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ASSOCIATION OF GSTO1 AND GSTO2 POLYMORPHISM WITH RISK OF END-STAGE RENAL DISEASE DEVELOPMENT AND PATIENT SURVIVAL

POVEZANOST POLIMORFIZMA GSTO1 I GSTO2 SA RIZIKOM OD TERMINALNE FAZE BUBREŽNE INSUFICIJENCIJE I PREŽIVLJAVANJEM BOLESNIKA

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Summary

Background: Oxidative stress in patients with end-stage renal disease (ESRD) is associated with long-term cardiovascular complications. The cytosolic family of glutathione S-transferases (GSTs) is involved in the detoxication of various toxic compounds and antioxidant protection. GST omega class members, GSTO1 and GSTO2 possess, unlike other GSTs, dehydroascorbate reductase and deglutathionylation activities. The aim of this study was to clarify the role of genetic polymorphisms of GSTO1 (rs4925) and GSTO2 (rs156697) as risk determinants for ESRD development, as well as in the survival of these patients.
Methods: A total of 199 patients and 199 healthy subjects were included in the study and genotyped for both GSTO1 and GSTO2 polymorphism. Protein thiol and carbonyl groups as markers of protein oxidative damage were determined spectrophotometrically. Cox proportional hazard model and Kaplan-Meier analysis were performed to investigate the role of GSTO1 and GSTO2 genetic polymorphism on mortality of ESRD patients during the follow-up period (36 month).
Results: Individuals carrying the variant *GSTO2 GG* genotype were at 2.45-fold higher risk of ESRD development compared to the wild type *GSTO2 AA* genotype (OR=2.45; 95%CI=1.18–5.07; p=0.016). The results of *GSTO1/*

Kratak sadržaj

Uvod: Oksidativni stres je kod pacijenata sa terminalnom slabošću bubrega (TSB) povezan sa brojnim kardiovaskularnim komplikacijama. Pored uloge u detoksikaciji, citosolne glutation transferaze (GST) poseduju i antioksidantnu aktivnost. Članovi GST klase omega, GSTO1 i GSTO2, katališu nekoliko reakcija koje nisu tipične za ostale GST, kao što su reakcija uklanjanja glutationa i dehidroaskorbat-reduktazna aktivnost. Cilj studije bio je da se ispita uloga polimorfizama gena GSTO1 (rs4925) i GSTO2 (rs156697) na razvoj terminalne bubrežne slabosti, kao i njihova uloga u preživljavanju ovih bolesnika.
Metode: *GSTO1* i *GSTO2* genotipovi određivani su kod 199 bolesnika sa TSB i 199 kontrola uparenih po polu i starosti. Proteinske tiol i karbonilne grupe, kao markeri oksidativnog oštećenja proteina, određivane su spektrofotometrijski. Uticaj *GSTO1* i *GSTO2* genotipova na rizik za smrtni ishod analiziran je pomoću Cox regresione analize, a verovatnoća preživljavanja je analizirana pomoću Kaplan-Meier metode tokom 36 meseci praćenja bolesnika.
Rezultati: Osobe sa varijantnim *GSTO2 GG* genotipom su bile pod 2,45 puta većim rizikom za razvoj TSB u odnosu na bolesnike sa referentnim *GSTO2 AA* genotipom (OR=2,45; 95%CI=1,18–5,07; p=0,016). Rezultati *GSTO1/GSTO2* haplotip analize pokazali su da haplotip

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List of abbreviations: GST – glutathione transferase, ESRD – end-stage renal disease, DHAR – dehydroascorbate reductase, SNP – single nucleotide polymorphism, HD – hemodialysis.

GSTO2 haplotype analysis showed that the haplotype combination of *GSTO1 (*A)/GSTO2 (*A)* (*GSTO1* variant/*GSTO2* wild type allele) was protective for ESRD (OR=0.23 95%CI=0.12-0.44, p=0.001). Patients carrying at least one *GSTO1* reference allele have shorter mean overall (Log rank=2.844, p =0.241) and cardiovascular survival probability (Log rank=4.211, p=0.122).
Conclusions: GSTO polymorphisms have been shown to act as significant markers in assessing the risk of ESRD development and patients' survival.

