Polymorphisms of glutathione-s-transferase M1, T1, and P1 genes in endometrial carcinoma

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Summary

Objective: To investigate the polymorphism rates and possible roles of glutathione-s-transferase M1, T1, and P1 gene polymorphisms in the predisposition to endometrial cancer in Caucasian women. *Materials and Methods:* Serum samples and medical records were collected from 53 Caucasian women with newly diagnosed endometrial cancer and 67 women of the same race without any known history of cancer. Multiplex polymerase chain reaction (PCR) analysis was used to assess glutathione-s-transferase M1 (GSTM1) and T1 gene polymorphisms. Polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) method was used in salvage of GSTP1 gene polymorphism. *Results:* Frequencies of GSTM1 and GSTT1 null genotypes were not significantly different between the controls and patients with endometrial cancer (56.7% vs 52.8%, p = 0.671; 32.8% vs 26.4%, p = 0.574, respectively). The authors were not able to demonstrate any association between neither GSTP1 genotypes nor allele frequencies and endometrial carcinoma in the population studied (p = 0.310, p = 0.318, respectively). Moreover, no significant association could be demonstrated with GSTM1 and GSTT1 polymorphisms and clinical stages of endometrial cancer. Nevertheless, there was a significant difference between the frequencies of GSTP1 AA and GG genotypes in relation to Stage I disease when compared with advanced stages of endometrial carcinoma (p = 0.03). In addition, no association was found between polymorphisms of GST suspergene family and non-endometrioid type endometrial carcinomas. *Conclusion:* These results suggest that GSTT1, GSTM1, and GSTP1 polymorphisms are not associated with endometrial cancer in the Caucasian population.

Key words: Polymorphism; Gene; Glutathione-s-transferase; Adenocarcinoma; Carcinoma; Endometrium.

Introduction

Endometrial cancer is the most common type of uterine cancer and the most common gynecologic cancer worldwide [1, 2]. Prolonged unopposed estrogen stimulation, partial estrogen agonist drugs like Tamoxifen, late menopause, nulliparity, and obesity are the major known risk factors in the development of endometrial cancer [1, 3]. As well as the influence of environmental and individual etiological factors, genetic predisposition plays an important role in multifactorial process of carcinogenesis [4]. Common DNA polymorphisms in low penetrance genes, one of which is glutathione-s-transferase (GST) supergene family are addressed as one of the genetic factors to modulate an individual's susceptibility to malignancy [5-7].

The GST supergene family encodes dimeric enzymes which constitute a significant part of cellular enzymatic defense against exogenous chemicals and endogenous toxins that have carcinogenic potential [8]. The GST enzymes are involved in phase II detoxification reactions by conjugating a wide variety of suspected carcinogens, including aliphatic aromatic heterocyclic radicals, epoxides, arene oxides, and facilitate elimination of them [9]. Protection of the cells against oxidative stress by conjugation of reactive oxygen species with glutathione is the other vital function of GST enzymes [7, 8]. Therefore, normal or increased GST enzyme activity might be considered to protect somatic mutations in the DNA of susceptible tissues. Some researchers have asserted that GST enzymes are likely to modulate the induction of other enzymes in the conjugation process [10, 11]. In addition, it was shown that they could bind steroid hormones noncovalently and minimize the effects of short-term fluctuations in hormone levels in the extracellular environment [12, 13]. Consequently, they are considered to have a role in carcinogenesis of endometrial carcinoma which is a steroid hormone dependent cancer. Based on the structural, biochemical, and distributional characteristics, seven classes of GST enzymes covering alpha, mu, omega, pi, sigma, theta, and zeta subclasses are identified [14]. Among them, mu (coded from GSTM1), pi (coded from GSTP1), and theta (coded from GSTT1) enzymes are extensively studied because of their potential to modulate individual vulnerability to cancer.

