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INFLUENCE OF *GSTT1* AND *GSTM1* NULL GENOTYPES ON DIFFERENTIATED THYROID CANCER RISK AND BASELINE AND RADIOIODINE INDUCED CYTOGENETIC DAMAGE IN PERIPHERAL BLOOD LYMPHOCYTES OF PATIENTS

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As it is known that genetic polymorphisms of glutathione S-transferases (GST) have been associated with a variety of human diseases including cancer, we have analyzed the impact of *GSTT1* and *GSTM1* null genotypes on the risk of development of differentiated thyroid cancer (DTC) and cytogenetic changes in peripheral blood lymphocytes of DTC patients before and after radioiodine therapy. The polymorphism of *GSTT1* and *GSTM1* genes were genotyped using multiplex polymerase chain reaction (PCR) and cytokinesis - block micronucleus (MN) assay to assess cytogenetic changes. *GSTT1* and *GSTM1* null were predominantly found in patients, but statistical significance was observed only for *GSTT1* null. The null genotypes increased risk of development of DTC; *GSTT1* null a 4.5 times ($p < 0.05$), *GSTM1* null about 3 times but on the border of statistical significance ($p = 0.057$), while combination of dual null genotypes almost 7 times ($p < 0.05$) increased risk. No significant effects of the null genotypes as well as their interactions with potential modifiers of MN (diagnose, age, gender and smoking habits) were observed on both baseline and radioiodine-induced values of MN and cytokinesis block proliferation index (CBPI) in DTC patients. Results suggest that both *GSTT1* and *GSTM1* null genotypes increased risk for DTC but to a greater extent *GSTT1* null. Null genotypes

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of *GSTT1* and *GSTM1* did not have potential to influence baseline and radioiodine-induced values of MN and CBPI, so that absence of *T1* and *M1* isoenzymes did not cause increased mutagen sensitivity of PBLs of DTC patients.

*Keywords***:** differentiated thyroid cancer, *GSTT1* and *GSTM1* null genotypes, micronuclei, lymphocytes, radioiodine

INTRODUCTION

Thyroid cancer is the most common endocrine cancer (JEMAL *et al.*, 2010). Incidence of this cancer continuously increases, which can be associated with greater exposure to numerous agents including the first exposure to ionizing radiation as well as numerous other agents which may act as genotoxic carcinogens (PELLEGRITI *et al.*, 2013). Numerous epidemiological studies examined the effect of ethic, geographic and dietary factors on the risk of thyroid cancer. Studies aimed at diet and lifestyle provided controversial results because these factors may change in the same individual over time (MACK *et al.*, 2003); interestingly, there are studies that showed a reduction of thyroid cancer risk in women smokers (KREIGER and PARKES, 2000; STRANGE *et al.*, 2000).

Differentiated thyroid cancers (DTCs) derive from follicular cells of the thyroid gland and include papillary and follicular cancers (BOYCE, 1995). Initial treatment of these patients includes total or near total thyroidectomy and radioiodine therapy (SCHLUMBERGER and SHERMAN, 2012). In the previous study using the micronucleus (MN) assay we demonstrated cytogenetic damage in lymphocytes in DTC patients before and after radioiodine therapy in comparison to healthy controls ($p < 0.001$). We also demonstrated that baseline cytokinesis block proliferation index (CBPI) in the patients were significantly lower than in controls ($p < 0.05$) and that after radioiodine therapy these values further reduced ($p < 0.001$) (VRNDIĆ *et al.*, 2013).

The role of MN as the intermediate endpoint of carcinogenesis occurs in a number of published papers, which showed a significant increase in MN frequencies (VARGA *et al*., 2006; BONASSI *et al.*, 2007; MILOŠEVIĆ-DJORDJEVIĆ *et al*., 2010). Recent studies have shown that changes in both metabolic capacity and DNA repair system can significantly change the sensitivity of individuals on the effect of genotoxic agents and thus significantly increase the risk of cancer (HO *et al.*, 2006; XIANG *et al*., 2012).

Glutathione S-transferase (GST) system consists of a large group of detoxifying enzymes involved in the metabolism of xenobiotics (JANACOVA *et al.*, 2010) whose activity is reflected in the catalyzing the glutathione conjugation of genotoxic compounds with electrophilic functional groups to prevent adduct formation and thus protect cells from DNA damage (MANNERVIK, 1985).

In humans, GSTs have been grouped into eight classes of isoenzymes Alpha, Mu, Pi, Sigma, Omega, Theta, Zeta and Kappa and each class can include several genes (PARL, 2005). Null genotypes of *GSTT1* and *GSTM1* genes result in total loss of enzyme activity and reactive metabolites will not detoxify and can act with DNA.

Many studies have investigated the effects of *GSTT1* and *GSTM1* null on cancer risk, but the results are controversial. So, JOSEPH *et al.* (2004) reported increased frequencies of *GSTT1* and *GSTM1* null genotypes in the children with acute lymphoblastic leukemia and CHACKO *et al.* (2005) reported similar results in the breast cancer patients. JAIN *et al.* (2006) analyzing association of *GSTT1* and *GSTM1* polymorphisms and environmental exposures on susceptibility for development of esophageal cancer in North Indian population indicated that deletions of

studied genes were not associated with higher risk for esophageal cancer, but interaction of smoking or alcohol and *GSTM1* null genotype moderately increased risk. An association of the *GSTT1* and *GSTM1* null genotypes and significantly increases the risk only for DTC but not for benign thyroid tumors, reported by HO *et al.* (2006).

