Medicinski podmladak

Medical Youth

ORIGINAL ARTICLE

THE ASSOCIATION OF *SOD2* AND *GST* GENE POLYMORPHISMS WITH THE RISK OF DEVELOPMENT AND PROGNOSIS OF PAPILLARY RENAL CELL CARCINOMA

POVEZANOST POLIMORFIZAMA *SOD2* I *GST* GENA SA RIZIKOM ZA NASTANAK I PROGNOZOM PAPILARNOG KARCINOMA BUBREŽNOG PARENHIMA

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Abstract

Introduction: Redox imbalance is an important factor in both carcinogenesis and progression of renal cell carcinoma. Numerous studies are focused on finding potential biomarkers that can aid in early detection, as well as in monitoring disease progression. Among the candidates there are genes coding for antioxidant enzymes - superoxide dismutase 2 (SOD2) and glutathione S -transferase (GST).

Aim: This study aims to assess the role of *SOD2* and *GST* genes polymorphisms as risk biomarkers for papillary renal cell carcinoma (pRCC), along with their impact on the survival of these patients.

Material and methods: This study included 39 patients and 336 controls. The following polymorphisms were determined by appropriate PCR methods: *SOD2* (*rs4880*), *GSTA1 C69T*, *GSTM1*, *GSTT1*, and *GSTP1* (*rs1695*) . ELISA method was used to measure 8-hydroxy-2'-deoxyguanosine (8-OHdG) and benzo(a)pyrene diol epoxide (BPDE)-DNA adducts plasma level. The effect of the polymorphisms on postoperative prognosis was examined using the available survival data.

Results: There was no significant difference in the distribution of *SOD2*, *GSTA1*, *GSTM1*, and *GSTT1* gene variants between patients and controls ($p > 0.05$). However GSTP1 variant (*GSTP1 * IleVal + ValVal*) genotype was statistically significantly more frequent in patients compared to controls ($p < 0.05$). Similarly, carriers of *GSTP1 variant* genotype were at significantly higher risk of developing carcinoma compared to carriers of *GSTP1 reference* genotype (OR = 16.103, 95% IP = 2.036 - 127.398). There was no association between the level of both 8-OHdG and BPDE-DNA adducts, and different genotypes ($p > 0.05$). The investigated polymorphisms did not show any prognostic significance ($p > 0.05$).

Conclusion: These results indicate that the *GSTP1 variant* genotype was related to an increased risk of papillary renal cell carcinoma development. In order to fully understand the effect of investigated polymorphisms as a potential risk and prognostic biomarkers of this cancer, further research with a bigger sample size and longer follow-up are required.

Keywords:
papillary renal cell carcinoma,
SOD2,
GST,
risk,
survival

doi:10.5937/mp73-35160
Editorial board: podmladak.med.bg@gmail.com

Sažetak

Uvod: Smatra se da karcinom bubrežnog parenhima pripada tumorima čijem nastanku, ali i progresiji, doprinosi narušena redoks ravnoteža. Mnoge studije su fokusirane na pronalaženje potencijalnih biomarkera rizika, kao i prognostičkih biomarkera za ove tumore, što bi doprinelo ranom otkrivanju i praćenju progresije ovog karcinoma. Među kandidatima su i polimorfizmi gena za enzime superoksid dizmutazu 2 (SOD2) i glutation S-transferaze (GST).

Cilj: Cilj rada bio je da se ispita uloga polimorfizama *SOD2* i pojedinih *GST* gena u nastanaku papilarnog karcinoma bubrežnog parenhima (pKBP), kao i njihov uticaj na preživljavanje obolelih.

Materijal i metode: U studiju je bilo uključeno 39 pacijenata i 336 kontrolnih ispitanika. Polimorfizmi *SOD2* (*rs4880*), *GSTA1 C69T*, *GSTM1*, *GSTT1* i *GSTP1* (*rs1695*) određeni su odgovarajućim metodoma polimerazne lančane reakcije (*PCR*). Nivo 8-hidroksi-2'-deoksiguanozina (8-OHdG) i benzo(a)piren diolepoksid (BPDE)-DNK adukata određivan je ELISA metodom. Uticaj ispitivanih polimorfizama na postoperativnu prognozu ispitivan je na osnovu raspoloživih podataka o preživljavanju.

