19. Grooteman MP, van den Dorpel MA, Bots ML *et al*. for the CONTRAST Investigators. Effect of online haemodiafiltration on all-cause mortality and cardiovascular outcomes. *J Am Soc Nephrol* 2012; 23: 1087–1096
20. Penne EL, Van der Weerd NC, Bots ML *et al*. on behalf of the CONTRAST investigators. Patient- and treatment-related determinants of convective volume in post-dilution haemodiafiltration in clinical practice. *Nephrol Dial Transplant* 2009; 24: 3493–3499
21. Bowry SK, Gatti E. Impact of haemodialysis therapy on anaemia of chronic kidney disease: the potential mechanisms. *Blood Purif* 2011; 32: 210–219
22. Vaslaki L, Major L, Berta K *et al*. On-line haemodiafiltration versus haemodialysis: stable haematocrit with less erythropoietin and improvement of other relevant blood parameters. *Blood Purif* 2006; 24: 163–173
23. Pedrini LA, De Cristofaro V, Comelli M *et al*. Long-term effects of high-efficiency on-line haemodiafiltration on uraemic toxicity. A multicentre prospective randomized study. *Nephrol Dial Transplant* 2011; 26: 2617–2624
24. Wizemann V, Lotz C, Techert F *et al*. On-line haemodiafiltration versus low-flux haemodialysis. A prospective randomized study. *Nephrol Dial Transplant* 2000; 15(Suppl 1): 43–48
25. Maduell F, Navarro V, Cruz MC *et al*. Osteocalcin and myoglobin removal in on-line haemodiafiltration versus low- and high-flux haemodialysis. *Am J Kidney Dis* 2002; 40: 582–589
26. Blankestijn PJ, Ledebo I, Canaud B. Hemodiafiltration: clinical evidence and remaining questions. *Kidney Int* 2010; 77: 581–587
27. Lin CL, Yang CW, Chiang CC *et al*. Long-term on-line haemodiafiltration reduces predialysis beta-2 microglobulin levels in chronic haemodialysis patients. *Blood Purif* 2001; 19: 301–307
28. Odell RA, Slowiaczek P, Moran JE *et al*. B2-microglobulin kinetics in end-stage renal failure. *Kidney Int* 1991; 39: 909–919
29. Ward RA, Greene T, Hartmann B *et al*. Resistance to intercompartmental mass transfer limits beta2-microglobulin removal by post-dilution haemodiafiltration. *Kidney Int* 2006; 69: 1431–1437.
30. Locatelli F, Marcelli D, Conte F *et al*. Comparison of mortality in ESRD patients on convective and diffusive extracorporeal treatments. The Registro Lombardo Dialisi E Trapianto. *Kidney Int* 1999; 55: 286–293
31. Locatelli F, Altieri P, Andrulli S *et al*. Hemofiltration and haemodiafiltration reduce intradialytic hypotension in ESRD. *J Am Soc Nephrol* 2010; 21: 1798–1807

Received for publication: 8.3.2012; Accepted in revised form: 31.7.2012

Nephrol Dial Transplant (2013) 28: 202–212
doi: 10.1093/ndt/gfs369
Advance Access publication 2 October 2012

Glutathione S-transferase A1, M1, P1 and *T1* null or low-activity genotypes are associated with enhanced oxidative damage among haemodialysis patients

Sonja Suvakov[1], Tatjana Damjanovic[2], Aleksandra Stefanovic[3], Tatjana Pekmezovic[4], Ana Savic-Radojevic, Marija Pljesa-Ercegovac[1,1], Marija Matic[1], Tatjana Djukic[1], Vesna Coric[1], Jovana Jakovljevic[1], Jasmina Ivanisevic[3], Steva Pljesa[5,6], Zorana Jelic-Ivanovic[3], Jasmina Mimic-Oka[1], Nada Dimkovic[2,6] and Tatjana Simic[1]

[1]Institute of Medical and Clinical Biochemistry, Faculty of Medicine, University of Belgrade, Belgrade, Serbia, [2]Clinical Department for Renal Diseases, Zvezdara University Medical Center, Belgrade, Serbia, [3]Department of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia, [4]Institute of Epidemiology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia, [5]Department of Nephrology and Hemodialysis, University Teaching Hospital Zemun, Belgrade, Serbia and [6]Faculty of Medicine, University of Belgrade, Belgrade, Serbia

Correspondence and offprint requests to: Tatjana Simic, E-mail: tatjanasimic@med.ac.bg.rs

Abstract

Background. Increased oxidative stress is a hallmark of end-stage renal disease (ESRD). Glutathione *S*-transferases (GST) are involved in the detoxification of xenobiotics and protection of oxidative damage. We hypothesized that genetic polymorphism in antioxidant enzymes *GSTA1*, *GSTM1*, *GSTP1* and *GSTT1* is more frequent in ESRD and modulates the degree of oxidative stress in these patients.

Methods. *GSTA1*, *GSTM1*, *GSTP1* and *GSTT1* genotypes were determined in 199 ESRD patients and 199 age- and gender-matched controls. Markers of protein and lipid oxidative damage [thiol groups, carbonyl groups, advanced oxidative protein products, nitrotyrosine, malondialdehyde (MDA) and MDA adducts], together with total oxidant status and pro-oxidant–antioxidant balance were determined.

Results. Individual GST polymorphisms influence vulnerability to both protein and lipid oxidation, with *GSTM1-null* gene variant having the most pronounced effect. Furthermore, a strong combined effect of null/low-activity *GSTM1*, *GSTT1*, *GSTA1* and *GSTP1* genotypes in terms of susceptibility towards oxidative and carbonyl stress was found in ESRD patients. When patients were stratified according to *GSTM1* and *GSTT1*, the highest oxidant damage was noted

in those with the *GSTM1-null*/*GSTT1-null* genotype. The observed effect was even stronger in patients with the third low-activity *GSTP1* or *GSTA1* genotype. Finally, the level of oxidative and carbonyl stress was most pronounced in the subgroup of patients with all four null or low-activity *GSTM1*, *GSTT1*, *GSTP1* and *GSTA1* genotypes.

Conclusions. According to the GST genotype, ESRD patients may be stratified in terms of the level of oxidative and carbonyl stress that might influence cardiovascular prognosis, but could also improve efforts towards individualization of antioxidant treatment.

Keywords: end-stage renal disease; glutathione *S*-transferases; haemodialysis; oxidative stress; polymorphism

Introduction

There is a mounting evidence for the presence of oxidative stress in patients undergoing maintenance haemodialysis (MHD) [1–6], contributing to poor cardiovascular (CV) and overall outcome [7]. Both increased free radicals production and down-regulated antioxidant enzymes activities contribute to protein, lipid and DNA oxidative damage by-products accumulation in dialysis patients [8–11]. In addition to the well-established link of oxidative stress with specific causes of renal failure, dialysis procedure and uraemic state, the role of genetic predisposition in enhanced oxidative damage and consequent worsening of MHD patients' prognosis has emerged recently.

Members of the glutathione transferase (GST) enzyme superfamily are able to detoxify accumulated uraemic toxins in MHD patients and posses strong antioxidant activity towards reactive oxygen species (ROS) and peroxides [12 –14]. However, as recently shown by Lin *et al.* [10], the capacity to evoke the GST response towards oxidative stress in MHD patients seems to be genetically determined. Namely, almost all members of GST family exhibit genetic polymorphism, resulting in complete lack or lowering of enzyme activity [15]. Approximately half of the population lacks GSTM1 enzyme activity, due to a homozygous deletion of the *GSTM1* gene [16]. The *GSTM1-null* genotype has attracted much attention as a result of risk linkage with lung and bladder cancer [17, 18] and increased susceptibility to coronary heart disease among smokers [19, 20]. Haemodialysis (HD) patients lacking GSTM1 activity exhibit enhanced oxidative DNA damage and higher mortality rate than those with active GSTM1 enzyme [10]. Despite the fact that protein and lipid oxidative modifications have a key role in the pathogenesis of CV complications in these patients, the question of whether the *GSTM1* genotype influences the level of protein and lipid oxidative damage by-products in MHD patients has not been well established as yet.

