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ORIGINAL ARTICLE

Association of *GPX1* Pro198Leu and *SOD1* A251G Polymorphisms With Risk of Acute Kidney Rejection

Shima Rayatpisheh,[1] Mohammad-Hossein Karimi,[2] Iraj Saadat[1]

1. Department of Biology, College of Science, Shiraz University, Shiraz, Iran.
2. Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

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Corresponding author:
IRAJ SAADAT
E: Iraj1972@yahoo.com,
E: isaadat@shirazu.ac.ir

Abstract

Background/Aim: Acute rejection is one factor threatening the success of kidney transplantation. One of the factors that can cause graft rejection is oxidative stress. The aim of this study was to investigate the association between *GPX1* Pro198Leu and *SOD1* A251G polymorphisms and acute renal allograft rejection.
Methods: A total of 262 healthy individuals and 262 patients who had undergone kidney transplantation were enrolled in the present study, of which 46 patients had acute rejection. The genotypes were determined using the PCR-RFLP technique. Case-control study between patients and control groups and cohort study between patients with and without acute rejection were then performed.
Results: Analysis showed that the incidence of kidney disease leading to kidney transplantation did not depend on the genotype of *GPX1* and *SOD1* polymorphisms. In the cohort study, no significant differences were observed between the cases with and without acute rejection for any of the two genes.
Conclusions: The genotypes of *GPX1* and *SOD1* polymorphisms were not found to be involved in the incidence of acute renal rejection. It is advised to continue further studies, because defects in the activities of these genes can be compensated by other members of their families.

Key words: Kidney transplantation; Polymorphism, genetic; Glutathione peroxidase; *GPX1*; Superoxide dismutase-1; *SOD1*.

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Introduction

For patients with end-stage renal disease (ESRD), kidney transplantation is a treatment option. Despite the expansion and improvement of this therapy, recipient immune rejection of donor tissue is common and the leading cause of graft failure.[1-3] Three types of transplant rejection have been classified based on the histopathological pattern of kidney transplantation: hyperacute rejection, acute rejection and chronic rejection.[4] The transplanted kidney is susceptible to acute rejection for several reasons. Oxidative stress is one of the reasons for noxious events during transplantation. Oxidative stress is caused by intra- and extracellular reactive oxygen species (ROS). Oxidative stress damages the kidney both before and after transplantation. The defence mechanism against OS damage is provided by antioxidants, which include both enzymatic and non-enzymatic molecules. The most important antioxidant enzymes that can reduce the reactive oxygen species are the superoxide dismutase (SOD) family, the glutathione peroxidase (GPX) family and cat-

alase. The main non-enzymatic antioxidant molecules that can reduce ROS levels are glutathione and vitamins A, C and E.[5-7]

The GPX family is made up of eight members and is located in the 3p21 region of the chromosome. The gene encoding GPX1 (OMIM: 138320), which is an intercellular selenium-dependent enzyme and reduces the levels of organic peroxides, especially hydrogen peroxide. A SNP at codon 198 of the *GPX1* gene has been reportedly associated with a decrease in enzyme activity. At this position, proline (C) is replaced by leucine (T).[8, 9] Studies on the genetic polymorphism of *GPX1* Pro198Leu with different diseases have shown an increased risk of chronic kidney disease (CHD), KBD, brain tumour and cardiovascular diseases (CVD) in the presence of leucine at this position.[10-13]

SOD1 (OMIM: 147450) is a member of a family with three members. SOD is located on chromosome 21q22.11. It codes a copper- and zinc-containing enzyme.[14] SOD1 reacts with superoxide to produce oxygen in its oxidised form. One of the reported SNPs for this gene is at nucleotide 251, replacing adenine with guanine.[15] Studies on the relationship between *SOD1* A251G polymorphism and various diseases show a protective effect of AA on NIHL in Han Chinese workers and the protective effect of G allele in non-syndromic myelomeningocele disease, on the other hand, studies show that the GG genotype increases the risk cataract.[14-16]

This study aimed to investigate whether *GPX1* Pro198Leu and *SOD1* A251G polymorphisms are associated with acute renal allograft rejection.

