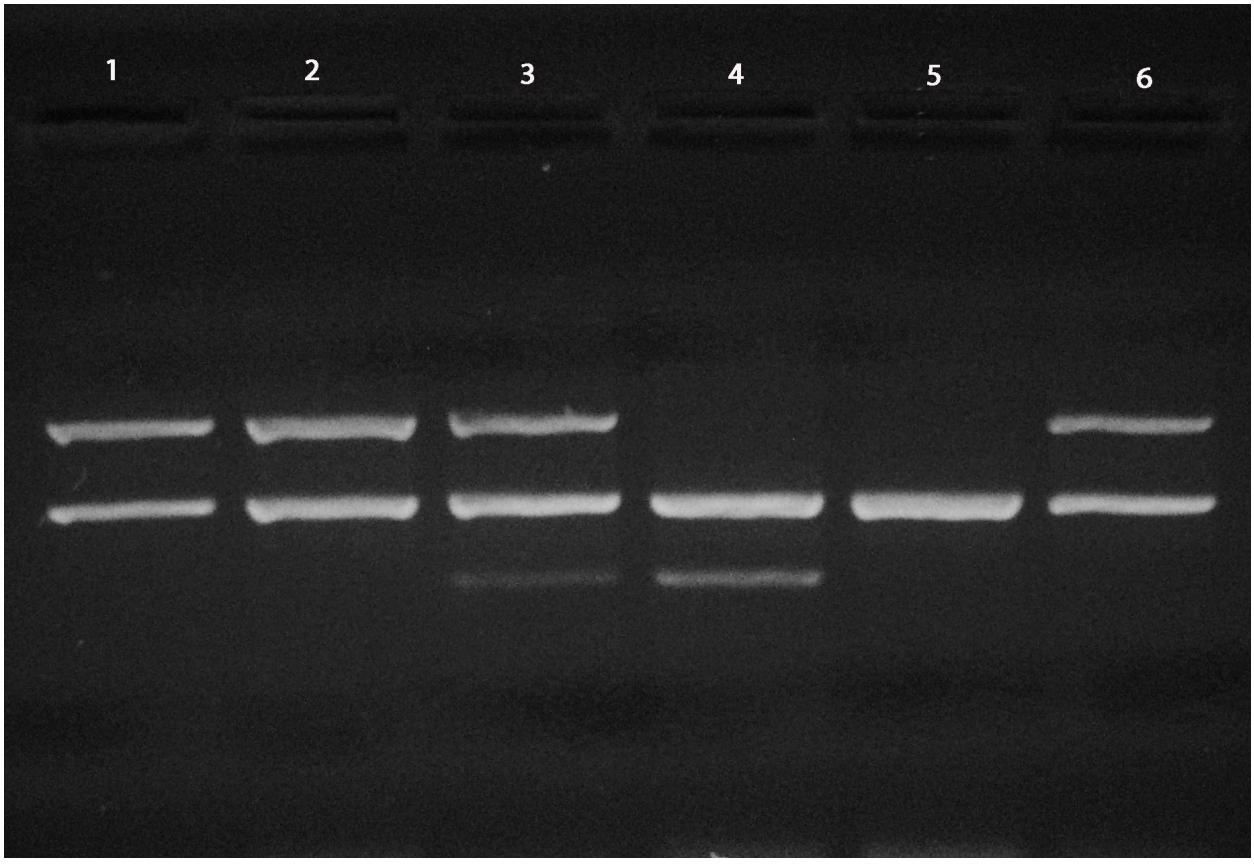

Fig. 1. Electrophoresis of the multiplex PCR amplified products showing individuals for the GSTT1 and GSTM1 polymorphisms. Homozygous: line 3 GSTT1 positive/GSTM1 positive genotypes, and line 5 GSTT1 null/GSTM1 null genotypes; Heterozygous: lines 1, 2 and 6 GSTT1 positive/GSTM1 null genotypes, and line 4 GSTT1 null/GSTM1 positive genotypes

The GSTT1 null genotype did not increase the risk of uterine myoma development (OR = 0.41, 95% CI = 0.13-1.26, p = 0.12)

The proportion of patients with GSTM1 null genotype was 73.5% and in the control sample it was 62.9%. No significant difference was found between the uterine myoma patients and healthy controls for the GSTM1 null genotype (p = 0.342) (Table 1). The GSTM1 null genotype did not increase the risk of uterine myoma development (OR = 1.64, 95% CI = 0.59-4.57, p = 0.34).

Considering different combinations of GSTT1 and GSTM1 genes among the uterine myoma patients, 14.7% (5/34) were homozygous GSTT1 null/GSTM1 null genotypes. Among the controls, 20% (7/35) were homozygous GSTT1 null/GSTM1 null; this was statistically non-significant in comparison to the patient sample.