We hypothesized that limited ability for detoxification of carcinogens due to absence of *GSTT1* and *GSTM1* isoenzymes causes increased sensitivity of cells to the mutagen effects of administered therapy. The aim of this study was to examine the association of total deletions (null) of *GSTT1* and *GSTM1* genes polymorphisms with DTC and cytogenetic damage in peripheral blood lymphocytes (PBLs) of DTC patients before and after radioiodine therapy treatment.

MATERIALS AND METHODS

Study population

The study population included 26 well-DTC patients of mean age 52.04 ± 14.18 years and 33 control subjects of mean age 45.91 ± 11.4 years (Table 1). All patients after thyroidectomy were treated at the Nuclear Medicine Department of the Clinical Center Kragujevac with fixed nominal activities of 3.7 GBq (100mCi) of orally administered radioiodine according to EANM guidelines (LUSTER *et al*., 2008). The controls were colleagues who were free of any kind of malignancy and who had not been exposed to radioactive sources or other known genotoxic agents of 3 months before. Peripheral blood samples were obtained twice from the patients, before and after radioiodine treatment, while blood samples from controls were taken only once. After collecting, blood samples were kept at 4^{o}C and for genotyping were stored at -20^{o}C until use. The study was approved by the Ethical Committee of the Clinical Centre Kragujevac. All patients and control subjects gave written informed consent according to the Helsinki Declaration.

Table 1. General characteristics of the study populations

Characteristics	DTC patients	Controls
No of subjects	26	33
Mean age (± S.D.)	52.04 ± 14.18	45.91 ± 11.40
Gender male/female	8/18	5/28
Smoking habit smokers/nonsmokers	15/11	15/18
Dietary habits yes/no	0/26	0/33
Radioiodine dose	3.7 GBq/26	0/33

DNA isolation and genotyping of GSTT1 and GSTM1 genes

Genomic DNA was isolated from 350 µl of whole blood using the EZI DNA Blood 350 µl Kit (Qiagen, Hilden, Germany) and BioRobot EZ1, following the manufacturer's instructions.

The genetic polymorphism analysis for the *GSTT1* and *GSTM1* genes was determined using the multiplex polymerase chain reaction (PCR) method described by ABDEL-RAHMAN *et al.* (1996) with some modifications. For detection of *GSTT1* gene polymorphism the amplified

product is 480 base pairs in length, while absence of this product is indicative of the deletion. For detection of the *GSTM1* gene polymorphism the amplified product is 215 base pairs in length, and absence of this product is indicative of the deletion. Positive control was exon 7 of *CYP1A1* and the amplified product is 312 base pairs in length.

The DNA products were amplified with primers (Invitrogen, California, USA):

5' - TTCCTTACTGGTCCTCACATCTC- 3' and 5'-TCACCGGATCATGGCCAGCA-3' for *GSTT1*;

5'-GAACTCCCTGAAAAGCTAAAGC-3' and 5' - GTTGGGCTCAAATATACGGTGG - 3' for *GSTM1*;

5' - GAACTGCCACTTCAGCTGTCT- 3' and 5' - CAGCTGCATTTGGAAGTGCTC - 3' for exon 7 of *CYP1A1*.

Each PCR reaction (50 µl) contained 30 pmol of each primers (*GSTT1*, *GSTM1* and *CYP1A1*), 200 µM of each deoxynucleotide triphosphate (dNTP, Invitrogen, California, USA), 1.5 mM of $MgCl_2$, 2 units of Taq polymerase (Invitrogen, California, USA), 50 µg of genomic DNA and 5% dimethyl sulfoxide (DMSO).

Amplification consists of initial denaturation at 94°C for 5 minutes, followed by 35 cycles at 94°C for 2 minutes, 58°C for 1 minute and 72°C for 1 minute. This was followed by a final extension step at 72°C for 10 minutes. PCR products were visualized on 2% agarose gel containing SYBR Safe DNA gel stain (Invitrogen, California, USA).

Cytokinesis-block micronucleus (MN) assay

Cytokinesis - block micronucleus (MN) assay was carried out using the procedure described by FENECH (2000). Whole heparinized blood (0.5 ml) was added to 5 ml of the RPMI medium contains fetal bovine serum, L-glutamine and phytohemaglutinin (complete GIBCO ™PB-Max™ karyotyping medium; Invitrogen, California, USA). Cultures were incubated at 37°C for 72 hrs. On the forty fourth hour after the beginning of incubation, cytochalasin B (Sigma, St. Louis, MO, USA) was added in the final concentration of 4 µg/ml. Cultures were harvested 28 hrs later. The cells were collected by centrifugation, treated with cold (4°C) hypotonic solution (0.56% KCl) and fixed with fixative methanol: glacial acetic acid = 3:1. The cell suspensions were dropped onto clean slides, lamp-dried and stained with 2% Giemsa (Alfapanon, Novi Sad, Serbia).

The analysis of MN was performed using a light microscope (Nikon E50i) at 400x magnification following the criteria for MN scoring in binucleated (BN) cells only, described by FENECH (2007). One thousand BN cells per subject examined for MN scoring and one thousand viable cells from each subject were scored to determine the number of cells with 1, 2, 3 and 4 nuclei for calculation of cytokinesis-block proliferation index (CBPI). CBPI was calculated using the formula CBPI = (M1 + [2 x M2] + 3 x [M3 + M4])/ N, where M1 - M4 are the numbers of cells with 1 to 4 nuclei and N is the total number of viable scored cells (FENECH, 2007).