Rezultati: Nije uočena statistički značajna povezanost *SOD2*, *GSTA1*, *GSTM1* i *GSTT1* genskih varijanti sa rizikom za nastanak pKBP ($p > 0,05$). Međutim, nosioci *GSTP1 varijantnog* genotipa imali su značajno veći rizik za nastanak karcinoma u odnosu na nosioce *GSTP1 referentnog* genotipa (OR = 16,103, 95% IP = 2,036 – 127,398). Nije uočena statistički značajna povezanost varijantnih genotipova sa sadržajem 8-OHdG i BPDE-DNK adukata ($p > 0,05$). Ispitivani polimorfizmi nisu se pokazali statistički značajnim prognostičkim faktorima u preživljavanju obolelih, iako je uočeno kraće preživljavanje kod nosilaca *SOD2 varijantnog* i *GSTM1 referentnog* genotipa ($p > 0,05$).

Zaključak: Rezultati ove studije pokazali da je GSTP1 varijantni genotip udružen sa povećanim rizikom od nastanka papilarnog karcinoma bubrežnog parenhima. Da bi se u potpunosti razjasnila uloga ispitivanih polimorfizama u nastanku i progresiji karcinoma, nezavisno ili udruženo sa već utvrđenim faktorima rizika, neophodna su dalja istraživanja na većem broju ispitanika.

Ključne reči:
papilarni karcinom bubrežnog parenhima,
SOD2,
GST,
rizik,
preživljavanje

Introduction

Renal cell carcinoma (RCC) is the most frequent adult renal cancer, representing over 90% of all renal malignancies (1). According to its genetic and pathologic characteristics, RCC includes three major histological subtypes (2). Papillary RCC comprises nearly 15% of all renal malignancies. This is a less common and less aggressive subtype compared to clear cell RCC, with a five-year survival rate of 80% to 85% (3). Based on its histological features, papillary RCC can be further classified into Type 1 and 2, where Type 1 is more common, but Type 2 is more aggressive (4).

Established RCC risk factors include cigarette consumption, hypertension and obesity (5). It is known that these risk factors manifest their harmful effects largely by causing increased exposure of cells to oxidative stress (6). However, since RCC occurs only in a minority of those who are exposed to the aforementioned risk factors, it is assumed that genetic variations among the populations have an impact on its development and progression as well.

Recent studies emphasized the role of genes encoding antioxidant enzymes involved in free radical protection and detoxification reactions. Single nucleotide polymorphisms (SNPs), as well as deletion polymorphisms in such genes, are known to affect enzyme activity and therefore impair redox balance (7). Among the antioxidant enzymes, involved in the first line of defense against oxidative stress, there is superoxide dismutase 2 (SOD2), an isoenzyme situated in the mitochondrial matrix. The *rs4880* polymorphism that occurs in the *SOD2* gene is a type of missense mutation, which results in a reduced transport of enzymes into the mitochondria, and thus reduced activity (8). On the other hand, the vast majority of carcinogens that are known to be associated with RCC development, such as benzo(a)pyrene (B(a)P) and its DNA-reactive metabolite, benzo(a)pyrene diolepoxide (BPDE), are detoxified by glutathione S-transferases (GST) (9). These represent a family of isoenzymes that participate in the conjugation of glutathione (GSH) to exogenous or endogenous electrophilic substances, thereby reducing their reactivity to nucleophilic groups in important biological macromolecules, at least in the majority of the reactions (10). Polymorphisms of *GST* genes can affect the level of transcription or catalytic function of these enzymes, and thus can determine the individual response to carcinogens.

The role of the polymorphic expression of *SOD2* and certain *GSTs*, has been already demonstrated in the most common RCC type - clear cell RCC (11–13). However, the aim of this particular study was to assess the association of *SOD2* and *GST A1*, *M1*, *T1* and *P1* gene polymorphisms with the development and progression of papillary RCC, as well as to determine whether these polymorphisms were associated with higher concentration of

DNA damage indicators: BPDE-DNA adducts and 8-hydroxy-2'–deoxyguanosine (8-OHdG).