Several GST loci exhibit genetic polymorphisms, many of which led to significant changes in GST enzyme activities. Polymorphisms of both subclasses GSTM1 and GSTT1 lead to alleles that are nonfunctional. Therefore, absence of enzyme activity is encountered with the presence of homozygous polymorphic alleles [15]. Similarly, 313A→G polymorphism on subclass GSTP1 resulting in Ile105Val substitution creates an allele that yields diminished GSTP1 enzyme activity [16]. There are ethnic variations in genotype frequencies of GST genes [6, 17-19]. Although the influences of GST genes were addressed in some cancers including lung, breast, and colorectal cancers so far [20-23], there have been very few reports on genotyping of these genes in endometrial cancer. The data concerning the role of polymorphisms of the GST genes in endometrial cancer were inconsistent [24-28].

Revised manuscript accepted for publication May 31, 2012

In this study, the authors investigated the relationship between the risk for development of endometrial cancer and polymorphisms of genes that play a role in detoxification reactions. Specifically, null polymorphisms on GSTM1 and GSTT1 genes and common GSTP1 Ile105Val mutation were studied in Caucasian patients with endometrial cancer and in controls. The possible additive effects of specific GST genotypes were also investigated.

Materials and Methods

Subjects

This study was conducted as a prospective case control study at the Department of Obstetrics and Gynecology, Uludağ University Medical School between November 2008 and October 2010. The study group consisted of 53 newly-diagnosed cases of endometrial cancer and 67 age-matched healthy volunteers with no cancer or chronic/devastating disorder history. Patients were recruited after histological diagnosis of endometrial carcinoma. Both groups visited the clinic during the same period. All the patients with endometrial cancer were primarily treated with surgical intervention. The clinical stages of endometrial cancer were staged and managed according to the recommendations of the International Federation of Gynecology and Obstetrics (FIGO) [29]. Demographic characteristics including age at diagnosis, height and weight were assessed in all women. Body mass index (BMI) was calculated as weight (in kilograms) divided by the square of the height (in meters). The patients and control subjects were from the same geographic region. Members of both the patient and control groups were asked to sign an informed consent form.

DNA extraction and GST genotyping

Blood samples of three ml from both the patient and the control groups were taken into ethylenediaminetetraacetic acid (EDTA) containing tubes. Genomic DNA was extracted from circulating leucocytes with salting out procedure by using DZ® DNA isolation kit (Dr. Zeydanlı Laboratories Ltd., Ankara, Turkey), and samples were stored in Tris EDTA buffer at -20°C until the time for polymerase chain reaction (PCR) analysis. All of the DNA samples collected from participants were studied and included in the present report without any elimination.

The GSTM1 and GSTT1 polymorphisms in the isolated DNAs were established by multiplex PCR method as described previously by Lin *et al*. [30]. For the GSTT1 polymorphism, forward 5'-TTC CTT ACT GGT CCT CAC ATC TC-3' and reverse 5'-TCA CCG GAT CAT GGC CAG CA-3' primers were used. To determine GSTM1 polymorphism, forward 5'-GAA CTC CCT GAA AAG CTA AAG C-3' and reverse 5'-GTT GGG CTC AAA TAT ACG GTG G-3' primers were used. Albumin forward 5'-GCC CTC TGC TAA CAA GTC CTA C-3' and reverse 5'-GCC CTA AAA AGA AAA TCC CCA ATC-3' primers were used as internal controls. PCR was conducted by using 100 ng of genomic DNA, 500 pmol of each primer, 0.5 mM each of the dNTPs, one unit of Taq DNA polymerase, three mM $MgCl_2$, 50 mM KCl, ten mM Tris-HCl, 0.001% gelatin, pH 8.3 in a total volume of 25 µl. PCR conditions required denaturation for five min at 94ºC, followed by a second denaturation for 30 sec at 94ºC. Thirty-five cycles of amplification were conducted as follows: one min at 94ºC (denaturation), one min at 58ºC (annealing), one min at 72ºC (elongation), and finally ten min at 72ºC (final elongation). Genotypes were determined by migration of the products in agarose gel with added 2% ethidium bromide. GSTT1 459 bp, GSTM1 219 bp, and albumin 350 bp PCR products were produced.

Table 1. — *Demographic characteristics of the study population*.

