

RESEARCH ARTICLE

Glutathione S-Transferase T1 and M1 Polymorphisms and Risk of Uterine Cervical Lesions in Women from Central Serbia

Ivana Stosic^{1*}, Darko Grujicic¹, Slobodan Arsenijevic², Marija Brkic³, Olivera Milosevic-Djordjevic^{1,2}

Abstract

The aim of this study was to investigate the frequencies of GSTT1 and GSTM1 deletion polymorphisms in newly-diagnosed patients with uterine cervical lesions from central Serbia. Polymorphisms of GST genes were genotyped in 97 patients with cervical lesions and 50 healthy women using a multiplex polymerase chain reaction (PCR). The GSTM1 null genotype was significantly more prominent among the patients than in controls (74.2% vs 56.0%), the risk associated with lesions being almost 2.3-fold increased (OR=2.26, 95% CI=1.10-4.65, p=0.03) and 3.17-fold higher in patients above >45 years old (95% CI=1.02-9.79, p=0.04). The analysis of the two genotypes demonstrated that GSTM1 null genotype significantly increased risk only for low grade squamous intraepithelial lesion-LSIL (OR=2.81, 95% CI=1.03-7.68, p=0.04). GSTT1 null genotype or different genotype combinations were not found to be risk factors, irrespective to lesion stages, age or smoking. We found that the risk of cervical lesions might be significantly related to the GSTM1 null genotype, especially in women aged above 45 years. Furthermore, the GSTM1 polymorphism might have greater role in development of early stage lesions.

Keywords: Cervical lesions - polymorphism - glutathione S - transferase T1 - glutathione S - transferase M1

Asian Pac J Cancer Prev, 15 (7), 3201-3205

Introduction

Cervical cancer is the third most common women cancer worldwide with ~530 000 new cases and 275 000 deaths in 2008 (Jemal et al., 2011). Invasive cervical cancer is developing gradually through the precancerous stages LSIL (Low squamous intraepithelial lesion) and HSIL (High squamous intraepithelial lesion).

So far, the Human Papilloma Virus (HPV) is the most well-established risk factor for cervical lesions development. Despite that the majority of diagnosed lesions are in the women with HPV (Evans et al., 2006; Zuna et al., 2007) a percent of women with cervical lesions is not HPV positive, suggesting that genetical and environmental factors may also play a role in the cervical lesions development.

Different individual susceptibility to the cancer may be due to polymorphism in genes involved into cellular metabolism and detoxification of carcinogens products. The Glutathione S Transferases (GSTs) are a super family of polymorphic phase II enzymes involved in the metabolism of xenobiotics (Jancova et al., 2010). The deletion polymorphism of theta (GSTT1) and mu (GSTM1) gene has been described and the homozygous deletion resulting in null genotypes, leading to the absence

of enzyme activity. Since these enzymes protect the cell, it is assumed that GSTT1 and GSTM1 null genotype, alone or in combination, may lead to increased susceptibility to cancer. However, in the relevant literature data there are different results on association of GSTT1 and GSTM1 polymorphism and cancer risk (Ates et al., 2005; Singh et al., 2008; Taspinar et al. 2008; Ansari et al., 2009; Kondo et al., 2009; Sivonova et al., 2009; Piao et al., 2013; Peng et al., 2014).

In the present study we have evaluated the frequencies GSTT1 and GSTM1 genotypes in the women with cervical lesions diagnoses (LSIL, HSIL and CC- cancer *in situ* or invasive cancer) and have compared them with the frequencies in the healthy women. Furthermore, we have evaluated the interaction of these genes with the risk factors (i.e. smoking, age) in modulating the susceptibility for cervical lesions development.

Materials and Methods

Patients

Our study has been approved by Ethics Committee of the Clinic of Kragujevac (No 2577) and Faculty of Medicine University of Nis (01-5518-1). The study population was composed of newly diagnosed 32 LSIL

¹Faculty of Science, University of Kragujevac, ²Faculty of Medical Sciences, University of Kragujevac, ³Department of Obstetrics and Gynecology, Clinical Center Kragujevac, Kragujevac, Serbia *For correspondence: stosicster@gmail.com

patients aged 24-64 years, 33 HSIL patients aged 24-65 years and 32 CC patients (cancer in situ or invasive cancer) aged 21-77 years, observed at the Department of Obstetrics and Gynecology of the Clinic Center Kragujevac and the Department of Obstetrics and Gynecology of the Clinic Center Nis. All patients underwent a regular gynecological examination.

