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THE ROLE OF GSTM1 AND GSTT1 POLYMORPHISM IN PATIENTS WITH RENAL CELL CARCINOMA

ULOGA POLIMORFIZMA GLUTATION S-TRANSFERAZA M1 I T1 KOD PACIJENATA SA KARCINOMOM BUBREŽNOG PARENHIMA

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Summary: Members of the glutathione S-transferase (GST) superfamily exhibit polymorphic expression. GSTs are investigated as biomarkers of risk for various cancers, including renal cell carcinoma (RCC). The aim of this study was to test the association between *GSTM1* and *GSTT1* polymorphism and susceptibility to RCC, independently or in conjunction with known risk factors. Genomic DNA was isolated from 182 controls and 76 patients with RCC. *GSTM1* and *GSTT1* genotypes were determined by multiplex PCR. Data obtained were analyzed with respect to RCC risk factors including smoking and occupational exposure. The frequency of *GSTM1*-null genotype was higher in patients with RCC (60.5%) compared to controls (47.2%). *GSTT1*-null genotype was found in 28.6% controls and 27.6% of cases. *GSTM1*-null individuals exhibit 1.9-fold increased risk of RCC (95% CI: 1.06–3.33). The presence of *GSTT1* active genotype was associated with increased risk of RCC in occupationally exposed subjects when unexposed *GSTT1*-null subjects were used as a comparison group (OR: 2.48; 95% CI: 1.05–5.86). No association was found between the inactive form of *GSTM1* and *GSTT1* and smoking in RCC patients. In a Serbian cohort of patients, the presence of a *GSTM1* active genotype is protective against RCC, whereas a *GSTT1* active genotype increases RCC risk in occupationally exposed subjects.

Keywords: glutathione S-transferase, polymorphism,renal cell carcinoma, risk factors

Kratak sadržaj: Genetski polimorfizam je prisutan kod mnogih članova superfamilije glutation-S transferaza. U toku su istraživanja koja ispituju ulogu GST kao biomarkera za nastanak različitih karcinoma, uključujući karcinom bubrežnog parenhima (KBP). U ovoj studiji je ispitivana uloga *GSTM1* i *GSTT1* polimorfizma u nastanku KBP, nezavisno ili udruženo sa poznatim faktorima rizika za ovaj karcinom. DNK je izolovana iz krvi 182 kontrolna subjekta i 76 bolesnika sa KBP. Polimorfizam *GSTM1* i *GSTT1* je određivan metodom PCR-a. Dobijeni rezultati su analizirani u odnosu na faktore rizika za KBP, uključujući pušenje i profesionalnu izloženost. Učestalost *GSTM1*-nultog genotipa je bila viša kod bolesnika sa KBP (60,5%) nego kod kontrola (47,2%). Prisustvo *GSTT1*-nultog genotipa je utvrđeno kod 28,6% kontrola i 27,6% bolesnika sa KBP. Nosioci *GSTM1*-nultog genotipa imaju 1.9-puta veći rizik za KBP (95% CI: 1,06–3,33). Prisustvo *GSTT1* aktivnog genotipa je udruženo sa povećanim rizikom za KBP kod profesionalno izloženih subjekata kada su kao referentna grupa uzeti neizloženi nosici *GSTT1*-nultog genotipa (OR: 2,48; 95% CI: 1,05–5,86). Nije otkrivena povezanost između nedostatka aktivne forme *GSTM1* i *GSTT1* i pušenja kod obolelih od KBP. Studija izvedena u Srbiji je pokazala da prisustvo *GSTM1* aktivnog genotipa štiti od nastanka KBP, dok prisustvo *GSTT1* aktivnog genotipa povećava rizik kod profesionalno izloženih osoba.

Ključne reči: faktori rizika, glutation S-transferaze, karcinom bubrežnog parenhima, polimorfizam

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List of non-standard abbreviations: GST – glutathione S-transferase; RCC – renal cell carcinoma; OR – odds ratio, CI – confidence interval

Introduction

Renal cell carcinoma (RCC) represents about 3% of all tumours in adults, with an increasing incidence in Europe (1) as well as in the United States (2). The etiology of RCC remains unclear, though epidemiological studies indicate that obesity (3), high blood pressure (4), smoking (5) and occupational exposure to chemicals (such as organic solvents, metals and pesticides) (6–8) may play a role in the development of this tumour.