Statistical analyses

The commercial SPSS version 16.00 for Windows was used for statistical analyses. For comparing distribution of *GSTT1* and *GSTM1* genotypes the chi-square (χ^2) or Fischer's (F) exact test were used. The Odds Ratio (OR) with 95% confidence interval (CI) calculated by binary logistic regression analyses was used to illustrate association between *GSTT1* and *GSTM1* null genotypes and DTC risk. All values for MN and CBPI were expressed as mean ± standard

deviation (S.D.). Student' s *t*-test was employed for comparison of mean values of baseline and radioiodine-induced cytogenetic biomarkers in patients. Additionally, multiple regression analyses were performed for analyzing the influence of variables such as diagnose, age, gender, smoking habits and GSTT1 null and GSTM1 null genotypes on MN and CBPI values. Probability less than 0.05 was set up as significant.

RESULTS AND DISCUSSION

Table 2 shows summarized distributions of both GST genes *T1* and *M1*, individually and in combinations, in DTC patients and controls. *GSTT1* and *GSTM1* null genotypes were predominantly found in DTC patients (38.5% and 61.5% in DTC patients vs. 12.1% and 36.4% in controls), but statistical significance was observed only for *GSTT1* null ($p < 0.05$). The *GSTT1* null genotype showed an increased risk for DTC (OR = 4.53, 95% CI = 1.22 – 16.8, $p < 0.05$) while *GSTM1* null increased risk for DTC, but on the border of statistical significance (OR = 2.8, 95% CI = 0.97 – 8.09, $p = 0.057$).

Table 2. Distribution of GSTT1 and GSTM1 genotypes in control subjects and DTC patients

Genotypes	Controls	DTC patients	*p*	OR	CI (95%)	*p*
GSTT1						
positive	29 (87.90%)	16 (61.50%)	0.018*[a]	4.53	1.22 - 16.80	0.024
null	4 (12.10%)	10 (38.50%)				
GSTM1						
positive	21 (63.60%)	10 (38.50%)	0.055*	2.80	0.97 – 8.09	0.057
null	12 (36.40%)	16 (61.50%)				
GSTT1/GSTM1						
positive/positive	20 (60.60%)	6 (23.10%)	0.009*[a]			
positive/null	9 (27.30%)	10 (38.50%)	0.09*	3.70	1.03 – 13.35	0.045[b]
null/positive	1 (3.00%)	4 (15.40%)	0.16**	13.33	1.24 – 143.15	0.032[b]
null/null	3 (9.10%)	6 (23.10%)	0.41**	6.67	1.27 – 35.04	0.025[b]

*statistical significance of differences in the frequency was assessed applying Pearson Chi-Square test to compare between controls and patients

**statistical significance of differences in the frequency was assessed applying Fisher's exact test to compare between controls and patients

[a] statistically significant difference in the frequency of GSTT1 null genotype between controls and patients ($p < 0.05$)

[b] statistically significant risk for DTC with the combinations of one and dual null genotypes in comparison to GSTT1p/GSTM1p genotype ($p < 0.05$)

Considering combinations of *GSTT1* and *GSTM1* genotypes about 23% of patients and 61% of controls had dual *GSTT1*/*GSTM1* positive combination, which was a significant difference ($p < 0.001$). There were no significant differences in the frequency of *GSTT1*positive/*GSTM1*null, *GSTT1*null/*GSTM1*positive and dual *GSTT1*/*GSTM1* null combinations between DTC patients and controls ($p > 0.05$), but the total of combinations with one and dual null genotypes was significantly higher in patients than in controls (76.9% vs. 39.4%, $p < 0.05$). Using the dual *GSTT1*/*GSTM1* positive combination as reference, there were significantly risk for DTC with combinations with one or both null *T1* and *M1* genotypes (OR =

3.7, 95% CI = 1.03 – 13.35, $p < 0.05$; OR = 13.33, 95% CI = 1.24 – 143.15, $p < 0.05$; OR = 6.67, 95% CI =1.27 – 35.04, $p < 0.05$).

Table 3 summarizes the influences of *GSTT1* and *GSTM1* genotypes on both values MN and CBPI in DTC patients. The MN frequencies and CBPI values were stratified relevant to status of genotypes. No significant effects of *GSTT1* and *GSTM1* null genotypes, alone and in combinations with one and dual null, were observed on baseline and radioiodine-increased MN frequencies. According to the genotype status on CBPI our results showed that neither of analyzed null genotypes, alone and in combinations, influenced the baseline and therapy-decreased CBPI values.

Table 3. Distribution of studied cytogenetic parameters in regards to status of GSTT1 and GSTM1 genotypes

	MN±S.D.				CBPI±S.D			
	before therapy	*p*	after therapy	*p*	before therapy	*p*	after therapy	*p*
GSTT1								
Positive	20.00±7.39		29.00±9.57		1.49±0.16		1.33±0.09	
Null	16.90±7.52	>0.05	22.90±7.72	>0.05	1.53±0.23	>0.05	1.43±0.22	>0.05
GSTM1								
Positive	18.60±7.09		26.00±9.40		1.48±0.13		1.35±0.08	
Null	19.50±6.71	>0.05	27.06±9.44	>0.05	1.52±0.22	>0.05	1.38±0.19	>0.05
GSTT1/GSTM1								
positive/positive	18.33±8.96	(ref)	26.50±11.62	(ref)	1.51±0.15	(ref)	1.33±0.08	(ref)
positive/null	21.30±5.81	>0.05	30.50±8.42	>0.05	1.49±0.17	>0.05	1.33±0.10	>0.05
null/positive	19.00±4.08	>0.05	25.25±6.24	>0.05	1.45±0.08	>0.05	1.39±0.05	>0.05
null/null	16.50±7.56	>0.05	21.33±8.76	>0.05	1.59±0.29	>0.05	1.47±0.28	>0.05

