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

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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 possess 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'-GTTAAACGCTGTACCCGTCCT-3'. The presence of restriction site resulting in two fragments (385 and 96 bp) indicated mutant allele (*T/T*)

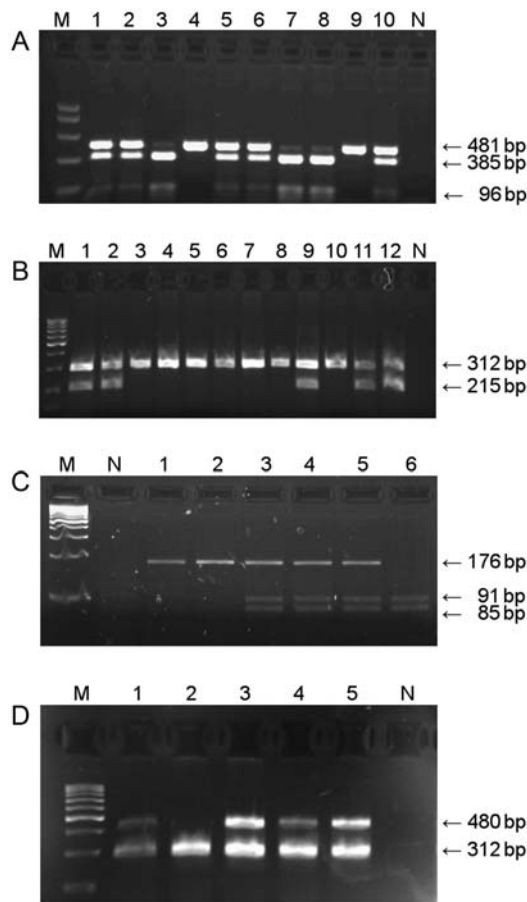


Fig. 1. Agarose gel electrophoretogram. PCR-RFLP products of the *GSTA1* gene. Lanes 4 and 9 represent the *GSTA1* CC genotype. Lanes 1, 2, 5, 6 and 10 represent the *GSTA1* CT genotype, while lanes 3, 7 and 8 represent the *GSTA1* TT genotype (A). PCR products of the *GSTM1* gene are showed in (B). 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'-CAGCTGCATTG 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'-ACCCAGGGCTCTATGGGAA-3' and *GSTP1 Ile105Val* reverse: 5'-TGAGGGCACAAGAAGCCCT-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'-TTCTTACTGGTCCTACATCTC-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

Spectrophotometrical 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 (OxiSelect™ ELISA kits, Cell Biolabs).

Statistical analysis

In descriptive statistics, we summarized all continuous variables by means \pm 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 \pm 12.1 | 59.3 \pm 10.9 |
| Time on haemodialysis (year) ^a | 6.3 \pm 4.4 | — |
| Biochemical serum parameters ^a | | |
| Albumin (g/L) | 39.6 \pm 4.0 | 43.5 \pm 2.6* |
| Urea (mmol/L) | 24.4 \pm 4.8 | 5.5 \pm 1.2* |
| Creatinine (μ mol/L) | 880.2 \pm 238.6 | 84.4 \pm 10.3* |
| Triacylglycerol (mmol/L) | 2.1 \pm 1.3 | 1.4 \pm 0.4* |
| Haemoglobin (g/L) | 108.1 \pm 15.4 | 144.0 \pm 13.7* |
| Serum iron (μ mol/L) | 11.3 \pm 5.6 | 21.1 \pm 4.8* |
| Ferritin (ng/mL) | 350.2 \pm 278.0 | 48.0 \pm 29.6* |
| Haematocrit (%) | 32.1 \pm 5.2 | 39.8 \pm 4.9* |

^aAll results are presented as mean \pm 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 |
|------------------------|------------------|---------------------|------------------|---------|
| <i>GSTA1</i> | | | | |
| 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 |
| <i>GSTM1</i> | | | | |
| *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 |
| <i>GSTP1</i> | | | | |
| 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 |
| <i>GSTT1</i> | | | | |
| *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.

^aReference category.

^bActive (present) if at least one active allele present.