Cytosolic glutathione *S*-transferases (GSTs) are a superfamily of enzymes that protect normal cells by catalyzing the conjugation of electrophilic compounds, including carcinogens, to glutathione, which decreases their toxicity and facilitates their excretion from the body (9). Cytosolic GSTs are divided into several classes on the basis of their primary structure (9). The best characterized classes have been named alpha (GSTA), mu (GSTM), pi (GSTP) and theta (GSTT); each class contains several isoenzymes. Several types of allelic variation have been identified within classes, with that in the genes *GSTM1* and *GSTT1* having the most clinical significance. Individuals homozygous for the *GSTM1*0* and *GSTT1*0* alleles (frequently referred to as *GSTM1*-null and *GSTT1*-null genotypes) exhibit loss of GSTM1 and GSTT1 enzymatic activity. The *GSTM1*-null and *GSTT1*-null genotypes are present in 50% and 11–18% of white populations, respectively (10–13).

GSTs are involved in the biotransformation of several compounds recognized as risk factors for RCC (9, 14). Namely, the carcinogenic metabolites of environmental pollutants and tobacco smoke (i.e. polycyclic aromatic hydrocarbon diol-epoxides) are detoxified by the members of GSTA, GSTM and GSTP classes (11, 15). GSTT1 substrates include halogenated solvents formed endogenously from ethane, which is also present at high levels in cigarette smoke (16). Not all reactions catalyzed by GST enzymes result in detoxification; for example, conjugation of certain compounds catalyzed by GSTT, such as *tert*-butylhydroquinone and trichloroethene, produces mutagenic species (17, 18).

As differential GST expression markedly influences the anticarcinogenic potential of tissues, GSTs are currently being investigated as biomarkers of risk for various cancers, including RCC. However, results from the previous studies of GST polymorphisms and RCC have been inconsistent (3, 19). Furthermore, according to the latest results of Karami et al. it seems that occupational exposure to pesticides is the only confirmed risk factor for RCC. The data regarding the role of GST polymorphism, independently or in combination with environmental agents, in the development of RCC, are lacking in Serbia. Therefore, the aim of the present study was to investigate whether *GSTM1* and *GSTT1* polymorphisms modify smoking and occupational exposure-related RCC risk in a small hospital-based case – control study in Belgrade, Serbia.

Materials and Methods

Study participants

Seventy six patients (mean age 60.4±12.01) with a histologically confirmed diagnosis of RCC were enrolled from the Clinic of Urology and Nephrology, Clinical Centre of Serbia, between the year of 2007 and 2009. The control group included 182 patients (mean age 56.2±11.2) with kidney stones, randomly selected from the same Clinic within the same period. The study protocol was approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade, Serbia, and was performed in conformance with the Declaration of Helsinki ethical guidelines (1983). All subjects gave their written and informed consent. Cases and controls were interviewed using a structured questionnaire identifying the socio-demographic variables, including smoking habits and occupational and medical histories. Concerning the smoking status, both cases and controls were dichotomized into »ever« and »never« groups. Regarding the exposure, both cases and controls were dichotomized into »exposed« and »unexposed«. The exposed group comprised subjects occupationally exposed for one year or more to at least one of the categories of agents (solvents, metals and pesticides) known to be associated with RCC.

Genotyping

The genotypes of *GSTM1* and *GSTT1* were characterized on DNA isolated from lymphocytes of whole peripheral venous blood by a commercial kit (Qiagen DNA mini kit). Genetic polymorphisms were determined by the multiplex PCR-based method of Garcia-Closas et al. (20). Three sets of primers were used to amplify a 215 bp sequence of the *GSTM1* gene, a 312 bp sequence of the *CYP1A1* gene and a 480 bp segment of the *GSTT1* gene. Presence or absence of the *CYP1A1* lane has been used as an inner control for failed PCR and its conditions. The PCR primers for *GSTM1* gene were: forward primer 5′-CTGCCCTACTTGATTGATGGG-3′ and reverse primer 5′-CTGGATTGTAGCAGATCATGC-3′, for *GSTT1* gene: forward primer 5′-TTCCTTACTGGTCCTCACATCTC-3′ and reverse primer 5′-TCACGGGATCA TGGCCAGCA-3′. Control primers for the *CYP1A1* gene were: forward primer 5′-GAACTGCCACTTCAGCTGTCT-3′ and reverse primer 5′-CAGCTGCATTTGGAAGTGCTC-3′. To determine successful amplification, the PCR products were separated by electrophoresis in TBE buffer on a 2.2% agarose gel and visualized with ethidium bromide by a UV transilluminator on 302 nm.

