Manganese superoxide dismutase, glutathione peroxidase and catalase gene polymorphisms and clinical outcomes in acute kidney injury

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ABSTRACT

Introduction The aim of this study was to evaluate the potential association of single gene polymorphisms of manganese superoxide dismutase (MnSOD), glutathione peroxidase 1 (GPX1) and catalase (CAT) with clinical outcomes of acute kidney injury (AKI). Materials and methods Ninety AKI patients and 101 healthy volunteers were included in the study. Determination of MnSOD rs4880, GPX1 rs1050450 and CAT rs769217 polymorphisms was performed using real-time polymerase chain reaction amplification. The duration of hospitalization of AKI patients, dialysis and intensive care requirements, sepsis, oliguria and in-hospital mortality rates were assessed. Results The MnSOD, GPX1 and CAT genotypes and allele frequencies of AKI patients did not differ significantly from those of healthy controls. In patients with a T allele in the ninth exon of the CAT gene, intensive care requirements were greater than those of patients with the CC genotype ($p = 0.04$). In addition, sepsis and in-hospital mortality were observed significantly more frequently in patients with a T allele in the ninth exon of the CAT gene ($p = 0.03$). Logistic regression analysis determined that bearing a T allele was the primary determinant of intensive care requirements and in-hospital mortality, independent of patient age, gender, presence of diabetes and dialysis requirements (OR 6.10, 95% CI 1.34–27.81, $p = 0.02$ and OR 10.25, 95% CI 1.13–92.80, $p = 0.04$, respectively). Conclusion Among AKI patients in the Turkish population, hospital morbidity and mortality were found to be more frequent in patients bearing a T allele of the rs769217 polymorphism of the CAT gene.

Introduction

In healthy individuals, there is a balance between antioxidants and the formation of reactive oxygen species (ROS); when this balance is disturbed, oxidative stress occurs.\textsuperscript{1,2} Oxidative stress plays a major role in the pathogenesis of atherosclerosis, diabetic nephropathy and diabetic retinopathy within the chronic kidney disease population.\textsuperscript{3–6} In addition, oxidative stress is an important determinant of acute kidney failure.\textsuperscript{7,8} In some studies, ROS has been advanced as a factor in the pathogenesis of both ischemic and nephrotoxic kidney damage.\textsuperscript{9–12} The antioxidant defense enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) play important roles in protecting cells from free oxygen radical damage.\textsuperscript{13} Manganese SOD (MnSOD), which is found in the form of SOD within mitochondria, has been found to be associated with the development of type 2 diabetes,\textsuperscript{14} diabetic nephropathy\textsuperscript{15,16} and dyslipidemia.\textsuperscript{13} MnSOD gene Ala16 Val polymorphism has been shown to be related to microvascular complications (retinopathy, nephropathy and neuropathy) in diabetics.\textsuperscript{17} Moreover, several single nucleotide polymorphisms (SNPs) in the CAT gene have been found to be associated with diabetic nephropathy and neuropathy.\textsuperscript{18,19}

However, it is unknown whether there is an association between antioxidant gene polymorphisms and clinical outcomes of acute kidney injury (AKI). Therefore, in the current study, we studied the association between...
Materials and methods

Study design

This cross-sectional study was conducted by the Isparta Suleyman Demirel University Faculty of Medicine in the Departments of Nephrology and Medical Genetics. The study was approved by the Suleyman Demirel University School of Medicine Clinical Studies Ethical Board, dated 2009 and numbered 4/15. Written informed consent was obtained from all participants.

Ninety AKI patients and 101 healthy volunteers were included in the study. AKI was described in accordance with the K-DIGO guidelines.20 Exclusion criteria were age below 18 years, pregnancy, chronic dialysis treatment, history of renal transplantation and presence of acute obstructive uropathy.

Collection of data

Demographic data, blood pressure, body mass index (BMI), baseline creatinine, enrollment creatinine, peak creatinine, exit creatinine values and the duration of hospitalization, AKI etiology, concomitant conditions, dialysis and intensive care requirements, sepsis, oliguria and mortality development were provided from hospital records.

Blood sampling and genetic analysis

Venous blood samples, collected in EDTA, were obtained during routine blood sampling for biochemical and hematological analyses from patients and controls. Genomic DNA was isolated from venous blood using the Promega DNA isolation kit (Madison, WI) according to the manufacturer's recommendations.