^cInactive (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/*GSTP1*-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 *GSTP1*-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 *GSTM1*- 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*/*GSTT1*-null and low-activity *GSTP1* and *GSTT1* 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

| | <i>GSTM1</i> | | <i>GSTA1</i> | | <i>GSTP1</i> | |
|---------------------------|----------------------|----------------------------|---------------------|---------------------------|---------------------|---------------------------|
| | Present ^a | Null ^b | Active ^a | Low activity ^c | Active ^a | Low activity ^c |
| <i>GSTA1</i> | | | | | | |
| 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) | | | | |
| <i>GSTP1</i> | | | | | | |
| 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) | | |
| <i>GSTT1</i> | | | | | | |
| 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.

^aActive (present) if at least one active allele present.

^bNull if no active alleles present.

^cLow activity if at least one lower activity allele present.

^dReference group.

^eStatistically significant difference when compared with the reference group.

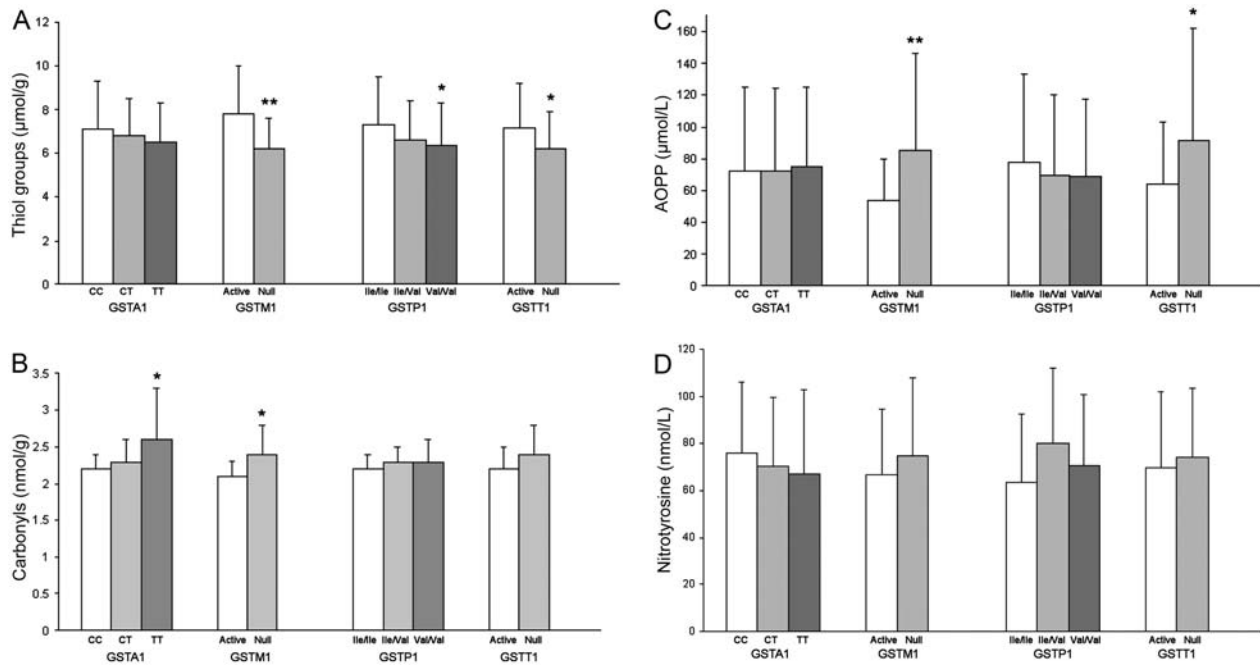


Fig. 2. Protein oxidative damage biomarkers in relation to the GST genotype in ESRD patients. Plasma thiol groups decreased in patients with null/low-activity GST genotype (A). Carbonyl groups, AOPP and nitrotyrosine content in plasma increased in patients with null/low-activity GST genotype (B–D, respectively).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