The distribution of a GSTT1 positive/GSTM1 null combination of genotypes was the highest in both patients and controls (58.8% vs. 42.9%). No statistically significant difference was found between the uterine myoma patients and the healthy controls for this combination of genotypes (p = 0.634).

Table 2 shows the OR of uterine myoma associated with each combination of genotypes. Using the GSTT1 positive/GSTM1 positive combination of genotypes as reference, there were no statistically

Table 1. Distribution of persons and ORs for GSTT1 and GSTM1 genotypes

Polymorphism	Patients (%)	Controls (%)	p	OR	95% CI
GSTT1					
positive	28 (82.4)	23 (65.7)			
null	6 (17.6)	12 (34.3)	0.116	0.41	0.13 - 1.26 (p = 0.12)
GSTM1					
positive	9 (26.5)	13 (37.1)			
null	25 (73.5)	22 (62.9)	0.342	1.64	0.59 – 4.57 (p = 0.34)

OR = odds ratio; CI = confidence interval

Table 2. Distribution of GSTT1 and GSTM1 genotypes and ORs in women with and without uterine myoma

Polymorphism	Patients (%)	Controls (%)	p	OR	95% CI
GSTT1/GSTM1 positive/positive	8 (23.5)	8 (22.9)			
positive/null	20 (58.8)	15 (42.9)	0.634	1.33	0.41 – 4.37 (p = 0.63)
null/positive	1 (2.9)	5 (14.3)	0.333	0.20	0.02 – 2.11 (p = 0.18)
null/null	5 (14.7)	7 (20.0)	0.662	0.71	0.16 – 3.23 (p = 0.66)

significant risk of uterine myoma with the combinations of the GSTT1 and GSTM1 null genotypes (OR = 1.33, 95% CI = 0.41-4.37, p = 0.63; OR = 0.20, 95% CI = 0.02-2.11, p = 0.18; OR = 0.71, 95% CI = 0.16-3.23, p = 0.66).

DISCUSSION

In the relevant literature there are a number of studies that evaluate the polymorphism of GSTT1 and GSTM1 genes and its association with different gynecologic diseases – endometriosis (Baranova et al., 1999; Hur et al., 2004; Aban et al., 2004), polycystic ovaries (Babu et al., 2004), cervical cancer (Sharma et al., 2004; Kiran et al., 2010), ovarian cancer (Spurdle et al., 2001), breast cancer (Gudmundsdottir et al., 2001; Ramalhinho et al., 2011). When it comes to uterine myoma, there is a study that investigated the relationship of GSTM1 polymorphism with susceptibility to this common disease (de Oliveira et al., 2009). However, in addition to GSTM1 gene polymorphism, a few recent studies have been investigating the relationship between uterine leiomyoma and polymorphism of the DNA repair gene XRCC1 (Jeon et al., 2005; Yang et al., 2010), CYP1A1 and CYP1B1 genetic polymorphism (Ye et al., 2008), as well CYP1A1 and CYP2A13 polymorphism (Herr et al., 2006).

In the present study, GST polymorphism associated with the risk of uterine myoma in women in central Serbia was evaluated using GSTT1 and GSTM1 genotyping for gene deletions by multiplex polymerase chain reaction. Our results showed no significant difference in the frequencies of GSTM1 null deletions between patients and controls (73.5% vs. 63.9%). These data are in agreement with recent reported studies; no significant differences in the frequency of GSTM1 polymorphism in control and uterine leiomyoma patients from Brazil was reported by de Oliveira et al. (2009) and in both fibroblastic breast conditions or breast cancer patients and healthy controls from China was reported by Sakoda et al. (2008).

In our study, no significant difference in the frequencies of GSTT1 null genotypes between uterine myoma patients and healthy controls was observed. Very recently, Whang et al. (2011) reported the lack of association of the GSTT1 null genotype and cervical cancer. Furthermore, similar results have been obtained in studies that considered the association of GSTT1 and GSTM1 polymorphism and gynecological diseases. Hur et al. (2004) did not find an association between the polymorphism of GSTT1 and GSTM1 and endometriosis in Korean women. In endometriosis patients from southeastern England the frequency of GSTM1 null was lower than in the control (Baxter et al., 2001). Babu et al. (2004) reported no significant difference in the frequencies of GSTT1 null and GSTM1 null genotypes between Indian women with polycystic ovaries and healthy controls.

In our study, the combination of carrying the GSTT1 null/GSTM1 null or GSTT1 null/GSTM1 positive and GSTT1 positive/GSTM1 null did not present an increased risk for uterine myoma in women from Central Serbia.

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