J Gynecol Oncol Vol. 21, No. 3:169-173, September 2010 DOI:10.3802/jgo.2010.21.3.169

Original Article

GST (*GSTM1*, *GSTT1*, and *GSTP1*) polymorphisms in the genetic susceptibility of Turkish patients to cervical cancer

Beray Kiran[1], Mutlu Karkucak[2], Hakan Ozan[1], Tahsin Yakut[2], Kemal Ozerkan[1], Sebnem Sag[2], Mehmet Ture[2]

Departments of [1]Obstetrics and Gynecology, [2]Medical Genetics, Uludag University Medical Faculty, Bursa, Turkey

Objective: This work investigates the role of glutathione S-transferase M1 (*GSTM1*), glutathione S-transferase T1 (*GSTT1*), and glutathione S-transferase P1 (*GSTP1*) enzymes and polymorphisms, which are found in phase II detoxification reactions in the development of cervical cancer.
Methods: This study was conducted with 46 patients diagnosed with cervical cancer and 52 people with no cancer history. Multiplex PCR methods were used to evaluate the *GSTM1* and *GSTT1* gene polymorphism. However, the *GSTP1* (Ile105Val) gene polymorphism was studied using a PCR-RFLP method. The patient and control groups were compared using a chi-square test with $p<0.05$.
Results: In the patient group, statistical significance was determined for gravidity ($p=0.03$), parity ($p=0.01$), and the number of living children ($p=0.01$) compared to the control group. The gene frequency of *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms was evaluated. We observed that *GSTM1* and *GSTT1* null genotype frequencies were 54.3% and 32.6% respectively, while *GSTP1* (Ile/Val), (Ile/Ile), (Val/Val) genotype frequencies were 52%, 44%, and 4%, respectively, in the cervical cancer patients. No statistical variation was determined between the control and patient groups in terms of *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms ($p>0.05$).
Conclusion: Our results demonstrate that *GSTT1*, *GSTM1*, and *GSTP1* polymorphisms are not associated with cervical cancer in Turkish patients.

Key Words: Cervical cancer, GST gene, Polymorphism

INTRODUCTION

Cervical cancer is one of the most common types of cancer observed in women. The largest contributing factor in its development is the progression of untreated, high-risk types of human papillomavirus (HPV) in cervical tissue. Oncoproteins E6 and E7 have been shown to be activated by HPV, disrupting the normal cell cycle and DNA structures and leading to the development of cervical cancer.[1-3] Epidemiological studies have shown that certain etiological factors other than HPV may also play a role in the development of cervical cancer. These may include marrying at a very early age, early childbirth, multiple births, low socio-economic status and heavy cigarette consumption.[4-7] Smoking cigarettes may also play a role in the development of high-risk human papillomaviruses (HR-HPVs), which appear to interact with active cigarette smoking to increase the risk of high-grade cervical squamous intraepithelial lesions (HSIL).[8,9] The nicotine in cigarettes has been found in cervical mucus, which may have a mutagenic impact.[4,10,11]

GST enzymes, which are encoded by *GST* genes, are responsible for the detoxification of chemicals found in the environment and naturally synthesized metabolites, and they play an important role in protecting tissue from oxidative damage. An increase or decrease in the tendency of certain types of cancer observed in a group of individuals is often linked to the genetic polymorphism observed in enzymes that play a role in the detoxification of xenobiotics. A significant relationship is observed between the risk of developing cancer and xenobiotic metabolism enzyme gene polymorphism. This relationship has highlighted the role of genetics in cancer etiology.[12-14] The relation between *GST* gene polymorphism and cervical cancer has been investigated in various studies, which demonstrated that the risk of cervical cancer increases in women with *GST* gene polymorphism.[15,16]

In this study, we aimed to investigate the relationship between the development of cancer and general polymorphisms that play a role in detoxification reactions. Specifically, glutathione S-transferase M1 (*GSTM1*), glutathione S-transferase T1 (*GSTT1*), and glutathione S-transferase P1 (*GSTP1*) polymorphisms were studied in Turkish patients with cervical cancer.

