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Investigation of association between donors' and recipients' NADPH oxidase p22^{phox} C242T polymorphism and acute rejection, delayed graft function and blood pressure in renal allograft recipients

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ABSTRACT

Background: Production of reactive oxygen species (ROS) and thereby induction of oxidative stress seem to be one of the major mediators of inflammatory adverse outcomes after renal transplantation. p22^{phox} is a polymorphic subunit of NAD(P)H-oxidase that is critical for activation and stabilization of the enzyme. This enzyme is involved in the production of superoxide that triggers inflammatory injuries to the kidney. So in this study, the association between donors and recipients' C242T polymorphism of p22^{phox} and acute rejection (AR), delayed graft function (DGF), creatinine clearance (CrCl), and blood pressure in renal-allograft recipients was studied.
Methods: One hundred ninety six donor–recipient pairs were studied. The C242T polymorphism of p22^{phox} was determined using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). According to p22 genotype, the subjects were divided in wild-type (CC) and T allele carriers (CT + TT). Transplantation outcomes were determined using acute rejection and delayed graft function criteria. The mean arterial pressure was also measured monthly after transplantation.
Results: There was a significant association between the recipients' p22^{phox} polymorphism and DGF occurrence (OR = 2.5, CI: 1.2–4.9, p = 0.0009). No significant association was detected between donors' p22^{phox} polymorphism and AR and DGF events. CrCl during the six months follow-up after transplantation was lower in the patients who received allograft from donors carrying 242T allele (B = −12.8, CI: −22.9–12.8 (−22.9 to −2.6)). Changes in the blood pressure were not different among the patients having different genotypes of p22^{phox}.
Conclusion: These results suggest that the recipients' p22^{phox} C242T polymorphism may be a major risk factor for DGF in renal transplantation. Moreover, the donors' 242T allele seems to affect the rate of CrCl in the renal allograft recipients.

1. Introduction

Delayed graft function (DGF) is a form of acute renal failure that results in post-transplantation oliguria, increased allograft immunogenicity and risk of acute rejection episodes, and decreased long-term survival [1,2]. The results of a meta-analysis have shown that the incidence of acute rejection is higher in the recipients with DGF in comparison to those with non-DGF [3]. It is also shown that DGF is strongly associated with chronic allograft nephropathy (CAN) [4]. The role of ischemic/reperfusion injury (IRI) as one of the major contributors in DGF is well described [2]. In the ischemic phase, reactive oxygen species (ROS) and an acidotic milieu result in phospholipolysis, endothelial membrane injury, and thrombin-mediated fibrin deposition. In the reperfusion phase, the host inflammatory response plays a critical role. In the early hours of reperfusion, the chemotactic signals released by the endothelial cells guide the migration of host neutrophils and macrophages to the transplant. After penetration of leucocytes to the proximal tubul cells, myeloperoxidase in the neutrophils and NAD(P)H oxidase in the macrophages contribute to ROS production [2]. ROS is known to trigger cytokine and chemokine cascades through NF-κB activation that causes a series of cellular processes, such as inflammation,

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immunity, cell proliferation and apoptosis [5]. NAD(P)H oxidases are the major sources of superoxide ROS in phagocytic and non-phagocytic cells like endothelial cells, vascular smooth muscle cells, renal mesangial cells, and renal tubular cells [6,7]. Vascular superoxide production by NAD(P)H oxidase and subsequent oxidative stress cause endothelial dysfunction and clinical risk factors like hypertension and atherosclerosis [7]. The $p22^{phox}$ is one of the essential subunits of NAD(P)H oxidase which is encoded by CYBA gene [7]. Several investigations have examined the association between the $p22^{phox}$ polymorphisms, especially C242T (His72Tyr), and cardiovascular diseases [8–13]. Considering the pivotal role of cardiovascular diseases in the mortality of patients with renal allograft transplantation [14,15], we hypothesized that C242T polymorphism in the $p22^{phox}$ gene may partly explain individual variability in allograft function after transplantation. To our knowledge based on a search of the literature only one study conducted in 2011 has shown a significant relation between the C24T $p22^{phox}$ polymorphism and risk of acute renal rejection [16].

2. Objectives

The aim of this study was to assess the association between the donors and recipients' $p22^{phox}$ C242T polymorphism and transplantation outcomes including the occurrence of acute rejection and delayed graft function, and also post-transplant creatinine clearance (CrCl). Meanwhile, the change in the post-transplant blood pressure of the recipients was shown according to the $p22^{phox}$ C242T genotypes.

3. Materials and methods

3.1. Patients

After getting the informed consent, 196 consecutive recipient–donor pairs who underwent renal transplantation at a single center (Afzalipour Hospital, Kerman, Iran) were recruited in this prospective cohort study. Four recipients were lost to follow-up for the determination of acute rejection.

DGF was defined by stringent criteria on the basis of Boom's definition and independent from the need of dialysis [17]. Based on this definition, an increase, no change, or a less than 10% decrease in the serum creatinine level in three consecutive days during the first week after transplantation indicates DGF. Acute rejection during at least six months post-transplantation was defined as a 20% increase in the creatinine level from the post-operative baseline in the absence of other causes of graft dysfunction that responded to anti-rejection therapy [18]. Creatinine clearance was calculated using Cockcroft–Gault formula, which in turn estimated GFR in ml/min. Mean arterial blood pressure (MAP) was calculated using the following formula: MAP = (diastolic pressure × 2) + systolic pressure) / 3.