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*

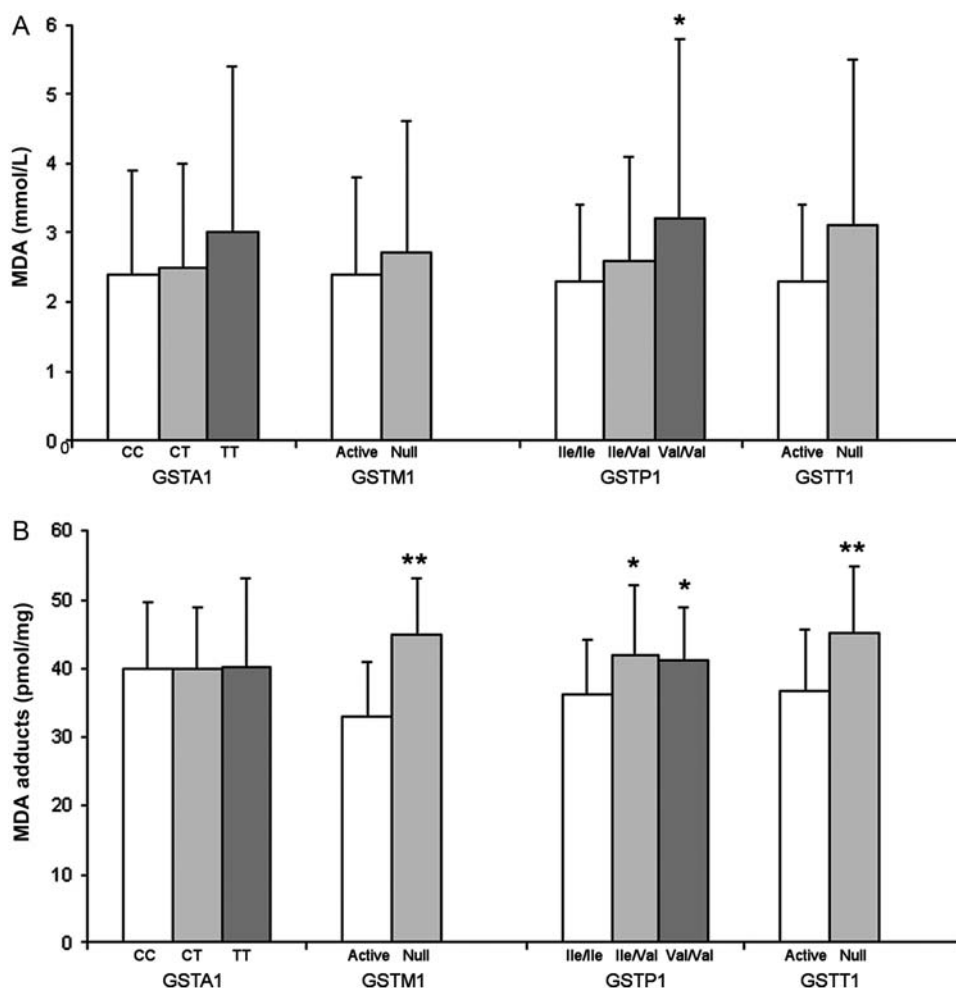


Fig. 3. Lipid oxidative damage biomarkers in relation to the GST genotype in ESRD patients. Plasma MDA concentrations and MDA adducts content increased in patients with null/low-activity GST genotype (A and B, respectively). * $P < 0.05$, ** $P < 0.01$.