In addition to the *GSTM1* polymorphism, *GSTT1, GSTP1* and *GSTA1* polymorphisms also gained a lot of attention. In the case of *GSTT1*, gene homozygous deletion present in ∼20% of Caucasians, leads to the lack of GSTT1 enzyme activity [21]. Single-nucleotide polymorphism (SNP) leading to amino acid substitution from isoleucine (*Ile*) to valine (*Val*) [22] changes catalytic activity of the GSTP1 enzyme [23]. Thus, if *Val* is present in GSTP1, specific substrates might accumulate and contribute to oxidative damage [23–25]. In healthy Caucasians, the frequencies of the genotype variants of *GSTP-Ile/Ile, -Ile/Val* and *-Val/Val* are 51.5, 39.4, and 9.1%, respectively [26]. *GSTA1* polymorphism is represented by three, apparently linked, SNPs: -567TOG, -69COT and -52GOA. These substitutions result in differential expression with lower transcriptional activation of the variant *GSTA1*B* (-567G, -69T, -52A) than common *GSTA1*A* allele (-567T, -69C,-52G) [27]. It seems reasonable to assume that *GSTT1-null* or *GSTA1-* or *GSTP1-low-activity* genotypes might also influence the level of oxidative stress in MHD patients and thus contribute to endogenous predisposition to oxidative damage in the setting of disrupted redox balance. Still, the role of polymorphic expression of *GSTA1*, *GSTP1* and *GSTT1* genes in increased oxidant-induced protein and lipid damage among MHD patients has to be established. This has prompted us to assess whether the null or low-activity *GSTM1, GSTT1, GSTP1* and *GSTA1* genotype alone or in combination correlate with eight biomarkers of oxidative stress, including protein thiol and carbonyl groups, advanced oxidation protein products (AOPP), nitrotyrosine, malondialdehyde (MDA), MDA adducts, total oxidant status (TOS) and pro-oxidant–antioxidant balance (PAB) in HD patients.

Materials and methods

Study subjects

A total of 199 patients (84 male and 115 female, mean age 60.0 ± 12.1 years) undergoing HD treatment for 12–15 h weekly in two dialysis facilities in Belgrade (Center for Renal Diseases, Zvezdara University Medical Center and Department of Nephrology and Hemodialysis, University Teaching Hospital Zemun) were included in this case–control study. All patients were stable, aged over 21 and with HD vintage >3 months before the study. Exclusion criteria were malignancy or infectious co-morbidity based on C-reactive protein values. Patients did not receive any antioxidant therapy (vitamin C or E), while 35.2% of patients received angiotensin-converting inhibitors therapy and 15.1% received statins.

The causes of end-stage renal disease (ESRD) were hypertensive nephrosclerosis ($n = 93$), glomerulonephritis ($n = 32$), diabetic nephropathy ($n = 25$), polycystic renal disease ($n = 19$), pyelonephritis ($n = 19$), Balkan endemic nephropathy ($n = 7$) and obstructive nephropathy ($n = 4$). Patients were treated with single-use dialysers equipped with low- and high-flux polysulphone membranes, with a membrane surface area of 1.3–2.1. m². A total of 199 controls (85 male and 114 female, mean age 59.3 ± 10.9 years) were recruited from individuals with nephrolithiasis and normal renal function who were admitted to the same hospitals during the same time period. All the participants provided written informed consent. This study protocol was approved by the Institutional Review Board, and the research was carried out in compliance with the Helsinki Declaration (as revised in 2000).

GST genotyping

Genomic DNA was isolated from whole blood using the QIAGEN QIAmp kit (Qiagen, Inc., Chatsworth, CA).

GSTA1 C-69T polymorphism was determined by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) by Ping *et al.* [28]. The primers used were *GSTA1 C-69T* forward: 5′-TGTTGATTGTTTGCCTGAAATT-3′ and *GSTA1 C-69T* reverse: 5′-GTTAAACGCTGTCACCCGTCCT-3′. The presence of restriction site resulting in two fragments (385 and 96 bp) indicated mutant allele (*T/T*)

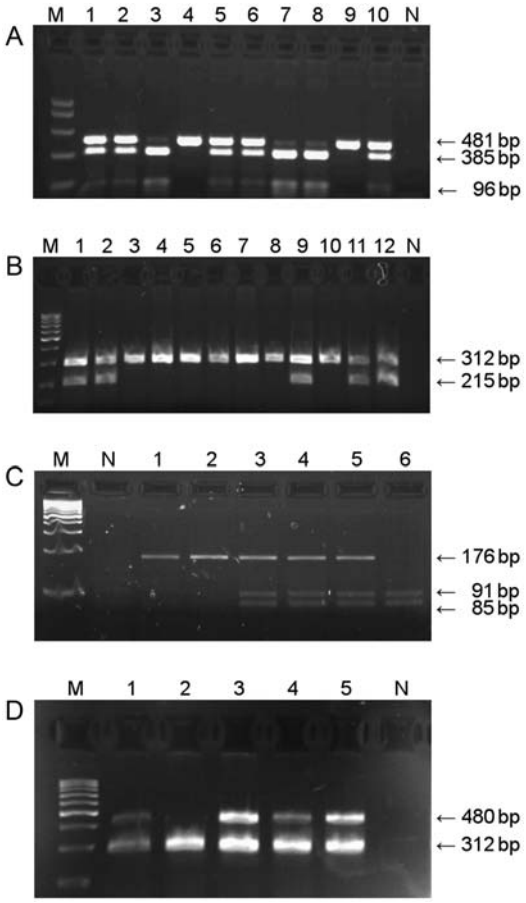

Fig. 1. Agarose gel electrophoretogram. PCR–RFLP products of the *GSTA1* gene. Lanes 4 and 9 represent the *GSTA1 CC* genotype. Lanes 1, 2, 5, 6 and 10 represent the *GSTA1 CT* genotype, while lanes 3, 7 and 8 represent the *GSTA1 TT* genotype (**A**). PCR products of the *GSTM1* gene are showed in (**B**). Lanes 1, 2, 9, 11 and 12 are patients with the *GSTM1 active* genotype and lanes 3 through 8 with lane 10 represent the *GSTM1 null* genotype (B). PCR–RFLP products of the *GSTP1* gene. Lanes 1 and 2 are wild-type (*Ile/Ile*), lanes 3, 4 and 5 are heterozygotes (*Ile/Val*) and lane 6 is homozygote (*Val/Val*) (**C**). PCR products of the *GSTT1* gene. Lanes 1, 3, 4 and 5 are patients with the *GSTT1 active* genotype and lane 2 represents the *GSTT1 null* genotype (**D**). M, DNA marker; N, negative control.

and if *C/T* polymorphism incurred, it resulted in one more fragment of 481 bp (Figure 1A).

GSTM1 genotyping was performed by multiplex PCR [29]. Primers used were *GSTM1* forward: 5′-GAACTCCCTGAAAAGCTAAAGC-3′ and *GSTM1* reverse: 5′-GTTGGGCTCAAATATACGGTGG-3′. Exon 7 of the *CYP1A1* gene was co-amplified and used as an internal control using the following primers: *CYP1A1* forward: 5′-GAACTGCCACTT CAGCTGTCT-3′ and *CYP1A1* reverse: 5′-CAGCTGCATTTG GAAGTGCTC-3′. The presence of the *GSTM1-active* genotype was detected by the band at 215 bp, since the assay does not distinguish heterozygous or homozygous wild-type genotypes (Figure 1B).