Statistical analysis

The chi-square test was applied to compare the distributions of the *GSTM1* and *GSTT1* genotypes and socio-demographic variables. The p value was

regarded as significant if it did not exceed 0.05. Logistic regression was used to estimate the odds ratio (OR) along with 95% confidence intervals (95% CI). Significantly increased ORs were referred to when the lower confidence interval exceeded 1.00. Also, significantly decreased ORs were referred to when the upper confidence interval did not exceed 1.00. In the analysis of the gene – smoking interaction, we used never smokers bearing either the *GSTM1* or *GSTT1* active genotype (at least one allele present) as a reference group. In the analysis of gene-occupational exposure interaction, we used non-exposed subjects (that is, those not exposed to solvents, metals and pesticides) bearing either the *GSTM1*-null or *GSTT1*-null genotype as the reference. All analyses were performed using the statistical package SPSS for Windows (Version 15.0).

Results

Demographic and clinical characteristics of the study participants are presented in *Table I*. As shown, renal cell carcinoma patients and respective controls did not differ with respect to age, sex, smoking and hypertension. However, the cases were more often occupationally exposed than the controls. The prevalence of being occupationally exposed to either organic solvents, metals or pesticides was 31.6% among the cases and 14.3% among controls (*Table I*). RCC risk was 2.8–fold elevated among the occupationally exposed subjects compared with those never exposed (*Table I*) and was not substantially altered after adjustment for BMI, age, sex and hypertension.

The distribution of *GST* polymorphisms among the cases and controls is presented in *Table II*. The

Table I Characteristics of patients with renal cell carcinoma and controls.

	RCC patients n (%)	Controls n (%)	OR	95% CI	P trend
Gender					
Male	48 (63.2%)	103 (56.6%)	1.0		
Female	28 (36.8%)	79 (43.4%)	0.76	0.44–1.32	> 0.05
BMI					
18.51 ≤ 24.9	32 (42.1%)	76 (41.7%)	1.00		
25 ≤ 29.9	33 (43.4%)	72 (39.6%)	1.09	0.61–1.95	
≥30	9 (11.8%)	32 (17.6%)	0.67	0.29–1.56	> 0.05
Smoking status					
Never	35 (46.1%)	82 (45.1%)	1.00		
Ever	41 (53.9%)	100 (54.9%)	0.96	0.56–1.64	> 0.05
Blood pressure					
Normal	54 (71.1%)	146 (80.2%)	1.00		
Increased	22 (28.9%)	36 (19.8%)	1.65	0.89–3.2	> 0.05
Ocupational exposure*					
No	52 (68.4%)	156 (85.7%)	1.00		
Yes	24 (31.6%)	26 (14.3%)	2.77	1.46–5.24	0.01

RCC – renal cell carcinoma*; BMI* – body mass index
* Exposed to solvents, metals and pesticides

Table II Distribution of *GSTM1* and *GSTT1* genotype in patients with RCC and controls.

GST status	Cases n (%)	Controls n (%)	OR (95%CI)
GSTM1			
GSTM1 active (present)[a]	30 (39.5%)	96 (52.8%)	1.0 (reference group)
GSTM1 inactive (null)[b]	46 (60.5%)	86 (47.2%)	1.88 (1.06–3.33)
P – trend [c]			0.031
GSTT1			
GSTT1 active (present)[a]	55 (72.4%)	130 (71.4%)	1.0 (reference group)
GSTT1 inactive (null)[b]	21 (27.6%)	52 (28.6%)	1.49 (0.66–3.34)
P – trend[c]			0.813

Adjusted for age, sex, smoking and occupational exposure.
[a]Active (present), if at least one active allele is present.
[b]Inactive (null), if no active alleles are present.
[c]P-value for interaction using the likelihood ratio test.
OR – odds ratio, *CI* – confidence interval

main effect for RCC risk associated with the null compared with the active *GSTM1* genotype was statistically significant for the *GSTM1* genotype (OR: 1.88; 95% CI: 1.06–3.33) (*Table II*). However, no significant effect was observed for the association between *GSTT1* genotype and RCC risk (OR: 1.49; 95% CI: 0.66-3.34) (*Table II*).

