Association of Programmed Cell Death 1 and Programmed Cell Death 1 Ligand Gene Polymorphisms With Delayed Graft Function and Acute Rejection in Kidney Allograft Recipients

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Introduction. The genetic variations of co-stimulatory molecules can affect the extent of T cell activity during T-cell mediated immunity, especially in transplant patients. This study aimed to investigate the association of programmed cell death 1 (*PDCD1*) and programmed cell death 1 ligand 1 (*PDCD1LG1*) gene polymorphisms with clinical outcome of kidney transplantation.

Materials and Methods. A total of 122 patients with a kidney transplant were included in this retrospective study. Patients were classified into two groups of biopsy-proven acute allograft rejection (AAR) and stable graft function (SGF) during the 5-year follow-up period. Four single nucleotide polymorphisms in *PDCD1* and *PDCD1LG1* were determined in the groups of patients as well as in 208 healthy control individuals.

Results. The frequencies of PD-1.3 (+7146 G > A), PD-1.9 (+7625 C > T), PD-L1 (8923 A > C), and PD-L1 (+6777 C > G) genotypes and alleles were not significantly different between the AAR and SGF groups. In comparison with healthy controls, PD-1.9 (+7625 C > T) genotype and T allele were significantly more frequent in all of the patients and in those with SGF. Overall, 27 of 122 kidney allograft recipients experienced delayed graft function, and a higher frequency of PD-1.9 (+7625 C > T) genotype and T allele was observed in this group versus those without delayed graft function. Similarly, a significant high frequency of this genotype was found among the AAR subgroup of patients with delayed graft function.

Conclusions. Our results indicate that potentially functional genetic variation in *PDCD1* can influence the outcome of kidney transplantation.

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INTRODUCTION

It is well documented that T cell activation is the main event in alloimmune response leading to allograft rejection. In fact, T lymphocytes play a central role in the induction and regulation of the adaptive immune response to foreign antigens.[1] Acute rejection as a major cause of morbidity in kidney transplant patients is considered to be an

important risk factor in development of chronic allograft nephropathy.[2] Recent investigations have clarified how T lymphocytes, the central players of acute allograft rejection (AAR), migrate into the allograft and recognize alloantigens. In this context, the influence of co-stimulatory molecules and cytokines as well as contribution of the innate immune responses to allograft rejection has been shown intelligibly.[3] Signalling through co-stimulatory molecules affect the regulation of T cell activation and therefore determines the outcome of virtually any primary and secondary immune responses.[4]

The important role of co-stimulatory molecules, particularly B7-CD28 family in development and progression of various autoimmune diseases have been shown clearly.[5] One of the members of B7-CD28 superfamily is known as programmed cell death 1 (PD1) molecule.[3,5,6] The *PDCD1* gene, mapping on chromosome 2q37.3, encodes a 55-kD type 1 transmembrane inhibitory receptor, which contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) in its cytoplasmic tail to induce downregulation of T cells responses in favor of peripheral tolerance.[5-7] Interaction of PD1 and PD1 ligands (*PDCD1LG1* and *PDCD1LG2* genes) plays a crucial role in these tolergenic responses.[8]

Two types of PD1 ligands differ in their expression patterns; *PDCD1LG1* is constitutively expressed on hematopoietic and nonhematopoietic cells as well as mast cells whereas, *PDCD1LG2* has an inducible expression on dendritic cells macrophages and bone marrow derived mast cells.[9,10] As the interactions between PD1 and PD1 ligands influence the extent of T cell activation, any changes in their structure and expression levels, mainly due to genetic variations, can affect T cells activity during autoimmune or alloimmune responses.[11] The *PDCD1* gene polymorphisms have been reported to be associated with several autoimmune diseases such as systemic lupus erythematus,[12,13] type 1 diabetes mellitus,[14,15] ankylosing spondylites,[16] and multiple sclerosis.[7]

