Effect of Glutathione-S-Transferase M1 and T1 Allelic Polymorphisms on HPV-induced Cervical Precancer Formation

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Abstract. *Aim: The effect of GSTM1 and GSTT1 allelic polymorphisms was studied on the HPV-induced cervical carcinogenesis. Patients and Methods: Two hundred and fifty-three women with persistent high-risk HPV infection were involved in the study; 117 of them developed cervical high-grade dysplasia and/or cervical intraepithelial neoplasia grade III during the 7-year follow-up period. Occurrence of GSTM1 and GSTT1 null genotypes was compared between women with and without dysplasia. Results: Presence of GSTM1 (OR=1.78, 95% CI=1.06-2.97; p=0.028) and GSTT1 (OR=1.89, 95% CI=1.10-3.26; p=0.022) null genotypes was statistically significantly more frequent among women with cervical dysplasia than in the group without dysplasia. Participants with dual null genotype had an even more elevated risk of precancerous lesion (OR=2.35, 95% CI=1.17-4.73; p=0.017). Conclusion: Our study demonstrated the role of both GSTM1 and T1 null genotypes in the development of high-grade cervical dysplasia in a Caucasian population.*

During recent decades the mortality from cervical cancer has decreased in developed countries, but the incidence is still high, as it that of precancerous lesions (1). Human papillomavirus (HPV) infection has been identified as being the strongest risk factor in cervical carcinogenesis (2-4). Intensive research has led to the identification of high-risk strains of HPV (*e.g.* 16, 18, 31, 45), (5-6). The risk of cervical cancer or dysplasia in women with persistent high-risk HPV infection is between 10-40% (7-9). Several other external risk factors have also been described to have an influence on cervical cancer/precancerous lesion formation, *e.g.* smoking, use of oral contraceptives, certain lifestyle factors (10-12). The role of different genetic risk modifiers has also been extensively studied. Allelic polymorphism of carcinogen-metabolizing enzymes has been found to influence the risk of cervical cancer, or formation of precancerous lesions in certain populations (13-16). In spite of the numerous studies in this field, there are still several open questions concerning the effect of these polymorphisms on the cervical carcinogenesis. The glutathione-S-transferases (GSTs) form one of the most important groups of phase II metabolizing enzymes: these enzymes conjugate carcinogenic compounds with glutathione, thus inactivating and making them more water soluble (17-19). The most frequently studied members of this enzyme superfamily are the GSTM1 and GSTT1 enzymes. The genes for both of these exhibit an insertion/deletion polymorphism, resulting in null (0, homozygous deletion) and + (with at least one functional allele) genotypes. *GSTM1* and/or *GSTT1* 0 genotypes have been found in some studies to increase the risk of cervical cancer or precancer (20-23). There was, however, an inconsistency between the results, primarily caused by heterogeneous study designs, non-comparable enrollment criteria, and different HPV status or genetic background of the study populations. Two recent meta-analyses have also been published, indicating the need for further research on this field, particularly in relation to the difference between Asian and Caucasian populations, and concerning the joint effect of the two polymorphisms (24-25).

In the present study we investigated the effect of *GSTM1* and *GSTT1* polymorphisms on the risk of precancerous cervical lesion formation in a Hungarian population with persistent high-risk HPV infection.

Patients and Methods

Two hundred and fifty-three women, with a persistent high-risk HPV positivity, were involved in the study. The participants were selected from women attending gynecological screening at the Fejér

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County Szent György Hospital or at the Diósgyőr Hospital, Hungary. They had no cervical cancer or dysplasia at the beginning of the observation period. Informed consent was collected from the participants at their enrollment in the study. Occurrence of precancerous cervical lesion (high-grade dysplasia or cervical intraepithelial neoplasia grade III) was registered during a 7-year follow-up period (with annual gynecological examination, cervix cytology, or biopsy if needed).

After DNA isolation (26) from samples taken for cervical cytology, a simultaneous genotyping of *GSTM1* and *GSTT1* was performed (27) by polymerase chain reaction (PCR). The amplification took place in the presence of an internal control (a 268 bp fragment of the β-globin gene), in 15 μl total volume containing 0.5 U Taq DNA polymerase (Go Taq, Promega, Madison, WI, USA), 1X Buffer (Promega), 2 μl DNA template, 200 μM dNTP and 1.5 mM $MgCl_2$, 30-30 pmol *GSTT1*-F and *GSTT1*-R primers, 50-50 pmol *GSTM1*-F and *GSTM1*-R primers, 20-20 pmol β-globin-F and β-globin-R primers. The primer sequences were as follows: *GSTM1*-F: GAACTCCCTGAAAAGCTAAAGC, *GSTM1*-R: GTTGGGCTCAAATATACGGTGG, *GSTT1*-F: TTCCTTACT GGTCCTCACATCTC, *GSTT1*-R: TCACCGGATCATGGCC AGCA, β-globin-F: CAACTTCATCCACGTTCACC, β-globin-R: GAAGAGCCAAGGACAGGTAC. After a 7 min denaturation at 94˚C, 35 PCR cycles were performed: 60 sec at 94˚C, 60 sec at 60˚C, 60 sec at 72˚C, followed by 5 min at 72˚C. The PCR products were visualized by electrophoresis in 2% agarose gel. The amplification resulted in a 215 bp fragment for the GSTM1+ genotypes and a 480 bp fragment in case of GSTT1+ individuals. Lack of the appropriate band indicated the 0 (null) genotype for the given polymorphism. Occurrence of 0 and + genotypes were compared between participants with and without cervical precancerous lesions.