Table 4. Results of multiple regression analysis for micronucleus (MN) frequency including GSTT1and GSTM status and potential modifiers of MN

	Unstandardized coefficients		Standardized coefficients		
	B	S.E.	B	t	Sig.
MN before therapy					
Constant	1.258	5.734		.219	.827
diagnose	12.704	1.357	.785	9.360	< 0.001
GSTT1	.484	1.380	-.0286	.351	.727
GSTM1	. 298	1.249	.019	. 239	.812
age	.075	.054	.119	1.400	.166
gender	1.415	1.581	.070	.895	.374
smoking	-.583	1.182	-.037	-.493	.623
MN after therapy					
Constant	9.496	7.085		1.340	.196
GSTT1	-2.760	1.581	-.148	-1.746	.097
GSTM1	-.521	1.569	-.028	-.332	.743
age	-.032	.056	-.049	-.583	.567
gender	-.429	1.557	-.022	-.275	.786
smoking	.138	1.621	.008	.085	.933
MN before Th	1.253	.121	.912	10.354	< 0.001

The multiple regression analyses for MN and CBPI in DTC patients which included interactions of *GSTT1* and *GSTM1* null genotypes with variables diagnose, age, gender and smoking habits (Tables 4 and 5), showed that both baseline and radioiodine-induced values of MN and CBPI in DTC patients were not affected by *GSTT1* and *GSTM1* null genotypes. Namely, the therapy-increased MN frequencies were associated only with baseline MN (Table 4), while the therapy-decreased CBPI values were affected by interaction between gender, smoking habits and baseline CBPI (Table 5).

Table 5. Results of multiple regression analysis for citokinesis-block proliferation index (CBPI) including GSTT1 and GSTM1 status and potential modifiers of CBPI

	Unstandardized coefficients		Standardized coefficients		
	B	S.E.	B	t	Sig.
CBPI before therapy					
Constant	1.830	.241		7.579	.000
diagnose	-.104	.044	-.327	-2.346	< 0.05
GSTT1	-.005	.050	-.012	-0.092	.927
GSTM1	-.049	.046	-.154	-1.065	.292
age	-.035	.042	-.111	-0.822	.415
gender	-.036	.051	-.095	-0.703	.485
smoking	.048	.041	.152	1.168	.248
CBPI after therapy					
Constant	.665	.197		3.368	.003
GSTT1	.113	.027	.356	4.175	.180
GSTM1	-.042	.027	-.132	-1.651	.136
age	-.037	.027	-.121	-1.396	.180
gender	-.076	.029	-.228	-2.604	< 0.05
smoking	.070	.026	.225	2.645	< 0.05
CBPI before Th	.593	.068	.716	8.676	< 0.001

DISCUSSION

Numerous literature data suggested importance of GST genes in defense against endogenously agents (CHEN *et al.*, 2010; KUMAR *et al.*, 2011) and that their mutations could associate with different diseases (MANFREDI *et al.*, 2007; MUSTAFA *et al.*, 2010) especially with cancer (ATES *et al.*, 2005; HO *et al*., 2006; SINGH *et al.*, 2008).

In this study we investigated the effects of *GSTT1* and *GSTM1* null genotypes on the risk of development of DTC and both baseline and radioiodine-induced cytogenetic damage in PBLs of DTC patients.

The study of the individual roles of *GSTT1* and *GSTM1* genes revealed significantly higher frequency of *GSTT1* null genotype in DTC patients than in controls (38.5% vs. 12.1%). We found that the *GSTT1* null genotype was significantly associated with high risk of DTC (OR = 4.53, $p < 0.05$). These findings are consistent with the report of SIRAJ *et al.* (2008) who concluded that the *GSTT1* null genotype showed a 3.48 times higher risk of DTC. However, although a greater percentage of patients had *GSTM1* null genotype, this genotype on the border of statistical significance was associated with almost three times increased risk for DTC (OR = 2.80, $p = 0.057$). Another thyroid cancer studies showed that only combination with dual *GSTT1*/*GSTM1* null genotype significantly increased the risk for thyroid cancer (MORARI *et al.*,

2002; HO *et al.*, 2006). Our results showed that all of the combinations with one or dual null genotype significantly increased the risk for DTC and that the individuals with dual null *GSTT1*/*GSTM1* genotypes had an almost 7-fold increased risk for DTC. DTC patients had significantly higher frequency of combinations with one null or dual null genotypes than controls ($p < 0.05$), whereas the frequency of combination with dual positive genotypes was significantly higher in controls than in patients ($p < 0.05$).

The role of genetic factors in the research of susceptibility to carcinogen effects of various agents has been the subject of many studies and micronuclei (MN) frequency is the most frequently used endpoints (BONASSI *et al.*, 2005). Some studies demonstrated the increase of baseline MN levels in *GSTT1* null subjects. Thus KADIOGLU *et al.* (2012) evaluated the relationship between chromosomal aberrations (CA), CBMN, and modified comet assay and metabolic GST genes, as well as mutagen sensitivity in cultured lymphocytes of healthy volunteers. They showed that the *GSTT1* null genotype significantly elevated baseline levels of CA and MN in respect to the positive genotype, and that *GSTM1* null and *GSTP1* mutant genotypes did not result in any differences in studied cytogenetic parameters. Similarly, TUIMALA *et al.* (2004) reported that *GSTT1* null genotype was associated with significant increase in baseline frequency of CA in unexposed individuals. On the contrary, HERNANDEZ *et al.* (2006) studied the association between GSTs (*T1*, *M1*, *P1*) and MN frequency in lymphocytes of thyroid cancer patients, reported that both baseline and increased MN frequency after therapy exposure were not associated with the GST genotypes.

