Role of Donors and Recipients’ Glutathione S-Transferase Gene Polymorphisms in Association of Oxidative Stress With Delayed Graft Function in Kidney Allograft Recipients

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Introduction. Oxidative stress contributes to delayed graft function (DGF). Glutathione S-transferases (GSTs) are polymorphic genes which produce enzymes with protective effect against oxidative stress. This study aimed to investigate the association between donors’ and recipients’ GSTM1 and GSTT1 polymorphisms and DGF, creatinine clearance, and oxidative stress parameters in kidney allograft recipients.

Materials and Methods. One hundred and eighty-two donor-recipient pairs were studied. Lipid peroxidation and total antioxidant capacity were measured in the recipients’ plasma as the parameters of oxidative stress. Delayed graft function was determined based on at least 10% increase, no change, or less than 10% decrease in the serum creatinine level in 3 consecutive days during the 1st week after transplantation.

Results. Lipid peroxidation was significantly greater in the recipients with DGF (P < .001). The frequency of GSTM1 null was significantly higher in the patients with DGF (odds ratio [OR], 0.38; 95% confidence interval [CI], 0.17 to 0.86; P = .02). There was also a significant association between the donors’ GSTM1 polymorphism and DGF (OR, 0.31; 95% CI, 0.14 to 0.68; P = .003). A significant association was detected between combination of recipients and donors’ GSTM1 polymorphism and DGF (OR, 0.31; 95% CI, 0.14 to 0.68; P = .003). A significant association was detected between combination of recipients and donors’ GSTM1 polymorphism and DGF (OR, 0.31; 95% CI, 0.14 to 0.68; P = .003). A significant association was detected between combination of recipients and donors’ GSTM1 polymorphism and DGF (OR, 0.31; 95% CI, 0.14 to 0.68; P = .003). A significant association was detected between combination of recipients and donors’ GSTM1 polymorphism and DGF (OR, 0.31; 95% CI, 0.14 to 0.68; P = .003). A significant association was detected between combination of recipients and donors’ GSTM1 polymorphism and DGF (OR, 0.31; 95% CI, 0.14 to 0.68; P = .003). A significant association was detected between combination of recipients and donors’ GSTM1 polymorphism and DGF (OR, 0.31; 95% CI, 0.14 to 0.68; P = .003).

Conclusions. These results suggest that the donors and recipients’ GSTM1 polymorphism may be a major risk factor for oxidative stress and poor kidney allograft transplantation outcomes.

INTRODUCTION

Delayed graft function (DGF) is a post-transplantation phenomenon which adversely influence the allograft functionality and survival. It is also shown that rejection episodes are more frequent in organs with DGF than it is in those that function immediately.1-4

Ischemic reperfusion injury (IRI) is a common
event in kidney transplantation, and it is considered as one of the main contributors to acute kidney injury and DGF in allografts.\textsuperscript{3,5} In the ischemic phase, reduction of oxygen supply triggers production of reactive oxygen species (ROS) and an acidic environment, which eventually result in phospholipolysis, endothelial membrane injury, and thrombin-mediated fibrin deposition.\textsuperscript{3,5} In the reperfusion phase, these injuries are deteriorated by migration of inflammatory cells and reintroduction of oxygen into the damaged tissue. Excess oxygen plus activity of inflammatory cells lead to production of more ROS and thereby more intense damages to the cells and finally their apoptosis.

Among the numerous endogenous defence mechanisms against ROS and oxidative injury, glutathione plays a critical role in homeostasis of cellular redox environment.\textsuperscript{6,7} Many studies have reported glutathione deficiency could intensify oxidative stress and IRI in different organs.\textsuperscript{5,8-11} Glutathione S-transferases (GSTs) are a family of enzymes that catalyse the conjugation of the reduced glutathione thiolate anion with a wide range of electrophiles including ROS.\textsuperscript{6} Glutathione S-transferases are highly polymorphic and these polymorphisms are likely to contribute to interindividual differences in response to oxidants. Recent studies emphasize the potentially distinctive roles of GST enzymes as crucial determinants of the development of IRI.\textsuperscript{12}