The demographic characteristics of the groups were compared by Student's *t*-test for independent samples or Pearson's chi-square test. Occurrence of the genotypes was compared by multivariate logistic regression analysis. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated, and chi-square analysis was performed to describe the association between GST genotypes and cervical dysplasia. The ORs were adjusted for age, age at menarche, age at first intercourse, parity, and number of abortions. $P<0.05$ was used as a level of statistical significance throughout the analysis. The statistical analysis was performed using the IBM SPSS Statistics software package version 19 (SPSS Inc., Chicago, IL, USA).

Results

Over the 7-year follow-up period, high-grade dysplasia or CIN grade III developed in 117 participants, while 136 had no such abnormalities. The main demographic characteristics of these women are shown in Table I. The demographic characteristics of the two groups did not differ statistically significantly from each other. The presence of the GSTM1 (OR: 1.78, 95% CI: 1.06-2.97; $p=0.028$) and GSTT1 (OR=1.89, 95% CI=1.10-3.26; $p=0.022$) 0 genotype was statistically significantly more frequent among women with precancerous lesions than in the healthy group. The association was even stronger for those individuals who belonged to the dual high-risk genotype (OR=2.35, 95% CI=1.17-4.73; $p=0.017$; Table II). These results demonstrate a risk-increasing effect of the null genotypes, particularly in combination with each other.

Discussion

Undoubtedly the most important factor in cervical carcinogenesis is persistent infection with a high-risk strain of HPV. This does not necessarily lead to cancer or formation of a precancerous lesion, but, particularly in case of HPV 16, the incidence of precancerous states can reach 40% (7-9) among persistent HPV carriers. In order to refine the present risk assessment possibilities, identification of HPV-infected women with very high probability of developing precancerous lesions would be essential. Beside external factors, genetic components also modify HPV-induced cervical carcinogenesis. Carcinogenic-metabolizing enzymes have been found to be important risk modifiers in human carcinogenesis (28). Phase I enzymes, such as like members of the cytochrome P450 superfamily, activate procarcinogens, while the reactive intermediate products are inactivated by phase II metabolizing enzymes (17-19). In contrast to the majority of metabolizing enzymes, where the typical form of the allelic variability is the single nucleotide polymorphism, the *GSTM1* and *GSTT1* genes possess an insertion/deletion polymorphism (19, 29). These polymorphisms have been suspected to affect the risk of different types of human cancer, since individuals with the null genotype have a somewhat reduced overall detoxifying capacity (17, 30, 31). Because of the huge overlap between the substrates of the GST enzymes, loss of GSTM1 or GSTT1 function will not cause serious problems in the metabolism of carcinogenic compounds, but it may lead to a decreased rate in the removal of carcinogenic compounds from the body.

The possible role of these enzymes has also been studied in cervical carcinogenesis. Certain publications have found a statistically significant association between *GSTM1* and/or *GSTT1* 0 genotype and an increased risk of cervical cancer or precancerous lesions (32, 33), but other studies did not confirm this correlation (34, 35). Unfortunately the distribution of 0/+ genotypes may differ between certain populations, most importantly in Caucasians compared to Asians (24), which makes it difficult to compare the results of such studies. Recently two meta-analyses have been published on the association between *GSTM1*/*GSTT1* and cervical cancer (24, 25). Wang *et al*. concluded that *GSTM1* 0 genotype was associated with the risk of cervical cancer in Asians (OR=1.47, 95% CI=1.11-1.94), but not in Caucasians (OR=0.96, 95% CI=0.73-1.27). However, according to the other meta-analysis by Economopoulos *et al*., *GSTM1* 0 genotype was a risk factor in the non-Chinese population (OR=1.392, 95% CI=1.003-1.932), and had no statistically significant effect in Chinese women (OR=1.08, 95%

Table I. *Characteristics of study participants*.