Received November 19, 2009, Revised May 21, 2010,
Accepted June 8, 2010

Correspondence to **Tahsin Yakut**
Department of Medical Genetics, Uludag University Faculty of Medicine, 16059 Gorukle, Bursa, Turkey
Tel: 90-224-295-4371, E-mail: tyakut@uludag.edu.tr

MATERIALS AND METHODS

1. Study subjects

This study was conducted as a prospective study in the Department of Obstetrics and Gynecology at the Medical School of Uludag University between 2008 and 2009. In this study, the patient group consisted of 46 cases diagnosed with cervical cancer, and the age-matched control group consisted of 52 cases with no cancer history. Both groups visited our clinic during same period. Members of both the patient group and the control group were asked to sign an informed consent form.

2. DNA extraction and *GST* genotyping

Blood samples from both the patient and the control groups were taken in EDTA tubes. DNA isolation was performed according to the procedures of the Dr. Zeydanlı (DZ) DNA isolation kit, and samples were stored at −20°C until PCR. Multiplex PCR method was used to determine *GSTM1* and *GSTT1* polymorphisms in the isolated DNAs. For the *GSTT1* polymorphism, forward 5'-TTCCTTACTGGTCCTCACATCTC-3' and reverse 5'-TCACCGGATCATGGCCAGCA-3' primers were used. For the *GSTM1* polymorphism, forward 5'-GAACTCCCT GAAAAGCTAAAGC-3' and reverse 5'-GTTGGGCTCAAATA TACGGTGG-3' primers were used. Albumin forward 5'-GCCC TCTGCTAACAAGTCCTAC-3' and reverse 5'-GCCCTAAAA AGAAAATCCCCAATC-3' primers were used as internal controls.[17] Albumin 350 bp, *GSTM1* 219 bp and *GSTT1* 459 bp PCR products were formed. PCR conditions required denaturation for 5 minutes at 94°C and then 35 cycles as follows: 1 minute at 94°C (denaturation), 1 minute at 58°C (annealing), one minute at 72°C (elongation) and finally 10 minutes at 72°C (final elongation). Genotypes were determined by migration of the products in agarose gel with added 2% ethidium bromide (Fig. 1).

GSTP1 (Ile105Val) gene polymorphism was determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. For the *GSTP1* polymorphism, forward 5'-ACCCCAGGGCTCTATGGGAA-3' and reverse 5'- TGAGGGCACAAGAAGCCCCT -3' primers were used.[17] To identify the *GSTP1* (Ile105Val) gene polymorphism among the products, the Alw 26 I (Genemark, Russia) enzyme was used. In the analysis conducted in 4% agarose gel after cutting the enzyme, genotypes were determined 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 bp, 91 bp and 85 bp, then the genotype was identified as Ile/Val; and if the product was 176 bp then the genotype was identified as Ile/Ile (Fig. 2).

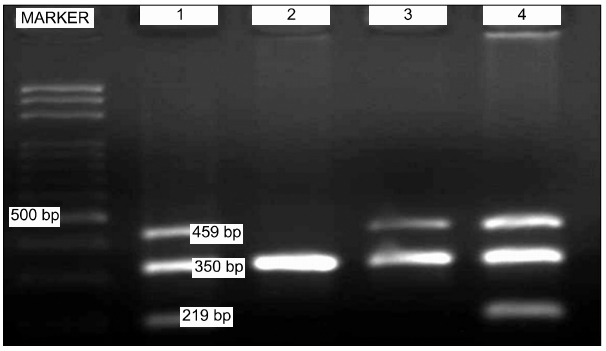

Fig. 1. A representative multiplex PCR analysis of glutathione S-transferase (*GST*) polymorphisms. Albumin (350 bp), glutathione S-transferase T1 (*GSTT1*; 459 bp) and glutathione S-transferase M1 (*GSTM1*; 219 bp) genes PCR products resolved by agarose gel electrophoresis. Line MARKER is 100 bp DNA ladder. Lines 1 and 4 are both *GSTT1* and *GSTM1* present genotype, line 2 both *GSTT1* and *GSTM1* null genotype, line 3 is *GSTT1* present genotype and *GSTM1* null genotype.