Although smoking was not associated with increased risk of RCC in this study, the combined effect of smoking and both *GST* genotypes was analyzed due to the fact that carcinogenic components of tobacco smoke are metabolized via the *GSTM1* or *GSTT1* pathway. The joint effects of smoking and both *GST* genotypes are shown in *Table III*. No associations were observed between smoking and the *GSTM1* or *GSTT1* genotype regarding RCC risk.

Table IV shows the joint effects of both *GST* genotypes and occupational exposure on RCC risk.

Table III Combined effect of smoking and *GSTM1* or *GSTT1* genotype on risk of RCC.

GST and smoking status	Cases n (%)	Controls n (%)	OR (95%CI)
GSTM1			
GSTM1 active (present) / non-smokers	18 (23.7%)	36 (19.8%)	1.00 (reference group)
GSTM1 inactive (null) / non-smokers	17 (22.3%)	46 (25.2%)	0.78 (0.33–1.88)
GSTM1 active (present) / smokers (ever)	12 (15.8%)	60 (33%)	0.38 (0.15–0.99)
GSTM1 inactive (null) / smokers (ever)	29 (38.2%)	40 (22%)	1.49 (0.66–3.39)
P – interaction[c]			0.008
GSTT1			
GSTT1 active (present) / non-smokers	26 (34.2%)	57 (31.3%)	1.00 (reference group)
GSTT1 inactive (null) / non-smokers	9 (11.8%)	25 (13.7%)	1.03 (0.38–2.81)
GSTT1 active (present) / smokers (ever)	29 (38.2%)	73 (40.2%)	0.97 (0.49–1.91)
GSTT1 inactive (null) / smokers (ever)	12 (15.8%)	27 (14.8%)	1.03 (0.38–2.81)
P – interaction[c]			0.946

Adjusted for age, sex, and occupational exposure.
[a]Active (present), if at least one active allele is present.
[b]Inactive (null), if no active alleles are present.
[c]P – value for interaction using the likelihood ratio test.
OR – odds ratio, *CI* – confidence interval

Table IV Combined effect of occupational exposure and *GSTM1* or *GSTT1* genotype on risk of RCC.

GST and occupational exposure	Cases n (%)	Controls n (%)	OR (95%CI)
GSTM1			
GSTM1 inactive (null) / no exposure	34 (44.7%)	72 (39.6%)	1.00 (reference group)
GSTM1 active (present) / no exposure	18 (23.7%)	84 (46.2%)	0.37 (0.18–0.75)
GSTM1 inactive (null) / exposure	12 (15.8%)	14 (7.7%)	1.54 (0.62–3.84)
GSTM1 active (present) / exposure	12 (15.8%)	12 (6.6%)	1.83 (0.71–4.70)
P – interaction [c]			0.001
GSTT1			
GSTT1 inactive (null) / no exposure	17 (22.4%)	46 (25.3%)	1.00 (reference group)
GSTT1 active (present) / no exposure	35 (46%)	110 (60.4%)	0.76 (0.38–1.53)
GSTT1 inactive (null) / exposure	4 (5.3%)	6 (3.3%)	1.05 (0.21–5.18)
GSTT1 active (present) / exposure	20 (26.3%)	20 (11%)	2.48 (1.05–5.86)
P – interaction [c]			0.02

Adjusted for age, sex and smoking.
[a]Active (present), if at least one active allele is present.
[b]Inactive (null), if no active alleles are present.
[c]P – value for interaction using the likelihood ratio test.
OR – odds ratio, *CI* – confidence interval

After consideration of occupational exposure, a significantly increased risk was observed only among the occupationally exposed subjects with the *GSTT1* active genotype (OR: 2.48; 95% CI: 1.05–5.86), whereas no excessive risk was observed for the unexposed subjects with an active genotype (OR: 0.76; 95% CI: 0.38–1.53) or exposed subjects with an inactive genotype (OR: 1.05; 95% CI: 0.21–5.18) when compared with unexposed subjects with the inactive genotype. However, concerning the effect of the *GSTM1* genotype, a significant effect was obtained only for the *GSTM1* active unexposed subjects who exhibited lower risk of RCC when compared with the *GSTM1* inactive unexposed persons (OR: 0.37; 95% CI: 0.18–0.75). Occupationally exposed *GSTM1* active subjects had a 1.8-fold higher risk of RCC when compared with the *GSTM1* inactive unexposed persons. However, this effect was not statistically significant (95% CI: 0.71–4.70) (*Table IV*).