To our knowledge, the role of GST polymorphisms in kidney allograft outcome has been reported in one study.\textsuperscript{13} In this study, like the most related studies, only the role of donors’ genetics in transplantation has been evaluated. Considering the crucial role of oxidative stress in the IRI, contribution of IRI to development of DGF after kidney allograft transplantation, and also the probable role of donors’ genetics in transplantation, we hypothesized that GSTM1 and GSTT1 polymorphisms may partly explain individual variability in allograft function after transplantation. The aim of this study was to investigate the association between donors’ and recipients’ GSTM1 and GSTT1 polymorphisms and DGF, creatinine clearance, and oxidative stress parameters in kidney allograft recipients.

MATERIALS AND METHODS

Patients

One hundred and eighty-two recipient-donor pairs who had undergone kidney transplantation at 1 center (Afzalipour Hospital, Kerman, Iran) were enrolled in this prospective cohort study. The inclusion criteria for patients were grafting transplant from a living person, the first kidney transplant, and signing the consent form.

Delayed graft function was defined by stringent criteria on the basis of the Boom definition and independent from the need for dialysis,\textsuperscript{2} as we reported in our previous articles.\textsuperscript{14-16} According to this definition, an increase, no change, or a less than 10% decrease in the serum creatinine level in 3 consecutive days during the 1st week after transplantation was considered as DGF. Creatinine clearance was calculated using the Cockcroft-Gault formula, which in turn estimated glomerular filtration rate in mL/min.

Glutathione S-transferase M1 and T1 Genotyping

Genomic DNA was isolated from ethylenediaminetetraacetic acid whole blood using a rapid salting out DNA extraction method. After measuring the quality and quantity of the extracted DNA by determination of A\textsubscript{260}/A\textsubscript{280}, aliquots of the DNA were stored in Tris-ethylenediaminetetraacetic acid buffer at -70°C until the analysis of genotypes. According to our previous protocol,\textsuperscript{17-19} a multiplex polymerase chain reaction (PCR) was performed to detect the null alleles of the \textit{GSTM1} and \textit{GSTT1} genes. The c-Abl gene was used as internal positive control. The primers used to amplify genotypes were 5’-GAA CTC CCT GAA AAG CTA AAG C-3’ and 5’-GTT GGG CTC AAA TAT ACG GTG G-3’ as forward and reverse primers, respectively, for the \textit{GSTM1} (X68676.1, GeneBank), resulting in a 219-bp band, and 5’-TTC CTT ACT GGT CCT CAC ATC TC-3’ and 5’-TCA CCG GAT CAT GGC CAG CA-3’ as forward and reverse primers, respectively, for the \textit{GSTT1} (AB057594.1, GeneBank) genotype, resulting in a 450-bp product. As internal control, the c-Abl gene was amplified using 5’-TTC AGC GGC CAG TAG CAT CTG ACT-3’ and 5’-TCA CGG GAT CAT GGC CAG CA-3’ as forward and reverse, respectively, for the \textit{GSTT1} (AB057594.1, GeneBank) genotype, resulting in a 450-bp product. As internal control, the c-Abl gene was amplified using 5’-TTC AGC GGC CAG TAG CAT CTG ACT-3’ and 5’-TGT GAT TAT AGC CTA AGA CCC GGA GCT TTT-3’ as forward and reverse primers, respectively, producing a 750-bp product. The PCR reactions were resolved on 2% agarose gel electrophoresis and the PCR products were detected with ethidium bromide. The absence of the \textit{GSTM1}- or \textit{GSTT1}-specific fragments indicated the corresponding
null genotype, whereas the c-Abl specific fragment confirmed the presence of amplifiable DNA in the reaction mixture. The reliability and validity of the PCR method were assessed through reconducting the genotype assays using at least a 10% sample of our DNA samples. The results for all reassessments were 100% concordant.