3. Statistical analysis

Data was recorded in ±standard deviations. A chi-square (χ^2) test was used to compare genotypes. p-values smaller than 0.05 were accepted as being statistically significant.

RESULTS

The characteristics of the study population are shown in Table 1. The age distribution was no different between patients and controls, the mean age being 53.73±10.35 and 51.32±8.86 years for patients and controls, respectively (p=0.11). No significant difference was seen between the two groups in terms of year of menopause and abortus. However, gravida, parity and number of living children were sig-

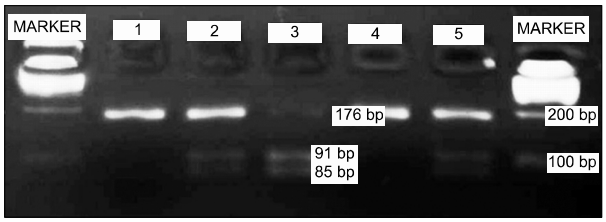

Fig. 2. Photograph of the PCR products of the glutathione S-transferase P1 (*GSTP1*) gene after Alw 26 I enzyme cutting and on 3.5% agarose gel. Line MARKER shows the 100 bp DNA ladder, line 1 and 4 shows individuals with Ile/Ile genotype (176 bp), line 2 and 5 shows Ile/Val genotype (176 bp, 91 bp, 85 bp), and line 3 shows the Val/Val genotype (91 bp, 85 bp).

Table 1. Demographic characteristics of both the patient group and control group

Demographic characteristics	Patient group mean±SD	Control group mean±SD	p-value
Age, yr	53.73±10.35	51.32±8.86	0.11
Age at menopause, yr	10.35±8.91	9.90±8.93	0.92
Gravida	4.32±2.17	3.59±2.14	0.03
Parity	3.26±1.65	2.40±1.05	0.01
Abortus	1.06±1.34	1.19±1.58	0.82
Live birth	2.89±1.49	2.17±0.75	0.01

nificantly different in the cervical cancer group and control group, respectively (p=0.03, p=0.01, and p=0.01).

Table 2 shows the distribution of *GSTM1*, *GSTT1*, and *GSTP1* (Ile105Val) genotype prevalence in patients and controls. We found a prevalence of *GSTM1* null genotype in 54.3% of patients compared with 57.7% in controls. The homozygous null genotype *GSTT1* was found in 32.6% of patients and in 30.8% of controls. When the groups were compared in terms of *GSTT1* and *GSTM1* genotype, no statistical significance was determined (p=0.84 and p=0.73, respectively). Using subject with the Val/Val homozygote as a reference group, we found no association between the Ile/Ile and Ile/Val genotypes and the risk of cervical cancer after statistical analysis (p=0.730 and p=0.053, respectively). The prevalence of Ile allele was 71.8% and 70.1% in the cervical cancer group and control group, respectively.

The frequency of *GSTM1*, *GSTT1*, and *GSTP1* (Ile105Val) genotype distributions in cervical cancer studies is summarized in Table 3 and Table 4.

Table 2. *GSTM1*, *GSTT1*, and *GSTP1* polymorphism rates among the patient group and control group

Genes	Genotype	Patient group no. (%)	Control group no. (%)	p-value
GSTM1	Null	25 (54.3)	30 (57.7)	0.730
	Present	21 (45.7)	22 (42.3)	1
GSTT1	Null	15 (32.6)	16 (30.8)	0.84
	Present	31 (67.4)	36 (69.2)	1
GSTP1	Ile/Ile	27 (58.7)	22 (44.0)	0.730
	Ile/Val	15 (32.6)	26 (52.0)	0.053
	Val/Val	4 (8.7)	2 (4.0)	1

GSTM1: glutathione S-transferase M1, *GSTT1*: glutathione S-transferase T1, *GSTP1*: glutathione S-transferase P1.