	Patients (n = 53)	Controls (n = 67)	*p*
Age (years)¥	58 (35 - 83)	54 (44 - 75)	0.185
BMI (kg/m²)¥	29.94 (18.73 - 50.68)	29.13 (16.90 - 45.61)	0.226
Age of menarche (years)¥	12 (11 - 15)	13 (11 - 16)	0.057
DM			
Present	16 (30.2%)	11 (16.4%)	0.116
Absent	37 (69.8%)	56 (83.6%)	
HT			
Present	31 (58.5%)	33 (49.3%)	0.411
Absent	22 (41.5%)	34 (50.7%)	
Smoking habit			
Present	6 (11.3%)	5 (7.5%)	0.534
Absent	47 (88.7%)	62 (92.5%)	
Endometriosis			
Present	8 (%)	4 (%)	0.178
Absent	45 (%)	63 (%)	

¥ Values are given as a median (minimum-maximum); BMI: body mass index.

GSTP1 gene exon 5 Ile105Val polymorphism was determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method as described in previous work by Harries *et al*. [31]. For the GSTP1 polymorphism analysis, forward 5'-ACC CCA GGG CTC TAT GGG AA-3' and reverse 5'- TGA GGG CAC AAG AAG CCC CT -3' primers were used. PCR was conducted by using 50 ng of genomic DNA, 200 pmol of each primer, 0.3 mM dNTPs, one unit of Taq DNA polymerase, 1.5 mM $MgCl_2$, 50 mM KCl, ten mM Tris-HCl, 0.001% gelatin, pH 8.3 in a total volume of 25 µl. PCR conditions required denaturation for five min at 94ºC. Thermocycling which consisted of 35 cycles was conducted as follows: thirty sec at 94ºC (denaturation), 30 sec at 58ºC (annealing), one min at 72ºC (elongation) and finally ten min at 72ºC (final elongation). To identify the Ile105Val polymorphism, the amplification product was digested with 5U of Alw26 I enzyme (Genemark, Russia) in 50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$ and one mM dithiothreitol and incubated at 55ºC for 16 hrs. Analysis conducted in 4% agarose gel after cutting with the enzyme. Genotypes were determined with the existence of distinct end products of 85, 91, and 176 bp. Interpretation of the results was as follows: if the 176 bp PCR product from the GSTP1 gene was cut into two distinct products of 85 bp and 91 bp, then the genotype was identified as Val/Val; if three distinct products formed as 176, 91, and 85 bp bands, then the genotype was identified as Ile/Val; and if the product was 176 bp then the genotype was identified as Ile/Ile.

Statistical analysis

Medians with minimum and maximum values were given for the descriptive variables of both groups. Statistics were performed using Statistical Package for the Social Sciences software version 17.0 (SPSS Inc., Chicago, IL, USA). Mann-Whitney U test was used for comparisons between the groups in terms of age, BMI, and age of menarche. In comparison of exposed risks and genotype frequencies, Pearson's chi-square test and Fisher's exact test were used, as appropriate. Hardy-Weinberg equilibrium was tested for allele and genotype frequencies of GSTP1 gene exon 5 Ile105Val polymorphism for the both groups. A p value of < 0.05 was accepted as a statistically significant difference.

Table 2. — *Association between GSTM1, GSTT1, GSTP1 genotypes and histological types, stages, and grades of endometrial carcinoma.*