Our results did not show the association between *GSTT1* and *GSTM1* null genotypes and both baseline and therapy-increased MN frequencies in PBLs of patients. Analyzing the association between CBPI values and GST polymorphisms, we concluded that both baseline and decreased CBPI values after therapy were not associated with *GSTT1* and *GSTM1* null genotypes.

Numerous studies showed that genetic instability of cells was based on the state of health and a causal association between MN frequency and a cancer risk (BONASSI *et al.*, 2007; VARGA *et al.*, 2006; MILOŠEVIĆ-DJORDJEVIĆ *et al.*, 2010).

We do not know of the previously reported data about the associated GST polymorphisms and radioiodine therapy-induced cytogenetic parameters except for one study, done by HERNANDEZ *et al.* (2006). The first report of the association between genetic polymorphism and cytogenetic response to genotoxic exposure *in vivo* was given by VAN POPPEL *et al.* (1992) who reported that slightly increased SCEs in heavy smokers was not expressed in *GSTM1* genotype. On the other hand, the same author (VAN POPPEL *et al.*, 1994) analyzed the MN frequency in sputum cells of smokers and concluded that this frequency was not influenced by *GSTM1*. More recent studies on occupationally or environmentally exposed individuals showed that higher MN frequency was associated with *GSTT1* null genotype (LAFFON *et al.*, 2006; KUMAR *et al.*, 2011). However, KIRSCH-VOLDERS *et al.* (2006) reported lower MN frequency in the occupationally exposed individuals with *GSTT1* and *GSTM1* null genotypes. Our results showed that patients with *GSTT1* null genotype had lower baseline and radioiodine-induced MN frequencies in comparison to their positive counterparts, but without statistical significance. On the contrary, patients with *GSTM1* null genotype had no significantly higher baseline and therapy induced MN frequencies in respect to the positive genotype.

When diagnose, age, gender and smoking habits implicated as the potential modifiers of MN frequency (BONASSI *et al.*, 2003; MILOŠEVIĆ-DJORDJEVIĆ *et al.*, 2005) in interacting with genotypes, we also found that *GSTT1* and *GSTM1* null genotypes were not associated with both

values MN and CBPI in PBLs of DTC patients before and after therapy. Multiple regression analyses showed that both baseline values, for MN significantly higher and for CBPI significantly lower than in controls (VRNDIĆ *et al.*, 2013), were associated only with diagnose (health condition), while increased values of MN after therapy were associated only with baseline values. On the contrary, CBPI values after therapy decreased and this decrease associated with interaction between genders, smoking habits and baseline CBPI.

The number of DTC patients analyzed in this study (n = 26) is a consequence of a relatively low incidence of thyroid carcinomas, which represent 1-2% of all malignant tumors (SIPOS and MAZZAFERRI, 2010) and criteria for their inclusion in the study (COOPER *et al*., 2009; SCHLUMBERGER *and* SHERMAN, 2012). Namely, in this study we analyzed only patients selected for radioiodine therapy. According to the American Thyroid Association (ATA) recommendations, radioiodine therapy is indicated in patients with moderate to high risk of recurrence based on age, tumor size, lymph node status, extra-thyroidal extension, and the histological type of the thyroid tumor. The association of *GSTT1* and *GSTM1* polymorphisms with both DT carcinomas and cytogenetic damage in PBLs of patients (baseline and induced) was demonstrated for the first time. This is especially important if one bears in mind that the incidence of DTCs is increased during last 30 years (DAVIES and WELCH, 2006; GRINIATSOS *et al.*, 2009; KOLFOY *et al*., 2009).

CONCLUSION

The general conclusion is that the presence of the *GSTT1* and *GSTM1* null genotypes is associated with an increased risk for development of DTC, but *GSTT1* null to a greater extent. Cytogenetic changes in PBLs of DTC patients before and after radioiodine therapy (MN frequency and CBPI) were not under the influence of *GSTT1* and *GSTM1* null genotypes (alone or in their combinations), so that the absence of *T1* and *M1* isoenzymes did not cause increased mutagen sensitivity of PBLs of DTC patients.

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REFERENCES

ABDEL-RAHMAN, S.Z., R.A. EL-ZEIN, W.A. ANWAR, W.W. AU (1996): A multiplex PCR procedure for polymorphic analysis of GSTM1 and GSTT1 genes in population studies. Cancer Lett., *107*(2):229-233.

ATES, N.A., L. TAMER, C. ATES, B. ERCAN, T. ELIPEK, K. OCAL, H. CAMDEVIREN (2005): Glutatione S-transferase M1, T1 P1 genotypes and risk for development of colorectal cancer. Bioch. Genet., *43*(3-4):149-163.

BONASSI, S., M. NERI, C. LANDO, M. CEPPI, Y.P. LIN, W.P. CHANG, N. HOLLAND, M. KIRSCH-VOLDERS, E. ZEIGER, M. FENECH, HUMN COLLABORATIVE GROUP (2003): Effect of smoking habit on the frequency of micronuclei in human lymphocytes: results from the Human MicroNucleus project. Mutat. Res., *543*(2):155-166.