Keywords: end-stage renal disease, genetic polymorphism, glutathione S-transferase, oxidative stress

kombinacija *GSTO1 (*A)/ GSTO2 (*A)* (*GSTO1* varijantni/*GSTO2* referentni alel) deluje protektivno na razvoj TSB (OR=0,23; 95%CI=0,12–0,44; p=0,001). Bolesnici koji su nosioci bar jednog *GSTO1* referentnog alela imaju kraće ukupno (log rank=2,844; p=0,241) i kardiovaskularno preživljavanje (log rank=4,211; p=0,122).
Zaključak: Polimorfizam gena za GSTO je povezan sa povećanim rizikom za razvoj terminalne slabosti bubrega kao i sa preživljavanjem ovih bolesnika.

Ključne reči: glutation S-transferaza; polimorfizam gena; oksidativni stres, terminalna slabost bubrega

Introduction

Oxidative stress is present in patients with end-stage renal disease (ESRD) (1). Moreover, there is a huge body of evidence implying that the prooxidant state is associated with long-term complications which include atherosclerosis and cardiovascular diseases (2–4), renal anemia (5), malnutrition (6), and bone disorders (7), and are the main cause of the increased morbidity and mortality of ESRD patients.

In the chronically hemodialyzed ESRD patients oxidative stress is primarily caused by accumulation of uremic toxins (8), phagocytes activation during the recurrent contact of blood with dialysis membranes, as well as dialyzer's membrane bioincompatibility (9), and use of intravenous iron preparations to correct anemia (10). Furthermore, ESRD patients frequently have lower levels of water soluble antioxidant vitamins which is a consequence of dietary restriction of fresh fruits and vegetables to avoid hyperkalaemia (11, 12). During the hemodialysis (HD) session, there is also a waste of low molecular antioxidants, such as ascorbic acid, vitamin C (13). In addition, it has been shown that low plasma vitamin C level predicts fatal and major non-fatal adverse cardiovascular events among ESRD patients (14, 15). In addition to the association of oxidative stress with renal failure development, hemodialysis and uremia, interest in the role of genetic predisposition towards oxidative damage and ESRD patients' prognosis has started to emerge recently. The family of glutathione S-transferases (GSTs) is a part of the cellular phase II detoxification program composed of multiple isoenzymes with functional polymorphisms in humans, that have the capacity to influence individual response to oxidative and electrophilic stresses. Their catalytic activity is expressed through GST dimer-mediated thioether conjugate formation with resultant detoxification of a variety of small molecule electrophils, including uremic toxins and reduction of accumulated hydro-peroxides (16–18). The family of cytosolic GSTs includes the Alpha (GSTA), Mu (GSTM), Pi (GSTP), Theta (GSTT) and Omega (GSTO) class and all these genetic polymorphisms occur within GST family members, resulting in complete lack or lowering of enzyme activity (19). *GSTM1-null*, *GSTT1-null* or *GSTP1-low activity* genotype are associated with both increased risk of ESRD development and more pronounced oxidative stress in hemodialyzed patients (20).

It should be emphasized that in addition to the classic catalytic functions, specific GST isoenzymes, such as GSTO1 and GSTO2, catalyze reactions that impact antioxidant defense in ways unrelated to detoxification. These reactions include facilitation of the addition of glutathione to cysteine residues in certain proteins (forward reaction, S-glutathionylation) as a protective mechanism of sensitive protein thiol groups in conditions of oxidative stress and reverse reaction of deglutathionylation (21), aimed at regenerating thiol groups after oxidant attack, catalyzed by glutaredoxins, thioredoxin and protein disulfide isomerase, as well as GSTO1. Moreover, deglutathionylation activity of GSTO1 is influenced by a single nucleotide polymorphism (SNP) (rs4925, 419C to A) reported at nucleotide 419 causing an alanine to aspartate substitution in amino acid 140 (A140D) of exon 4 of *GSTO1*, resulting in lower deglutathionylation activity in carriers of *GSTO1*A* gene variant (21). Another reaction atypical for other GSTs but catalyzed by both GSTO1 and GSTO2 is dehydroascorbate reductase (DHAR) activity, a reaction important for ascorbic acid level preservation. GSTO2 is suggested to have the highest DHAR activity in the cells, which is up to 100 times higher than GSTO1 DHAR activity (22). An SNP of *GSTO2* gene was found at nucleotide 424 causing an asparagine to aspartate substitution in amino acid 142 (N142D) of exon 4 (rs156697, 424 A to G) (23). Although the functional significance of *GSTO1* and *GSTO2* gene SNP is still under investigation, attempts were made to establish whether variant alleles confer higher risk of various types of malignancies (24, 25) and non-malignant diseases (26–29).