GSTP1 Ile105Val polymorphism was analysed using the PCR–RFLP method by Harries *et al.* [30]. Primers used were: *GSTP1 Ile105Val* forward: 5′-ACCCCAGGGCTCTATGGGAA-3′ and *GSTP1 Ile105Val* reverse: 5′-TGAGGGCACAAGAAGCCCCT-3′. The presence of restriction site resulting in two fragments (91 and 85 bp) indicated mutant allele (*Val/Val*), while if *Ile/Val* polymorphism incurred, it resulted in one more fragment of 176 bp (Figure 1C).

GSTT1 genotyping was performed by multiplex PCR [29]. Primers used were *GSTT1*-forward: 5′-TTCCTTACTGGTCCTCACATCTC-3′ and *GSTT1*-reverse: 5′-TCACGGGATCATGGCCAGCA-3′. The assay does not distinguish between heterozygous or homozygous wild-type genotypes; therefore, the presence of 480 bp bands was indicative for the *GSTT1-active* genotype (Figure 1D).

Biomarkers of oxidative damage in plasma

Spectrohotometrical analysis was performed for protein thiol groups (method of Jocelyn) [31], AOPP (modified method of Witko-Sarsat *et al.*) [5], MDA (method of Dousset *et al.*) [32], TOS (method of Erel) [33] and PAB (method of Alamdari *et al.*) [34].

Carbonyl protein derivatives content, MDA protein adducts and nitrotyrosine were measured by enzyme immunoassay (OxiSelectTM ELISA kits, Cell Biolabs).

Statistical analysis

In descriptive statistics, we summarized all continuous variables by means ± standard deviations (SD). Differences in investigated parameters were assessed by using analysis of variance (ANOVA) for continuous variables and χ^2 for categorical variables. The associations between the genotypes and ESRD risk were calculated by using logistic regression to compute odds ratios (ORs) and corresponding 95% confidence intervals (CIs), adjusted according to age and gender as potential confounding factors.

In the first step of statistical evaluation of relationships between biomarkers of oxidative damage (SH groups, carbonyls, AOPP, nitrotyrosine, MDA, MDA adducts, TOS, PAB) and combined *GSTA1, GSTM1, GSTP1* and *GSTT1* genotypes in ESRD patients, distribution was tested by using the Kolmogorov–Smirnov test. For normally distributed data, we performed ANOVA and, if necessary, the Bonferroni *post hoc* test for locating differences between multiple groups. For data with a non-normal distribution, we used the Mann–Whitney rank-sum test (for between-two-group comparisons) and the Kruskal–Wallis non-parametric test that compared three unpaired groups.

Two-tailed P-values of <0.05 were considered significant. Data were analysed using the Statistical Package for the Social Sciences (SPSS) (version 17.0, Chicago, IL).

Results

Baseline patients' characteristics are presented in Table 1. No significant difference was observed, except for biochemical parameters of renal function.

GST genotypes and ESRD risk

The distribution of GST genotypes in ESRD patients and controls is presented in Table 2. The frequency of *GSTA1*,

Table 1. Clinical characteristics of patients with ESRD and control group

Characteristic	Cases	Controls
Clinical parameters		
Male (%)	84 (42)	85 (43)
Female (%)	115 (58)	114 (57)
Age (year)[a]	60.0 ± 12.1	59.3 ± 10.9
Time on haemodialysis (year)[a]	6.3 ± 4.4	—
Biochemical serum parameters[a]		
Albumin (g/L)	39.6 ± 4.0	43.5 ± 2.6*
Urea (mmol/L)	24.4 ± 4.8	5.5 ± 1.2*
Creatinine (μmol/L)	880.2 ± 238.6	84.4 ± 10.3*
Triacylglycerol (mmol/L)	2.1 ± 1.3	1.4 ± 0.4*
Haemoglobin (g/L)	108.1 ± 15.4	144.0 ± 13.7*
Serum iron (μmol/L)	11.3 ± 5.6	21.1 ± 4.8*
Ferritin (ng/mL)	350.2 ± 278.0	48.0 ± 29.6*
Haematocrit (%)	32.1 ± 5.2	39.8 ± 4.9*

[a]All results are presented as mean ± SD.
*P < 0.001.

Table 2. *GSTA1, GSTM1, GSTP1* and *GSTT1* genotypes in relation to the risk of ESRD

GST genotype	Cases [*n* (%)]	Controls [*n* (%)]	OR (95% CI)	P-value
GSTA1				
CC	70 (35.2)	78 (39.2)	1.0[a]	
CT	90 (45.2)	94 (47.2)	1.1 (0.7–1.7)	0.110
TT	39 (19.6)	27 (13.6)	1.6 (0.9–2.9)	0.746
CT + TT	129 (64.8)	121 (60.8)	1.2 (0.8–1.8)	0.401
GSTM1				
**1 active*[b]	80 (40.2)	102 (51.3)	1.0[a]	
**0 null*[c]	119 (59.8)	97 (48.7)	1.6 (1.1–2.4)	0.024
GSTP1				
Ile/Ile	75 (37.7)	82 (41.2)	1.0[a]	
Ile/Val	77 (38.7)	84 (42.2)	1.0 (0.6–1.5)	0.939
Val/Val	47 (23.6)	33 (16.6)	1.6 (0.9–2.7)	0.112
Ile/ Val + Val/Val	124 (62.3)	117 (58.8)	1.1 (0.8–1.7)	0.531
GSTT1				
**1 active*[b]	132 (66.3)	142 (71.4)	1.0[a]	
**0 null*[c]	67 (33.7)	57 (28.6)	1.2 (0.8–1.9)	0.319

OR, odds ratio; CI, confidence interval; Ca, number of patients; Co, controls.
[a]Reference category.
[b]Active (present) if at least one active allele present.
[c]Inactive (null) if no active alleles present.

GSTM1, *GSTP1* and *GSTT1 null/low-activity* genotypes was higher in ESRD patients than in controls. Significant association between the GST genotype and risk of ESRD development was found only for the *GSTM1* genotype. Individuals with *GSTM1-null* were at 1.6-fold higher risk of ESRD development (OR = 1.6, 95% CI = 1.1–2.4, P = 0.024) than individuals carrying the *GSTM1-active* genotype. When GST genotypes were analysed in combination (Table 3), the highest risk of ESRD development was obtained in subjects who carried both *GSTM1* and *GSTT1-null* genotypes (OR = 2.0, 95% CI = 1.1–3.7, P = 0.025). Combination of *GSTM1-null* with the *GSTP1-low-activity* genotype was also found to be significant (OR = 1.8, 95% CI = 1.0–3.1, P = 0.042). The *GSTM1-null/GSTA1-low-activity* combination had certain effect on ESRD risk (OR = 1.8; 95% CI = 0.9–3.3, P = 0.058).

Association between GST genotype and biomarkers of oxidative damage

The degree of oxidative and nitrosative protein damage in ESRD patients stratified according to GST genotypes is presented in Figure 2. Plasma protein thiol groups concentration was significantly lower in patients with both *GSTM1* or *GSTT1-null*, as well as, *GSTP1-low-activity* genotype in comparison to corresponding active genotype (P = 0.001, 0.002 and 0.042, respectively) (Figure 2A). Significantly higher plasma carbonyl groups levels were found in patients with low-activity *GSTA1-TT* or *GSTM1-null* genotype (P = 0.007 and 0.005, respectively) (Figure 2B). AOPP concentrations were increased in patients carrying the *GSTM1-* or *GSTT1-null* genotype (P = 0.001 and 0.037, respectively) (Figure 2C). As presented, the most pronounced effect regarding protein oxidative damage was observed for the *GSTM1* genotype (Figure 2). Furthermore, nitrotyrosine, a reliable marker of nitrosative damage of proteins, was found to be higher in patients with the *GSTP1-low-activity* genotype (P = 0.07) (Figure 2D).

MDA, a commonly used biomarker of lipid oxidative damage, exists both as free and bound to proteins, nucleic acids and lipoproteins which are designated as MDA adducts. Both free and MDA adduct levels were significantly increased in patients with GST null/low-activity genotypes (Figure 3).