BONASSI, S., S. BONASSI, D. UGOLINI, M. KIRSCH-VOLDERS, U. STRONBERG, R. VERMEULEN, J.D. TUKER (2005): Human population studies with cytogenetic biomarkers: review of the literature and future prospective. Environ. Mol. Mutagen., *45*(2-3):258-270.

BONASSI, S., A. ZNAOR, M. CEPPI, C. LANDO, W.P. CHANG, N. HOLLAND, M.KIRSCH-VOLDERS, E. ZEIGER, S. BAN, R. BARALE, M.P. BIGATTI, C. BOLOGNESI, A. CEBULSKA-WASILEWSKA, E. FABIANOVA, A. FUCIC, L. HAGMAR, G. JOKSIC, A. MARTELLI, L. MIGLIORE, E. MIRKOVA, M.R. SCARFI, A. ZIJNO, H. NORPPA, M. FENECH (2007): An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. Carcinogenesis, *28*(3):625-631.

BOYCE, V. (1995): Differentiated thyroid cancer papillary and follicular carcinomas. Radiology, *195*:100.

CHACHO, P., T. JOSEPH, B.S. MATHEW, B. RAJAN, M.R. PILLAI (2005): Role of xenobiotic metabolizing gene polymorphisms in breast cancer susceptibility and treatment outcome. Mutat. Res.**,** *581*(1-2):153-163.

CHEN, L., H. GUO, J. YUAN, M. HE, D. CHEN, J. SHI, J. YANG, Y. BAI, Y. JU, A. LIU, Z. YU, L. LI, G. SHENG, J. FU, T. WU, X. CHEN (2010): Polymorphisms of GSTT1 and GSTM1 and Increased micronucleus frequencies in peripheral blood lymphocytes in residents at an e-waste dismantling site in China. J. Environ. Sci. Health Tox. Hazard Subst. Environ. Eng., *45*(4):490-497.

COOPER, D.S., G.M. DOHERTY, B.R. HAUGEN, R.T. KLOOS, S.L. LEE, S.J. MANDEL, E.L. MAZZAFERRI, B. MCIVER, F. PACINI, M. SCHLUMBERGER, S.I. SHERMAN, D.L. STEWARD, R.M. TUTTLE (2009): Revised American thyroid association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid, *19*:1167–1214.

DAVIES, L. and H.G. WELCH (2006): Increasing incidence of thyroid cancer in the United States 1973-2002. JAMA, *295*:2164-2167.

FENECH, M. (2000): The in vitro micronucleus technique. Mutat. Res., *455*(1-2):81-95.

FENECH, M. (2007): Cytokinesis-block micronucleus cytome assay. Nat. Protoc., *2*:1084-1104.

GRINIATSOS, J., C. TSIGRIS, M. KANAKIS, G. KALTSAS, O. MICHAIL, N. DIMITRIOU, G. ARGYRAKOPOULOU, I. DELLADETSIMA, V. KYRIAKOU, V. SYRIOU, K. ALEXANDRAKI, E. PIKOULIS, A. GIANNOPOULOS, G. KOURAKLIS, E. DIAMANTI-KANDARAKI, E. FELEKOURAS (2009): Increased incidence of papillary thyroid cancer detection among thyroidectomies in Greece between 1991 and 2006. Anticancer Res., *29*: 5163-5169.

HERNANDEZ, A., N. XEMENA, S. GUTIERREZ, A. VELAZGUEZ, A. CREUS, J. SURRALLES, P. GALOFRE, R. MARCOS (2006): Basal and induced micronucleus frequencies in human lymphocytes with different GST and NAT2 genetic backgrounds. Mutat. Res.**,** *606*(1-2):12-20.

HO, T., C. ZHAO, R. ZHENG, Z. LIU, Q. WEI, E.M. STURGIS (2006): Glutatione S-transferase polymorphisms and risk of differentiated thyroid carcinomas. Arch. Otolaryngol. Head Neck. Surg., *132*(7):756-761.

JAIN, M., S. KUMAR, N. RASTOGI, P. LAL, U.C. GHOSHAL, A. TIWARI, M.C. PANT, M.Q. BAIQ, B. MITTAL (2006): GSTT1, GSTM1 and GSTP1 genetic polymorphisms and interaction with tobacco, alcohol and occupational exposure in esophageal cancer patients from North India. Cancer Lett.**,** *242*(1):60-67.

JANACOVA, P., P. ANZENBACHER, E. ANZENBACHER (2010): Phase II drug metabolizing enzymes. Biomed. Pap. Med. Univ. Palacky Olomouc Czech Repub., *154*(2):103-116.

JEMAL, A., R. SIEGEL, J. XU, E. WARD (2010): Cancer statistics, 2010. CA. A Cancer J. Clin., *60*(5):277-300.

JOSEPH, T., P. KUSUMAKUMARY, P. CHACHO, A. ABRAHAM, M.R. PILLAI (2004): Genetic polymorphism of CYP1A1, CYP2D6, GSTM1 and GSTT1 and susceptibility to acute lymphoblastic leukemia in Indian children. Pediatric Blood Cancer, *43*(5):560-567.

KADIOGLU, E., N.A. KOCABAS, G.C. DEMIRCIGIL, E. COSKUN, E. OZCAGLI, E. DURMAZ, B. KARAHALIL, S. BURGAZ, S. SARDAS (2012): Assessment of individual susceptibility to baseline DNA and cytogenetic damage in a healthy Turkish population: evaluation with lifestyle factors. Genet. Test Mol. Biomarkers, *16*(10):1157-1164.