In view of the important roles that both members of GST omega class play in redox homeostasis, it may be reasonable to assume that their polymorphic expression might be associated with ESRD development and prognosis. Therefore, in this study, we assessed whether *GSTO1* and *GSTO2* polymorphisms have a modifying effect in terms of ESRD development, as well as overall and cardiovascular mortality of hemodialyzed patients.

Materials and Methods

Study subjects

The case-control study included 199 stable patients (84 male and 115 female, mean age 59.9± 12.2 years) hemodialyzed 3 times/week and recruited from two dialysis centers (Center for Renal Diseases, Zvezdara University Medical Center and Department of Nephrology and Hemodialysis, University Teaching Hospital Zemun). Inclusion criteria were that patients were older than 21 and at least 3 months on hemodialysis before entering the study. Patients with malignancy or infectious co-morbidity were excluded from the study. Patients were not prescribed vitamin C or E as antioxidant therapy. Control group included 199 age and gender matched individuals with nephrolithiasis and normal renal function. All the participants gave written informed consent and the ethical approval was obtained by the local Ethics Committee. The research was carried out in compliance with the Helsinki Declaration (as revised in 2000).

Both overall and cardiovascular mortality were prospectively registered during a follow-up period of 36 months. Zero time in this study is defined as the time when patients started regular HD treatment. Data regarding patients' survival were obtained from hospital documents. For death to be classified as cardiovascular, a myocardial infarction and/or stroke should have occurred. A cardiologist diagnosed myocardial infarction based on clinical presentation, ECG and specific enzyme analysis. Stroke was diagnosed by a neurologist based on clinical presentation and CT scan.

GST genotyping

DNA was isolated from whole blood using the QIAGEN QIAmp kit (Qiagen, Inc., Chatsworth, CA). *GSTO1* (rs 4925) and *GSTO2* (rs156697) polymorphisms were determined by the PCR-RFLP method as previously described by Djukic et al. (25, 30).

Measurement of protein oxidative damage in plasma

In order to determine the degree of protein oxidative damage, the level of protein thiol groups (30) and carbonyl derivatives were measured (OxiSelect TM ELISA kits, Cell Biolabs).

Statistical analysis

For the statistical analysis, an SPSS program (version 17.0, Chicago, IL) was used. The continuous variables were presented by means ± standard deviations (SD). Logistic regression was used to test the associations between the genotypes and ESRD. All data were adjusted according to age and gender. The individual haplotype and its frequencies were estimated using the *haplo.stats* program. For survival analysis, the Kaplan-Meier method and long rank were used. We used three different models. The variables for Model 1 were adjusted to age and gender. Smoking status was added as an additional covariate for Model 2 while the Model 3 further obtained patients' diabetes and cholesterol status.

Results

Baseline characteristics of 199 patients with end-stage renal disease were presented in *Table I*. Average HD vintage was 6.3±4.5 years. The main causes of death were cardiovascular diseases, infection and cachexia.

The associations between *GSTO1* and *GSTO2* genotypes and ESRD risk were presented in *Table II*. *GSTO1* polymorphism was not significantly associated with risk of ESRD. However, individuals carrying variant *GSTO2 GG* genotype were at 2.45-fold higher risk of ESRD development compared to wild-type *GSTO2 AA* genotype carrying individuals (OR= 2.45; 95%CI=1.18–5.07; p=0.016).

The results regarding haplotype *GSTO1* and *GSTO2* polymorphisms analysis were presented in *Table III*. *GSTO1*C* and *GSTO2*A* haplotype had the highest frequency among both patients (55.1%) and controls (45.5%). Furthermore, the results of haplotype analysis showed that the haplotype combination of one copy of variant *GSTO1*A* and one copy of wildtype *GSTO2*A* allele was protective for ESRD (OR=0.23; 95%CI=0.12–0.44; p=0.001).

Table I Baseline characteristics of patients with ESRD.