Table 3. Frequency of glutathione S-transferase M1 (*GSTM1*) and glutathione S-transferase T1 (*GSTT1*) null genotypes in cervical cancer studies

	Cervical cancer cases (N)	*GSTM1*-null (%)	*GSTT1*-null (%)	p-value
Sobti et al.[4]	103	40.7	15.5	<0.05*
Kim et al.[12]	181	52.5	66.3	<0.05†
Warwick et al.[27]	70	-	12.9	NS‡
de Carvalho et al.[28]	43	-	51	NS
Settheetham-Ishida et al.[29]	90	60	46.7	NS
Chen et al.[30]	190	53.2	-	NS
Sharma et al.[31]	142	57.1	19.7	NS
Present study	46	54.3	32.6	NS

**GSTM1* (null) (odds ratio [OR], 7.0; 95% confidence interval [CI], 2.19 to 22.36; p=0.0005) and *GSTT1* (null) (OR, 10.2; 95% CI, 1.23 to84.18; p=0.02). †Null genotypes of *GSTT1* and *GSTM1* in cervical carcinoma were significantly overrepresented in the younger age subgroup (age 40 years or younger) compared with those of controls. ‡NS: not significant (p>0.05).

DISCUSSION

Genetic variation in susceptibility to chemical carcinogens among individuals is one of the main factors leading to cancer development among human beings. Genetic variations among the genes forming the enzymes that play a role in metabolism, such as CYP and *GST*, have been shown to lead to susceptibility to the development of various cancers.[18,19] The mu and theta classes of *GST* isozymes (*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.[12,20] The null genotype developing from homozygote deletion of *GSTM1* or *GSTT1* genes is frequently observed in lung and bladder cancers.[21-26]

When patients with cervical cancer were reviewed, 54.3% of them showed the *GSTM1* null genotype. The *GSTM1* null genotype among the control group was 57.7%. While the *GSTM1* null genotype rate among the patients was lower than that of the control group, this difference is not statistically significant (p=0.73). On the other hand, while the *GSTT1* null genotype rate among patients with cervical cancer was determined to be 32.6%, it was determined to be 30.8% in the control group. While the *GSTT1* null genotype rate among patients was higher than that of the control group, this difference is not statistically significant (p=0.84).

Our study seems to be more in compliance with the studies of Warwick et al.,[27] de Carvalho et al.,[28] Settheetham-Ishida et al.,[29] and Chen et al.,[30] which did not demonstrate any relationship between the *GSTM1* and *GSTT1* null genotype and cervical cancer (p>0.05). Warwick et al.[27] reported that no difference was found in the frequency of *GSTM1* and *GSTT1* null genotypes between controls and cervical carcinoma cases, including cervical intraepithelial neoplasia. Chen et al.[30] did not observe any increase in incidences of cervical cancer in patients with the *GSTM1* null genotype. In the study conducted by Sharma et al.[31] on Indian patients with cervical cancer, the combined analysis of both *GSTM1* null and *GSTT1* null genotypes did not appear to influence the susceptibility. Contrary to these studies, Kim et al.[12] reported that, among patients who carried HPV, the risk of developing cervical can-

Table 4. Frequency of glutathione S-transferase P1 (*GSTP1*) genotypes in cervical cancer studies

	Cervical cancer cases (N)	*GSTP1* (Ile/Ile) (%)	*GSTP1* (Ile/Val) (%)	*GSTP1* (Val/Val) (%)	p-value
Sobti et al.[4]	103	30	66	4	<0.05*
Jee et al.[32]	342	64.3	31.6	4.1	<0.05†
Present study	46	58.7	32.6	8.7	NS‡

**GSTP1* (ile/val) (odds ratios, 6.4; 95% confidence interval, 2.25 to 18.38; p=0.0005). †Polymorphism of *GSTP1* in women who smoke cigarettes was associated with a higher risk of developing cervical cancer. ‡NS: not significant (p>0.05).

cer before 40 is high ($p<0.05$) (Table 3).

The presence of the *GSTP1* (Ile105Val) polymorphism produced no difference between the patient and control groups. Jee et al.[32] reported that the polymorphism of *GSTP1* in women who smoke cigarettes was associated with a higher risk of developing cervical cancer. In a study by Sobti et al.,[4] it was reported that, in women with *GSTM1* (null), *GSTP1* (null) and *GSTP1* (Ile105Val) genotype, the cervical cancer development rate was elevated in passive smokers. In our study, no relation could be found between the *GSTP1* polymorphism and the development of cervical cancer. Since the number of cases in our study was limited, our findings would need to be supported with studies conducted on a larger number of cases (Table 4).