The presence of a regulatory single-nucleotide polymorphism (SNP) in the intronic region of *PDCD1* gene (rs 11568821, PD-1.3 A > T) was shown to be correlated with susceptibility to systemic lupus erythematus in Europeans and Mexicans.[13] The A allele for this SNP alters regulation of gene expression through disruption of binding of the Runt-related transcryption factor 1 to the enhancer of this gene. Another SNP in *PDCD1* but in the exon region (PD-1.9 C > T, rs 2227982) affects protein synthesis through changing from valine to alanine amino acid.[15] Preclinical studies on the role of PD1 and PD1 ligands in cardiac and islet transplantation have shown that PD1 and PD1 ligands expression are induced within cardiac allografts undergoing rejection.[17]

Similarity between autoimmunity and alloimmune responses to allograft antigens in the sense of inflammatory reactions indicates the essential role of T cells in initiation and regulation of these deleterious immune responses to foreign or self-antigens. This in turn highlights the contribution of regulatory molecules in T cell activation in these situations. Therefore, elucidation of the impact of genetic variation of these molecules on their functional activity has an importance in evaluation of T cell responses and it could be clinically relevant in transplant patients.[18-20]

Based on the literature, few studies have investigated the correlation between *PDCD1* and *PDCD1LG1* genetic variations and outcome of liver and kidney transplants,[4,21] which is not adequate to make a clear conclusion. Hence, this retrospective multicenter study aimed to investigate the association of *PDCD1* and *PDCD1LG1* gene polymorphisms with occurrence of AAR in kidney allograft recipients.

MATERIALS AND METHODS

Study Population

A total of 122 kidney allograft recipients who underwent transplantation between June 2007 and September 2012 at 3 transplant centers of Sina Hospital, Emam Khomeini Hospital, and Labbafi-Nejad Medical Center, affiliated to Tehran University of Medical Sciences and Shahid Beheshti University of Medical Sciences, were enrolled in this retrospective study. Patients were classified into 2 groups based on the occurrence of biopsy-proven AAR (AAR group) and having clinically stable graft function (SGF) without any previous episode during the 5 years of follow-up (SGF group). The data of 208 healthy control participants from our previous study[16] were included for comparison. Acute rejection was defined based on clinical finding and confirmed by a biopsy protocol based on the Banff classification.[22] Inclusion criteria

consisted of being first transplant recipients and having stable graft function for at least 18 months posttransplantation without any clinical or paraclinical evidences of rejection. Exclusion criteria were having non-biopsy-proven AAR and second transplantation.

All of the patients received human leukocyte antigen (HLA)-mismatched kidney transplants (1 to 6 mismatches) from cadaveric (n = 38), living unrelated (n = 77), and living related donors (n = 7). Other clinical data including cytomegalovirus infection status, delayed graft function (DGF), graft loss, and serum creatinine levels were considered for comparison between the two groups of patients. Delayed graft function was defined as rising of serum creatinine levels (> 500 µm/L) and the need for dialysis during the 1st week posttransplant.[23,24] The allograft was considered lost upon return of the patient to dialysis. Patients with no history of clinical or biopsy-proven rejection and with good functioning graft, as judged by serum creatinine level (< 1.5 mg/dL), were considered as SGF. Immunosuppressant regimen for all of the recipients consisted of conventional triple drug therapy with cyclosporine A or tacrolimus, mycophenolate mofetil or azathioprine, and methyl prednisone. None of the patients from either of the groups received antibody induction therapy. The study protocol was approved by the Research Ethics Committee of Tehran University of Medical Sciences and informed consents were obtained from all of the participants.