Since the effects of different oxidant molecules are additive, we determined the TOS and PAB. Similar to previous results, patients with GST null or low-activity genotypes had increased plasma TOS and PAB levels, with the *GSTM1-null* genotype carriers having the highest levels (P = 0.001) (Figure 4A). Increased TOS and PAB concentrations were also found in patients with *GSTT1-null* genotype, but did not reach statistical significance.

To assess whether the effects of null/low-activity GST genotypes are more pronounced when combined, we compared the level of oxidative damage between ESRD patients stratified according to the various combinations of GST gene variants (Table 4). The level of oxidative damage of proteins and lipids was lowest in patients with both *GSTSM1-* and *GSTT1-active* genotypes, then gradually increased in *GSTM1-active/GSTT1-null* and *GSTM1-null/GSTT1-active* ESRD patients, reaching the highest values in patients with both *GSTM1-* and *GSTT1-null* genotypes (Table 4). The observed effect was even more obvious if patients were stratified according to the combination of three GST genotypes (Table 4). Furthermore, when a combination of all four GST genotypes tested was analysed, we found striking evidence in favour of increased susceptibility to lipid peroxidation and protein oxidative and nitrosative damage in ESRD patients carrying combined *GSTM1/GSTT-null* and *low-activity GSTA1* and *GSTP1* genotypes (Table 4). On the other hand, carriers of combined active *GSTM1, GSTT1, GSTP1* and *GSTT1* genotypes had the lowest level of oxidative stress among ESRD patients.

Discussion

High levels of free radicals that occur in the course of chronic renal failure (CRF) are associated with the disease pathogenesis, its progression and complications [11, 35]. Based on this premise, we speculated that variations in detoxifying and antioxidant activities of GST modulate individual tendency towards ESRD development, regardless of its specific cause. Among four common GST polymorphisms analysed in this study, only the *GSTM1-null* genotype has shown a significant association with ESRD that was more pronounced if any of the other three null/low-activity GST genotypes was also present. Our results on the association between *GSTM1-null* genotype and increased risk of ESRD development are in accordance with several studies performed in Indian population [36–38].

Table 3. Combined effects of *GSTA1, GSTM1, GSTP1* and *GSTT1* genotypes in relation to the risk of ESRD

	GSTM1		*GSTA1*		*GSTP1*	
	Present[a]	Null[b]	Active[a]	Low activity[c]	Active[a]	Low activity[c]
GSTA1						
Active[a]						
Ca/Co	29/36	41/42	—	—	—	—
OR (95% CI)	1.0[d]	1.2 (0.6–2.3)				
Low activity[c]						
Ca/Co	48/64	78/55	—	—	—	—
OR (95% CI)	0.9 (0.5–1.8)	1.8 (0.9–3.3)				
GSTP1						
Active[a]						
Ca/Co	34/50	41/32	30/33	45/49	—	—
OR (95% CI)	1.0[d]	2.1 (1.1–4.0)[e]	1.0[d]	1.0 (0.5–2.0)		
Low activity[c]						
Ca/Co	45/52	78/65	40/45	84/72	–	–
OR (95% CI)	1.3 (0.7–2.4)	1.8 (1.0–3.1)[e]	1.0 (0.5–1.9)	1.2 (0.7–2.3)		
GSTT1						
Present[a]						
Ca/Co	51/73	81/69	48/56	85/85	55/62	77/80
OR (95% CI)	1.0[d]	1.7 (1.1–2.8)[e]	1.0[d]	1.2 (0.7–1.9)	1.0[d]	1.1 (0.7–1.7)
Null[b]						
Ca/Co	28/30	38/27	22/22	45/35	20/20	47/37
OR (95% CI)	1.3 (0.7–2.5)	2.0 (1.1–3.7)[e]	1.1 (0.5–2.3)	1.5 (0.8–2.7)	1.2 (0.6–2.5)	1.4 (0.8–2.5)

OR, odds ratio; CI, confidence interval.
[a]Active (present) if at least one active allele present.
[b]Null if no active alleles present.
[c]Low activity if at least one lower activity allele present.
[d]Reference group.
[e]Statistically significant difference when compared with the reference group.

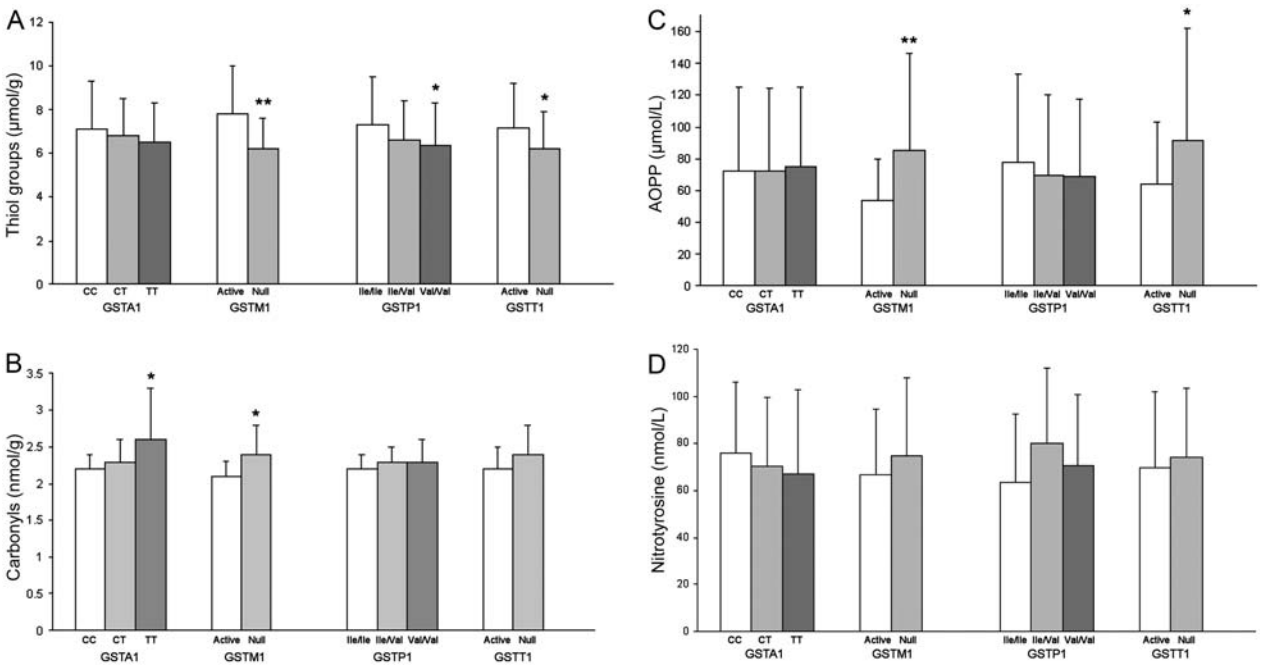

Fig. 2. Protein oxidative damage biomarkers in relation to the GST genotype in ESRD patients. Plasma thiol groups decreased in patients with null/low-activity GST genotype (**A**). Carbonyl groups, AOPP and nitrotyrosine content in plasma increased in patients with null/low-activity GST genotype (**B**–**D**, respectively). *$P < 0.05$, **$P < 0.01$.