To conclude, demonstrating a relation between cancer types and genes that code the enzymes that act in detoxification metabolism is of great importance for determining risk groups for various cancer types. While the *GST* polymorphism is related to the development of cervical cancer in certain studies, no relation is seen in other studies. Many more studies should be conducted with larger patient and control groups to resolve this conflict.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. Pate Capps N, Stewart A, Burns C. The interplay between secondhand cigarette smoke, genetics, and cervical cancer: a review of the literature. Biol Res Nurs 2009; 10: 392-9.
2. Moodley M. Update on pathophysiologic mechanisms of human papillomavirus. Curr Opin Obstet Gynecol 2005; 17: 61-4.
3. Trunk MJ, Wentzensen N, von Knebel Doeberitz M. Molecular pathogenesis of cervical cancer and its first steps. Pathologe 2005; 26: 283-90.
4. Sobti RC, Kaur S, Kaur P, Singh J, Gupta I, Jain V, et al. Interaction of passive smoking with GST (GSTM1, GSTT1, and GSTP1) genotypes in the risk of cervical cancer in India. Cancer Genet Cytogenet 2006; 166: 117-23.
5. La Vecchia C, Negri E, Franceschi S, Parazzini F. Long-term impact of reproductive factors on cancer risk. Int J Cancer 1993; 53: 215-9.
6. Capalash N, Sobti RC. Epidemiology of cervical cancer: a case control study on north Indian population. Indian J Cancer 1999; 36: 179-85.
7. Winkelstein W Jr. Smoking and cancer of the uterine cervix: hypothesis. Am J Epidemiol 1977; 106: 257-9.
8. Settheetham-Ishida W, Singto Y, Yuenyao P, Tassaneeyakul W, Kanjanavirojkul N, Ishida T. Contribution of epigenetic risk factors but not p53 codon 72 polymorphism to the development of cervical cancer in Northeastern Thailand. Cancer Lett 2004; 210: 205-11.
9. Coker AL, Bond SM, Williams A, Gerasimova T, Pirisi L. Active and passive smoking, high-risk human papillomaviruses and cervical neoplasia. Cancer Detect Prev 2002; 26: 121-8.
10. Winkelstein W Jr. Smoking and cervical cancer: current status: a review. Am J Epidemiol 1990; 131: 945-57.
11. Gram IT, Austin H, Stalsberg H. Cigarette smoking and the incidence of cervical intraepithelial neoplasia, grade III, and cancer of the cervix uteri. Am J Epidemiol 1992; 135: 341-6.
12. Kim JW, Lee CG, Park YG, Kim KS, Kim IK, Sohn YW, et al. Combined analysis of germline polymorphisms of p53, GSTM1, GSTT1, CYP1A1, and CYP2E1: relation to the incidence rate of cervical carcinoma. Cancer 2000; 88: 2082-91.
13. Malats N, Camus-Radon AM, Nyberg F, Ahrens W, Constantinescu V, Mukeria A, et al. Lung cancer risk in nonsmokers and GSTM1 and GSTT1 genetic polymorphism. Cancer Epidemiol Biomarkers Prev 2000; 9: 827-33.
14. Reszka E, Wasowicz W, Gromadzinska J. Genetic polymorphism of xenobiotic metabolising enzymes, diet and cancer susceptibility. Br J Nutr 2006; 96: 609-19.
15. Joseph T, Chacko P, Wesley R, Jayaprakash PG, James FV, Pillai MR. Germline genetic polymorphisms of CYP1A1, GSTM1 and GSTT1 genes in Indian cervical cancer: associations with tumor progression, age and human papillomavirus infection. Gynecol Oncol 2006; 101: 411-7.
