Chemokine Receptor 2-V64I and Chemokine Receptor 5-Δ32 Polymorphisms and Clinical Risk Factors of Delayed Graft Function and Acute Rejection in Kidney Transplantation

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Introduction. Chemokines and chemokine receptors have a pivotal role in immunity and inflammation. We aimed to evaluate their role in kidney transplant rejection.
Materials and Methods. The association of chemokine (C-C motif) receptor 2 (*CCR2*)-*V64I* and *CCR5*-Δ*32* gene polymorphisms with acute rejection (AR) and delayed graft function (DGF) were examined in 100 donor-recipient pairs. The *CCR2-V64I* and *CCR5-Δ32* alleles were determined using polymerase chain reaction and polymerase chain reaction-restriction fragment length polymorphism, respectively.
Results. No associations were found between donors or recipients' *CCR2-V64I* and *CCR5-Δ32* gene polymorphisms and AR or DGF. Of the characteristics of the donors, recipients, and transplantation, glomerulonephritis as a cause of kidney failure in the recipients was weakly associated with AR (relative risk, 6.1; 95% confidence interval, 0.8 to 46.0; $P = .07$). Transplantation of kidney from females to males was weakly associated with DGF (relative risk, 5.5; 95% confidence interval, 0.9 to 33.0; $P = .06$). There was a significant association between AR, but not DGF, and graft loss in the patients (relative risk, 28.6; 95% confidence interval, 1.7 to 487.0; $P = .03$).
Conclusions. Our study failed to suggest *CCR2-V64I* or *CCR5-Δ32* gene polymorphisms as risk factors for AR and DGF in kidney transplantation. Sex-matching between donors and recipients should be considered for living donor kidney transplantation.

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INTRODUCTION

Despite many improvements in the prevention of allograft injury after transplantation, delayed graft function (DGF) and acute rejection (AR) are still the major obstacles to achieving successful organ transplantation. Lines of evidence have demonstrated that AR is one of the most important risk factors for chronic allograft rejection and long-term graft survival.[1-3] Also, it is shown that AR is more frequent in organs with DGF than it is in those that function immediately.[4-6] Although AR is one of the most severe outcomes studied in kidney transplantation, DGF that is a measure of graft functionality requires the most intense consideration.[4]

There are different reports about the rate of DGF in various sources of the graft,[7,8] but most centers report a DGF rate of 20% to 40%.[9] The initial event leading to kidney allograft injury and organ failure is reperfusion injury. The cause

of reperfusion injury is the re-establishment of blood flow to ischemic tissues, causing various intracellular events to occur, which in turn leads to cellular dysfunction, apoptosis, and cell death.[4,10,11] Among factors modulating DGF and AR, cytokines including chemotactic cytokines or chemokines play an important role.[12-15] Because of their essential role in host defense, the role of chemokine receptors and their ligands in rejection of grafts is predictable.[16]

It is revealed that monocyte chemotactic protein 1 gene (*MCP1*) plays a key role in the pathogenesis of renal reperfusion injury via chemokine (C-C motif) receptor (CCR2) signalling by infiltration and activation of macrophages, and it offers a therapeutic target for ischemia-reperfusion.[17,18] During AR, a variety of chemokines are produced in the graft, including chemokine (C-X-C motif) ligand 10 (CXCL10), chemokine (C-C motif) ligand 2 (CCL2), CCL3, CCL4, CCL5, and lymphotactin.[16,19] Tubules are also a source of chemokines CCL2, CCL3, CCL4, CCL5, CXCL8 (interleukin-8), and chemokine (C-X3-C motif) ligand 10 (CX3CL1) and cytokines including tumor necrosis factor-α, transforming growth factor-β, and interleukin-6.[16,20] Chemokines and their receptors have polymorphic gene sequences that cause interindividual differences in their production and functions.[13] This provides an excellent explanation for variation in susceptibility to disease and patterns of disease progression.[21,22] The effects of genetic polymorphisms of *CCR1*, *CCR2*, *CCR5*, *CXCR3*, *CX3CR1* genes on allograft rejection have been shown in many studies.[23-29]

In the present study we aimed to determine the relationship of chemokine receptor polymorphism in *CCR2-V64I* and *CCR5-Δ32* and human kidney transplant rejection and DGF in Kerman, Iran. In addition, the association of donor source and recipients' and transplant characteristics with AR and DGF were evaluated.