	With high-grade dysplasia	Without high-grade dysplasia
Age (years)		
At the beginning of follow-up (SD)	40.56 (13.61)	42.54 (13.21)
At menarche (SD)	13.11 (1.05)	13.01 (1.00)
At first intercourse (SD)	17.88 (1.65)	17.63 (1.80)
Parity		
0-1	75 (64.1%)	80 (58.8%)
≥2	42 (35.9%)	56 (41.2%)
Number of abortions		
0-1	106 (90.6%)	121 (89.0%)
≥2	11 (9.4%)	15 (11.0%)

Table II. *Distribution of GSTM1 and GSTT1 genotypes among women with persistent high-risk HPV infection*.

Genotype		With dysplasia	Without dysplasia	OR (95% CI)	*p*-Value
GSTM1	+	54 (46.2%)	83 (61.0%)	1.00 (reference)	-
	0	63 (53.8%)	53 (39.0%)	1.78 (1.06-2.97)	0.028
GSTT1	+	70 (59.8%)	101 (74.3 %)	1.00 (reference)	-
	0	47 (40.2%)	35 (25.7%)	1.89 (1.10-3.26)	0.022
GSTM1 and/or *GSTT1*	+	90 (76.9%)	121 (89.0 %)	1.00 (reference)	-
Both	0	27 (23.1%)	15 (11.0%)	2.35 (1.17-4.73)	0.017

CI=0.87-1.34) (25). No statistically significant association was found between the *GSTT1* 0 genotype and cervical cancer, with the exception of Latinos, where quite a strong association was identified (OR=4.58, 95% CI=2.04-10.28). Wang *et al*. (24) also found an interaction between *GSTM1/T1* 0 genotypes in Asian studies: individuals with dual null genotypes had an elevated risk (OR=1.77, 95% CI=1.14-2.75), higher than women with either *GSTM1* or *GSTT1* 0 genotype alone. The comparability of the studies was limited by differences in the categorization of HPV infections, outcome (cancer and/or various precancerous stages), and confounders.

One of the major issues is that most of the previous studies compared the HPV status of the participants at the time of the diagnosis of the cancer/precancerous lesion, and there was no information on the length of its persistence. The duration of a persisting high-risk HPV infection is, however, critical in the risk assessment, since it is definitively associated with the risk of cancer. The other crucial problem is the use of different outcomes, *i.e*. in certain papers cervical cancer (33-35), in some publications precancerous lesions (36, 37), and in other articles, cancer or precancerous lesions (in mixed groups) (16, 21) were used as outcome. The main goal of our study was to address these problems, and to analyze the effect of GST null genotypes in relation to these important factors. In order to eliminate the possibility of error concerning the unknown length of HPV persistence, only women with a high-risk HPV infection persistent for at least 7 years were included in the study. In our study, high-grade cervical dysplasia or CIN grade III were chosen as outcomes, since the immediate precancerous state is probably the best marker for risk measurement. This approach is also useful from a practical point of view, since, according to the present guidelines, women with a precancerous lesion receive therapy, and there is no waiting for a spontaneous regression. The further conversion to cervical cancer depends on several prognostic factors as well, so it is not a clean and direct risk measure.

In our study 46.2% of women developed a precancerous cervical lesion during the observation period, which is also an important result, since the previous studies – giving incidences up to 40% – relied on shorter follow-up periods. The length of the persistence of the HPV infection seems to be in a correlation with the risk of a precancerous lesion. In such a situation, the *GSTM1* or *T1* 0 genotypes seem to be important risk-modifying factors. In order to ensure an adequate size of the groups we did not use matching for smoking or other possible confounders. It would probably have been unnecessary anyway, since the vast majority of the previous studies did not find any interaction between

smoking habits and the polymorphisms of *GSTM1* or *GSTT1* genes (16, 21, 35, 38, 39).

We found lower parity and less abortions among participants with a precancerous lesion than in the group of women without dysplasia, but this difference was not statistically significant. These factors have already been found to be in correlation with the risk of cervical cancer (3, 40-42). Our findings underline the importance of the long-standing persistence of HPV infection, which seems to be stronger than the possible risk-modifying effect of the studied demographic variables.

This study demonstrated the effect of *GSTM1* and *GSTT1* polymorphisms on the risk of HPV-induced cervical dysplasia/CIN. In the case of women with long-standing persistent high-risk HPV infection, the null genotype for both enzymes proved to be a statistically significant risk factor. The dual null genotype further increased the risk of high-grade dysplasia, thus demonstrating an interaction between the two studied polymorphisms. The results demonstrate that neither the association between null genotypes and risk of precancerous lesions, nor the interaction between the two GST polymorphisms is limited to Asian populations. In cases of persistent HPV infection, both of these genetic risk factors should be included in the risk assessment in Caucasian populations as well in Asians.

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