Statistical Analysis
Continuous variables, including oxidative stress parameters, were compared using the unpaired t test according to the DGF occurrence and GSTM1 and GSTT1 polymorphisms. Individuals with at least 1 GSTM1 and GSTT1 active gene were coded 1 in analysis, and the second category included persons who were GSTM1 and GSTT1 null (coded zero). The logistic regression model was used to determine the association between the GSTM1 and GSTT1 polymorphisms and DGF in a univariable model. Odds ratios (ORs) and 95% confidence interval (CI) were used to estimate the risk of the association between DGF and a specific polymorphism. Backward regression analyses evaluated the independent predictors of the creatinine clearance at the discharge day. The association between the dependent variables (DGF and creatinine clearance at the day discharge) and the GST polymorphisms was adjusted using multivariable regression in the presence of potential confounders.15,20 For all the tests, a P value less than .05 was considered significant. All the analyses were conducted using the SPSS software (Statistical Package for the Social Sciences, version 16.0, SPSS Inc, Chicago, IL, USA).

RESULTS
Demographics, Clinical, and Laboratory Parameters
Eighty-two percent of the donors and 62% of the recipients were men (Table 1). The mean values of age for the donors and recipients were 28.6 ± 5.8 years and 40.7 ± 15.4 years old, respectively. Of all of the recipients, 11% and 20% suffered from acute rejection and DGF, respectively. Demographics of the recipients and donors according to their GSTM1 and GSTT1 polymorphisms are shown in Table 1.

Oxidative Stress Parameters and Delayed Graft Function
As it is shown in the Figure, the level of lipid peroxidation was significantly higher in the recipients who had DGF than those who had a normal functioning allograft.
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The relationship between the donors and recipients’ GSTM1 polymorphisms and the incidence of DGF is shown in Table 2. The frequency of GSTM1 null was significantly higher in the patients with DGF (OR, 0.38; 95% CI, 0.17 to 0.86; \(P = .02\)). There was also a significant association between the donors’ GSTM1 polymorphism and DGF events in the recipients (OR, 0.31; 95% CI, 0.14 to 0.68; \(P = .003\)). Considering the role of confounders in association between the GSTM1 polymorphism and DGF,20 multivariable logistic regression was performed in the presence of age, sex, body mass index, and mean arterial pressure variables. The adjusted association between the recipients’ GSTM1 polymorphism and DGF abolished in the presence of these confounders (OR, 0.37; 95% CI, 0.37 to 1.06; \(P = .06\)).

**GSTM1 Polymorphism and Delayed Graft Function**

The relationship between the donors and recipients’ GSTT1 polymorphisms and the incidence of DGF is shown in Table 3. The frequency of GSTT1 null was not significantly different in the patients with and without DGF (OR, 1.67; 95% CI, 0.64 to 4.35; \(P = .29\)). There was also no significant association between the donors’ GSTT1 polymorphism and DGF events in the recipients (OR, 1.17; 95% CI, 0.48 to 2.80; \(P = .73\)). These associations remained insignificant in multivariable logistic regression in the presence of age, sex, body mass index, and mean arterial pressure variables.

**Combination of Donors and Recipients’ GSTM1 Polymorphisms and Delayed Graft Function**

Combinations of recipients and donors’ GSTM1 polymorphisms were analysed in order to determine if their interaction had a joint effect on DGF or not. There was a significant association between combinations of recipient and donor GSTM1 and DGF (OR, 0.20; 95% CI, 0.07 to 0.64; \(P = .006\); Table 4).

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**Table 2. Association Between Kidney Allograft Donors and Recipients’ GSTM1 Polymorphism and Delayed Graft Function**

<table>
<thead>
<tr>
<th>GSTM1 Polymorphism</th>
<th>Delayed Graft Function</th>
<th>Unadjusted Analysis</th>
<th>Adjusted Analysis*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>Odds Ratio (95% Confidence Interval)</td>
</tr>
<tr>
<td>Recipients</td>
<td>19</td>
<td>115</td>
<td>0.38 (0.17 to 0.86)</td>
</tr>
<tr>
<td>Donors</td>
<td>15</td>
<td>111</td>
<td>0.31 (0.14 to 0.68)</td>
</tr>
</tbody>
</table>