Genotyping

For both patients and controls, DNA was isolated from whole peripheral blood using the EZ1 DNA Blood 350µl Kit (Qiagen, Hilden, Germany) and BioRobot EZ1.

An analysis for GST polymorphism was performed by multiplex PCR method described by Abdel-Rahman et al. (1996), with some modification. Briefly, 50ng of template DNA was amplified in 50µl of PCR reaction mix containing 30 pmol of each GSTT1 primers (Invitrogen, California, USA) and GSTM1 primers (Invitrogen, California, USA), 200µM of each deoxynucleotide triphosphate (Invitrogen, California, USA), 1.5mM of MgCl₂, 1X PCR buffer and 2U of Taq polymerase (Invitrogen, California, USA). In order to improve PCR reaction, glycerol and dimethyl sulfoxide (DMSO) in the final concentration of 5% were added in reaction mixture. Exon 7 of CYP1A1 gene was co-amplified and serves as internal control. After initial denaturation at 94°C for 5 minutes, 35 cycles of denaturation at 94°C for 2 minutes, annealing for 1 minute at 58°C and extension at 72°C for 1 minute, followed by a final extension step for 10 minutes at 72°C, were performed.

PCR products were separated and analyzed in 2% agarose gel containing SYBR Safe DNA gel stain (Invitrogen, California, USA). The absence or presence of GSTT1 and GSTM1 was detected by the presence

or absence of appropriate bands (480bp for GSTT1 and 215bp for GSTM1), while the band corresponding to internal control CYP1A1 (312bp) was always present. The absence of band was considered as a null genotype, while the presence was considered as a positive genotype.

Statistical analysis

The chi-square (χ²) or Fischer's (F) exact test were used to compare distribution of GSTT1 or GSTM1 genotypes between variables. The Odds Ratio (OR) with 95% confidence interval (CI) calculated by binary logistic regression analyses was used to illustrate the association. The probability p<0.05 was considered as statistically significant.

Results

The results of GST polymorphism analyses in the patients with cervical lesions and healthy controls are presented in Tables 1-4.

General demographics, lifestyle and medical characteristics of the study population are presented in Table 1.

Table 2 shows distributions of GSTT1 and GSTM1 polymorphism, alone or in combinations, and the OR with 95%CI, in the analyzed patients with cervical lesions and healthy women. GSTT1 null genotype has not been associated with increased risk for cervical lesions (OR=0.97, 95%CI =0.48-1.94, p=0.92). In the contrary, the frequency of GSTM1 null genotype was significantly more prominent among the patients in comparison to the controls (74.2% vs 56.0%) and the risk was almost 2.3-fold increased (OR=2.26, 95%CI=1.10-4.65, p=0.03). There were no significant differences in the frequency of GSTT1positive/GSTM1null, GSTT1null/GSTM1positive and GSTT1null/GSTM1null combinations between patients and controls.

Considering different stages of cervical lesions, GSTM1 null genotype has been significantly associated with increased risk only for LSIL (OR=2.81, 95%CI =1.03-7.68, p=0.04), while there were no association for HSIL and for CC. GSTT1 null genotype was not found to be a risk factor for LSIL, HSIL or CC. Similarly, compared to GSTT1positive/GSTM1positive combination, neither of the analyzed combinations were associated with the risk for developing LSIL, HSIL or CC (Table 3).

The distribution of GSTT1 and GSTM1 according to age and smoking habits in the patients and controls are

Table 1. General Characteristics of The Study Population

	Patients	Controls
Total No	97	50
Mean age±S.D (years, range)	44.54±12.19 (21-77)	42.98±8.26 (24-62)
Smoking habit		
smokers	52	27
nonsmokers	45	23
Reproductive history (range)		
miscarriages	27 (1-4)	10 (1-2)
abortions	55 (1-7)	18 (1-5)
HPV infection		
positive	14	-
negative	5	-

Table 2. Distribution of GSTT1 and GSTM1 Genotypes in Patients with Cervical Iesions and Controls with Odd Ratio (OR) and 95% Confidence Interval (CI)

		Controls (%)	Patientsa (%)	p	OR	CI (95%)	p
Total No	Genotype	50	97				
	GSTT1						
	positive	30 (60.0)	59 (60.8)		1 (ref.)		
	null	20 (40.0)	38 (39.2)	0.92	0.97	0.48-1.94	0.92
	GSTM1						
	positive	22 (44.0)	25 (25.8)		1 (ref.)		
	null	28 (56.0)	72 (74.2)	0.03	2.26	1.10-4.65	0.03
	GSTT1/GSTM1						
	positive/positive	11 (22.00)	17 (17.5)		1 (ref.)		
	positive/null	19 (38.0)	42 (43.3)	0.45	1.43	0.56-3.63	0.45
	null/positive	11 (22.0)	8 (8.2)	0.21	0.47	0.14-1.54	0.21
	null/null	9 (18.0)	30 (30.9)	0.15	2.16	0.74-6.24	0.16

de Carvalho et al., 2008; Singh et al., 2008; Ueda et al., 2010; Ches et al., 2011).