Material and methods

Study population

A number of 39 patients with papillary renal cell carcinoma (pRCC) was enrolled in this case-control study in the period from 2011 to 2014. The diagnosis was histopathologically confirmed and the assessment was performed in accordance with the WHO classification of tumors and TNM staging system (14). There were 336 control subjects admitted to the Urology department of the University Clinical Center of Serbia in the same period, without prior diagnosis of malignant disease. Their DNA and genotyping results are part of the biobank of the Institute of Medical and Clinical Biochemistry at the Faculty of Medicine University of Belgrade. The study was conducted respecting the ethical standards given in the Declaration of Helsinki and the rules of the Ethics Committee of the Faculty of Medicine University of Belgrade. All participants signed informed consent to participate in the study. A structured questionnaire was used to collect data on exposure to established risk factors.

DNA extraction

DNA was extracted from EDTA blood, using a commercial kit (Qiagen, Chatsworth CA, USA), aliquoted and stored at -20 ° C until PCR reaction was performed.

Genotyping

The *SOD2 rs4880* polymorphism was identified using Taqman Genotyping Assay (ThermoFisher Scientific, C___8709053_10), whereas GSTP1 rs1695 using Taqman Genotyping Assay (ThermoFisher Scientific, C___3237198_20). Additionally, *GSTA1 C69T* polymorphism was identified by the PCR-restriction fragment length polymorphism (RFLP), by the method of Coles et al. (15). *GSTM1* and *GSTT1* genotyping were performed by the multiplex PCR method of Abdel-Rahman et al. (16) for simultaneous analysis. Polymorphic deviations from Hardy-Weinberg equilibrium for each SNP were calculated using the Chi-square test, in both patient and control group.

Measuring the level of BPDE-DNA adducts and 8-OHdG

The level of BPDE-DNA adducts and 8-OHdG in the plasma samples was determined by ELISA-method using commercial kits (Cell Biolabs, Inc., San Diego, California, USA).

Statistical analysis

According to the normality of the distribution and the homogeneity of the variance, continuous data were interpreted as the mean ± standard deviation or as the median with range. The results were then analyzed with an appropriate statistical test. In addition to descriptive statistics, the analysis included the $\chi 2$ test to assess the significance of the difference in the frequency of the obtained genotypes, but also certain demographic characteristics between the patient and the control group. The Mann – Whitney test was performed to calculate the significance of the difference in 8-OHdG and BPDE-DNA adduct levels concerning different genotypes. The effect of a variable on the risk for carcinoma was assessed through odds ratio (OR) with a confidence interval of 95% (CI 95%) by logistic regression analysis. The overall survival was determined as the time from surgical intervention to the day of death or the last day of follow-up (The 1st of March, 2015). Consequently, survival analysis was performed using the Kaplan-Meier method to assess the probability of cumulative survival. The Log-rank test was used to calculate the significance of the difference in the survival according to *SOD2* and *GST* genotypes.

Results

The results of the macroscopic and microscopic assessment of the tumors are shown in **Table 1**.

Table 1. Macroscopic and microscopic features of tumor

Tumor characteristics[1]	Patients
Side, n (%)	
Right	18 (50)
Left	18 (50)
Surgery type, n (%)	
Radical	19 (70)
Partial	8 (30)
Tumor type, n (%)	
Type 1	10 (29)
Type 2	20 (59)
Oncocitoid	4 (12)
Tumor maximal diameter (cm)[2]	7.00 (2.20-13.00)
Tumor grade, n (%)	
I	2 (5)
II	14 (36)
III	21 (54)
IV	2 (5)
Tumor stage (pT), n (%)	
1	15 (39)
2	4 (10)
3	18 (46)
4	2 (5)
Tumor necrosis, n (%)	
Yes	22 (71)
No	9 (29)
Microvasular invasion, n (%)	
Yes	22 (71)
No	9 (29)

[1] Available data; [2] Median (min-max).

Table 2. Baseline characteristic of patients and respective controls

Baseline characteristic	Patients	Controls	p-value	OR (95% CI)
Age (years)[1]	59.153±10.225	60.443±10.846	0.480[8]	-
Sex, n (%)				
Female	13 (33)	138 (41)		1.00[2]
Male	26 (67)	198 (59)	0.351[4]	0.756 (0.248-2.307)[3]
Hypertension, n (%)[5]				
No	17 (50)	211 (65)		1.00[2]
Yes	17 (50)	116 (35)	0.095[4]	1.439 (0.516-4.015)[6]
Obesity, n (%)[5]				
BMI < 25	13 (38)	110(35)		1.00[2]
BMI > 25	21 (62)	204 (65)	0.711[4]	0.981 (0.352-2.736)[7]
Smoking, n (%) [5]				
Never	12 (34)	164 (49)		1.00 [2]
Ever[9]	23 (66)	171 (51)	0.098[4]	1.651 (0.763-3.571)[10]
Pack-years[5,11]	33.00 (7.20-92.00)[12]	30.00 (0.20-88.00)[12]	0.185[13]	-