Samples and Genotyping

Five milliliters of peripheral blood was collected in vacutubes containing ethylenediaminetetraacetic acid and then DNA extraction was performed by phenol-chloroform method. After assessment of quantity and quality of DNA samples by ultraviolet spectrophotometers, polymerase chain reaction (PCR)-restriction fragment length polymorphism method was carried out to determine the single nucleotide polymorphisms in *PDCD1* gene PD-1.3 (+7146 G > A), PD-1.9 (+7825 C > T), PD-L1(8923 A > C), and PD-L1 (+6777 C > G). The specific primers for detection of each SNPs were used according to the previous studies.[8,16,24] Amplification reaction was prepared in a total volume of 20 µL containing 10 pm of each primers, 1.5 mM mgcl2, 1x PCR buffer, 0.5 U Taq DNA polymerase, and 100 ng of the genomic DNA.

The PCR conditions for all SNPs, were as follows: initial denaturation at 94°C for 2 minutes and 10 cycles of denaturation at 94°C for 10 seconds, annealing at 66°C for 1 minute followed by 20 cycles of denaturation at 94°C for 10 seconds, annealing at 59°C for 45 seconds and extension at 72°C for 2 minutes. The PCR products were run on 2% gel agarose electrophoresis and confirm PCR product-specific bands using gel documentation system (Vilber, USA) and there after restriction fragment length polymorphism reactions on these products were performed using restriction enzymes (BanII for PDL-1 8923 A > C, BsrI for PD-L1 6777 C > G, PstI for PD-1.3 G > A, and Bpu10 for PD-1.9 C > T) according to manufactures' instructions (Fermentas, Russia). Finally, digested products were analysed on 3% agarose gel electrophoresis.

Statistical Analysis

The Hardy-Weinbrg equilibrium was assessed in cases using the chi-square test or the Fisher exact test. The maximum likelihood estimation of haplotype frequencies from unphased genotypes were calculated by the expectation-maximization algorithm implemented in haplo.stats package within the R statistical software. Global test for comparison of haploytpe frequencies between the two groups was done by the likelihood ratio test. In order to compare genotype, allele, and haplotype frequencies between the two groups, we used the chi-square test or the Fisher exact test, and the results reported as *P* values as well as odds ratios and their 95% confidence intervals (95% CIs) by using the univariable logistic regression. Additionally, multivariable logistic regression analysis was done to identify correlations between covariates and kidney graft failure. *P* values less than .05 were considered significant. All computations were done using R package version 2.15.2.[25]

RESULTS

Demographic characteristics of all of the patients are summarized in Table 1. Among 122 kidney allograft recipients, 66% were men and 34% were women. Both groups of the patients were followed for 5 years. Although the patients with SGF had a longer follow-up period than the patients with AAR (*P* = .01, Table 1), the difference in age between the groups was not significant (*P* = .29, Table 1). Overall,

Table 1. Characteristics of Kidney Allograft Recipients With Acute Allograft Rejection (AAR) and Stable Graft Function (SGF)

Parameters	AAR Group (n = 61)	SGF Group (n = 61)	*P*
Follow-up duration, mo	43.8 ± 36.2	61.3 ± 22.4	.01
Age, y	33.9 ± 13.2	31.4 ± 10.9	.29
Sex			
Male	42 (68.9)	32 (52.5)	
Female	19 (31.1)	29 (47.5)	.09
Donor Source			
Cadaver	23 (37.7)	15 (24.6)	.17
Living unrelated	33 (54.1)	42 (68.85)	.26
Living related	3 (4.9)	4 (6.55)	> .99
Cytomegalovirus infection	18 (29.5)	14 (23.0)	.53
Rejection type			
Cell-mediated	53 (86.9)	…	…
Antibody-mediated	7 (11.5)	…	…
Cellular and humoral	1 (1.6)	…	…
Graft loss	21 (34.4)	…	…
Delayed graft function	18 (29.5)	9 (14.8)	.08
Serum creatinine level, mg/dL	3.06 ± 1.39	1.08 ± 0.13	< .001

77 patients (63%) received HLA-mismatched kidney transplants from living unrelated donors, 38 (31%) received allografts from cadavers, and 7 (6.0%) received allografts from living related donors. The differences in donor sources were not significant. Panel reactive antibodies levels for all recipients in the two groups were between zero and 10% before transplantation. Cytomegalovirus infection was more frequent in the AAR group than the SGF group, although it was not significant (P = .53, Table 1).