Considering joint effect of these two polymorphisms, the combination of GSTT1 null and GSTM1 null genotypes did not influence susceptibility to CIN or SCC (Warwick et al., 1994a). A similar result for cancer risk was obtained in the study of Sharma et al. (2004). Nevertheless, in the study of Cseh et al. (2011) patients with dual null genotype had significantly increased risk for precancerous lesion.

Since the obtained results for association between GSTs polymorphism and cervical lesions are inconsistent, several meta-analyses were recently performed. Analyzing the total population samples, published meta-analyses indicate the possible significant role of GSTM1 null genotype in development of cervical neoplasia (Economopoulos et al., 2010; Gao et al., 2011; Wang et al., 2011; Zhang et al., 2012), while GSTT1 null genotype was associated with cervical neoplasia in the study of Gao et al. (2011). In GSTT1/GSTM1 interaction analysis, women carrying dual polymorphism were associated with increased risk for cervical neoplasia (Gao et al., 2011; Wang et al., 2011).

In our study, homozygous deletion of GSTM1 has been prominent in patients with cervical lesions (74.2% vs 56.0%) and carrying these polymorphisms significantly increase the risk for cervical lesions development (OR=2.26, 95%CI=1.10-4.65, p=0.03). On the contrary, there was no statistically significant association between GSTT1 null genotype and cervical lesions, suggesting that carrying this genotype is not a risk factor for cervical lesions. In our sample no significant differences have been revealed between patients and controls for different combination of genotypes.

Divided into lesion stages the results obtained in the meta-analyses by Sui et al. (2011) suggest that GSTM1 null may be important in the very early stages of cervical cancerogenesis while no association was found for GSTT1 polymorphism and different stages of cervical lesions. Although Gao et al. (2011) suggested that GSTM1 and GSTT1 null genotypes may play role for cervical neoplasia development, GSTT1 or GSTM1 null genotypes were not associated with LSIL, HSIL, SCC (squamous cell carcinoma) or AC (adenocarcinoma and adenosquamous carcinoma). On the other hand, some of meta-analyses provide evidence of associating the GSTM1 null genotype and cervical cancer (Economopoulos et al., 2010; Wang et al. 2011; Zhang et al., 2012). Due to the nature of results, it is necessary to conduct further research in this field in order to obtain a better understanding of the role of GSTT1 and GSTM1 null polymorphism in different stages of cervical lesions.

Analyzing our data for different cervical lesions stages, results showed significant difference in GSTM1 distribution between LSIL cases and controls (78.1% vs 56.0%), corresponding to a~3-fold increase in risk for LSIL development (p=0.04), but not for HSIL or CC. On the contrary, no association was found for GSTT1 polymorphism and different stages of cervical lesions. These results might indicate possible important role of GSTM1 deletion in development of early stage of

precancerous lesions.

Tobacco smoke contains carcinogens and they may be found in the cervical mucus. McCann et al. (1992) found both nicotine and cotinine in the cervical mucus of smokers. Similarly, the concentrations of potent carcinogen NNK in smokers were significantly higher than in nonsmokers (Prokopczyk et al., 1997). Because some of the tobacco related carcinogens are substrate for GSTs and that smoking is a one of the risk factor for cervical lesions development it is important to examine the tobacco/gene interaction as a modulating factor. After stratification, in smokers and non-smokers, we did not find significant interaction between polymorphisms and smoking habit in the lesions development risk. Palma et al. (2010) did not find any positive correlation between GSTs polymorphism and smoking habit in the cervical lesions risk, but they explained that these results were probably the consequence of the small sample size. Settheetham-Ishida et al. (2009) have not observed the effect of the GST null genotype on the increased risk for cervical cancer among smokers. However, Sobti et al. (2006) found that the absence of GSTT1 and GSTM1 genes in passive smokers increased the risk for developing cervical cancer.

At the end, we divided patients and controls by age. Similar to Sharma et al. (2004), we found significant difference in GSTM1 null genotype distribution between patients with cervical lesions and controls in individuals >45 years (p=0.04), but difference was not significant in individuals ≤45 years (p=0.23). On the contrary, while studying the association between age and GSTT1 polymorphism in cervical lesions risk, we found no significant differences in both age groups. Our results are in accordance with Sharma et al. (2004).