Methods

Patients

A total of 262 renal transplant patients aged 42.3 ± 14.2 years were enrolled. During three years, whole blood samples were collected from Shiraz Organ Procurement and Transplantation Centre. Control samples, mean age 42.9 ± 13.6, were collected from Shiraz Blood Transfusion Centre. Age and sex were matched between patients and controls. Biopsy was used as a diagnostic method after serum creatinine and blood urea nitrogen (BUN) elevations.

Genotyping

For both case and control samples, DNA extraction was performed using the standard method.[17] Genotypes were then determined by PCR-RFLP method for both (*GPX1* and *SOD1*) genes as previously described.[18] In this study, a case-control study was conducted among healthy individuals and patients. A cohort study was also conducted among kidney transplant patients who experienced acute rejection and those who didn't experience acute rejection of the transplanted kidney.

Statistical analysis

Data analysis was performed with SPSS statistical software version 16 and Chi-squared, logistic regression and t-test applications for means with significance level at $p < 0.05$.

Results

Age, sex and blood group were the parameters considered in the study. Patients and controls were matched for age and gender. There was no significant relationship between acute and non-acute rejection samples in these aspects.

Hardy-Weinberg equilibrium in the control population was tested and confirmed for both genes ($\chi^2 = 3.04$, df = 1, $p > 0.05$ for *GPX1* and $\chi^2 = 3.040.359$, df = 1, $p > 0.05$ for *SOD1*). For the *GPX1* Pro198Leu variants with base CC genotype, the p-values were 0.509, 0.084 and 0.291 for CT, TT and CT+TT, respectively. This shows no significant association between the patient and control groups (Table 1).

In the study of *SOD1* A251G variants with basing AA genotype, the p-value was 0.654, 0.575 and 0.739 for AG, GG and AG+GG, respectively. It showed no significant relationship between patient and control groups (Table 2). The findings showed no association between the polymorphic status of these two genes and the risk of developing a disease leading to kidney transplantation.

In the second study, two groups of patients, those with and without acute rejection were compared. For the *GPX1* Pro198Leu variants with base CC genotype, the p-values were 0.196, 0.676 and 0.202 for the CT, TT and CT+TT genotypes, respectively. Thus, no significant association was found (Table 3).

Table 1: Genotype and allele frequency distribution analysis for the GPX1 gene in kidney transplant patients and healthy controls

Parameter	Healthy control group	Kidney transplanted patients	OR	95 % CI	p-value
Genotype					
CC	154 (58.78 %)	142 (54.20 %)	1.00		
CT	100 (38.17 %)	104 (39.70 %)	1.13	0.79—1.61	0.509
TT	8 (3.05 %)	16 (6.10 %)	2.17	0.90—5.22	0.084
CT+TT vs CC	108 (41.22 %)	120 (45.80 %)	1.20	0.85—1.70	0.291
Allele					
C	408 (77.86 %)	388 (74.05 %)	1.00		
T	116 (22.14 %)	136 (25.95 %)	1.23	0.93—1.64	0.149

OR: odds ratio; CI: confidence interval; C: cytosine; T: thymine;

Table 2: Genotype and allele frequency distribution analysis for the SOD1 gene in kidney transplant patients and healthy controls

Parameter	Healthy control group	Kidney transplanted patients	OR	95 % CI	p-value
Genotype					
AA	222 (85.72 %)	221 (84.35 %)	1.00		
AG	35 (13.51 %)	39 (14.89 %)	1.12	0.68—1.83	0.654
GG	2 (0.77 %)	1 (0.38 %)	0.50	0.04—5.58	0.575
AG+GG vs AA	37 (14.28)	40 (15.27 %)	1.09	0.67—1.76	0.739
Allele					
A	479 (92.47 %)	481 (92.15 %)	1.00		
G	39 (7.53 %)	41 (7.85 %)	1.05	0.66—1.65	0.844

OR: odds ratio; CI: confidence interval; A: adenine; G: guanine;

Table 3: Genotype and allele frequency distribution analysis for GPX1 gene among groups with or without acute rejection