Variable	ESRD patients
Age (years)[a]	59.9±12.2
Gender, n (%)	
Female	84 (42)
Male	115 (58)
Time on hemodialysis (years)	6.3±4.5
Diabetes, n (%)	
Present	25 (13)
Absent	174 (87)
Smoking, n (%)	
Current + Former (ever)	55 (28)
Never	144 (72)
BMI (kg/m^2)	24.5
Total cholesterol (mmol/L)	4.7±1.1
Triacylglycerol (mmol/L)	2.1±1.3

[a] All results are presented as mean ± SD.
ESRD – end-stage renal disease; BMI – body mass index

Table II *GSTO1* and *GSTO2* genotypes in relation to risk of chronic renal failure.

GST genotype	ESRD pts[b] [n (%)]	Controls[c] [n (%)]	OR (95% CI)	p value
GSTO1				
CC	81 (41)	65 (33)	1.00[a]	
CA	94 (47)	117 (59)	0.69 (0.45–1.07)	0.098
AA	23 (12)	16 (8)	1.24 (0.60–2.58)	0.561
				Global association p value 0.110
GSTO2	ESRD pts[d] [n (%)]	Controls[e] [n (%)]	OR (95% CI)	
AA	66 (34)	82 (42)	1.00	
AG	97 (51)	100 (51)	1.19 (0.77–1.85)	0.427
GG	28 (15)	14 (7)	2.45 (1.18–5.07)	0.016
				Global association p value 0.046

OR, odds ratio; CI, confidence interval; [a]reference category; adjusted by age and gender; ESRD – end-stage renal disease; [b]GSTO1 genotyping was successful in 198 patients; [c]GSTO1 genotyping was successful in 198 controls; [d]GSTO2 genotyping was successful in 191 patients; [e]GSTO2 genotyping was successful in 196 controls.

Table III Association of haplotype frequencies in patients with ESRD and controls.

Genotype		Haplotype frequencies (%)			
GSTO1	*GSTO2*	ESRD pts	Controls	OR (95% CI)	p value
C	A	55.1	45.5	1.00*	
A	G	30.6	15.9	1.47 (0.97–2.23)	0.071
C	G	9.6	16.7	0.62 (0.38–1.03)	0.068
A	A	4.7	21.9	0.23 (0.12–0.44)	0.001
				Global haplotype association p value	0.001

OR, odds ratio; CI, confidence interval
*Reference category
Adjusted by age and gender
ESRD – end-stage renal disease

Table IV represented the level of protein oxidative damage in ESRD patients regarding *GSTO1* and *GSTO2* genotypes. Plasma protein thiol groups' concentrations were decreased in ESRD patients carriers of at least one *GSTO1* or *GSTO2* wild-type allele compared to *GSTO1* or *GSTO2* variant allele homozygotes, although statistically insignificantly (p=0.195; p=0.169). On the other hand, plasma protein carbonyl groups' concentration in ESRD patients did not differ between ESRD patients with different *GSTO1* or *GSTO2* genotypes.

The Kaplan-Meier survival analysis demonstrated shorter mean overall (log rank=2.844, p=0.241) and cardiovascular survival probability (log rank= 4.211, p=0.122) after the initiation of dialysis in patients carriers of at least one *GSTO1* wild-type allele (*Figure 1A* and *2A*) compared to *GSTO1 AA* homozygotes, although statistically insignificant. As presented in *Figure 1B* and *2B*, there is no difference between *GSTO2* gene variants and mean overall and cardiovascular survival probability.

Table IV Plasma thiol and carbonyl groups concentration in relation to GSTO1 and GSTO2 genotype in ESRD patients.

GST	GSTO1			GSTO2		
Genotype	CC+CA	AA	p	AA+AG	GG	p
SH groups (μmol/g)	6.31 (3.27–10.91)	6.94 (3.32–10.64)	0.195	6.31 (3.76–10.90)	7.36 (3.27–10.91)	0.169
Carbonyl groups (nmol/mg)	2.25 (1.62–3.74)	2.13 (1.54–2.92)	0.441	2.25 (1.54–3.74)	2.20 (1.82–2.92)	0.784

Data are presented as medians with 95% confidence intervals
SH – thiol, GST – glutathione transferase, ESRD – end-stage renal disease