For assessment of SNPs of MnSOD, CAT and GPX1 genes, the polymerase chain reaction (PCR)-restriction fragment length polymorphism (PCR-RFLP) method was used. An alanine/valine polymorphism (rs4880) in the signal peptide of the MnSOD gene was evaluated using the Promega DNA isolation kit (Madison, WI) according to the manufacturer's recommendations, and digestion products were analyzed following electrophoresis on 3% agarose gel stained with ethidium bromide (0.5 µg/mL). Restriction enzyme digestion resulted in a 107-bp product (allele 1 C) or 108- and 94-bp products (allele 2 T) for CAT, and 360-bp product (allele 1 Val-9) or 89- and 18-bp products (allele 2 Ala-9) for MnSOD; followed by 35 cycles of amplification at 94°C for 30 s (melting), 59°C for 30 s (annealing), 72°C for 30 s, and a final extension at 72°C for 6 min and at 95°C for 5 min for CAT; followed by 35 cycles of amplification at 95°C for 45 s (melting), 57°C for 45 s (annealing), 72°C for 1 min, and a final extension at 72°C for 7 min and at 94°C for 5 min for MnSOD; followed by 35 cycles of amplification at 94°C for 30 s (melting), 59°C for 30 s (annealing), 72°C for 30 s, and a final extension at 72°C for 6 min and at 95°C for 5 min for CAT; followed by 35 cycles of amplification at 95°C for 45 s (melting), 58°C for 45 s (annealing), 72°C for 1 min, and a final extension at 72°C for 7 min for GPX1. Following PCR amplification, for the determination of related polymorphisms of MnSOD, CAT and GPX1 enzymes, NgoMV, BstXI and Dde cross-section enzymes was used. The resulting 107-bp, 202-bp and 360-bp PCR products were digested with the restriction endonuclease according to the manufacturer’s recommendations, and digestion products were analyzed following electrophoresis on 3% agarose gel stained with ethidium bromide (0.5 µg/mL). Restriction enzyme digestion resulted in a 107-bp product (allele 1 Val-9) or 89- and 18-bp products (allele 2 Ala-9) for MnSOD, 202-bp product (allele 1 C) or 108- and 94-bp products (allele 2 T) for CAT, and 360-bp product (allele 1 Prolin) or 300- and 60-bp products (allele 2 Leucine) for GPX1.

In order to ensure the reliability of genotyping, the results of genotyping were evaluated by two observers (A.Y. and B.Y.). No interobserver variability was observed. Ten percent of the samples was analyzed as blinded repeats, without any discordance.

Statistical analysis

Clinical and laboratory values were expressed as mean ± standard deviation (SD) or number of patients. Genotype distribution was tested for the Hardy–Weinberg equilibrium. A chi-square test was used to compare genotype frequencies between the patient and control groups. The possible predictor effect of CAT gene polymorphism on clinical outcomes (intensive care
requirements, sepsis and hospital mortality) was assessed with logistic regression analysis. The models were adjusted for several baseline covariates, including age, gender, dialysis requirements and presence of diabetes. p values less than 0.05 were considered statistically significant. All statistical analyses were performed using the statistical software package SPSS 15.0 for Windows (SPSS Inc., Chicago, IL).

Results

The clinical and laboratory characteristics for AKI and control group patients are given in Table 1. The average age of AKI patients included in the study was 67.6 ± 14.0 years, whereas the average age of patients in the control group was 55.4 ± 13.7 years (p = 0.001). The male:female ratio in the AKI group was 53:37; in the control group it was 49:52 (p > 0.05). In both the AKI group and the control group, genotype distribution was inconsistent with the Hardy–Weinberg equation (p < 0.05).

The genotype distributions of the MnSOD rs4880, CAT rs769217 and GPX1 rs1050450 gene polymorphisms are given in Table 2. No significant difference was observed between the AKI and control groups (p > 0.05).

As seen in Tables 3 and 4, in the AKI group, no significant association was found between MnSOD and GPX1 genotype distributions and negative clinical outcomes of the patients (p > 0.05). A significant difference was seen between CAT genotype distributions with respect to sepsis (p = 0.03). Although no sepsis was seen in the TT genotype, sepsis was seen in the CC and CT genotypes (Table 5). Allele assessment revealed that in patients with the CAT gene T allele, intensive care requirements were greater than those of patients with the CC genotype (p = 0.04). In addition, sepsis and in-hospital mortality were observed significantly more frequently (p = 0.03) (Table 6). Logistic regression analysis determined that carrying a T allele was a determinant of intensive care requirements and hospital mortality, independent of patient age, gender, presence of diabetes or dialysis requirements (Table 7).