[1]Mean ±SD; [2]Reference group; [3]OR, odds ratio adjusted to age and risk factors; [4]p-value for Chi-square test; [5]based on the data available; [6]OR, odds ratio adjusted to age, gender, pack-years, BMI (body mass index); [7]OR, odds ratio adjusted to age, gender, and remaining risk factors; [8]p-value for Student's T test; [9]minimum of 60 days; [10]OR, odds ratio adjusted to age, gender, and remaining risk factors; [11] pack-yeas formula: number of cigarettes smoked per day divided by 20, times years of cigarette consumption;[12]Median (Min-Max); [13]p-value for Mann-Whitney test.
CI, confidence interval.

Certain characteristics of patients and respective controls are presented in **Table 2**. The results indicate that there was no statistically significant difference between these two groups regarding gender, age, obesity and body mass index ($p > 0.05$). Also, no statistically significant difference was found regarding blood pressure, pack-years, as well as the distribution of smokers and nonsmokers ($p > 0.05$).

Genotype distribution of *SOD2* and *GST* in patients and control subjects, as well as the risk of tumor development, are shown in **Table 3**. The frequency of *SOD2 variant* (*SOD2 *AG + AA*) genotype in the patient group was 79%, and in members of the control group 67%, however, this difference was not statistically significant ($p > 0.05$). Carriers of *SOD2 variant* genotype had 3 times higher risk of pRCC than carriers of the SOD2 reference genotype (*SOD2 * GG*), without statistical significance.

Similarly, there was no statistical difference in the distribution of *GSTM1*, *GSTT1* and *GSTA1* genotypes

Table 3. *SOD2* and *GST* genotypes in relation to the risk of pRCC

Genotypes	Patients n, %	Controls n, %	p- value[7]	OR (95%CI)[8]
SOD2 (*rs4880*)				
GG[1]	6 (21)	111 (33)		1.00[9]
AG+AA[2]	23 (79)	225 (67)	0.172	2.743 (0.687-10.941)
GSTM1				
Active [3]	19 (49)	163 (50)		1.00 [9]
Null[4]	20 (51)	163 (50)	0.880	1.514 (0.561-4.087)
GSTT1				
Null[4]	12 (31)	89 (27)		1.00[9]
Active [3]	27 (69)	243 (73)	0.647	0.869 (0.281-2.689)
GSTA1 (*rs 3957357*)				
CC (active)	11 (28)	134 (41)		1.00[9]
CT+TT [5]	28 (72)	192 (59)	0.120	1.454 (0.479-4.410)
GSTP1 (*rs1695*)				
IleIle	10 (26)	141 (43)		1.00[9]
IleVal+ValVal [6]	29 (74)	185 (57)	**0.035**	**16.103 (2.036-127.398)**

[1] *GG* –referent alleles; [2]*AG+AA* –at least one variant allele; [3]*Active*, present enzyme; [4] *Null*– no enzyme; [5] Low activity, T allele present; [6] *Variant*, *Val* allele present; [7] p-value for Chi-square test; [8] OR, odds ratio adjusted to age, gender, and risk factors; [9] Reference group.

between patients and controls (p > 0.05). By contrast, the frequency of *GSTP1 variant* (*IleVal* + *ValVal*) genotype was significantly higher (p = 0.035) among patients (74%) compared to the frequency of this genotype among controls (57%). In addition, carriers of *GSTP1 variant* genotype were at significantly higher risk of cancer development compared to carriers of *GSTP1* (*IleIle*) reference genotype (OR = 16.103, 95% IP = 2.036 - 127.398).

No statistically significant effect of *SOD2* genotype on 8-OHdG level (p> 0.05) was observed (**Table 4**).

Similarly, there was no statistically significant effect (p > 0.05) of *GST* genotypes on the level of BPDE-DNA adducts (**Table 5**).