*Multivariable regression adjusted for age, sex, body mass index, and mean arterial pressure variables

**Table 3. Association Between Kidney Allograft Donors and Recipients’ GSTT1 Polymorphism and Delayed Graft Function**

<table>
<thead>
<tr>
<th>GSTT1 Polymorphism</th>
<th>Delayed Graft Function</th>
<th>Unadjusted Analysis</th>
<th>Adjusted Analysis*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>Odds Ratio (95% Confidence Interval)</td>
</tr>
<tr>
<td>Recipients</td>
<td>26</td>
<td>104</td>
<td>1.67 (0.64 to 4.35)</td>
</tr>
<tr>
<td>Donors</td>
<td>24</td>
<td>108</td>
<td>1.17 (0.48 to 2.80)</td>
</tr>
</tbody>
</table>

*Multivariable regression adjusted for age, sex, body mass index, and mean arterial pressure variables
**GSTM1 and GSTT1 Polymorphisms and Oxidative Stress Parameters**

The relationships between the donors and recipients’ GSTM1 and GSTT1 polymorphisms and the levels of lipid peroxidation and total antioxidant capacity are shown in Table 5. There was a significant association between GSTM1 polymorphism and lipid peroxidation as the level of lipid peroxidation was higher in the recipients with GSTM1 null polymorphisms. Adjustment for the potential confounding factors did not change the associations.

**GSTM1 and GSTT1 Polymorphisms and Creatinine Clearance on Discharge Day**

Changes in the creatinine clearance at the discharge is shown in Table 6. Among the polymorphisms, only the recipient GSTM1 polymorphism correlated with creatinine clearance at discharge. This association was abolished when analysis was adjustment for the potential confounding factors.

**DISCUSSION**

The results of this study showed that lipid peroxidation was significantly higher in the allograft recipients who underwent DGF. There was also an association between both donors and recipients’ GSTM1 polymorphism and DGF as the frequency of DGF was significantly higher in the patients with GSTM1 null allele or those who received allograft from GSTM1-null donors. Furthermore, linear regression demonstrated that lipid peroxidation in the allograft after transplantation was significantly linked to both donors and recipients’ GSTM1 polymorphism. There was no significant association

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**Table 4. Association of Combination of Kidney Allograft Donors and Recipients’ GSTM1 Polymorphisms With Delayed Graft Function**

<table>
<thead>
<tr>
<th>GSTM1 Polymorphism</th>
<th>Delayed Graft Function</th>
<th>Unadjusted Analysis</th>
<th>Adjusted Analysis*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>Odds Ratio (95% Confidence Interval)</td>
</tr>
<tr>
<td>Recipient null-donor null</td>
<td>7 (22)</td>
<td>13 (9)</td>
<td>1 (referent)</td>
</tr>
<tr>
<td>Recipient null-donor M1</td>
<td>6 (19)</td>
<td>17 (12)</td>
<td>0.65 (0.18 to 2.42)</td>
</tr>
<tr>
<td>Recipient M1-donor null</td>
<td>9 (28)</td>
<td>25 (17)</td>
<td>0.67 (0.20 to 2.21)</td>
</tr>
<tr>
<td>Recipient M1-donor M1</td>
<td>10 (31)</td>
<td>90 (62)</td>
<td>0.20 (0.07 to 0.64)</td>
</tr>
</tbody>
</table>

*Multivariable regression adjusted for age, sex, body mass index, and mean arterial pressure variables.

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**Table 5. Association Between Kidney Allograft Donors and Recipients’ GSTM1 and GSTT1 Polymorphisms and Lipid Peroxidation and Total Antioxidant Capacity**

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Lipid Peroxidation</th>
<th>Total Antioxidant Capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Recipient GSTM1</td>
<td>-27 (-40 to -15)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Recipient GSTT1</td>
<td>-3.2 (-16 to 9.6)</td>
<td>.61</td>
</tr>
<tr>
<td>Donor GSTM1</td>
<td>-19 (-31 to -8)</td>
<td>.001</td>
</tr>
<tr>
<td>Donor GSTT1</td>
<td>-13.3 (-25 to -1)</td>
<td>.03</td>
</tr>
</tbody>
</table>

*CI indicates confidence interval.
†Multivariable regression adjusted for age, sex, body mass index, and mean arterial pressure variables.