KIRSCH-VOLDERS, M., R.A. MATEUCA, M. ROELANTS, A. TREMP, E. ZEIGER, S. BONASSI, N. HOLLAND, W.P. CHANG, P.V. AKA, M. DEBOECK, L. GODDERIS, V. HAUFROID, H. ISHIKAWA, B. LAFFON, R. MARCOS, L. MIGLIORE, H. NORPPA, J.P. TEIXEIRA, A. ZIJNO, M. FENECH (2006): The effects of GSTM1 and GSTT1 polymorphisms on micronucleus frequencies in human lymphocytes in vivo, Cancer Epidemiol. Biomarkers Prev., *15*: 1038-1042.

KOLFOY, B.A., T. ZHENG, T.R. HOLFORD, X. HAN, M.H. WARD, A. SJODIN, Y. ZHANG, Y. BAI, C. ZHU, G.L. GUO, N. ROTHMAN, Y. ZHANG (2009): International patterns and trends in thyroid cancer incidence, 1973 - 2002. Cancer Causes Control, *20*: 525-531.

KREIGER N. and R. PARKES (2000): Cigarette smoking and the risk of thyroid cancer. Eur. J. Cancer**,** *36*(15):1969-1973.

KUMAR, A., A. YADAV, S.K. GIRI, K. DEV, S.K. GAUTAM, R. GUPTA, N. AGGARWAL (2011): Influence of GSTM1 and GSTT1 genotypes and confounding factors on the frequency of sister chromatid exchange and micronucleus among road construction workers. Chemosphere**,** *84*(5):564-570.

LAFFON, B., J.P. TEIXEIRA, S. SILVA, J. ROMA-TORRES, B. PÉREZ-CADAHIA, J. MÉNDEZ, E. PÁSARO, O. MAYAN (2006): Assessment of occupational genotoxic risk in the production of rubber tyres. Ann. Occup. Hyg., *50*: 583-592.

LUSTER, M., S.E. CLARKE, M. DIETLEIN, M. LASSMANN, P. LIND, W.J. OYEN, J. TENNVALL, E. BOMBARDIERI, EUROPEAN ASSOCIATION OF NUCLEAR MEDICINE (EANM) (2008): Guidelines for radioiodine therapy of differentiated thyroid cancer. Eur. J. Nucl. Med. Mol. Imaging, *35*: 1941-1959.

MACK, W.J., S. PRESTON-MARTIN, L. DAL MASO, R. GALANTI, M. XIANG, S. FRANCESCHI, A. HALLQUIST, F. JIN, L. KOLONEL, C. LA VECCHIA, F. LEVI, A. LINOS, E. LUND, A. MCTIEMAN, K. MABUCHI, E. NEGRI, G. WINGREN, E. RON (2003): A pooled analysis of case-control studies of thyroid cancer: cigarette smoking and consumption of alcohol, coffee, and tea. Cancer Causes Control, *14*(8):773-785.

MANFREDI, S., C. FEDERICI, E. PICANO, N. BOTTO, A. RIZZA, M.G. ANDREASSI (2007): GSTM1, GSTT1 and CYP1A1 detoxification gene polymorphisms and susceptibility to smoking-related coronary artery disease: a case-only study. Mutat. Res., *621*(1-2):106-112.

MANNERVIK, B. (1985): The isozymes of glutathione S-transferase. Adv. Enzymol., *57*:357-417.

MILOŠEVIĆ-DJORDJEVIĆ, O., D. GRUJIČIĆ, Ž. VASKOVIĆ, D. MARINKOVIĆ (2010): High micronucleus frequency in peripheral blood lymphocytes of untreated cancer patients irrespective of gender, smoking and cancer sites. Tohoku J. Exp. Med., *220*:115-120.

MILOŠEVIĆ-DJORDJEVIĆ, O., D. GRUJIČIĆ, S. ARSENIJEVIĆ, D. MARINKOVIĆ (2005): Monitoring among newborns from Kragujevac in Central Serbia before and after environmental contamination. Tohoku J. Exp. Med., *205*(1):1-9.

MORARI, E.C., J.L.P. LEITE, L.V.M. DA ASSUMPCAO, L.S. WARD (2002): The null Genotype of Glutathione S-transferase M1 and T1 locus increases the risk for thyroid cancer. Cancer Epidemiol. Biomark. Prev., *11*(11):1485-1488.

MUSTAFA, M.D., R. PATHAK, R.S. AHMED, A.K. TRIPATHI, K. GULERIA, B.D. BANERJEE (2010): Association of glutathione S-transferase M1 and T1 gene polymorphisms and oxidative stress markers in preterm labor. Clin. Biochem.**,** *43*(13-14):1124-1128.

PARL, F.F. (2005): Glutatione S transferase genotypes and cancer risk. Cancer Lett., *221*(2):123-129.

PELLEGRITI, G., F. FRASCA, C. REGALBUTO, S. SQUATRITO, R. VIGNERI (2013): World increasing incidence of thyroid cancer: update on epidemiology and risk factors. J. Cancer Epidemiol., DOI 10.1155/2013/965212.

SINGH, M., P.P. SHAH, A.P SINGH, M. RUWALI, N. MATHUR, M.C. PANT, D. PARMAR (2008): Association of genetic polymorphisms in glutathione S-transferases and susceptibility to head and neck cancer. Mutat. Res., *638*(1-2):184-194.

SIPOS, J.A. AND E.L. MAZZAFERRI E.L. (2010): Thyroid cancer epidemiology and prognostic variables. Clin. Oncol. (R. Coll Radiol.), *22*: 395-404.