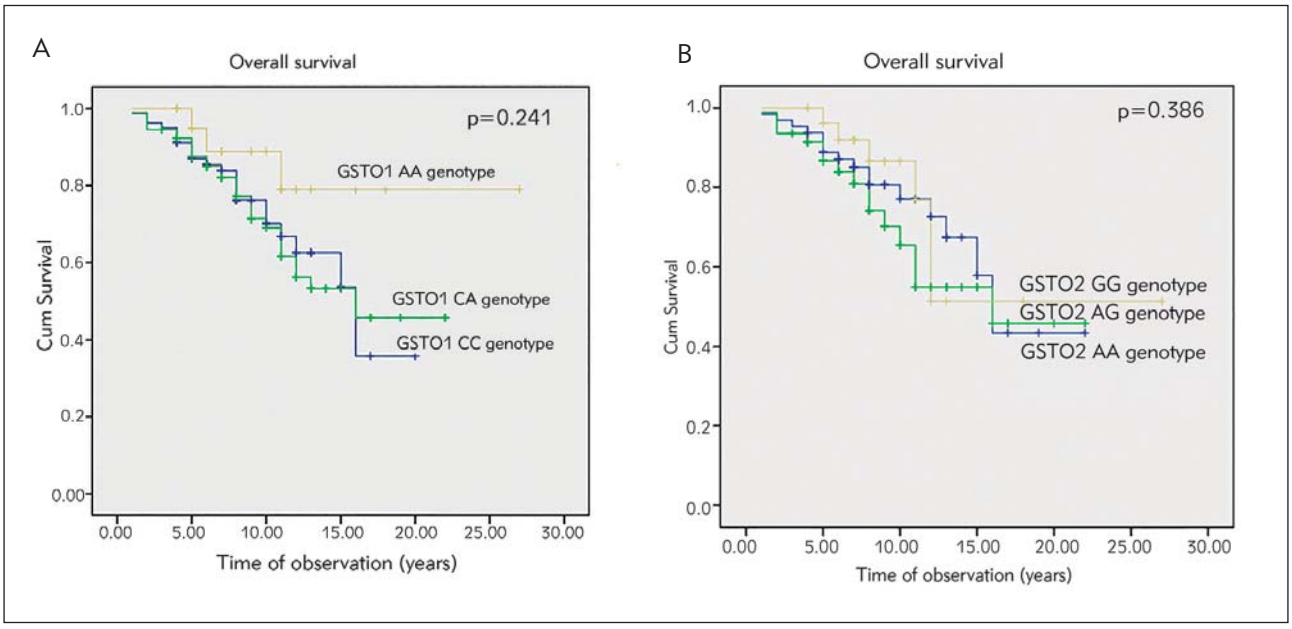

Figure 1 Kaplan-Meier survival analysis for overall mortality according to *GSTO1* and *GSTO2* genotype.