		GSTM1		GSTT1		GSTP1			
	No. of patients	Active	*p*	Active	*p*	AA	AG	GG	*p*
Histological type									
Endometrioid adenocarcinoma	41	19 (46.0%)	0.965[§]	29 (70.7%)	0.480[§]	21 (51.2%)	18 (43.9%)	2 (4.9%)	1.00[◊]
Mucinous adenocarcinoma	6	1 (16.7%)		5 (83.3%)		3 (50.0%)	3 (50.0%)	0	0.225[†]
Clear cell adenocarcinoma	2	2 (100%)		1 (50.0%)		1 (50.0%)	0	1 (50%)	
Serous adenocarcinoma	1	0		1 (100%)		1 (100%)	0	0	
Mixed adenocarcinoma	3	2 (66.7%)		3 (100%)		0	2 (66.7%)	1 (33.3%)	
Stage									
I	39	19 (48.7%)	0.488*	28 (71.8%)	1.00*	20 (51.3%)	19 (48.7%)	0	1.00[#]
II	4	0	0.948[†]	3 (75.0%)	0.735[†]	2 (50.0%)	0	2 (50.0%)	0.501[¥]
III	6	4 (66.7%)		4 (66.7%)		2 (33.3%)	3 (50.0%)	1 (16.7%)	0.701[ø]
IV	4	2 (50.0%)		4 (100%)		3 (75.0%)	1 (25.0%)	0	0.03[ɣ]
Grade									
G1	19	10 (52.6%)	0.790[ɥ]	15 (78.9%)	NA[ɥ]	11 (57.9%)	8 (42.1%)	0	NA[ɥɥ]
G2	17	8 (47.0%)	0.758[ß]	11 (64.7%)	0.736[ß]	8 (47.0%)	8 (47.0%)	1 (6.0%)	NA[ßß]
G3	17	7 (41.2%)		13 (76.5%)		8 (47.0%)	7 (41.2%)	2 (11.8%)	
> ½ Myometrial invasion									
Present	24	10 (41.7%)	0.650	19 (79.2%)	0.599	13 (54.2%)	9 (37.5%)	2 (8.3%)	0.723[**]
Absent	29	15 (51.7%)		20 (68.9%)		14 (48.3%)	14 (48.3%)	1 (3.4%)	1[##]
Pelvic lymph node (LN) metastasis									
Present	5	3 (12%)	0.668	4 (10.2%)	1.00	2 (7.4%)	3 (13%)	0	0.651[**]
Absent	48	22 (88%)		35 (89.8%)		25 (92.6%)	20 (87%)	3 (100%)	1[##]
Para-aortic LN metastasis									
Present	4	2 (8%)	1.00	3 (7.7%)	1.00	1 (3.7%)	2 (8.7%)	1 (33%)	0.588[**]
Absent	49	23 (92%)		36 (92.3%)		26 (96.3%)	21 (91.3%)	2 (67%)	0.193[##]

NA, not applicable.
p value was calculated using Pearson chi square test with Yates correction to compare; [§]patients with endometrioid adenocarcinoma and non-endometrioid endometrial carcinomas; [◊]AA genotype and AG genotype frequencies in patients with endometrioid adenocarcinoma and non-endometrioid carcinoma of endometrium; [†]AA genotype and GG genotype frequencies in patients with endometrioid adenocarcinoma and non-endometrioid carcinoma of endometrium; *Stage I and II patients compared with Stage III and IV patients, [†]Stage I patients compared with advanced stages of disease; [#]AA genotype and AG genotype frequencies between patients in Stage I and II and patients in Stage III and IV; [¥]AA genotype and GG genotype frequencies between patients in Stage I-II and patients in Stage III-IV; [ø]AA genotype and AG genotype frequencies between patients in Stage I and patients in other stages; [ɣ]*p* value for analysis of AA genotype and GG genotype frequencies between patients in Stage I and patients in other stages; [ɥ]*p* value for the comparison of all grades of endometrial carcinoma, ɥɥin patients with AA genotype, and AG genotype; [ß]*p* value for the comparison of patients with G1 endometrial carcinoma and the other grades; [ɥß]in patients with AA genotype, and AG+GG genotype; **p value for the comparison of AA genotype with AG genotype; [##]*p* value for the comparison of AA genotype with AG+GG genotype.