Discussion

In our study, the associations between MnSOD rs4880, GPX1 rs1050450 and CAT rs769217 polymorphisms and dialysis requirements, intensive care support requirements, sepsis, oliguria and hospital mortality in AKI patients were assessed. No significant difference was seen between AKI and control groups with respect to genotype distributions. In the AKI group, no significant association was identified between MnSOD and GPX1 genotype distributions and the negative clinical outcomes of AKI. We observed that in the AKI group, in patients with the CAT gene T allele, intensive care requirements were greater than those of patients with the CC genotype. In addition, sepsis and hospital mortality were also significantly greater in patients carrying a T allele. Logistic regression analysis determined that carrying a T allele was a determinant of intensive care requirements and hospital mortality, independent of patient age, gender, presence of diabetes or dialysis requirements.

ROS and oxidative stress have been shown to play a role in AKI pathophysiology.25–27 In post-traumatic AKI in obese rats, SOD activity was reported to be suppressed.28 It has been reported that in AKI patients, the NADPH oxidase p22phox gene polymorphism was associated with dialysis requirements and death.29 In our study, it was similarly determined that gene polymorphism of CAT, an antioxidant enzyme, was also related to intensive care requirements and in-hospital mortality.

In addition, a study related to MnSOD gene polymorphisms showed that the gene polymorphisms in this enzyme were related to kidney functions.30–32 A study conducted by Crawford et al. on stage 2–4 renal disease patients found that during a 12-month follow-up period, patients with the Ala/Ala genotype in MnSOD Ala16Val polymorphism had less decrease in kidney function than patients with the Ala/Val and Val/Val genotypes.30 In a study comparing 478 Japanese patients with type 2 diabetes with a non-diabetic healthy control group, it was found that MnSOD Ala16Val polymorphism was related to diabetic nephropathy.31 Lee et al. have

Table 1. Clinical characteristics of AKI patients.

| Characteristics                        | Patients (n = 90) | Controls (n = 101) |
|----------------------------------------|------------------|--------------------|
| Age, (yr)                              | 67.6 ± 14.0      | 55.4 ± 13.7        |
| Male/female (n)                        | 53/37            | 49/52              |
| Contributing cause of AKI (%)          |                  |                    |
| Ischemic                               | 35.6             |                    |
| Nephrotoxic                            | 25.6             |                    |
| Multifactorial/other                   | 38.9             |                    |
| Coexisting conditions (%)              |                  |                    |
| Diabetes mellitus                      | 41.1             | 0                  |
| Hypertension                           | 56.7             | 0                  |
| Heart failure                          | 16.7             | 0                  |
| Chronic lung disease                   | 8.9              | 0                  |
| Chronic kidney disease                 | 42.2             | 0                  |
| Cirrhosis                              | 2.2              | 0                  |
| BMI (kg/m²)                            | 26.6 ± 4.8       | 26.1 ± 4.5         |
| Systolic BP (mmHg)                     | 122 ± 19         | 119 ± 18           |
| Diastolic BP (mmHg)                    | 74 ± 11          | 70 ± 10            |
| Serum creatinine (mg/dL)               |                  |                    |
| Baseline value                         | 1.3 ± 0.7        | 0.84 ± 0.14        |
| Enrollment value                       | 4.5 ± 2.5        |                    |
| Peak value                             | 5.1 ± 2.6        |                    |
| Discharge value                        | 2.2 ± 1.3        |                    |

Notes: AKI, acute kidney injury; BMI, body mass index; BP, blood pressure. Values are given as mean ± SD or number.
reported that in Korean patients with type 2 diabetes, MnSOD V16A polymorphism was related to albuminuria, and patients with the A allele had lower rates of albuminuria.\(^{32}\) In our study, no significant relationship between MnSOD Ala9Val gene polymorphism and AKI was determined. However, this conclusion may have been due to the fact that a different genotype of MnSOD was studied.

Zhang et al. have reported that in the East Asian population, GPX1 gene Pro198Leu T allele carriers had greater cardiovascular disease risk than C allele carriers.\(^{33}\) Another study, contrary to this study, found that GPX1 gene Pro198Leu T allele carriers lived longer lives.\(^{34}\) In our study, it was determined that GPX1 genotype distributions were not associated with negative clinical outcomes of AKI.

A study conducted by Perianayagam et al. on 200 AKI patients found that C \(\rightarrow\) T polymorphism at position 262 of the CAT gene was not associated with AKI.\(^{29}\) However, in our study, the genotype distributions of the rs769217 polymorphism of the CAT gene were similar in the AKI and the control groups. It was determined that carrying a T allele in the ninth exon position of the CAT gene was associated with a six-fold increase in intensive care requirements and a ten-fold increase in hospital mortality. This issue, observed in our study, may be explained by different ethnic origins and different genotype analysis. The limitations of this study are its relatively small sample size, its reliance on observational data and the lack of any tests on antioxidant enzyme levels.