However, such association was not found among Taiwan Chinese [39], population of Taipei [10] and Asian Indians ESRD patients [40]. With regard to the *GSTT1-null* genotype, two studies revealed that the *GSTT1-null* genotype influences higher risk for ESRD development [37, 39]. The role of low-activity *GSTP1-Val/Val*

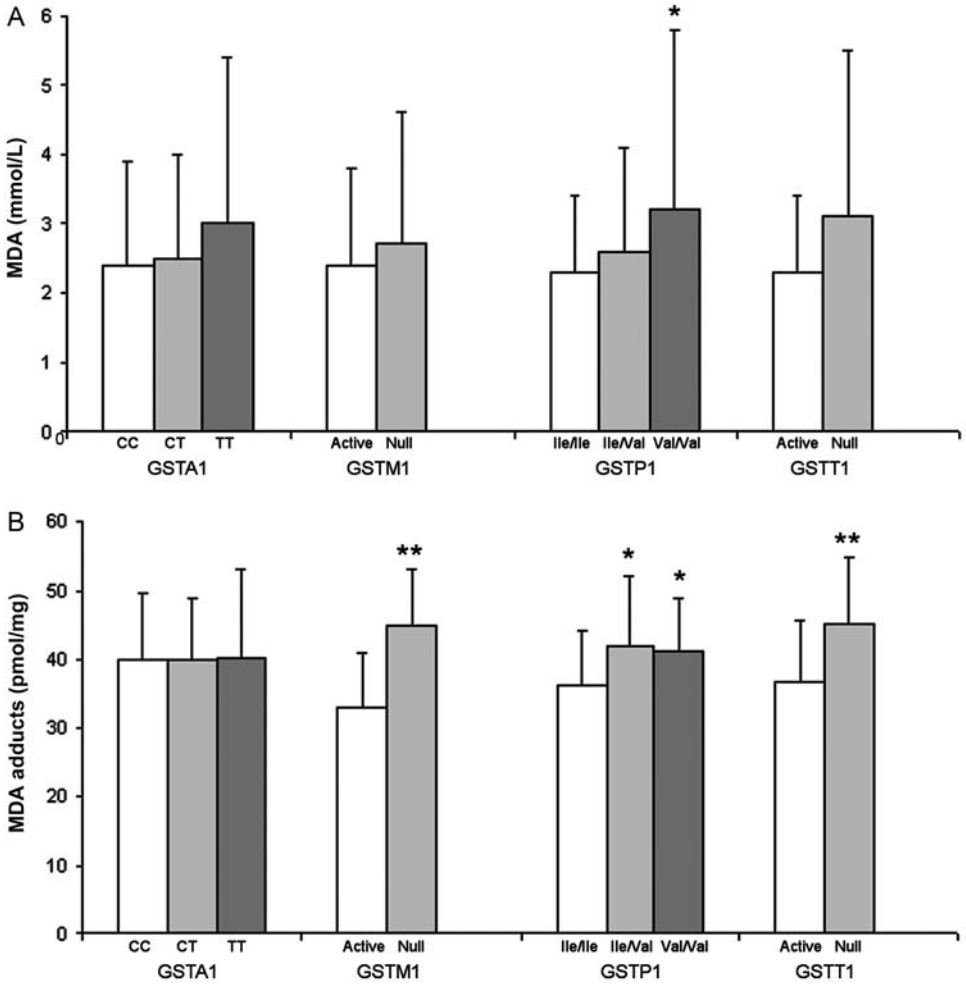

Fig. 3. Lipid oxidative damage biomarkers in relation to the GST genotype in ESRD patients. Plasma MDA concentrations and MDA adducts content increased in patients with null/low-activity GST genotype (**A** and **B**, respectively). *P < 0.05, **P < 0.01.

polymorphism was addressed in only two studies [36, 40], in which it was also associated with increased ESRD risk. Taken together, these data suggest the existence of ethnic-specific GST genetic susceptibility to ESRD development. To our knowledge, this is the first investigation that addressed the susceptibility to ESRD in European Caucasians in association to common GST polymorphisms. Our data on combined effect of various GST genotypes on ESRD development are in accordance with previous investigations of *GSTM1/GSTT1* [37] and *GSTM1/T1/P1* [36] genotype combinations in Northern Indian patients, although the magnitude of this association was lower in our population. Differential susceptibility to ESRD in various populations might not only be the consequence of differences in the genetic distribution of GSTs among ethnic groups, but also due to the different aetiology of ESRD in various regions. Since ESRD among our patients had distinct aetiology and could hardly be associated with environmental agents metabolized by GST enzymes, except for Balkan endemic nephropathy, weak association obtained for *GSTM1-null* and its absence with other GST forms is not unexpected. It is reasonable to assume that GST polymorphic expression may be much more important in the course of CRF progression, since accumulated end-products of endogenous and exogenous origin and ROS in these patients act as GST substrates [41]. This is a situation in which gene interaction with disease-specific mechanisms influences further course of disease.

The assumption that lack or low-activity GST gene variants contribute to increased susceptibility to oxidative and carbonyl stress in ESRD has been confirmed in the next phase of this investigation, in which the influence of polymorphic *GSTA1*, *GSTM1*, *GSTP1* and *GSTT1* genes was analysed with respect to oxidative phenotype. Oxidative stress in ESRD has multifactorial origin, including low molecular weight uraemic toxins, elevated homocysteine level, increased carbonyl stress, with decreased expression of extracellular glutathione peroxidase produced by renal parenchymal cells, as well as, exacerbations of oxidative stress by dialysis sessions and severe chronic inflammation [11, 35, 42]. Oxidative stress in ESRD patients is considered the cornerstone of atherosclerotic process. Carotid artery intima-media thickness in

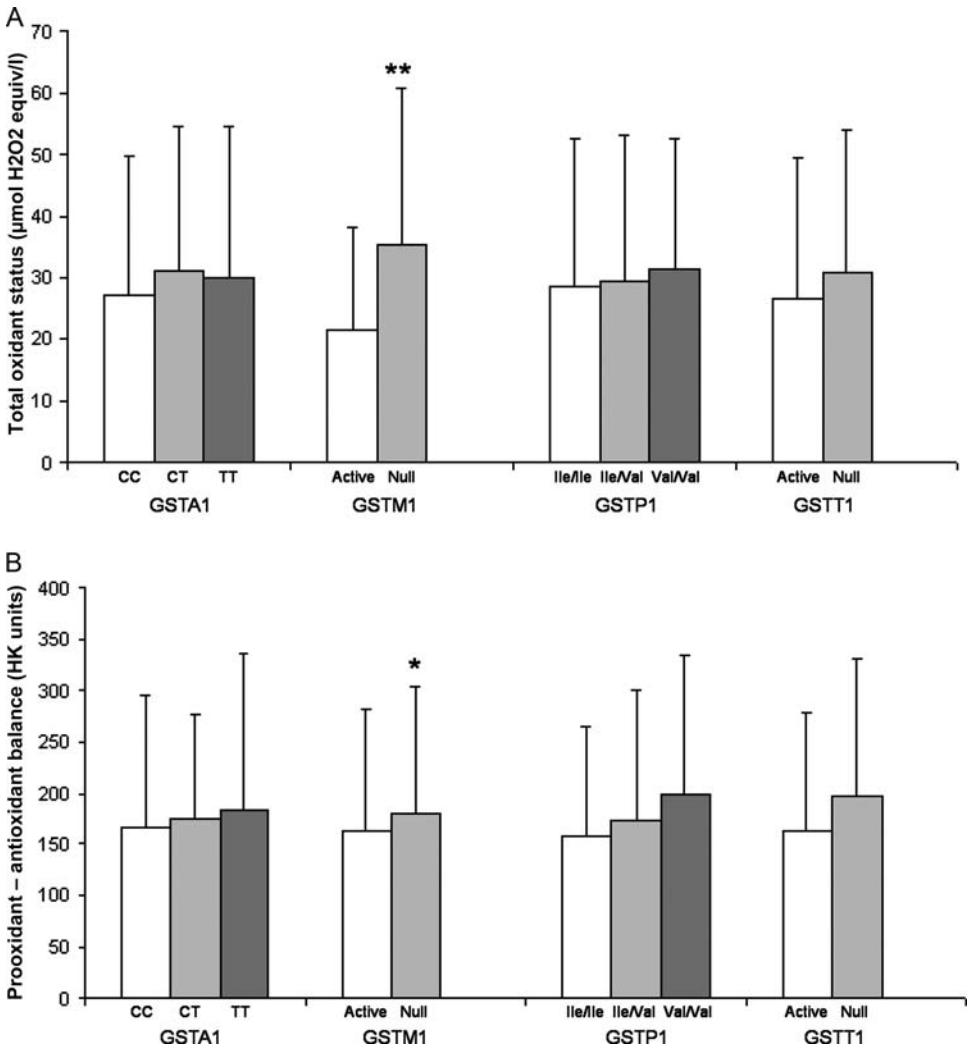

Fig. 4. TOS and PAB in relation to the GST genotype in ESRD patients. TOS and PAB values increased in patients with null/low-activity GST genotype (**A** and **B**, respectively). *P < 0.05, **P < 0.01.