Discussion

The results of this study showed that the deletion polymorphism of *GSTM1* has an impact on RCC risk. Namely, *GSTM1*-null individuals exhibit a 1.9-fold increased risk of RCC. On the other hand, the *GSTT1* genotype modifies the risk of RCC depending on the type of exposure. Presence of the *GSTT1* active genotype was associated with increased risk of RCC in the occupationally exposed subjects when the unexposed *GSTT1*-null subjects were used as a comparison group.

The frequency of *GSTM1*-null genotypes in the control population (47.2%) in the present study was similar to what was published previously in meta-analyses and pooled analyses among Caucasians (21–23). However, the frequency of *GSTT1*-null genotype in our cohort (27.6%) was higher than the values reported among Caucasians (18.1%) (24).

Results from the previous studies of *GST* polymorphisms and RCC have been inconsistent. Our preliminary results on the effect of *GSTM1*-null genotype in a Serbian cohort of RCC patients are in contrast with the data of several investigators (25) who did not find any differences in the distribution of the *GSTM1* genotype between RCC cases and controls. Various types of cancer including lung, larynx, stomach, colon and urinary bladder are more frequent among individuals with the *GSTM1*-null genotype (26). Findings obtained in this study speculate for the role of a common variation within the *GSTM1* gene to modify the cancer risk. Additional studies with detailed information not only on specific types of exposures but also on geographical distribution of patients would be important to confirm such an assumption.

The data regarding an association between the *GSTT1* polymorphism and RCC risk are also conflicting. Our results, that the presence of an active *GSTT1* genotype modifies the risk of RCC in occupationally exposed subjects, are similar to those of Buzio et al. (3) and Karami et al. (27) who reported an increased risk of RCC in participants occupationally exposed to pesticides with active *GSTM1* and *GSTT1* genotypes. However, the USA study of 130 renal cancer cases and 505 controls found a significant increase in RCC risk among unexposed subjects with the *GSTT1*-null genotype (2). A French case-control study (173 RCC and 211 controls) did not observe an effect modified by genotype (28). The effect of *GSTT1* genotype might vary according to the patterns of exposure to environmental risk factors for RCC. As already mentioned, in individuals exposed to metals and pesticides, the *GSTT1* positive genotype was also associated with an increased risk for RCC. Brüning et al. (18) suggested that high occupational exposure to the solvent trichloroethene also increased the risk for RCC among *GSTT1* positive individuals. More recent investigation by the same group could not confirm these findings. These inconsistencies may be attributable to several aspects of design, such as sample size or misclassification of exposures. All studies to date, including ours, have been underpowered to observe the main effects and interactions and have failed to identify the exact types of occupational exposures.

The finding that elevated RCC risk was only observed among individuals with active *GST* genotypes is biologically plausible. The GST enzyme is required for the metabolism of some groups of compounds through GST conjugation and excretion (28, 29). Generally, conjugation of foreign compounds with glutathione leads to the formation of less reactive products that are readily excreted. However, in specific tissues and with certain exposures, the glutathione conjugate is more reactive than the parent compound and there is evidence that this is particularly true in the kidney (11, 30). For halogenated compounds, in particular, the glutathione conjugate mediated by the GST serves as a substrate for a subsequent enzyme, renal cysteine conjugate beta-lyase (31). Metabolism occurs in the kidney and has been shown to form reactive chlorothioketones that are directly damaging to the kidney. Therefore, an active GST enzyme will be required to conjugate substrates and form more reactive intermediates that directly damage kidney tissues. Conversely, the deleted variant *GST* genotype will form an inactive enzyme, and therefore the metabolism of halogenated compounds will occur through oxidation, without the formation of reactive intermediates in the kidney (11, 32–35). The same mechanism of GSTT1 dependent bioactivation might be responsible for increased risk of upper urothelial tumors and urinary bladder tumors (36).

In conclusion, the results of this study suggest that occupational exposures may increase RCC risk. Active *GSTT1* variant significantly modified the RCC risk among participants occupationally exposed to organic solvents, metals and pesticides, whereas the active *GSTM1* genotype significantly decreased the RCC risk among unexposed subjects. Additional studies with

detailed information on specific types of exposures and larger numbers of exposed subjects will be important to replicate and extend these findings.

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Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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