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**Table 6. Association Between Kidney Allograft Donors and Recipients’ GSTM1 and GSTT1 Polymorphisms and Creatinine Clearance* **

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Creatinine Clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient (95% Confidence Interval)</td>
</tr>
<tr>
<td>Recipient GSTM1</td>
<td>8.3 (0.6 to 15.9)</td>
</tr>
<tr>
<td>Recipient GSTT1</td>
<td>-0.5 (-8.8 to 7.8)</td>
</tr>
<tr>
<td>Donor GSTM1</td>
<td>4.1 (-3.6 to 11.7)</td>
</tr>
<tr>
<td>Donor GSTT1</td>
<td>2.7 (-5.3 to 10.8)</td>
</tr>
</tbody>
</table>

*Multivariable regression adjusted for age, sex, body mass index, and mean arterial pressure variables.
between GSTT1 polymorphism and DGF and oxidative stress parameters.

Evidence suggests that oxidative stress is a common mechanism of injury in acute and chronic rejections. By altering the redox environment, ROS modulate the activation of transcription factors and cytokine genes involved in acute cellular rejection.

The role of GST polymorphisms in the intensity of lipid peroxidation and kidney transplant outcome was also investigated by using both recipients and their donors. Frequency of GSTM1 null polymorphism was higher in the recipients who had DGF and those who received allograft from the donors with GSTM1 null genotype. There were also association between recipients’ GSTM1 and donors’ GSTM1 and lipid peroxidation. Glutathione S-transferases are a family of enzymes that protect the living system against electrophilic substances such as ROS through conjugating them with glutathione. Although the effect of GST polymorphisms with oxidative stress and lipid peroxidation in kidney disease has been reported by several authors, there are few studies regarding the association of GST polymorphisms and kidney allograft functions. Singh and colleagues showed that patients with variant genotype of GSTM1 and GSTP1 were at a higher risk for rejection and DGF, respectively, supporting the hypothesis for involvement of GST isoform variants in allograft outcome in kidney transplant recipients. However, Azarpira and colleagues reported no association between GSTM1 and GSTT1 gene polymorphisms and acute rejection. One interesting aspect of our findings was that the donors’ GSTM1 polymorphism was also involved in the allograft functions. In fact this finding which highlights the role of donors’ genetics in transplantation has usually been overshadowed in most of the related studies.

Delayed graft function, as one of the main risk factors for acute rejection, is a multifactorial condition which is affected by the donor and recipient factors. The main factor for developing DGF is IRI of kidney allografts. Free radicals including ROS are extensively generated in the early stage of reperfusion that cause allograft dysfunction during the first postransplant week in various organs, including the liver, brain, heart, and kidney. Reactive oxygen species is known to trigger cytokine and chemokine cascades through nuclear factor-κB activation. The transcription factor nuclear factor-κB is crucial in a series of cellular processes such as inflammation, immunity, cell proliferation and apoptosis. Consistent with these findings, Danilovic and associates showed that treatment of recipients with N-acetyl cysteine, as an antioxidant, could decrease DGF in recipients and made those recipients required fewer days of dialysis.

CONCLUSIONS

Our study demonstrated that donors and recipients’ GSTM1 polymorphism could determine the occurrence of DGF in kidney transplantation. Meanwhile, lipid peroxidation may play an important role in pathophysiology of DGF. Administration of antioxidants before kidney transplantation and considering both the donor and recipient polymorphisms of antioxidant genes can help to improve kidney allograft transplantation outcomes. Although the research has reached its aims, there are some avoidable limitations. Because of time limit, this research was conducted on a relatively small sample size. Meanwhile, there was no possibility for confirming the acute rejection by biopsy.

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CONFLICT OF INTEREST

None declared.

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