Table 3. — *Association between GSTM1, GSTT1, and GSTP1 polymorphisms and endometrial carcinoma.*

	Patients n (%)	Controls n (%)	*p*
GSTM1			
active	25 (47.2%)	29 (43.3%)	0.671
null	28 (52.8%)	38 (56.7%)	
GSTT1			
active	39 (73.6%)	45 (67.2%)	0.574
null	14 (26.4%)	22 (32.8%)	
GSTP1			
AA	27 (50.9%)	28 (41.8%)	0.310
AG	23 (43.4%)	30 (44.8%)	
GG	3 (5.7%)	9 (13.4%)	
AA	27 (50.9%)	28 (41.8%)	0.318
AG + GG	26 (49.1%)	39 (58.2%)	
A allele	77 (72.6%)	86 (64.18%)	0.163
G allele	29 (27.3%)	48 (35.82%)	

Results

The demographic characteristics and exposure histories of the study population are shown in Table 1. The median age and median BMI between study and control groups did not differ significantly ($p = 0.185$ and $p = 0.226$, respectively). The authors analyzed 53 patients with endometrial adenocarcinoma and 67 healthy volunteers. Median age of menarche was insignificant between patients and control group (12 and 13-years-old, respectively; $p = 0.057$). The presence of diabetes mellitus (DM) and hypertension were not statistically significant between the patient and the control groups ($p = 0.116$ and $p = 0.411$, respectively). Similarly, smoking status and presence of endometriosis were not associated with the risk of developing endometrial adenocarcinoma ($p = 0.534$ and $p = 0.178$, respectively). Histological diagnosis of 41 patients was pure endometrioid carcinoma, while the histologic diagnoses of the remaining patients were as follows: six mucinous adenocarcinoma, two clear cell carcinoma, one high grade papillary serous carcinoma, and three mixed serous or clear cell type (Table 2). Of the patients with endometrial adenocarcinoma, 39 (73.6%) were in Stage I, four (7.5%) were in Stage II, six (11.4%) were in Stage III, and four (7.5%) were in Stage IV. Among the cases, 19 (35.8%) were in grade I, 17 (32.1%) were in grade II, and 17 (32.1%) were in grade III.

Table 4. — *Combined risk of GSTM1, GSTP1 genotypes with GSTT1 genotypes for developing endometrial carcinoma.*

	GSTT1 Active				GSTT1 Null			
	Patients	Controls	*p*	OR (95% CI)	Patients	Controls	*p*	OR (95% CI)
GSTM1 active, GSTP1 AA	6	6		1.0 (ref.)	5	4	1.000	1.25 (0.16 - 10.09)
GSTM1 active, GSTP1 AG/GG	10	12	0.916	0.83 (0.16 - 4.25)	4	7	0.680	0.57 (0.08 - 4.08)
GSTM1 null, GSTP1 AA	15	15	0.733	1.00 (0.22 - 4.64)	1	3	0.585	0.33 (0.01 - 5.99)
GSTM1 null, GSTP1 AG/GG	8	12	0.854	0.67 (0.12 - 3.54)	4	8	0.679	0.50 (0.07 - 3.45)

Table 5. — *Association between GSTM1, GSTT1 and GSTP1 genotypes and stages of endometrial carcinoma.*

	Stage I	Stage II	Stage III	Stage IV	$p^{\dagger}$	p*
GSTM1						
active	19	0	4	2	0.948	0.488
null	20	4	2	2		
GSTT1						
active	28	3	4	4	0.735	1.00
null	11	1	2	0		
GSTP1						
AA	20	2	2	3	1.0 (Ref)	1.0 (Ref)
AG	19	0	3	1	0.701[#]	1.00[#]
GG	0	2	1	0	0.03[$]	0.50[$]
GSTP1						
AA	20	2	2	3	0.685	0.627
AG + GG	19	2	4	4		

[†] Stages I was compared with Stages II+III+IV; *Stages I+II were compared with Stages III+IV.
[#] GSTP1 AA genotype was compared with AG genotype; [$]GSTP1 AA genotype was compared with GG genotype.