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

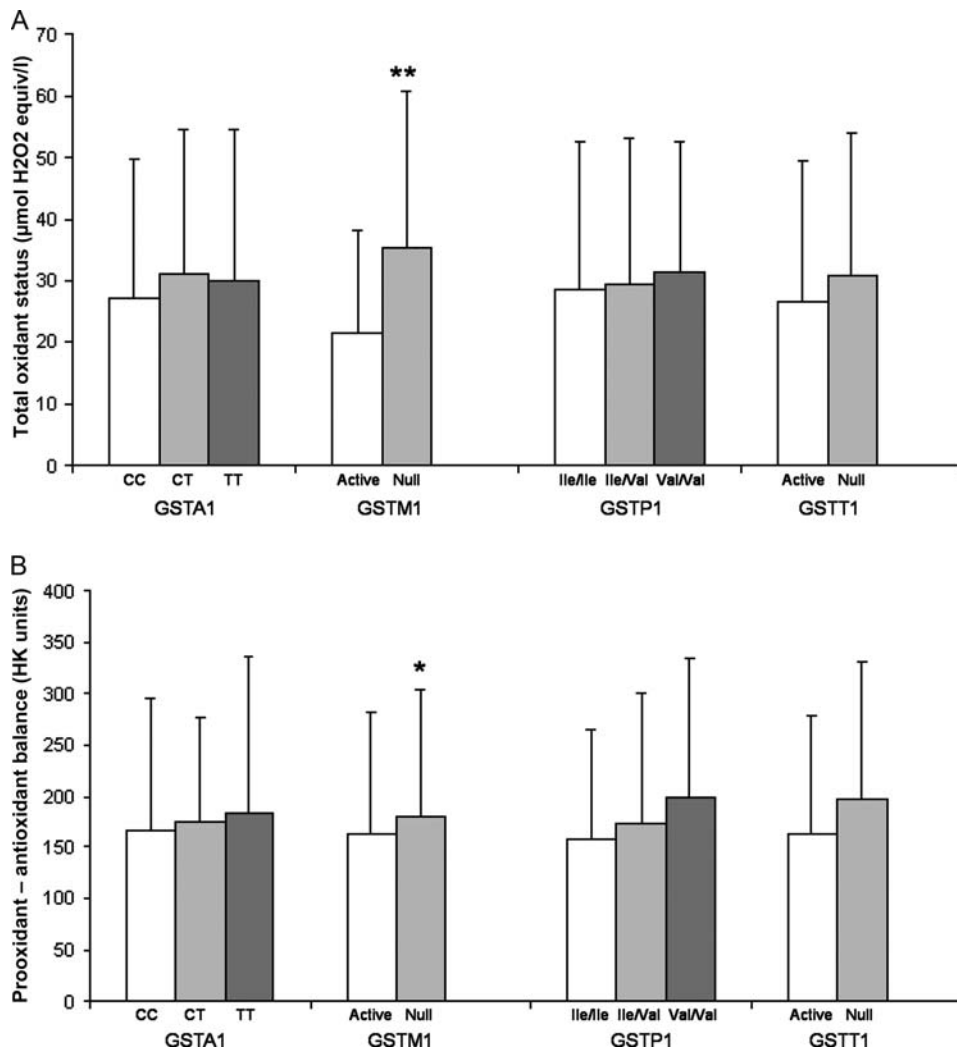


Fig. 4. TOS and PAB in relation to the GST genotype in ESRD patients. TOS and PAB values increased in patients with null/low-activity GST genotype (A and B, respectively). * $P < 0.05$, ** $P < 0.01$.

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) | | Carbonyls (nmol/mg prot) | | AOPP (mmol/L) | | Nitrotyrosine (nmol/l) | | MDA (mmol/l) | | MDA adducts (pmol/mg prot) | | TOS (μmol H ₂ O ₂ Equiv./l) | | PAB (HK units) | |
|--|-------------------------|-------|--------------------------|-------|---------------|-------|------------------------|-------|--------------|-------|----------------------------|-------|---|-------|----------------|-------|
| | | p | | p | | p | | p | | p | | p | | p | | p |
| <i>GSTM1/T1</i> 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% | |
| <i>GSTM1</i> active/ <i>T1</i> null | 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 |
| | 86.6% | | 109.5% | | 106.6% | | 111.1% | | 113.6% | | 136.1% | | 122.3% | | 156.8% | |
| <i>GSTM1</i> null/ <i>T1</i> active | 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 |
| | 79.3% | | 114.3% | | 135.2% | | 114.9% | | 127.3% | | 142.9% | | 239.9% | | 137.9% | |
| <i>GSTM1/T1</i> 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% | |
| <i>GSTM1/T1/A1</i> active | 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 | |
| | 100% | | 100% | | 100% | | 100% | | 100% | | 100% | | 100% | | 100% | |
| <i>GSTM1/T1/A1</i> null or low activity | 5.5 ± 1.2 | 0.001 | 2.9 ± 0.5 | 0.002 | 133.2 ± 89.6 | 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 |
| | 62.5% | | 131.8% | | | | 104.6% | | 150% | | 171.1% | | 679.6% | | 125.6% | |
| <i>GSTM1/T1/P1</i> active | 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 | |
| | 100% | | 100% | | 100% | | 100% | | 100% | | 100% | | 100% | | 100% | |
| <i>GSTM1/T1/P1</i> null or low activity | 5.2 ± 1.2 | 0.001 | 2.6 ± 0.5 | 0.024 | 116.0 ± 76.0 | 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 |
| | 61.9% | | 118.2% | | | | 130.26% | | 180.9% | | 164.7% | | 438.8% | | 128.1% | |
| <i>GSTM1/T1/A1/P1</i> active | 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 | |
| | 100% | | 100% | | 100% | | 100% | | 100% | | 100% | | 100% | | 100% | |
| <i>GSTM1/T1/A1/P1</i> null or low activity | 5.3 ± 1.2 | 0.001 | 2.9 ± 0.6 | 0.037 | 123.5 ± 84.0 | 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 |
| | 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 Fasset 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.

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