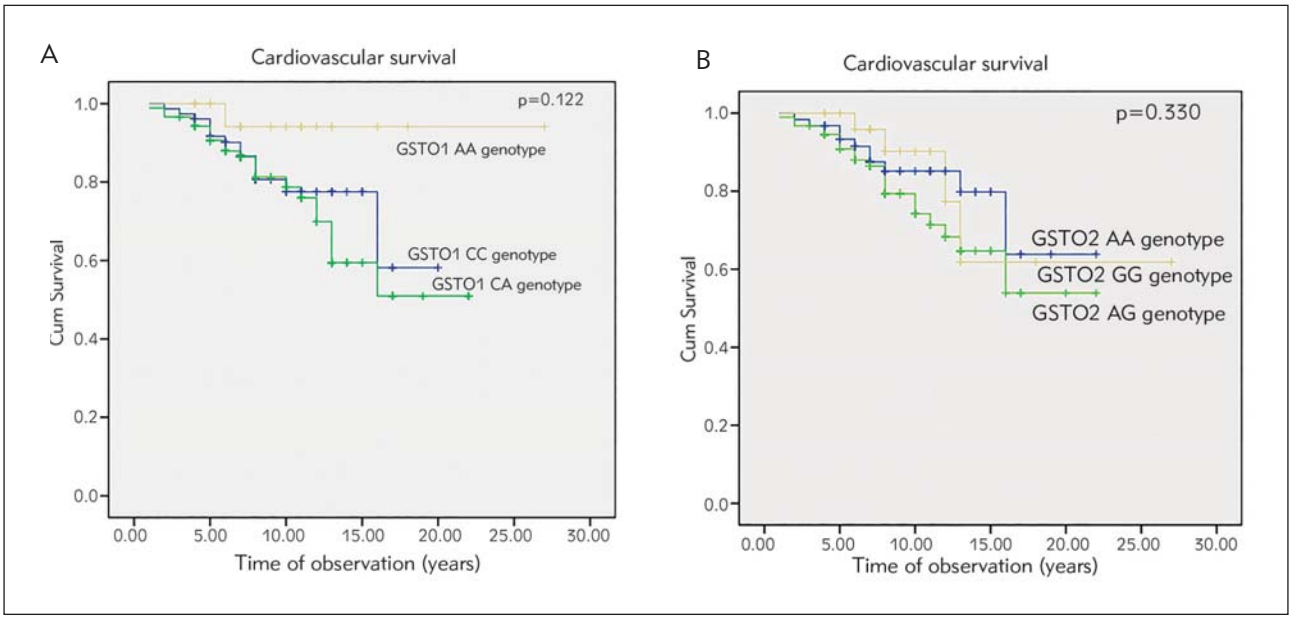

Figure 2 Kaplan-Meier survival analysis for cardiovascular mortality according to *GSTO1* and *GSTO2* genotype.

The results obtained by Cox regression analysis demonstrated that none of the *GSTO1* gene variants were predictors for overall mortality in all three models tested (*Table V*). However, *GSTO1* gene variants were predictors of a higher risk of cardiovascular mortality among ESRD patients in all tested models (global association p=0.052, p=0.04, p=0.036, respectively).

Regarding the prognostic role of *GSTO2* polymorphism among ESRD patients (*Table VI*), the results obtained demonstrated that none of the *GSTO2* gene variants were predictors for neither overall nor cardiovascular mortality in the models tested.

Further analysis of *GSTO1* and *GSTO2* haplotype gene variants with overall and cardiovascular mortality (*Table VII*) showed association only in case of overall mortality, still statistically insignificant (global haplotype association p=0.077).

Table V GSTO1 polymorphism as a predictor for overall and cardiovascular mortality among ESRD patients according to linear regression analysis.

GSTO1 genotype	Model 1[a]		Model 2[b]		Model 3[c]	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
	Risk for overall mortality among carriers of various GSTO1 genotypes					
C/C	1.00*	0.19	1.00	0.17	1.00	0.30
C/A	1.28 (0.65–2.52)		1.39 (0.70–2.76)		1.24 (0.55–2.79)	
A/A	0.43 (0.11–1.61)		0.45 (0.12–1.73)		0.43 (0.10–1.80)	
	Risk for cardiovascular mortality among carriers of various GSTO1 genotypes					
C/C	1.00	0.052	1.00	0.04	1.00	0.036
C/A	1.60 (0.74–3.46)		1.64 (0.76–3.55)		1.86 (0.73–4.70)	
A/A	0.23 (0.03–1.88)		0.22 (0.03–1.78)		0.20 (0.02–1.84)	

HR, hazard ratio; CI, confidence interval
*Reference category
ESRD – end-stage renal disease
[a]Adjusted for age and gender; [b]adjusted for the covariates in Model 1 plus an additional adjustment for smoking status; [c]adjusted for the covariates in Model 2 plus an additional adjustment for diabetes and cholesterol, TAG and HDL levels.

Table VI GSTO2 polymorphism as a predictor for overall and cardiovascular mortality among ESRD patients according to linear regression analysis.

GSTO2 genotype	Model 1[a]		Model 2[b]		Model 3[c]	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
	Risk for overall mortality among carriers of various GSTO2 genotypes					
A/A	1.00*	0.43	1.00	0.32	1.00	0.26
A/G	1.41 (0.68–2.93)		1.59 (0.75–3.36)		1.73 (0.69–4.31)	
G/G	0.79 (0.27–2.35)		0.86 (0.29–2.60)		0.74 (0.21–2.66)	
	Risk for cardiovascular mortality among carriers of various GSTO2 genotypes					
A/A	1.00	0.36	1.00	0.36	1.00	0.092
A/G	1.62 (0.69–3.78)		1.59 (0.68–3.74)		2.70 (0.87–8.39)	
G/G	0.83 (0.23–3.00)		0.79 (0.22–2.87)		0.87 (0.18–4.23)	

HR, hazard ratio; CI, confidence interval
*Reference category
ESRD – end-stage renal disease
[a]Adjusted for age and gender; [b]adjusted for the covariates in Model 1 plus an additional adjustment for smoking status; [c]adjusted for the covariates in Model 2 plus an additional adjustment for diabetes and cholesterol, TAG and HDL levels.