3.2. $p22^{phox}$ C242T genotyping

Genomic DNA was isolated from EDTA whole blood using a rapid salting out DNA extraction method. After measuring the quality and quantity of the extracted DNA by the determination of A_{260}/A_{280}, aliquots of the DNA were stored in Tris–EDTA buffer at −70 °C until the analysis of genotypes. The polymorphism of $p22^{phox}$ C242T (NG_007291.1, GenBank) was determined using PCR-restriction fragment length polymorphism (PCR-RFLP) as previously described by some others [12,19,20]. Briefly, a 348-bp fragment of $p22^{phox}$ gene was amplified using the following primers: sense 5′-TGCTTGTGGGTAAACCAAGG CCGGTG-3′ and antisense: 5′-AACACTGAGGTAAGTGGGGGTGGCTCCT GT-3′. The PCR product was subsequently digested by 1 unit of the restriction enzyme *RsaI* (Takara, Japan) for 3 h at 37 °C. The digests were then analyzed by electrophoresis in 3% agarose gels and visualized by ethidium bromide staining. The digestion of the PCR products yielded bands of 348 bp in CC homozygotes, 188 and 160 bp in TT homozygotes, and all three bands in heterozygotes. The reliability and validity of the RFLP method were assessed through reconducting the genotype assays using at least a 10% sample of our DNA samples. The results for all re-assessments were 100% concordant.

3.3. Statistical analysis

Continuous variables were compared using unpaired t-test according to the $p22^{phox}$ CC and CT/TT genotypes. According to the polymorphism and because of the dominancy of T alle [8,10,21], the study population was divided into two categories. The first category consisted of individuals who were wild type (CC: coded 0 in analysis, reference genotype), and the second category included persons who carried mutant (CT and TT) (coded 1). The logistic regression model was used to determine the association between the $p22^{phox}$ C242T genotypes and DGF and AR in a univariate model. Odd ratios and 95% confidence interval were used to estimate the risk of the association between DGF and AR and a specific genotype. Backward regression analyses evaluated the independent predictors of the creatinine clearance at the day of discharge and the sixth month. The association between the dependent variables (DGF, acute rejection, CrCl at the day of discharge and the sixth month) and the $p22^{phox}$ C242T polymorphism was ajdusted using multivariate regression in the presence of potential confounders [22,23]. For all the tests, a p value of less than 0.05 was considered significant. All the analyses were conducted using Statistics Package for Social Sciences (SPSS Inc., USA) software for Windows.

4. Results

4.1. Demographics, clinical and laboratory parameters

Eighty-two percent of the donors and 62% of the recipients were male (Table 1). The means of age for the donors and recipients were 28.6 (±5.8) and 40.7 (±15.4) years old, respectively. Of the total patients, 11% and 20% suffered from AR and DGF, respectively. Demographics of the recipients and donors according to their $p22^{phox}$ C242T genotypes are shown in Table 1. There was no significant difference between variables according to CC and CT/TT genotypes. The laboratory characteristics of the donors and recipients (both at the baseline and at the day of discharge) according to the $p22^{phox}$ C242T polymorphism are presented in Table 2. At the day of discharge, the patients carrying CT/TT genotypes had lower fast blood sugar (FBS) than wild types. Linear regression also showed a significant association between the recipients' C242T polymorphism and FBS at the day of discharge (B = −16.9, CI: −31.9 to −1.8, p = 0.028). Meanwhile, uric acid concentration was higher in the patients with CT/TT genotype in comparison with that of the patients with wild

Table 1
Donor, recipient and transplant characteristics and $p22^{phox}$ genotypes.

Parameters	Total	CC (n = 113)	CT/TT (n = 74)	p*
Donors				
Age (years)	28.6	28.6	28.7	ns
BMI (kg/m^2)	23.2	23.5	22.5	ns
Source (cadaver/living)	6/199	4/116	2/77	ns
Gender (M/F)	167/38	96/24	66/12	ns
Recipients				
Age (years)	40.7	40.4	41.3	ns
BMI (kg/m^2)	22.8	23.1	22.5	ns
Gender (M/F)	128/74	48/78	26/49	ns
SBP (mm Hg)	159.4	157.0	163.1	ns
DBP (mm Hg)	95.6	94.3	98.3	ns
MAP (mm Hg)	112.2	111.2	114.5	ns
Recipient diagnosis				
ESRD	72	45	27	ns
Diabetic nephropathy	25	18	7	ns
Hypertension	15	9	6	ns
PCKD	9	6	3	ns
GN	10	7	3	ns
Others	20	15	5	ns

BMI: body mass index, M: male; F: female, MAP: mean arterial pressure, ESRD: end stage renal disease, PCKD: polycystic kidney disease, GN: glomerulonephritis, SBP: systolic blood pressure, DBP: diastolic blood pressure.