Survival data were available for 37 (95%) patients. The follow-up time was 39 months (6-81 months). During the follow-up, 14 patients (38%) died. The analysis of cumulative survival for patients by *SOD2* genotype is presented in **Figure 1**. The Kaplan-Meier analysis did not show a significant impact of the genotype on survival of patients (p > 0.05), although the survival curve indicated that carriers of the *SOD2 reference* (*SOD2 * GG*) genotype had better cumulative survival.

However, Kaplan-Meier analysis did not demonstrate any significant difference (p > 0.05) in the cumulative survival regarding different *GST* genotypes (**Figure 2**) probably due to the limited sample size. However, the survival curve suggested that patients with the *GSTM1-null* genotype had longer cumulative survival.

Discussion

Previous data suggest that renal cell carcinoma might represent a tumor with significant changes in redox balance. Therefore, it is considered that oxidative stress can play a significant role in both carcinogenesis and progression of this cancer (17). Genetic variants of genes encoding antioxidant enzymes and enzymes involved in xenobiotics biotransformation may result in altered enzyme activity, and by interacting with environmental factors, may affect tumor development and progression. In this study, for the first time, the association of *SOD2* polymorphism and risk for the development and progression of a rare type RCC – papillary RCC was examined. Increased risk and poorer postoperative prognosis were observed in carriers of *SOD2*AG+AA variant* genotype. By contrast, the impact of the *GSTM1*, *GSTT1*, *GSTP1* and *GSTA1* gene polymorphisms on the predisposition for the formation of clear cell RCC, independently or in association with environmental factors, has been shown in several studies (13,18). However, the effect of these polymorphisms on the development of papillary RCC has not been investigated yet. This study showed that *GSTP1 variant* genotype (*IleVal* + *ValVal*) is a significant risk factor for papillary RCC development.

Risk factors for RCC comprise cigarette consumption, obesity, hypertension, and occupational exposure to certain chemical agents (6). For many years, these factors have been known to manifest their harmful effects mainly by causing increased exposure of cells to free radicals (19).

Table 4. *SOD2* genotypes and the level of 8-OHdG (ng/ml) in pRCC patients

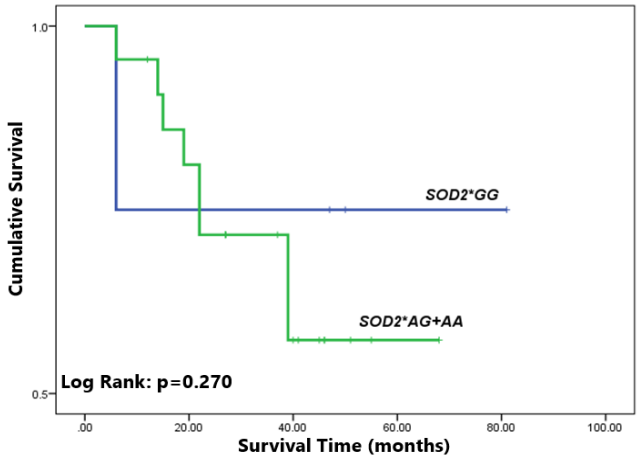
SOD2 (*rs4880*) genotype	8-OHdG (ng/ml)[3]	p-value[4]
GG[1]	1.436 (1.026-2.814)	0.661
AG+AA[2]	1.358 (0.468-3.983)	

[1] *GG* – carriers of both *referent* alleles; [2] *AG+AA* – carriers of at least one *variant* allele; [3] Median (Min-Max); [4] p-value for Mann-Whitney test.

Table 5. *GST* genotypes and the level of BPDE-DNA adducts in pRCC patients

Genotype		BPDE-DNA adducts (ng/ml)[5]	p-value[6]
GSTM1	*GSTM1-active*[1]	2.257 (1.64-5.34)	0.762
	GSTM1-null[2]	2.251 (1.51-4.23)	
GSTT1	*GSTT1-null*[2]	2.315 (1.51-5.34)	0.633
	GSTT1-active[1]	2.132 (1.64-4.11)	
GSTA1 (*rs 3957357*)	*GSTA1-active*	2.132 (1.89-5.34)	1.00
	GSTA1-low activity[3]	2.315 (1.51-4.23)	
GSTP1 (*rs1695*)	*GSTP1-wild type*	3.490 (2.13-4.23)	0.203
	GSTP1-variant[4]	2.255 (1.51-5.34)	

[1] *Active*, present enzyme; [2] *Null*,absent enzyme; [3] Low activity, *T* allele present; [4] *Variant*, *Val* allele present; [5] Median (Min-Max); [6] p-value for Mann-Whitney test.