SIRAJ, A.K., M. IBRAHIM, M. AL-RASHEED, J. ABUBAKER, R. BU, S.U. SIDDIQUI, F. AL-DAYEL, O. AL-SANEA, A. AL-NUAIM, S. UDDIN, K. AL-KURAYA (2008): Polymorphisms of selected xenobiotic genes contribute to the development of papillary thyroid cancer susceptibility in Middle Eastern population. BMC Medical Genetics, 9:61.

SCHLUMBERGER M. and S.I. SHERMAN (2012): Endocrine tumors: approach to the patient with advanced differentiated thyroid cancer. Eur. J. Endocrinol., *166*: 5-11.

STRANGE, R.C., P.W. JONES, A.A. FRYER (2000): Glutathione S-transferase: genetic and role in toxicology. Toxicol. Lett.**,** *112-113*:357-363.

TUIMALA, J., G. SZEKELY, H. WIKMAN, H. JARVENTAUS, A. HIRVONEN, S. GUNDY, H. NORPPA (2004): Genetic polymorphisms of DNA repair and xenobiotic-metabolizing enzymes: effects on levels of sister chromatid exchanges and chromosomal aberrations. Mutat. Res., *554*:319-333.

VAN POPPEL, G., N. DE VOGEL, J. VAN BALDERREN, F.J. KOK (1992): Increased cytogenetic damage in smokers deficient in glutathione S-transferase isozyme μ. Carcinogenesis, *13*: 303-305.

VAN POPPEL, G., H. VERHAGEN, P. VANT VEER, P.J. VAN BLADEREN (1994): Markers for cytogenetic damage in smokers: associations with plasma antioxidants and glutathione S-transferase μ. Cancer Epidemiol. Biom. Prev., *2*: 441-447.

VARGA, D., J. HOEGEL, C. MAIER, S. JAINTA, M. HOEHNE, B. PATINO-GARCIA, I. MICHEL, U. SCHWARZ-BOEGER, M. KIECHIE, R. KEIENBERG, W. VOGEL (2006): On the difference of micronucleus frequencies in peripheral blood lymphocytes between breast cancer patients and controls. Mutagenesis, *21*(5):313-320.

VRNDIĆ, O.B., O.M. MILOŠEVIĆ-DJORDJEVIĆ, LJ.C. MIJATOVIĆ-TEODOROVIĆ, M.Z. JEREMIĆ, I.M. STOŠIĆ, D.V. GRUJIČIĆ, S.T. ŽIVANČEVIĆ-SIMONOVIĆ (2013): Correlation between micronuclei frequency in peripheral blood lymphocytes and retention of 131-I in thyroid cancer patients. Tohoku J. Exp. Med., *229*:115-124.

XIANG, M., L. AO, H. YANG, W. LIU, L. SUN, X. HAN, D. LI, Z. CUI, N. ZHOU, J. LIU, J. CAO (2012): Chromosomal damage and polymorphisms of metabolic genes among 1,3-butadiene-exposed workers in a matched study in China. Mutagenesis, *27*(4):415-421.

UTICAJ *GSTT1* I *GSTM1* NULTIH GENOTIPOVA NA RIZIK ZA RAZVOJ DIFERENTOVANOG TIROIDNOG KARCINOMA I CITOGENETIČKA OŠTEĆENJA U LIMFOCITIMA PERIFERNE KRVI PACIJENATA PRE I NAKON TERAPIJE RADIOAKTIVNIM JODOM

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Izvod

Kako se polimorfizmi gena za glutation S-transferazu (GST) dovode u vezu sa pojavom različitih bolesti kod ljudi, uključujući i kancer, analiziran je uticaj *GSTT1* i *GSTM1* nultih genotipova na rizik za razvoj diferentovanog karcinoma štitaste žlezde (DTC) i citogenetičke promene u limfocitima periferne krvi. Polimorfizmi *GSTT1* i *GSTM1* gena detektovani su primenom multipleks lančane reakcije polimerizacije (PCR), a citogenetičke promene su analizirane primenom citokinezis-blok mikronukleus (MN) testa. *GSTT1* i *GSTM1* nulti genotipovi su češće bili zastupljeni kod pacijenata, ali sa statističkom značajnošću samo za *GSTT1* nulti gen. Oba nulta genotipa su povećavala rizik za razvoj DTC, i to *GSTT1* nulti za 4,5 puta ($p < 0,05$), *GSTM1* za oko 3 puta ali na granici statističke značajnosti ($p = 0,057$), a kombinacija oba nulta genotipa za skoro 7 puta ($p < 0,05$). *GSTT1* i *GSTM1* nulti genotipovi kao i njihove interakcije sa potencijalnim modifikatorima analiziranih citogenetičkih biomarkera (dijagnoza, godine starosti, pol, pušenje cigareta) nisu pokazali značajan efekat na bazalnu i terapijom indukovanu MN frekvencu, kao ni na vrednosti citokinezis blok proliferacionog indeksa (CBPI) kod pacijenata. Rezultati ukazuju da su oba nulta genotipa (*T1* i *M1*) povećavala rizik za razvoj DTC, ali *GSTT1* nulti u većoj meri. Oba nulta genotipa nisu pokazala značajan efekat na analizirane citogenetičke biomarkere (MN frekvencu i CBPI vrednosti) u limfocitima periferne krvi DTC pacijenata pre i nakon terapije, tako da nedostatak izoenzima *T1* i *M1* nije uzrokovao povećanu osetljivost limfocita periferne krvi DTC pacijenata na mutagena dejstva.

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