To the best of our knowledge, this is the first study of association of GSTT1 and GSTM1 genotypes and cervical lesions risk in the population of the women of central Serbia. In conclusion, we found that the risk of cervical lesions might be significant related to the GSTM1 null genotype, especially in women aged above 45 years. Furthermore, GSTM1 polymorphism might have greater role in developing the early stage of lesions.

Acknowledgements

This work was supported by the by Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 41010). We are most grateful to the women who participated in our study. Lastly, we would like to thank all the medical staff at Department of Obstetrics and Gynecology of Clinical Center Nis and Clinical Center Kragujevac for their kindness and dedication.

References

- Abdel-Rahman SZ, El-Zein RA, Anwar WA, Au WW (1996). A multiplex PCR procedure for polymorphic analysis of GSTM1 and GSTT1 genes in population studies. *Cancer Lett*, **107**, 229-33.
- Agorastos T, Papadopoulos N, Lambropoulos AF, et al (2007). Glutathione-S-transferase M1 and T1 and cytochrome P1A1 genetic polymorphisms and susceptibility to cervical

- intraepithelial neoplasia in Greek women. *Eur J Cancer Prev*, **16**, 498-504.
- Ansari BS, Vasudevan R, Mirinargesi M, et al (2009). Lack of association of glutathione S-transferase gene polymorphisms in Iranian prostate cancer subjects. *Am J Biochem Biotechnol*, **5**, 30-4.
- Ates NA, Tamer L, Ates C, et al (2005). Glutathione S-transferase M1, T1, P1 genotypes and risk for development of colorectal cancer. *Biochem Genet*, **43**, 149-63.
- Cseh J, Pazsit E, Orsos Z, et al (2011). Effect of glutathione-S-transferase M1 and T1 allelic polymorphisms on HPV-induced cervical precancer formation. *Anticancer Res*, **31**, 3051-5.
- de Carvalho CR, da Silva ID, Pereira JS, et al (2008). Polymorphisms of p53, GSTM1 and GSTT1, and HPV in uterine cervix adenocarcinoma. *Eur J Gynaecol Oncol*, **29**, 590-3.
- Economopoulos KP, Choussein S, Vlahos NF, Sergentanis TN (2010). GSTM1 polymorphism, GSTT1 polymorphism, and cervical cancer risk: a meta-analysis. *Int J Gynecol Cancer*, **20**, 1576-80.
- Evans MF, Adamson CS, Papillo JL, et al (2006). Distribution of human papillomavirus types in thinprep papanicolaou tests classified according to the Bethesda 2001 terminology and correlations with patient age and biopsy outcomes. *Cancer*, **106**, 1054-64.
- Gao LB, Pan XM, Li LJ, et al (2011). Null genotypes of GSTM1 and GSTT1 contribute to risk of cervical neoplasia: an evidence-based meta-analysis. *PLoS One*, **6**, 20157.
- Goodman MT, McDuffie K, Hernandez B, et al (2001). CYP1A1, GSTM1, and GSTT1 polymorphisms and the risk of cervical squamous intraepithelial lesions in a multiethnic population. *Gynecol Oncol*, **81**, 263-9.
- Jancova P, Anzenbacher P, Anzenbacherov E (2010). Phase II drug metabolizing enzymes. *Biomed Pap*, **154**, 103-16.
- Jemal A, Bray F, Center MM, et al (2011). Global cancer statistics. *CA Cancer J Clin*, **61**, 69-90.
- Kiran B, Karkucak M, Ozan H, et al (2010). GST (GSTM1, GSTT1, and GSTP1) polymorphisms in the genetic susceptibility of Turkish patients to cervical cancer. *J Gynecol Oncol*, **21**, 169-73.
- Kondo S, Sturgis EM, Li F, Wei Q, Li G (2009). GSTM1 and GSTT1 null polymorphisms and risk of salivary gland carcinoma. *Int J Clin Exp Med*, **2**, 68-75.
- McCann MF, Irwin DE, Walton LA, et al (1992). Nicotine and cotinine in the cervical mucus of smokers, passive smokers, and nonsmokers. *Cancer Epidemiol Biomarkers Prev*, **1**, 125-9.
- Palma S, Novelli F, Padua L, et al (2010). Interaction between glutathione-S-transferase polymorphisms, smoking habit, and HPV infection in cervical cancer risk. *J Cancer Res Clin Oncol*, **136**, 1101-9.