Parameter	Without acute rejection	With transplant rejection	OR	95 % CI	p-value
Genotype					
CC	121 (56.02 %)	21 (45.65 %)	1.00		
CT	82 (37.96 %)	22 (47.83 %)	1.54	0.80—2.99	0.196
TT	13 (6.02 %)	3 (6.52 %)	1.33	0.35—5.07	0.676
CT+TT vs CC	95 (43.98 %)	25 (54.35 %)	1.52	0.80—2.87	0.202
Allele					
C	324 (75.00 %)	64 (69.57 %)	1.00		
T	108 (25.00 %)	28 (30.43 %)	1.31	0.80—2.15	0.281

OR: odds ratio; CI: confidence interval; C: cytosine; T: thymine;

Table 4: Genotype and allele frequency distribution analysis for SOD1 gene between groups with and without acute rejection

Parameter	Without acute rejection	With acute rejection	OR	95 % CI	p-value
Genotype					
AA	184 (85.19 %)	37 (80.43 %)	1		
AG	30 (13.98 %)	9 (19.57 %)	1.49	0.65—3.40	0.342
GG	1 (0.46 %)	0 (0.00 %)			
AG+GG vs AA	31 (14.44 %)	9 (19.57 %)	1.44	0.63—3.20	0.381
Allele					
A	398 (92.56 %)	83 (90.22 %)	1		
G	32 (7.44 %)	9 (9.78 %)	1.35	0.62—2.93	0.450

OR: odds ratio; CI: confidence interval;

The p-values were 0.342 and 0.381 for the AG and AG+GG variants of *SOD1* A251G with the base genotype of AA, respectively and as a result no significant association was found (Table 4).

Discussion

As mentioned in the introduction, polymorphisms that have been studied are associated with the risk of many diseases. This is due to their effect on the antioxidant activity of enzymes. Indeed, few studies have shown that these polymorphisms correlate with transplantation. One of these studies between polymorphisms of *GPX*, *SOD* and catalase genes in patients with post-transplant diabetes mellitus has shown that the Pro200Leu polymorphism of the *GPX1* gene is associated with PTDM.[19] In another study, the *CAT* C262T gene polymorphism was found to be associated with DGF in patients with renal transplantation.[20] Oxidative stress can negatively affect the survival and function of transplanted tissues. It may be one of the factors that cause acute renal rejection. Oxidative stress occurs as a result of the imbalance between oxidants and antioxidants.[5, 6] Recent research in the syngeneic kidney transplant model has shown that free radicals play a role in upregulating adhesion molecules, cytokines, MHC class I and II antigens and inducible nitric oxide synthase, in addition to adversely affecting transplanted tissues.[21] Antioxidant enzymes such as GPX1 and SOD1 have a role in neutralising free radicals. They are two fundamental players in the oxidative stress process.

Conclusion

Two types of studies have been conducted in this research. One was a case-control study between kidney transplant patients and healthy individuals and the other was a cohort study between patients with and without acute renal rejection. In the first study, no significant association was observed between the *GPX1* Pro-198Leu and the *SOD1* A251G polymorphisms and the incidence of kidney disease leading to kidney transplantation. Again, no significant association was found in the second study between individuals with acute rejection and those without acute renal failure. There are eight members of the glutathione peroxidase family and three members of the superoxide dismutase family. The overlapping activity of the enzyme family members may explain this observation. The function of other members of these enzyme families may compensate the decrease in performance due to the considered polymorphisms of *GPX1* and *SOD1*. Therefore, it is better to investigate these family members simultaneously in future studies.

Ethics

The study was approved by the Ethics Committee of the Biology Department of Shiraz University, decision No ECBD-SU-9133360- 3, dated 3 February 2020.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

Author ORCID numbers

Shima Rayatpisheh (SR):
0000-0003-3388-5776
Mohammad-Hossein Karimi (MHK):
0000-0002-2435-6277
Iraj Saadat (IS):
0000-0002-8169-4707

Author contributions

Conceptualisation: IS
Methodology: IS
Formal analysis: SR, MHK
Investigation: SR, MHK
Data curation: SR, MHK
Writing - original draft: SR, MHK
Writing - review and editing: IS
Visualisation: SR, MHK
Supervision: IS

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