Table VII Haplotype association with risk for overall and cardiovascular mortality among ESRD patients.

Haplotype		Haplotype frequencies (%)		OR (95% CI)	*p* value
GSTO1	*GSTO2*	alive	deceased		
		Haplotype association with risk for overall mortality in ESRD patients			
C	*A*	53.7	58.5	1[a]	
A	*G*	30.3	31.6	0.97 (0.52–1.80)	0.92
C	*G*	9.9	8.8	0.79 (0.32–1.92)	0.60
A	*A*	6.1	1.1	0.00 (0.00–NA)	1
					Global haplotype association p value 0.077
	Haplotype association with risk for cardiovascular mortality in ESRD patients				
C	*A*	54.2	57.1	1	
A	*G*	30.2	31.3	1.01 (0.50–2.02)	0.99
C	*G*	10.0	10.0	1.00 (0.36–2.77)	1
A	*A*	5.6	1.6	0.00 (0.00–NA)	1
					Global haplotype association p value 0.260

OR, odds ratio; CI, confidence interval; [a]reference category; adjusted for age and gender; ESRD – end-stage renal disease

Discussion

In our study, frequencies of *GSTO* genotypes are within the range observed in the European population. Namely, the reported range for *GSTO1* variant allele is 0.302–0.399 according to Polimanti et al. and Paiva L et al. (32, 33). We reported that the frequency of this allele was 0.37. For *GSTO2* variant allele, the reported range of frequency in the European population is 0.31–0.341 (32, 34). The frequency obtained in our study was 0.33.

Our study demonstrated that individuals with variant *GSTO2 GG* genotype were at higher risk of ESRD development compared to the carriers of *GSTO2 AA* genotype. Additionally, the haplotype combination of one copy of variant *GSTO1*A* and one copy of wild-type *GSTO2*A* allele was protective for ESRD. The Kaplan-Meier survival analysis demonstrated shorter mean overall and cardiovascular survival probability, after the initiation of dialysis, in patients carriers of at least one *GSTO1* wild-type allele compared to *GSTO1 AA* homozygotes, although statistically insignificant.

Polymorphisms of the omega class of GST in non-malignant diseases have been studied so far in several studies. The study of Allen et al. has shown that carriers of *GSTO2* variant allele had increased risk for development of Alzheimer disease (26). The influence of *GSTO2* polymorphism has also been investigated in the risk of hypothyroidism development and it has been reported that the carriers of variant *GSTO2* genotype had almost five times higher risk for developing hypothyroidism (27). The study of Stamenkovic et al. (29) addressed the influence of *GSTO1* polymorphism Ala140Asp and *GSTO2* polymorphism Asn142Asp on the risk of age-related cataract. The results indicate that mutant *GSTO2*Asp* genotype is associated with increased risk of age-related cataract in smokers and ultraviolet-exposed subjects. However, these polymorphisms have not been widely studied in ESRD. Interestingly, there is only one study of *GSTO2* genetic polymorphism in patients with ESRD done in Iran (35). They reported that *GSTO2* genetic variant had no significant association with the ESRD risk. This result is in contrast to our findings, since we have reported that individuals with variant *GSTO2 GG* genotype were at higher risk of ESRD development compared to carriers of *GSTO2 AA* genotype. Since it has been speculated that rs156697 polymorphism of GSTO2 may influence its DHAR activity, our findings are biologically plausible due to the role of GSTO2 in regeneration of dehydroascorbate. Namely, chronically hemodialyzed patients show depletion of low molecular antioxidants, such as ascorbic acid (14). On the other hand, the reduction of dehydroascorbate to ascorbic acid is a vital cellular function. It is well established that various causes of ESRD, such as hypertension, diabetes and inflammation (e.g. glomerulonephritis), activate different molecular pathways which increase free radical production in the kidney. In subjects with both variant *GSTO2* alleles, low enzyme activity probably causes lower DHAR activity and consequently low vitamin C level in the serum, as well as insufficient antioxidant protection. Polymorphic GSTO2 expression may result in inter-individual differences in antioxidant capacity in hemodialyzed patients. Unfortunately, we failed to show a correlation between *GSTO2* polymorphism and oxidative phenotype determined as levels of protein oxidative stress byproducts.