16. Singh H, Sachan R, Devi S, Pandey SN, Mittal B. Association of GSTM1, GSTT1, and GSTM3 gene polymorphisms and susceptibility to cervical cancer in a North Indian population. Am J Obstet Gynecol 2008; 198: 303.e1-6.
17. Abbas A, Delvinquiere K, Lechevrel M, Lebailly P, Gauduchon P, Launoy G, et al. GSTM1, GSTT1, GSTP1 and CYP1A1 genetic polymorphisms and susceptibility to esophageal cancer in a French population: different pattern of squamous cell carcinoma and adenocarcinoma. World J Gastroenterol 2004; 10: 3389-93.
18. Idle JR. Is environmental carcinogenesis modulated by host polymorphism? Mutat Res 1991; 247: 259-66.
19. Nebert DW. Role of genetics and drug metabolism in human cancer risk. Mutat Res 1991; 247: 267-81.
20. Mannervik B, Danielson UH. Glutathione transferases: structure and catalytic activity. CRC Crit Rev Biochem 1988; 23: 283-337.
21. Nakachi K, Imai K, Hayashi S, Kawajiri K. Polymorphisms of the CYP1A1 and glutathione S-transferase genes associated with susceptibility to lung cancer in relation to cigarette dose in a Japanese population. Cancer Res 1993; 53: 2994-9.
22. Zhong S, Howie AF, Ketterer B, Taylor J, Hayes JD, Beckett GJ, et al. Glutathione S-transferase mu locus: use of genotyping and phenotyping assays to assess association with lung cancer susceptibility. Carcinogenesis 1991; 12: 1533-7.
23. Brockmoller J, Kerb R, Drakoulis N, Staffeldt B, Roots I. Glutathione S-transferase M1 and its variants A and B as host factors of bladder cancer susceptibility: a case-control study. Cancer Res 1994; 54: 4103-11.
24. Hosgood HD 3rd, Berndt SI, Lan Q. GST genotypes and lung cancer susceptibility in Asian populations with indoor air pollution exposures: a meta-analysis. Mutat Res 2007; 636: 134-43.
25. Shi X, Zhou S, Wang Z, Zhou Z. CYP1A1 and GSTM1 polymorphisms and lung cancer risk in Chinese populations: a meta-analysis. Lung Cancer 2008; 59: 155-63.
26. Srivastava DS, Mishra DK, Mandhani A, Mittal B, Kumar A, Mittal RD. Association of genetic polymorphism of glutathione S-transferase M1, T1, P1 and susceptibility to bladder cancer. Eur Urol 2005; 48: 339-44.
27. Warwick A, Sarhanis P, Redman C, Pemble S, Taylor JB, Ketterer B, et al. Theta class glutathione S-transferase GSTT1 genotypes and susceptibility to cervical neoplasia: interactions with GSTM1, CYP2D6 and smoking. Carcinogenesis 1994; 15: 2841-5.
28. de Carvalho CR, da Silva ID, Pereira JS, de Souza NC, Focchi

GR, Ribalta JC. Polymorphisms of p53, GSTM1 and GSTT1, and HPV in uterine cervix adenocarcinoma. Eur J Gynaecol Oncol 2008; 29: 590-3.
29. Settheetham-Ishida W, Yuenyao P, Kularbkaew C, Settheetham D, Ishida T. Glutathione S-transferase (GSTM1 and GSTT1) polymorphisms in cervical cancer in Northeastern Thailand. Asian Pac J Cancer Prev 2009; 10: 365-8.
30. Chen C, Madeleine MM, Weiss NS, Daling JR. Glutathione S-transferase M1 genotypes and the risk of squamous carcinoma of the cervix: a population-based case-control study. Am J Epidemiol 1999; 150: 568-72.
31. Sharma A, Sharma JK, Murthy NS, Mitra AB. Polymorphisms at GSTM1 and GSTT1 gene loci and susceptibility to cervical cancer in Indian population. Neoplasma 2004; 51: 12-6.
32. Jee SH, Lee JE, Kim S, Kim JH, Um SJ, Lee SJ, et al. GSTP1 polymorphism, cigarette smoking and cervical cancer risk in Korean women. Yonsei Med J 2002; 43: 712-6.