MATERIALS AND METHODS

Patients

In this cohort study, 100 consecutive kidney transplant recipients and their donors were recruited from a single center. The study conforms to the national guidelines for conducting human/animal studies (ethic committee permission No 89/35KA; Kerman University of Medical Sciences, Kerman, Iran). The eligibility criteria for the recipients were age between 15 to 75 years and transplantation from living donors. Immunosuppression administration consisted of cyclosporine A, steroids, and mycophenolate mofetil. Episodes of AR were treated with anti-rejection therapy (pulse methylprednisolone and antithymocyte globulin).

Genomic DNA of the participants was isolated from ethylenediaminetetraacetic acid whole blood using a rapid salting-out DNA extraction method.[30] After measuring the quality and quantity of the extracted DNA by determination of A_{260}/A_{280}, aliquots of DNA were stored in Tris-ethylenediaminetetraacetic acid buffer at -70°C until analysis of genotypes. Polymorphism of *CCR5-Δ32* (NG-012637, GenBank) was detected by a simple polymerase chain reaction (PCR) as described elsewhere.[23] This technique identified *CCR5-Δ32* genotypes, corresponding to a 32-base-pair (bp) deletion in the *CCR5* gene. Polymorphism of V64I in the *CCR2* gene (NG-021428.1, GenBank) was determined using PCR-restriction fragment length polymorphism as described by Abdi and colleagues.[23] Briefly, after amplifying a 173-bp fragment of the *CCR2* gene, the PCR product was subsequently digested by 10 units of the restriction enzyme *BsaBI* (Frementase, Lithuania) for 6 hours at 65°C. The samples were then analyzed by electrophoresis in 3% agarose gels and visualized by ethidium bromide staining. The homozygous *Val* genotype was identified by a 173-bp band, the homozygous *Ile* genotype was identified by the presence of 149-bp and 24-bp bands. The heterozygous type exhibited all the three bands. The PCR reaction mixture for both genes contained 1.5 mM of magnesium chloride, 0.2 mM of dNTP, 1x PCR buffer, 1.2 U of Taq DNA polymerase, 100 ng of DNA template and 5 pmol/mL of both forward and reverse primers in de-ionized sterile water in total volume of 50 mL. Amplification was carried as follows: 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, for 35 cycles, and 72°C for 10 minutes as final extension.

Definitions

Delayed graft function was defined by stringent criteria on the basis of Boom and colleagues' definition and independent from the need of dialysis.[4] In this definition, to exclude patients who were dialyzed for reasons other than impaired graft function, diagnose of DGF is made retrospectively if the serum creatinine level increased, remained

unchanged, or decreased by less than 10% per day immediately after surgery during 3 consecutive days for more than 1 week. Acute rejection was defined as an increase in serum creatinine level of 20% from the postoperative baseline in the absence of other causes of graft dysfunction, with a positive biopsy (if available), that responded to antirejection therapy.[23] Kidney function at 6 months was calculated using the Cockroft-Gault formula.[31]

Statistical Analyses

Testing deviation from the Hardy-Weinberg equilibrium (HWE) was performed using the Pearson chi-squared test using the observed genotype frequencies obtained from the data and the expected genotype frequencies obtained using the HWE.[32] For the comparison of continuous variables, we firstly checked the assumption that they were normally distributed. If the distribution was normal, results were expressed as mean and standard error of mean, and the *t* test or 1-way analysis of variance was used. According to *CCR2-V64I* and *CCR5-Δ32* polymorphisms, the study population was divided into 2 categories. The 1st category consisted of individuals who were wild type for *CCR2* (*V64V*) and CCR5 (+/+), coded 0 in analysis, reference genotype. The 2nd category included persons who carried on mutant (*V64I* and/or *I64I* for *CCR2*) or deleted alleles (*Δ32* for *CCR5*), coded 1.

The logistic regression model was used to determine the factors significantly related to DGF and AR in a univariable model. Relative risk and 95% confidence interval were used for estimating the risk of association between DGF and AR with a specific genotype. For all the tests, a *P* value less than .05 was considered significant. All analyses were conducted using the SPSS software (Statistical Package for the Social Sciences, version 13.0, SPSS Inc, Chicago, Ill, USA).