*GG –referent alleles;
*AG+AA –least one variant allele.

Figure 1. Kaplan-Meier Survival Curve for overall mortality in pRCC patients with respect to *SOD2* genotype

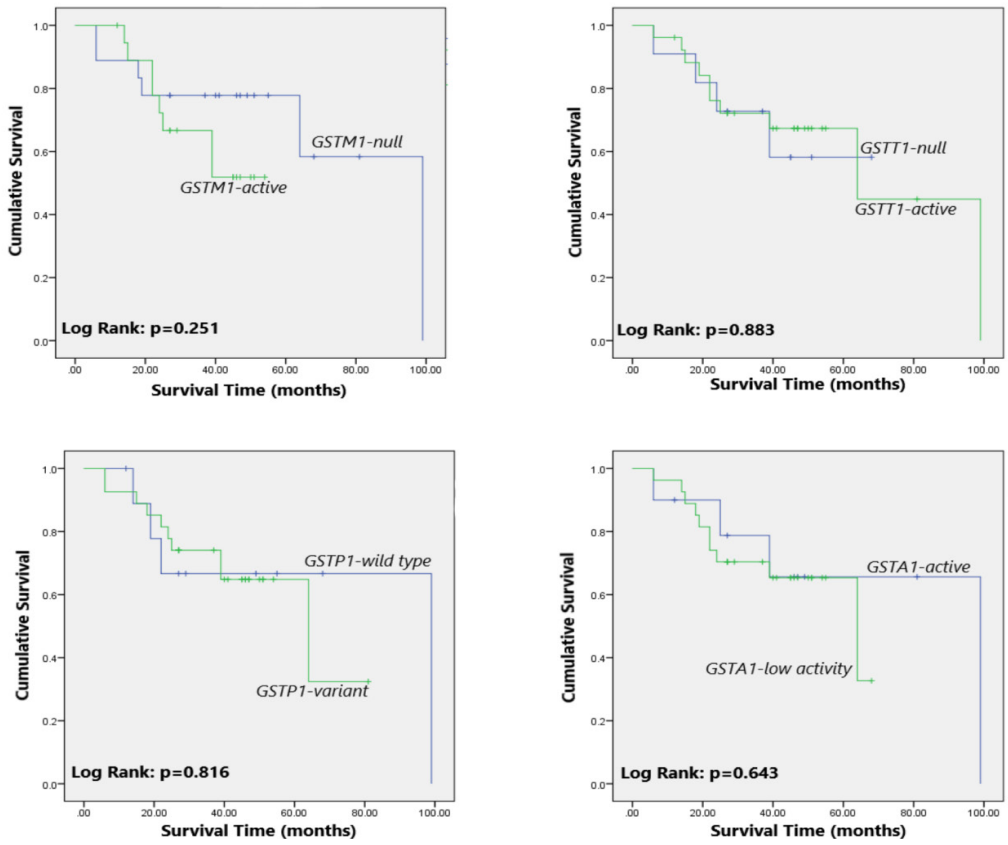


Figure 2. Kaplan-Meier Survival Curves for overall mortality according to GST genotype

Although cigarette consumption is believed to be one of the most important risk factors for RCC development, this has not been confirmed in this study. There was no difference between patients and controls regarding the distribution of smokers and non-smokers. This can be due to the nature of the control group (hospital-based), where an extremely high frequency of smokers was observed. For the same reason, there was no significant impact of obesity on the RCC development. In addition, the results of this study did not indicate a significant association of hypertension with RCC development.

The effect of rs4880 polymorphism on susceptibility to cancer has been extensively investigated; however, due to the existence of contradictory results, this effect has not been fully understood yet. For example, Atilgan et al. (20) observed that the risk of developing RCC, regardless of histological subtype, is higher in carriers of the *reference SOD2*GG* genotype, as well as the *variant SOD2*AG* genotype. However, in our study, it was observed that carriers of *variant SOD2*AG+AA* genotype were exposed to a higher but insignificant risk of developing papillary RCC, which is in accordance with a recently published study in the ccRCC group of patients (11). It is known that the occurrence of a v*ariant SOD2*A* allele results in slowing down the transport of SOD2 enzyme into the mitochondrial matrix, due to the impaired interaction with the Tim23 transporter on the inner mitochondrial membrane. Consequences comprise reduced dis-mutation of the superoxide anion to oxygen and hydrogen peroxide (H2O2) (8). However, Dasgupta et al. (21) showed that overproduction of H_2O_2, in the presence of the *reference SOD2*G allele*, leads to reduced apoptosis mediated by tumor necrosis factor alpha (TNF-α) and hence, longer survival of malignant cells. Because of this, it is still unclear whether high or low SOD2 enzyme activity is a risk factor for cancer development.