chronic HD patients correlates with lipid peroxidation by-products [43], while serum MDA is a strong predictor of prevalent cardiovascular disease in these patients [44]. Besides, the content of AOPP independently predicts atherosclerotic CV events in non-diabetic pre-dialysis patients [45]. It is important to note that GST family members play a dual role in defence mechanisms that counteract complex biochemical changes present in uraemic state. Namely, all products of analysed GST genes possess both glutathione conjugating and antioxidant enzymatic activity [18, 46]. According to the results presented in this study, the level of protection against both oxidative and carbonyl stress in uraemic syndrome is influenced by common GST polymorphisms which determine the quantity and/or activity of GST protein 'echelons' that act upon organic hydroperoxides and accumulated harmful compounds.

The data concerning genetic predisposition to worse oxidative phenotype in patients with CRF are limited. Lin *et al*. [10] showed that HD patients with the *GSTM1-null* genotype are more vulnerable to oxidative DNA damage and are at greater risk for death compared with those who possess active *GSTM1* genotype. In this study, we addressed the most relevant classes of oxidative protein and lipid damage by-products encountered in ESRD, as well as, TOS and PAB in relation to *GSTA1, GSTM1, GSTP1* and *GSTT1* genotypes. We found that individual GST polymorphisms influence vulnerability to both protein and lipid oxidation, with *GSTM1-null* gene variant having the most pronounced effect. Besides, our results have shown a strong combined effect of *null* or *low-activity GSTM1, GSTT1, GSTA1* and *GSTP1* genotypes in terms of susceptibility towards oxidative and carbonyl stress in patients with ESRD. Our results confirmed similar findings presented in patients with benign prostate hyperplasia in which combined *GSTM1-null/GSTT1-null* genotype was associated with increased plasma MDA level [47]. Our study is also consistent with the results of Tang *et al*. [48], who showed that coronary artery disease patients with the *GSTM1-null/GSTT1-null* genotype have lower total antioxidant capacity than those with both active genotypes [48]. Besides, this genotype combination was

Table 4. Biomarkers of oxidative damage in relation to combined *GSTA1*, *GSTM1*, *GSTP1* and *GSTT1* genotypes in ESRD patients (mean ± SD)

Combined Genotype	SH groups (μmol/g prot)	p	Carbonyls (nmol/mg prot)	p	AOPP (mmol/L)	p	Nitrotyrosine (nmol/l)	p	MDA (mmol/l)	p	MDA adducts (pmol/mg prot)	p	TOS (μmol H_2O_2 Equiv./l)	p	PAB (HK units)	p
GSTM1/*T1* active	8.2 ± 2.4		2.1 ± 0.3		52.6 ± 28.2		64.13 ± 27.55		2.2 ± 1.0		29.4 ± 6.8		18.8 ± 13.4		140.7 ± 118.1	
	100%		100%		100%		100%		100%		100%		100%		100%	
GSTM1	7.1 ± 1.7	0.029	2.3 ± 0.3	0.29	56.1 ± 21.9	0.450	71.24 ± 27.68	0.279	2.5 ± 1.3	0.269	40.0 ± 5.9	0.001	23.0 ± 18.4	0.315	220.6 ± 153.7	0.127
active/*T1* null	86.6%		109.5%		106.6%		111.1%		113.6%		136.1%		122.3%		156.8%	
GSTM1 null/*T1*	6.5 ± 1.2	0.001	2.4 ± 0.0.4	0.586	71.1 ± 43.4	0.021	73.67 ± 34.19	0.168	2.8 ± 1.8	0.132	42.0 ± 6.0	0.001	45.1 ± 45.4	0.001	194.1 ± 149.7	0.08
active	79.3%		114.3%		135.2%		114.9%		127.3%		142.9%		239.9%		137.9%	
GSTM1/*T1* null	5.5 ± 1.4	0.001	2.6 ± 0.5	0.003	123.5 ± 81.9	0.001	77.41 ± 30.89	0.073	3.3 ± 2.9	0.098	49.6 ± 9.6	0.001	109.7 ± 123.3	0.002	186.3 ± 147.7	0.181
	67.1%		123.8%		234.8%		120.7%		150%		168.7%		573.5%		132.4%	
GSTM1/T1/A1	8.8 ± 1.2		2.2 ± 0.2		46.5 ± 25.9		66.45 ± 29.2		2.4 ± 1.1		31.1 ± 8.3		14.7 ± 5.3		128.7 ± 107.8	
active	100%		100%		100%		100%		100%		100%		100%		100%	
GSTM1/T1/A1	5.5 ± 1.2	0.001	2.9 ± 0.5	0.002	133.2 ± 89.6286.4%	0.001	69.51 ± 28.04	0.718	3.6 ± 3.2	0.188	53.2 ± 8.9	0.001	99.9 ± 120.7	0.044	161.7 ± 110.9	0.402
null or low activity	62.5%		131.8%				104.6%		150%		171.1%		679.6%		125.6%	
GSTM1/T1/P1	8.4 ± 2.8		2.2 ± 0.2		57.9 ± 25.0		59.8 ± 26.1		2.1 ± 0.9		30.0 ± 6.4		21.4 ± 17.2		158.6 ± 124.5	
active	100%		100%		100%		100%		100%		100%		100%		100%	
GSTM1/T1/P1	5.2 ± 1.2	0.001	2.6 ± 0.5	0.024	116.0 ± 76.0200.3%	0.022	77.90 ± 29.71	0.040	3.8 ± 3.3	0.026	49.4 ± 9.9	0.001	93.9 ± 116.1	0.068	203.1 ± 154.3	0.361
null or low activity	61.9%		118.2%				130.26%		180.9%		164.7%		438.8%		128.1%	
GSTM1/T1/A1/P1	9.5 ± 2.3		2.3 ± 0.1		50.2 ± 23.7		56.08 ± 20.80		2.0 ± 0.8		33.5 ± 6.2		13.1 ± 3.4		152.2 ± 104.3	
active	100%		100%		100%		100%		100%		100%		100%		100%	
GSTM1/T1/A1/P1	5.3 ± 1.2	0.001	2.9 ± 0.6	0.037	123.5 ± 84.0246%	0.038	72.43 ± 29.29	0.169	3.8 ± 3.5	0.111	52.6 ± 8.4	0.001	103.3 ± 127.5	0.096	153.0 ± 109.0	0.791
null or low activity	55.8%		126.1%				127.5%		190%		157%		788.5%		100.5%	

associated with enhanced DNA damage in many studies that investigated the role of GST polymorphisms in relation to cancer risk [49–52]. In the light of the role that GST enzymes have in detoxification and as antioxidant enzymes, results of this and other studies on association between *null/low-activity GSTM1, GSTT1, GSTP1* and *GSTA1* genotypes and increased oxidant damage of proteins and lipids are biologically plausible. According to the presence of various GST gene variants in combination, ESRD patients may be stratified in terms of level of oxidative, carbonyl stress and nitrosative stress. Concerning the latter, imbalance in the relative levels of SOD and GPX, already observed in HD patients [8, 9, 13], influences the ratio of nitric oxide: superoxide anion leading to an increase in peroxynitrite production [53]. Although the association between GST null or low-activity gene variants and plasma nitrotyrosine level in ESRD patients was relatively mild, our findings suggest that their antioxidant activities at least partially affect the level of nitrosative damage. Since oxidative stress parameters correlate with CV complications and mortality [10, 45, 54, 55], it may be speculated that interaction between the uraemic state and combined GST genotype would represent at least one of the potential mechanisms explaining inter-individual differences in terms of CV outcome in these patients. Certain limitations might be considered in our study. The case–control design was used for estimation of associations between GST genotypes and ESRD development, and therefore, selection bias might influence the results. Our control group was hospital-based, thus the use of population controls may have been more appropriate. The cross-sectional design performed for analyses of influence of GST genotypes on by-products of oxidative stress did not allow us to investigate the role of GST polymorphisms in the development of CV complications in ESRD patients. Nevertheless, this study may offer some essential information that could be the base for future longitudinal research.