RESULTS

Baseline Parameters

Seventy-nine percent of the donors and 60% of the recipients were men. The mean age for donors and recipients were 29.5 ± 0.6 years and 39.9 ± 1.5 years, respectively. Of all the patients, 11.2% and 27.6% suffered from AR and DGF, respectively. The patient's characteristics according to the occurrence of AR are summarized in Table 1. Among the factors, glomerulonephritis as the cause of kidney

Table 1. Characteristics of Donor, Recipient, and Transplantation By Development of Acute Rejection*

Parameters	Acute Rejection	No Acute Rejection	Relative Risk	95% Confidence Interval	*P*
Donors					
Age, y	28	30	0.87	0.71 to 1.06	.42
Body mass index, kg/m^2	24	22	1.13	0.96 to 1.33	.23
Cadaveric donor	1 (8.3)	7 (7.6)	0.88	0.09 to 7.86	.90
Male gender	9 (81.8)	66 (75.9)	1.03	0.20 to 5.34	.97
Recipients					
Age, y	32	41	0.98	0.92 to 1.07	.12
Body mass index, kg/m^2	22	22	0.13	0.96 to 1.36	.98
Male gender	8 (72.7)	54 (62.1)	2.30	2.01 to 26.00	.80
Gender match					
Matched	9 (81.8)	53 (64.6)	1	reference	...
Male donor for female recipient	2 (18.2)	22 (26.8)	0.68	0.13 to 3.51	.68
Female donor for male recipient	0	7 (8.5)	...	...	.99
Blood group mismatch	1 (9.1)	13 (16.0)	0.82	0.07 to 9.94	.57
MAP < 100 mm Hg before transplantation	1 (11.1)	15 (23.8)	3.20	0.36 to 28.20	.30
Living unrelated donor	10 (90.9)	73 (89.0)	1.32	0.15 to 11.60	.80
Kidney failure cause					
Unknown	4 (36.4)	48 (55.8)	1	reference	...
Diabetic nephropathy	2 (18.2)	11 (12.8)	3.40	0.50 to 23.40	.21
Hypertension	1 (9.1)	7 (8.1)	2.20	0.20 to 24.20	.52
Polycystic kidney	1 (9.1)	8 (9.3)	1.92	0.17 to 20.80	.59
Glomerulonephritis	2 (18.2)	5 (5.8)	6.13	0.82 to 45.92	.07
Others	1 (9.1)	7 (8.1)	2.55	0.23 to 28.71	.45

*Values in parentheses are percents. MAP indicates mean arterial pressure and ellipses, not calculated.

failure was weakly associated with AR (relative risk [RR], 6.1; 95% confidence interval [CI], 0.8 to 46.0; $P = .07$). Table 2 summarizes characteristics of the recipients and donors in the patients according to the occurrence of DGF. Transplantation of kidney from women to men was weakly associated with DGF (RR, 5.5; 95% CI, 0.9 to 33.0; $P = .06$). There was a significant association between graft loss and AR (RR, 28.6; 95% CI, 1.7 to 487.0; $P = .03$), but not DGF (RR, 3.5; 95% CI, 0.2 to 67.7; $P = .40$; Figure).