In most of the *GST* genes, there are observed polymorphisms that gene transcription level or altered enzyme function, and thus can determine the pharmacogenomic or even individual response to carcinogens. The *GSTP1* polymorphism (*rs1695*) has been the most investigated so far. This polymorphism comprises a replacement of A (adenine) to G (guanine) at codon 105 (*GSTP1 A1578G*) and consequently, isoleucine is replaced by valine in the synthesized enzyme (*Ile105Val*) (22). Results showed that *GSTP1 variant* (*IleVal + ValVal*) genotype is a significant risk factor for papillary RCC development, which is consistent with previous studies (12, 23). It is considered, that *GST* genotypes, independently or combined, could potentially identify patients with a defective detoxification system for electrophilic compounds. Such patients are more prone to DNA conjugate formation and genetic mutations, and eventually cancer development (24). Also, *GSTs* are involved in detoxification of benzopyrene and its reactive metabolite, benzo(a)pyrene diolepoxide (BPDE). On the other side, BPDE can covalently bind to guanosine in the DNA molecule and form BPDE-DNA conjugates. Authors such as Zhu et al. (25) showed that in the culture of peripheral lymphocytes after treatment with BPDE, the frequency of chromosome 3p deletions was much higher

in RCC patients than in the control group. This implies that sensitivity of chromosome 3p to BPDE may be an indicator of an individual's genetic predisposition to RCC. In addition, the results of Miyake et al. (26) showed a significantly higher level of DNA oxidative damage markers (8-OHdG) in tumor tissue than in adjacent non-tumor tissue in RCC patients. This ratio may also have prognostic significance. However, our results did not show that the plasma levels of BPDE-DNA conjugates were significantly higher in patients, carriers of variant *GST* genotypes compared to carriers of *reference GST* genotypes. Similarly, our results did not show any differences in 8-OHdG plasma level of patients regarding different *SOD2* genotypes.

Therapy for localized RCC is partial and radical nephrectomy, and for the more advanced stages systemic therapy is the mainstay of treatment (27). In this study, the influence of the *GST* and *SOD2* polymorphisms on the survival of patients was also investigated. Investigated *GST* polymorphisms did not show a significant impact on patients' prognosis, probably due to the small sample. However, our results indicate better survival in *GSTM1-null* carriers. Previous studies on the role of *GST* polymorphisms as prognostic factors in papillary RCC are lacking and have been limited only to *GSTM1* polymorphism in overall RCC pathology where patients with *GSTM1-null* genotype showed significantly better survival than those with *GSTM1-active* genotype (12, 23). In their research, Searchfield et al. tried to connect cumulative survival with the level of expression of members of GST super family including GSTα and GSTπ expressed in tumor tissue of clear cell RCC patients. The results indicated that patients with tumors expressing GSTα had longer survival. However, the effect of the most common *GST polymorphisms* was not assessed within these classes (28). Based on our results, overall survival was shorter among patients, carriers of *variant SOD2*AG+AA* genotypes, but still without statistical significance. Similar results were obtained for the group of ccRCC patients (11).

This study has several limitations that need to be addressed. The pRCC sample size was small. However, given the lack of literature data on this topic, these results provide a valuable basis for further studies. The study subjects were Caucasians only, limiting the evaluation of the possible effect of ethnicity. The case-control study was performed in order to assess the associations between *GST* genotypes and the risk of pRCC with an inherent risk of selection bias. The hospital-based control instead of population controls was used. In addition, recall bias regarding the risk factors for RCC development might have influenced the results as well. Moreover, the BPDE-DNA adducts levels and 8-OHdG levels were not determined in the control population.

Conclusion

In conclusion, further research on a bigger sample is necessary to fully understand the role of polymorphisms occurring in antioxidant and detoxification enzymes concerning the development and progression of papillary RCC, independently or in conjunction with known risk factors.

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