In addition to prognosis, our results may have implications in new approaches to the antioxidant therapy in HD patients. The suggested therapeutic interventions aimed at reducing oxidative stress in HD patients include biocompatible membranes, administration of antioxidants and substances indirectly affecting oxidative stress [11]. Recently, vitamin E-bonded membranes for haemodialysers have been developed, which have a wide spectrum of positive effects on antioxidant status in dialysis patients [56–58]. The results obtained in this study suggest that the use of dialysers with vitamin E-bonded membranes would be of most benefit for patients with combined *GST* null or low-activity genotypes. Besides, the GST genotype determination in HD patients could be a step forward to individualization of antioxidant administration. In their recent review, Coumbes and Faset reported findings of more than 50 studies in which the effects of antioxidant therapy were investigated in HD patients. Only α-tocopherol and *N*-acetylcysteine treatment consistently decreased oxidative stress. Besides, the authors indicated that future studies need first to develop valid oxidative stress biomarkers before evaluating the efficiency of antioxidant therapy [59]. Since susceptibility to oxidative damage differs with respect to the GST genotype, it seems reasonable to assume that individuals' GST 'genetic profile', in addition to plausible oxidative stress biomarkers, should be also considered in the optimization of form, dose and time course of antioxidant treatment.

Based on the results of this investigation, it may be concluded that the *GSTM1-null* genotype is associated with increased risk of ESRD development and enhanced susceptibility to oxidative stress in dialysis patients. The presence of *GSTT1-null* or *GSTP1-low-activity* (*Val/Val* and *Ile/Val*) genotypes in ESRD patients also significantly influences the level of oxidative damage by-products, but not as much as *GSTM1-null*. However, the effect modification with regard to oxidative phenotype in HD patients is most pronounced if *GSTM1-* and *GSTT1-null* as well as low-activity *GSTA1* and *GSTP1* genotypes are present in combination. Taken together, these results suggest a possibility for GST genotype-based stratification of ESRD patients which could improve the attempts towards individualization of antioxidant treatment.

Acknowledgements. This study was supported by a Grant 175052 from Serbian Ministry of Education and Science.

Conflict of interest statement. None declared.

References

1. Tarng DC, Huang TP, Wei YH *et al*. 8-Hydroxy-2-deoxyguanosine of leukocyte DNA s a marker of oxidative stress in chronic hemodialysis patients. *Am J Kidney Dis* 2000; 36: 934–944
2. Tarng DC, Huang TP, Liu TY *et al*. Effect of vitamin E-bonded membrane on the 8-hydroxy 2′-deoxyguanosine level in leukocyte DNA of hemodialysis patients. *Kidney Int* 2000; 58: 790–799
3. Peuchant E, Carbonneau MA, Dubourg L *et al*. Lipoperoxidation in plasma and red blood cells of patients undergoing haemodialysis: vitamins A, E and iron status. *Free Radic Biol Med* 1994; 16: 339–346
4. Maccarrone M, Taccone-Gallucci M, Meloni C *et al*. Activation of 5-lipoxygenase and related cell membrane lipoperoxidation in hemodialysis patients. *J Am Soc Nephrol* 1999; 10: 1991–1996
5. Witko-Sarsat V, Friedlander M, Capeillere-Blandin C *et al*. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney Int* 1996; 49: 1304–1313
6. Miyata T, Ueda Y, Yamada Y *et al*. Accumulation of carbonyls accelerates the formation of pentosidine, an advanced glycation end product: carbonyl stress in uremia. *J Am Soc Nephrol* 1998; 9: 2349–2356
7. Locatelli F, Canaud B, Eckardt KU *et al*. Oxidative stress in end-stage renal disease: an emerging threat to patient outcome. *Nephrol Dial Transplant* 2003; 18: 1272–1280
8. Mimic-Oka J, Simic T, Ekmescic V *et al*. Erythrocyte glutathione peroxidase and superoxide dismutase activities in different stages of chronic renal failure. *Clin Nephrol* 1995; 44: 44–48
9. Mimic-Oka J, Simic T, Djukanovic L *et al*. Alteration in plasma antioxidant capacity in various degrees of chronic renal failure. *Clin Nephrol* 1999; 51: 233–241
10. Lin YS, Hung SC, Wei YH *et al*. GST M1 polymorphism associates with DNA oxidative damage and mortality among hemodialysis patients. *J Am Soc Nephrol* 2009; 20: 405–415
11. Libetta C, Sepe V, Esposito P *et al*. Oxidative stress and inflammation: implications in uremia and hemodialysis. *Clin Biochem* 2011; 44: 1189–1198
12. Carmagnol F, Sinet PM, Rapin J *et al*. Glutathione-S-transferase of human red blood cells; assay, values in normal subjects and in two pathological circumstances: hyperbilirubinemia and impaired renal function. *Clin Chim Acta* 1981; 117: 209–217