- Peng J, Liu HZ, Zhu YJ (2014). Null glutathione S-transferase T1 and M1 genotypes and oral cancer susceptibility in China and India-a Meta-analysis. *Asian Pac J Cancer Prev*, **15**, 287-90.
- Piao JM, Shin MH, Kim HN, et al (2013). Glutathione-S-transferase (GSTM1, GSTT1) null phenotypes and risk of lung cancer in a Korean population. *Asian Pac J Cancer Prev*, **14**, 7165-9.
- Prokopczyk B, Cox JE, Hoffmann D, Waggoner SE (1997). Identification of tobacco-specific carcinogen in the cervical mucus of smokers and nonsmokers. *J Natl Cancer Inst*, **89**, 868-73.
- Settheetham-Ishida W, Yuenyao P, Kularbkaew C, Settheetham D, Ishida T (2009). Glutathione S-transferase (GSTM1 and GSTT1) polymorphisms in cervical cancer in northeastern Thailand. *Asian Pac J Cancer Prev*, **10**, 365-8.
- Sharma A, Sharma JK, Murthy NS, Mitra AB (2004). Polymorphisms at GSTM1 and GSTT1 gene loci and susceptibility to cervical cancer in Indian population. *Neoplasma*, **51**, 12-6.
- Sierra-Torres CH, Au WW, Arrastia CD, et al (2003). Polymorphisms for chemical metabolizing genes and risk for cervical neoplasia. *Environ Mol Mutagen*, **41**, 69-76.
- Singh H, Sachan R, Devi S, Pandey SN, Mittal B (2008). Association of GSTM1, GSTT1, and GSTM3 gene polymorphisms and susceptibility to cervical cancer in a north Indian population. *Am J Obstet Gynecol*, **198**, 3031-6.
- Singh M, Shah PP, Singh AP, et al (2008). Association of genetic polymorphisms in glutathione S-transferases and susceptibility to head and neck cancer. *Mutat Res*, **638**, 184-94.
- Sivonova M, Waczulikova I, Dobrota D, et al (2009). Polymorphisms of glutathione-S-transferase M1, T1, P1 and the risk of prostate cancer: a case-control study. *J Exp Clin Cancer Res*, **28**, 32.
- Sobti RC, Kaur S, Kaur P, et al (2006). Interaction of passive smoking with GST (GSTM1, GSTT1, and GSTP1) genotypes in the risk of cervical cancer in India. *Cancer Genet Cytogenet*, **166**, 117-23.
- Sui Y, Han W, Yang Z, Jiang M, Li J (2011). Association of glutathione S-transferase M1 and T1 null polymorphisms with the development of cervical lesions: a meta-analysis. *Eur J Obstet Gynecol Reprod Biol*, **159**, 443-8.
- Taspinar M, Aydos SE, Comez O, et al (2008). CYP1A1, GST gene polymorphisms and risk of chronic myeloid leukemia. *Swiss Med Wkly*, **138**, 12-7.
- Ueda M, Toji E, Nunobiki O, et al (2010). Germline polymorphisms of glutathione-S-transferase GSTM1, GSTT1 and p53 codon 72 in cervical carcinogenesis. *Hum Cell*, **23**, 119-25.
- Wang D, Wang B, Zhai JX, Liu DW, Sun GG (2011). Glutathione S-transferase M1 and T1 polymorphisms and cervical cancer risk: a meta-analysis. *Neoplasma*, **58**, 352-9.
- Warwick A, Sarhanis P, Redman C, et al (1994a). Theta class glutathione S-transferase GSTT1 genotypes and susceptibility to cervical neoplasia: interactions with GSTM1, CYP2D6 and smoking. *Carcinogenesis*, **15**, 2841-5.
- Warwick AP, Redman CW, Jones PW, et al (1994b). Progression of cervical intraepithelial neoplasia to cervical cancer: interactions of cytochrome P450 CYP2D6 EM and glutathione S-transferase GSTM1 null genotypes and cigarette smoking. *Br J Cancer*, **70**, 704-8.
- Zhang ZY, Jin XY, Wu R, et al (2012). Meta-analysis of the association between GSTM1 and GSTT1 gene polymorphisms and cervical cancer. *Asian Pac J Cancer Prev*, **13**, 815-9.
- Zuna RE, Allen RA, Moore WE, et al (2007). Distribution of HPV genotypes in 282 women with cervical lesions: evidence for three categories of intraepithelial lesions based on morphology and HPV type. *Mod Pathol*, **20**, 167-74.