GSTO2 polymorphism seems not to be involved in the course of prognosis of ESRD patients. However, *GSTO1* polymorphism influences overall and cardiovascular survival probability of these patients, which is not in accordance with the findings of Peddareddygari et al. who showed that *GSTO1* gene C419A polymorphism was not involved in cerebral response to ischemia (36), while Kölsch et al. (37) showed that the carriers of at least one variant *GSTO1*A* allele had an increased risk of stroke. Although it has been pointed out that this SNP causes reduced enzyme activity and therefore tissue susceptibility to oxidative stress, recent findings revealing the functional role of this polymorphism in the glutathionylation/deglutathionylation cycle might provide additional explanations for the accelerated death in patients with *GSTO1*C* wild-type allele (21). Thus, Menon and Board (21) suggested that *GSTO1* genetic variants may influence regulation of glutathionylation, indicating *GSTO1*C* wild-type allele as a protein with higher deglutathionylation activity than the variant one. With an idea that glutathionylation impacts both protein structure and function, the differences in polymorphic expression of *GSTO1* could provide a plausible mechanism to explain the link between this genetic polymorphism and different diseases (24–29). In our study, the haplotype combination of one copy of wild-type *GSTO1*C* and one copy of variant *GSTO2*G* allele was deleterious for ESRD risk, while the carriers of wild-type *GSTO1 CC* had lower survival. It should be emphasized that in ESRD progression, altered redox regulation shifts biomolecules towards a more oxidized state (20, 38). Thiol groups are among the most sensitive targets of prooxidant compounds in ESRD, which reflect the level of oxidative stress in these patients (38, 39). Impaired homeostasis of blood thiols in ESRD includes a disturbance of redox pairs, such as GSH/GSSG and cysteine/cystine, in plasma and blood cells (40). Protein thiols (P-SH) are also modified, while albumins act as sacrificial antioxidants, as confirmed in this and other investigations which found that titrable P-SH are decreased in plasma specimens (20, 41). Among the posttranslational modifications of P-SH of relevance for these effects in ESRD patients, there are alkylation reactions produced by a series of electrophilic carbonyls and oxidation to sulfenic acid by the action of reactive species (41). Further oxidation of sulfenic acid to sulfuric and sulfonic acid leads to protein degradation. Such an unfavorable sequence of reactions is prevented by the so-called S-glutathionylation cycle, in which glutathione molecules are added to protein cysteins forming mixed disulfides P-S-S-G (42). This so-called forward gluthationylation reaction might occur spontaneously, but is also catalyzed by another GST enzyme family member, GSTP1 (42). Indeed, our previous results showed decreased thiol group concentration in ESRD patients in homozygous carriers of *GSTP1 Val/Val* compared to carriers of at least one *GSTP1 Ile* wildtype allele (20). Although changes in protein S-glutathionylation have still not been documented in ESRD patients, a recent work by Galli et al. (39) showed increased plasma protein thiolation. It might be speculated that the presence of a *GSTO1*C* allelic variant might also be in favor of protein deglutathionylation, and higher susceptibility to oxidation by oxidant species, resulting in more protein modifications and enhanced vascular oxidant stress. Thus, in our study, plasma protein thiol groups were decreased in ESRD patients who were carriers of at least one *GSTO1*C* wild-type allele. This probably reflects the overall process of oxidative damage, and the role of albumins as sacrificial targets. In order to establish the potential role of *GSTO1* polymorphism in the glutathionylation/deglutathionylation cycle, a wide range of cellular proteins should be targeted.

Limitations of this study include a hospital-based control group, a relatively small sample size, its cross-sectional design and short follow-up period. Use of population controls may have been more appropriate in this study. The case-control design was used to assess the associations between glutathione transferases omega genotypes and ESRD development and, therefore, selection bias might impact the results.

In conclusion, this study offers some essential information that could be the base for future longitudinal research. Additionally, with these combined methodological approaches, we also confirmed the value of the *GSTO* polymorphisms as significant markers in assessing the risk of ESRD development and patients' survival.

Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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