The distribution of GSTM1, GSTT1, and GSTP1 genotype frequencies in patients and controls are shown in Table 3. The GSTM1 active genotype was present in 25 (47.2%) cases and in 29 (43.3%) participants; the difference in their frequencies between both groups was not statistically significant (p = 0.671). Multivariate logistic regression model was applied for age, BMI, age of menarche, DM, hypertension, smoking, and endometriosis. Even after adjusting with the mentioned parameters, the authors did not find an association between presence of null GSTM1 genotype and endometrial adenocarcinoma (GSTM1 active versus null, OR = 1.514, 95% CI = 0.626-2.632). The GSTT1 active genotype was present in 39 (73.6%) and 45 (67.2%) of endometrial cancer patients and controls, respectively. When the groups were compared in terms of GSTT1 genotype frequencies, no statistical significance was determined (p = 0.574). Even after adjusting for age, BMI, age of menarche, DM, hypertension, smoking, and endometriosis in multivariate logistic regression model, presence of null GSTT1 genotype did not influence endometrial adenocarcinoma risk (GSTT1 active versus null, OR = 1.283, 95% CI = 0.735-3.197). As shown in Table 3, for the GSTP1 Ile105Val polymorphism, AA, AG, and GG genotype frequencies in endometrial cancer group were 27 (50.9%), 23 (43.4%), and three (5.7%), and in the control group were 28 (41.8%), 30 (44.8%), and nine (13.4%), respectively. There was no difference in frequencies of GSTP1 genotypes among the cases and the control group (p = 0.310). Furthermore, combined GSTP1 AG and GG genotypes were not associated with increased risk of developing endometrial adenocarcinoma when compared to AA genotype (p = 0.318). Even after adjusted age, BMI, age of menarche, DM, hypertension, smoking, and endometriosis, bearing AG or GG genotype, did not increase the risk of developing endometrial adenocarcinoma when compared to AA genotype (AG/GG vs AA, OR = 0.832, 95% CI = 0.548-1.975). GSTP1 A allele was present in 77 (72.6%) of the cases and 86 (64.18%) of the controls, whereas G allele was present in 29 (27.3%) and 48 (35.82%) of the cases and controls, respectively. Nevertheless, the difference in allele frequencies of GSTP1 gene did not reach statistical significance (p = 0.163).

The possible additive roles of combined GSTM1, T1, and P1 genotypes on the risk of developing endometrial carcinoma were calculated. Frequencies of cases and control individuals who were mapped according to the specific GST genotype display are shown in Table 4. The category including GSTM1, GSTT1, and GSTP1 AA genotypes was taken as the reference category. Nevertheless, the authors could not establish any significant association between any category and increased endometrial carcinoma risk.

Patients with endometrial carcinoma were also analyzed according to the clinicopathological characteristics of the cancer. Genotype statuses of the patients are shown with the stages of disease in Table 5. The differences in frequencies of GSTM1 and GSTT1 genes were not statistically significant in patients with Stage I disease when compared to patients in other stages (Stages II-IV) (p = 0.948, p = 0.735, respectively). Similarly, there was no difference in frequencies of GSTM1 and GSTT1 genes between patients with Stages I + II and patients with Stages III + IV (p = 0.488, p = 1.00, respectively). There was no significant difference in frequency of GSTP1 genotypes between Stage I and Stages II-IV, and Stages I + II and Stages III + IV, except for the frequency of GSTP1 GG genotype which was significantly higher among patients in advanced stage (Stages II-IV) disease than patients with Stage I disease when compared to AA genotype (p = 0.03).

Relationship between the frequencies of GSTM1, T1, and P1 genotypes and histological types, clinical stages and grades together with pelvic and para-aortic lymph node metastases in endometrial cancer are shown in Table 2. Frequencies of GSTM1, T1, and P1 genotypes between endometrioid and non-endometrioid histological types

were insignificant. Similar to the clinical stages of cancer, there was no significant difference between grade of endometrial carcinoma and frequency of all GST genotypes between Stage I and Stages II-IV, and Stages I + II and Stages III + IV. It is further shown in Table 5 that the presence of invasion in more than half of the myometrium, pelvic, and para-aortic lymph node metastases were not related to the GST genotypes.

Discussion

Genetic variation in susceptibility to chemical carcinogens among individuals is one of the main contributing factors leading to cancer development among human beings. Genetic variations of genes which produce enzymes that play a role in intracellular metabolism such as GST have shown to lead susceptibility in the development of various cancers [6-8]. The mu and theta classes of GST isoenzymes (GSTM1 and GSTT1, respectively) have a common and broad range of substrate specificities, and they detoxify the reactive metabolites of benzo-a-pyrene and other polycyclic aromatic hydrocarbons [32]. Expression of GST pi enzyme was found to correlate with the histological grade and chemoresistance of endometrial carcinoma [33]. The null genotype developing from homozygote deletion of GSTM1 or GSTT1 genes was frequently observed in lung, colorectal, and bladder cancers [20, 21, 34-37]. GSTP1 polymorphism was found in association with colorectal [38] and breast cancer [39].