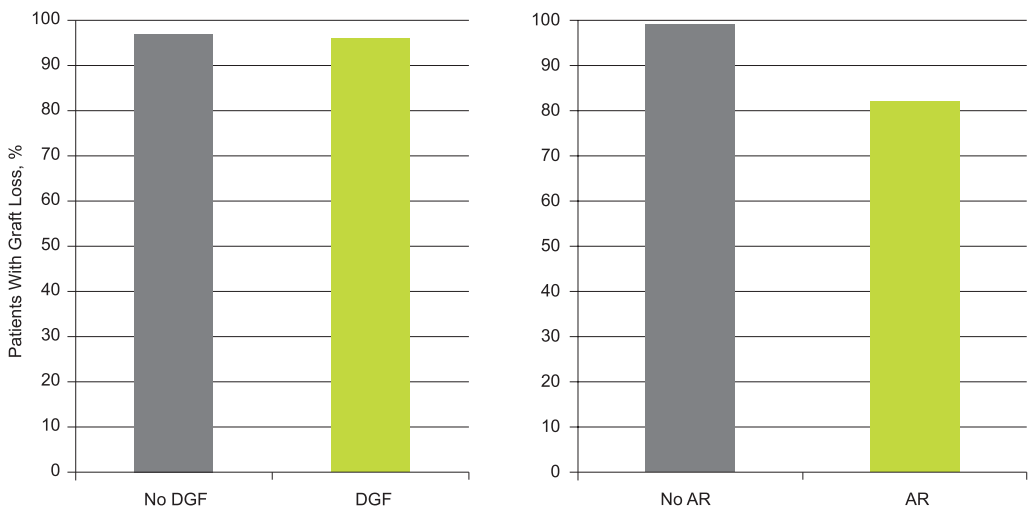
Gene Polymorphisms

The relationship of the donors and recipients' *CCR2-V64I* and *CCR5-Δ32* genotypes with AR and DGF are shown in Table 3. There were no significant differences between *CCR2-V64I* in the

Table 2. Characteristics of Donor, Recipients, and Transplantation By Development of Delayed Graft Function*

Parameters	DGF	No DGF	Relative Risk	95% Confidence Interval	*P*
Donors					
Age, y	31.4	28.8	1.07	0.99 to 1.15	.09
Body mass index, kg/m^2	23	23	0.97	0.86 to 1.11	.58
Cadaveric donor	3 (11.1)	6 (8.2)	0.60	0.13 to 2.73	.51
Male gender	21 (77.8)	58 (73.6)	0.52	0.13 to 2.15	.52
Recipients					
Age, y	40	39	1.00	0.95 to 1.60	.98
Body mass index, kg/m^2	22.9	22	1.02	0.92 to 1.13	.66
Male gender	20 (74.1)	45 (61.6)	0.97	0.08 to 10.80	.21
Gender match					
Matched	17 (63.0)	47 (67.1)	1	reference	...
Male donor for female recipient	6 (22.2)	20 (28.6)	0.65	0.18 to 2.21	.45
Female donor for male recipient	4 (14.8)	3 (4.3)	5.50	0.92 to 32.98	.06
Blood group mismatch	3 (88.9)	11 (17.4)	1.14	0.14 to 9.40	.56
MAP < 100 mm Hg before transplantation	1 (7.6)	14 (33.3)	6.00	0.7 to 50.92	.10
Living unrelated donor	24 (88.9)	63 (70.0)	0.91	0.08 to 10.46	.84
Kidney failure cause					
Unknown	17 (63.0)	39 (52.7)	1	reference	...
Diabetic nephropathy	4 (14.8)	9 (12.2)	2.08	0.55 to 7.95	.28
Hypertension	2 (7.4)	6 (8.1)	0.83	0.15 to 4.64	.84
Polycystic kidney	1 (3.7)	8 (10.8)	0.31	0.04 to 2.74	.29
Glomerulonephritis	2 (7.4)	5 (6.8)	1.00	0.17 to 5.77	> .99
Others	1 (3.7)	7 (9.5)	...	...	.99

*Values in parentheses are percents. DGF indicates delayed graft function; MAP, mean arterial pressure; and ellipses, not calculated.



The percentage of graft loss according to presence or absence of acute rejection (AR) and delayed graft function (DGF).

Table 3. Analysis of Recipient and Donor Gene Polymorphisms on Acute Rejection and Delayed Graft Function*

Genotype	Total (%)	AR			DGF		
		Number (%)	RR (95% CI)	*P*	Number (%)	RR (95% CI)	*P*
Recipient							
*CCR2*I* positive	22 (26.8)†	2 (9.1)	reference		8 (36.4)	reference	
*CCR2*I* negative	60 (73.2)	4 (6.7)	1.3 (0.2 to 7.7)	.60	16 (26.7)	1.4 (0.5 to 4.1)	.40
Recipients							
*CCR5*Δ32* positive	11 (12.6)¶	1 (9.1)	reference		4 (36.4)	reference	
*CCR5*Δ32* negative	76 (87.4)	8 (10.5)	0.8 (0.1 to 7.5)	> .99	19 (25.0)	1.7 (0.4 to 6.5)	.50
Donor							
*CCR2*I* positive	20 (25.6)‡	2 (10.0)	reference		8 (40.0)	reference	
*CCR2*I* negative	58 (74.4)	6 (10.3)	0.9 (0.2 to 5.2)	> .99	15 (25.9)	1.9 (0.6 to 5.6)	.20
Donors							
*CCR5*Δ32* positive	3 (3.5)¶	0	reference		3 (100)	reference	
*CCR5*Δ32* negative	83 (96.5)	10(12.0)	0.9 (0.8 to 1.0)	> .99	20 (24.1)	5.2 (0.7 to 33.8)	.10