Consequently, in the Turkish populations that participated in our study, no significant association between MnSOD rs4880 and GPX1 rs1050450 polymorphism and

| Table 2. The distributions of MnSOD, CAT and GPX1 genotypes in patients with AKI and controls. |
|-----------------------------------------------|
| **Genotype** | **MnSOD (rs4880)** | **CAT (rs769217)** | **GPX1 (rs1050450)** |
|--------------|---------------------|---------------------|---------------------|
| Control      | Val/Val (n=32)      | Ala/Val (n=55)      | Ala/Ala (n=3)       |
|              | 32 (31.7)           | 60 (59.4)           | 9 (8.9)             |
|              | p 0.41              | 0.69                | 0.69                |
| AKI          | Val/Val (n=27)      | Ala/Val (n=46)      | Ala/Ala (n=7)       |
|              | 22 (24.5)           | 56 (62.2)           | 12 (13.3)           |
|              | p 0.37              | 0.37                | 0.37                |

| Table 3. The distributions of negative clinical outcomes among MnSOD (rs4880) genotypes. |
|-----------------------------------------------|
| **Genotype** | **Val/Val (n=22)** | **Ala/Val (n=56)** | **Ala/Ala (n=12)** |
|--------------|--------------------|--------------------|--------------------|
| Dialysis requirement (n) | 7 19 3 | 0.83 |
| ICU setting (n) | 5 8 3 | 0.54 |
| Sepsis (n) | 4 5 1 | 0.48 |
| Oliguria (n) | 1 5 0 | 0.48 |
| Hospital length of stay (days) | 12±8 12±9 10±7 | 0.70 |
| Hospital death (n) | 4 4 2 | 0.31 |

| Table 4. The distributions of negative clinical outcomes among GPX1 (rs1050450) genotypes. |
|-----------------------------------------------|
| **Genotype** | **Pro/Pro (n=32)** | **Pro/Leu (n=55)** | **Leu/Leu (n=3)** |
|--------------|---------------------|---------------------|---------------------|
| Dialysis requirement (n) | 9 20 0 | 0.35 |
| ICU setting (n) | 5 10 1 | 0.74 |
| Sepsis (n) | 5 5 0 | 0.54 |
| Oliguria (n) | 0 6 0 | 0.13 |
| Hospital length of stay (days) | 12±5 12±10 9±8 | 0.83 |
| Hospital death (n) | 3 6 1 | 0.45 |

| Table 5. The distributions of negative clinical outcomes among of CAT (rs769217) genotypes. |
|-----------------------------------------------|
| **Genotype** | **CC (n=37)** | **CT (n=46)** | **TT (n=7)** |
|--------------|---------------|---------------|---------------|
| Dialysis requirement (n) | 12 16 1 | 0.56 |
| ICU setting (n) | 3 11 2 | 0.13 |
| Sepsis (n) | 1 9 0 | 0.03 |
| Oliguria (n) | 1 4 1 | 0.39 |
| Hospital length of stay (days) | 12±9 12±8 11±12 | 0.91 |
| Hospital death (n) | 1 7 2 | 0.06 |

Notes: AKI, acute kidney injury; MnSOD, manganese superoxide dismutase; CAT, catalase; GPX1, glutathione peroxidase 1. Data are number of patients (%) for genotypes.

| Table 6. In AKI patients comparison of cat (rs769217) gene T allele bearers with CC genotype bearers with respect to negative clinical outcomes. |
|-----------------------------------------------|
| **CC (n=37)** | **CT or TT (n=53)** |
|--------------|---------------------|
| Dialysis requirement (n) | 12 17 | 0.97 |
| ICU setting (n) | 3 13 | 0.04 |
| Sepsis (n) | 1 9 | 0.03 |
| Oliguria (n) | 1 5 | 0.21 |
| Hospital length of stay (days) | 12±9 12±8 | 0.89 |
| Hospital death (n) | 1 9 | 0.03 |

Notes: ICU, intensive care unit; CAT, catalase.

was determined. However, this conclusion may have been due to the fact that a different genotype of MnSOD was studied.

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Consequently, in the Turkish populations that participated in our study, no significant association between MnSOD rs4880 and GPX1 rs1050450 polymorphism and
AKI clinical results was observed. However, we observed that carrying a T allele in the CAT gene had an independent predictor effect for intensive care requirements and hospital mortality. This finding must be verified in larger patient groups. If this finding is confirmed, the efficacy of antioxidant treatment for patients carrying a T allele in the CAT gene who are in the early stages of AKI and those who have a high risk of developing AKI may be studied.

Declaration of interest
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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