In this population-based case-control study, the authors investigated the role of the GSTM1, GSTT1, and GSTP1 gene polymorphisms in endometrial cancer patients of Caucasian origin. When compared with other studies, this current study performed in a Caucasian population showed similar genotype and allelic frequency distribution with the previous studied populations [19, 40]. The presence of the GSTM1 and GSTT1 null genotype did not influence the risk of developing endometrial carcinoma in this study population. Also, the authors showed that GSTP1 genotype frequencies did not differ significantly between patients with endometrial cancer and the control group. In addition, the presence of GSTP1 G allele did not influence the risk of developing endometrial cancer in patients with endometrial cancer when compared to the control group. Nevertheless, when the authors considered that the etiology of most commonly occurring cancers could not be simply explained by allelic variability at a single locus, the significance of attributed risk could be reached when interactions of polymorphisms of aforementioned genes with each other and the other identified etiological factors were considered. From this point of view, the authors calculated combined risk of GSTM1 and GSTP1 genotypes with GSTT1 genotypes between patient and control groups. However, the authors could not demonstrate any specific genotype combination that increased the risk of endometrial carcinoma development. Additionally, environmental and genetic factors including the presence of DM, HT, smoking, and history of endometriosis did not have an impact on endometrial cancer development in this study population.

The authors did not find any association between GSTM1, GSTT1, and GSTP1 polymorphisms and histological subtypes of the endometrial carcinoma. There was also no association between polymorphisms of all three GST genes and histological grade of the endometrial cancer. Frequencies of GSTM1 and GSTT1 genotypes were not statistically different between patients with Stage I+II and Stage III+IV, early stage (Stage I), and advanced stage (Stage II-IV) endometrial cancer, whereas the frequencies of GSTP1 AA genotype and GSTP1 GG genotype were statistically significant between patients with early and advanced stage disease. There were no other statistical differences in genotype frequencies concerning deep myometrial invasion, pelvic, and para-aortic lymph node metastasis between cases and the controls.

Previous studies concerning the role of GST genes in endometrial cancer have yielded mixed results. There have been conflicting studies regarding the role of GSTM1, GSTT1, and GSTP1 polymorphisms in endometrial carcinoma. Ueda *et al*. found that GSTT1 null genotype was dominant in endometrial carcinoma cells and also the frequency of GSTM1 null genotype was not associated with increased risk for endometrial carcinoma development [27]. The study by Chan *et al*., reported a strong association between endometrial adenocarcinoma and GSTP1 AG polymorphism in a Chinese cohort [40]. However, interestingly they found no statistical difference in frequency of GSTP1 GG genotype. Therefore, the latter result was contradictory to the expected outcome, as in this hypothesis much more abolished enzyme production should entail unconjugated substrates in target tissue which is thought to increase endometrial cancer development risk. In another study by Chan *et al*., post-transcriptional modification of GSTP1 gene in endometrial carcinoma patients was investigated [41]. In that study, they found statistically significant correlations between reduced GSTP1 mRNA and enzyme expression and hypermethylation of the GSTP1 gene in endometrial carcinoma. The researchers concluded that GSTP1 gene expression was altered at the transcriptional level. When the present findings are interpreted and compared with those results, it is also possible that altered expression of GSTP1 enzymes may be a result from factors other than polymorphisms of the GSTP1 gene.

Conclusion

The authors did not find any association between GSTM1, GSTT1, and GSTP1 polymorphisms and histological subtype, histological grade, lymph node status and clinical stage of endometrial carcinoma. Furthermore, there is no evidence supporting the clinical importance of GSTM1, GSTT1, and GSTP1 polymorphisms and increased risk of endometrial carcinoma development in Caucasian population.

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