*values in parentheses are percents. AR indicates acute rejection; DGF, delayed graft function; RR, relative risk; CI, confidence interval; and CCR, chemokine (C-C motif) receptor.
†*CCR2-V64I*, 20 (24.4)
‡*CCR2-V64I*, 19 (24.7)
¶All heterozygote

recipients who had a rejection episode or DGF. There were also no differences in AR or DGF among the recipients when stratified by the presence or absence of the *CCR5-Δ32* allele in the recipients and their donors (Table 3). The associations of donor and recipients' *CCR2-V64I* and *CCR5-Δ32* with graft loss were nonsignificant either.

DISCUSSION

Considering the crucial role of chemokines and their receptors in the immune response and transplantation rejection, we studied the role of *CCR2-V64I* and *CCR5-Δ32* polymorphisms in DGF and AR. Meanwhile, some other factors of donors and recipients which might affect the occurrence of DGF and AR were studied. We did not detect any association between donor and recipient's *CCR2-V64I* and *CCR5-Δ32* polymorphisms and development of AR and DGF in 100 donor-recipient pair in our center. The *CCR5-Δ32* is a 32-bp deletion in the *CCR5* gene resulting in a nonfunctional protein which cannot be detected at the cell surface.[33] It is shown that homozygosity for the *CCR5-Δ32* gene had a protective effect on the survival of kidney transplants than in the control group.[34,35] In agreement with our results, no influence of *CCR5-Δ32* gene polymorphism on AR episodes was observed in several studies.[23-26] There is no study, however, in the literature about the role of *CCR5-Δ32* gene polymorphism and development of DGF. We also found no association between *CCR2-64I* allele and AR or DGF. This result is consistent with the findings of Kang and colleagues.[29] The lack of beneficial association between *CCR2-64I* allele and AR or long-term liver allograft survival has been also reported by Schroppel and colleagues.[36] However, most of the studies about the effect of *CCR2-64I* polymorphism on AR are in favor of the renoprotective effect of *CCR2-64I* allele in kidney transplantation as the risk of AR was reduced significantly in recipients who possessed the *I* allele.[23-26]

In addition to the role of chemokine receptor polymorphism, the association of some other factors related to the donors, recipients, and transplantation with AR and DGF was evaluated. Among the studied factors, only glomerulonephritis as the cause of kidney failure was weakly associated with AR (RR, 6.1; 95% CI, 0.8 to 46.0; $P = .07$). In univariable analysis, transplantation of a kidney from a female donor to a male recipient was associated with DGF (RR, 5.5, 95% CI, 0.9 to 33.0; $P = .06$). These results are consistent with the previous reports which noticed female donor to male recipient as a risk factor for DGF and AR.[4,37,38] In the cadaveric donor kidney transplantation, several risk factors including donor age of more than 50 years, mean arterial blood pressure of less than 100 mm Hg, cold ischemia time, transplantation of a kidney from a female donor to a male recipient, peak panel reactive antibodies of over 50%, cold ischemic time, and donor serum creatinine have been noticed as independent risk factors for development of DGF.[7,9,37,39-44] Increased donor age, which has

been widely considered to be the most potent risk factor for DGF after cadaveric donor kidney transplantation,[40-42,44-47] did not achieve significance in our cohort of medically healthy live donors. This result is supported by some other studies on living donors.[7,44,46]

Lastly, we assessed the impact of AR and DGF on graft failure. Our analyses indicated that there was a significant association between AR and graft failure (RR, 28.6; 95% CI, 1.7 to 487.0; $P = .03$). Rejection has been identified as an independent risk factor for graft failure after living donor kidney transplantation.[7,44,46,48]

CONCLUSIONS

Our study failed to suggest *CCR2-V64I* or *CCR5-Δ32* gene polymorphisms as risk factors for AR and DGF in kidney transplantation. Because of the low frequency of the interested polymorphisms, studying a larger cohort with a longer follow-up is needed to confirm our results.

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CONFLICT OF INTEREST

None declared.

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