13. Mimic-Oka J, Simic T, Djukanovic Lj *et al*. Glutathione and its associated enzymes in peripheral blood cells in different stages of renal insufficiency. *Amino Acids* 1992; 2: 215–224
14. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferase—the first enzymatic step in mercapturic acid formation. *J Biol Chem* 1974; 249: 7130–7139
15. Hayes JD, Strange RC. Glutathione S-transferase polymorphisms and their biological consequences. *Pharmacology* 2000; 61: 154–166
16. Board P, Coggan M, Johnston P *et al*. Genetic heterogeneity of the human glutathione transferases: a complex of gene families. *Pharmacol Ther* 1990; 48: 357–369
17. Carlsten C, Sagoo GS, Frodsham AJ *et al*. Glutathione S-transferase M1 (GSTM1) polymorphisms and lung cancer: a literature-based systematic HuGE review and meta-analysis. *Am J Epidemiol* 2008; 167: 759–774
18. Simic T, Savic-Radojevic A, Pljesa-Ercegovac M *et al*. Glutathione S-transferases in kidney and urinary bladder tumors. *Nat Rev Urol* 2009; 6: 281–289
19. Miller EA, Pankow JS, Millikan RC *et al*. Glutathione-S-transferase genotypes, smoking, and their association with markers of inflammation, hemostasis, and endothelial function: the atherosclerosis risk in communities (ARIC) study. *Atherosclerosis* 2003; 171: 265–272
20. Li R, Boerwinkle E, Olshan AF *et al*. Glutathione S-transferase genotype as a susceptibility factor in smoking-related coronary heart disease. *Atherosclerosis* 2000; 149: 451–462
21. Wiencke JK, Pemble S, Ketterer B *et al*. Gene deletion of glutathione s-transferase theta: correlation with induced genetic damage and potential role in endogenous mutagenesis. *Cancer Epidemiol Biomarkers Prev* 1995; 4: 253–259
22. Kellen E, Hemelt M, Broberg K *et al*. Pooled analysis and metaanalysis of the glutathione S-transferase P1 ile105val polymorphism and bladder cancer: a HuGe-GSEC review. *Am J Epidemiol* 2007; 165: 1221–1230
23. Dusinská M, Ficek A, Horská A *et al*. Glutathione S-transferase polymorphisms influence the level of oxidative DNA damage and antioxidant protection in humans. *Mutat Res* 2001; 482: 47–55
24. Zimniak P, Nanduri B, Pilula S *et al*. Naturally occurring human glutathione S-transferase GSTP1.1 isoforms with isoleucine and valine at position 104 differ in enzymatic properties. *Eur J Biochem* 1994; 244: 893–899
25. Matsui A, Ikeda T, Enomoto K *et al*. Increased formation of oxidative DNA damage, 8-hydroxy-2′-deoxyguanosine, in human breast cancer tissue and its relationship to GSTP1 and COMT genotypes. *Cancer Lett* 2000; 151: 87–95
26. Garte S, Gaspari L, Alexandrie AK *et al*. Metabolic gene polymorphism frequencies in control populations. *Cancer Epidemiol Biomarkers Prev* 2001; 10: 1239–1248
27. Coles FB, Kadlubar FF. Human alpha class glutathione S-transferases: genetic polymorphism, expression, and susceptibility to disease. *Methods Enzymol* 2005; 401: 9–42
28. Ping J, Wang H, Huang M *et al*. Genetic analysis of glutathione S-transferase A1 polymorphism in the Chinese population and the influence of genotype on enzymatic properties. *Toxicol Sci* 2006; 89: 438–443
29. Abdel-Rahman SZ, el-Zein RA, Anwar WA *et al*. A multiplex PCR procedure for polymorphic analysis of GSTM1 and GSTT1 genes in population studies. *Cancer Lett* 1996; 107: 229–233
30. Harries LW, Stubbins MJ, Forman D *et al*. Identification of genetic polymorphisms at the glutathione S-transferase Pi locus and association with susceptibility to bladder, testicular and prostate cancer. *Carcinogenesis* 1997; 18: 641–644
31. Jocelyn PC. Spectrophotometric assay of thiols. *Methods Enzymol* 1987; 143: 44–67
32. Dousset JC, Trouilh M, Foglietti MJ. Plasma malonaldehyde levels during myocardial infarction. *Clin Chim Acta* 1983; 129: 319–322
33. Erel O. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem* 2005; 38: 1103–1111
34. Alamdari DH, Paletas K, Pegiou T *et al*. A novel assay for the evaluation of the prooxidant–antioxidant balance, before and after antioxidant vitamin administration in type II diabetes patients. *Clin Biochem* 2007; 40: 248–254
35. Kao MP, Ang DS, Pall A *et al*. Oxidative stress in renal dysfunction: mechanisms, clinical sequelae and therapeutic options. *J Hum Hypertens* 2010; 24: 1–8
36. Agrawal S, Tripathi G, Khan F *et al*. Relationship between GSTs gene polymorphism and susceptibility to end stage renal disease among North Indians. *Ren Fail* 2007; 29: 947–953
37. Datta SK, Kumar V, Pathak R *et al*. Association of glutathione S-transferase M1 and T1 gene polymorphism with oxidative stress in diabetic and nondiabetic chronic kidney disease. *Ren Fail* 2010; 32: 1189–1195
38. Datta SK, Kumar V, Ahmed RS *et al*. Effect of GSTM1 and GSTT1 double deletions in the development of oxidative stress in diabetic nephropathy patients. *Indian J Biochem Biophys* 2010; 47: 100–103
39. Yang Y, Kao MT, Chang CC *et al*. Glutathione S-transferase T1 deletion is a risk factor for developing end-stage renal disease in diabetic patients. *Int J Mol Med* 2004; 14: 855–859
40. Tiwari AK, Prasad P, B KT *et al*. Oxidative stress pathway genes and chronic renal insufficiency in Asian Indians with Type 2 diabetes. *J Diabetes Complications* 2009; 23: 102–111
41. Klemm A, Voigt C, Friedrich M *et al*. Determination of erythrocyte antioxidant capacity in haemodialysis patients using electron paramagnetic resonance. *Nephrol Dial Transplant* 2001; 16: 2166–2171
42. Galli F. Protein damage and inflammation in uraemia and dialysis patients. *Nephrol Dial Transplant* 2007; 22: 20–36
43. Dursun B, Dursun E, Suleymanlar G *et al*. Carotid artery intima-media thickness correlates with oxidative stress in chronic haemodialysis patients with accelerated atherosclerosis. *Nephrol Dial Transplant* 2008; 23: 1697–1703
44. Boaz M, Matas Z, Biro A *et al*. Serum malondialdehyde and prevalent cardiovascular disease in hemodialysis. *Kidney Int* 1999; 56: 1078–1083
45. Descamps-Latscha B, Witko-Sarsat V, Nguyen-Khoa T *et al*. Advanced oxidation protein products as risk factors for atherosclerotic cardiovascular events in nondiabetic predialysis patients. *Am J Kidney Dis* 2005; 45: 39–47
46. Hayes JD, Strange RC. Potential contribution of glutathione S-transferase supergene family to resistance to oxidative stress. *Free Radic Res* 1995; 22: 193–207
47. Kumar V, Yadav CS, Datta SK *et al*. Association of GSTM1 and GSTT1 polymorphism with lipid peroxidation in benign prostate hyperplasia and prostate cancer: a pilot study. *Dis Markers* 2011; 30: 163–169
48. Tang JJ, Wang MW, Jia EZ *et al*. The common variant in the GSTM1 and GSTT1 genes is related to markers of oxidative stress and inflammation in patients with coronary artery disease: a case-only study. *Mol Biol Rep* 2010; 37: 405–410
49. Kato S, Bowman ED, Harrington AM *et al*. Human lung carcinogen-DNA adduct levels mediated by genetic polymorphisms *in vivo*. *J Natl Cancer Inst* 1995; 87: 902–907
50. Butkiewicz D, Cole KJ, Phillips DH *et al*. GSTM1, GSTP1, CYP1A1 and CYP2D6 polymorphisms in lung cancer patients from an environmentally polluted region of Poland: correlation with lung DNA adduct levels. *Eur J Cancer Prev* 1999; 8: 315–323
51. Rojas M, Cascorbi I, Alexandrov K *et al*. Modulation of benzo[a] pyrene diolepoxide-DNA adduct levels in human white blood cells by CYP1A1, GSTM1 and GSTT1 polymorphism. *Carcinogenesis* 2000; 21: 35–41
52. McCarty KM, Santella RM, Steck SE *et al*. PAH-DNA adducts, cigarette smoking, GST polymorphisms and breast cancer risk. *Environ Health Perspect* 2009; 117: 552–558
53. Köse FA, Seziş M, Akçiçek F *et al*. Oxidative and nitrosative stress markers in patients on hemodialysis and peritoneal dialysis. *Blood Purif* 2011; 32: 202–208
54. Bayes B, Pastor C, Bonal J *et al*. Homocysteine, C-reactive protein, lipid peroxidation and mortality in haemodialysis patients. *Nephrol Dial Transpl* 2003; 18: 106–112

55. Kalantar-Zadeh K, Brennan ML, Hazen SL. Serum myeloperoxidase and mortality in maintenance hemodialysis patients. *Am J Kidney Dis* 2006; 48: 59–68
56. Cruz DN, De Cal M, Garzotto F *et al*. Effect of vitamin E-coated dialysis membranes on anemia in patients with chronic kidney disease: an Italian multicenter study. *Int J Artif Organs* 2008; 31: 545–552
57. Galli F, Rovidati S, Benedetti S *et al*. Lipid peroxidation, leukocyte function and apoptosis in hemodialysis patients treated with vitamin-E modified filters. *Contrib Nephrol* 1999; 127: 156–171
58. Schiecke G, Gwinner W, Radermacher J *et al*. Long-term effect of vitamin E-bonded dialysis membrane on mononuclear cell activation, malondialdehyde generation and endothelial function in ESRD patients. *Contrib Nephrol* 1999; 127: 243–250
59. Coombes JS, Fassett RG. Antioxidant therapy in hemodialysis patients: a systematic review. *Kidney Int* 2012; 81: 233–246

Received for publication: 6.4.2012; Accepted in revised form: 3.7.2012