Among 61 patients with biopsy-proven AAR, 21 (34.4%) lost their allograft and returned to dialysis or received a second transplant. This group also showed a higher frequency of DGF (29.4% versus 14.75% in the SGF group, P = .08; Table 1) and higher serum creatinine levels during the follow-up (P < .001, Table 1). The frequencies of PD-1.3 (+7146 G > A), PD-1.9 (+7625 C > T), PD-L1 (8923 A > C), and PD-L1 (+6777 C > G) genotypes and alleles were not significant between the two groups (Table 2). However, in comparison with the healthy controls, PD-1 +7625 C > T genotype was significantly more frequent in all of the patients and in those with SGF (P = .001 and P = .001, respectively; Table 2). The frequency of PD-1 +7625 T allele was also high in all of the patients and those with SGF compared to healthy control s (P < .0001 and P = .001 respectively, Table 2).

Overall, 27 of 122 the recipients (22.1%)

Table 2. Alleles and Genotypes of *PDCD1* and *PDCD1LG1* Gene in Kidney Allograft Recipients and Healthy Controls*

Genotype	AAR Group (n = 61)	SGF Group (n = 61)	Control Group (n = 208)	*P*			
				AAR vs SGF	AAR vs Control	SGF vs Control	AAR and SGF vs Control
PDCD1							
+7146							
G > G	50 (81.9)	52 (85.2)	164 (78.8)	.80	.72	.35	.36
A > G	11 (18.0)	9 (14.7)	41 (19.7)	.80	.91	.35	.54
A > A	0	0	3 (1.4)				.29
G	111 (90.9)	113 (92.6)	369 (88.7)	.81	.58	.28	.25
A	11 (9.0)	9 (7.4)	47 (11.2)	.81	.58	.28	.25
+7625							
C > C	55 (90.1)	51 (83.6)	198 (96.5)	.42	.08	.001	.001
C > T	6 (9.8)	10 (16.4)	7 (3.4)				
T > T	0	0	0				
C	116 (95.0)	112 (91.8)	403 (98.3)	.43	.08	.001	< .001
T	6 (4.9)	10 (8.2)	7 (1.7)	…	…	…	…
PDCD1LG1							
+8923							
A > A	17 (27.8)	20 (32.8)	…	.69	…	…	…
A > C	43 (70.5)	40 (65.6)	…	.69	…	…	…
C > C	1 (1.6)	1 (1.6)	…	> .99	…	…	…
A	77 (63.1)	80 (65.6)	…	.78	…	…	…
C	45 (36.8)	42 (34.4)	…	.78	…	…	…
+6777							
C > C	61 (100)	61 (100)	…	> .99	…	…	…

*AAR indicates acute allograft rejection and SGF, stable graft function.

experienced DGF. Comparison of *PDCD1* and *PDCD1LG1* alleles and genotypes between the two groups of patients demonstrated a higher frequency of PD1.9 +7625 C > T genotype and T allele in the recipients with DGF (P = .04 and P = .05, respectively; Table 3). Similarly, a significant high frequency of this genotype was observed among the AAR subgroup of patients with DGF (P = .05, Table 3). However, PD-1.9 T allele was also more frequent in this group compared to those without DGF although it was marginally significant (P = .06, Table 3). Considering the type of AAR based on the biopsy protocol in the AAR group, higher but nonsignificant frequencies of PD-1.3 +7146 A > G, PD-1.9 +7625 C > C, and PDL1 8923 A > C genotypes were observed in the recipients with cell-mediated compared to antibody-mediated AAR (Table 4).

Haplotype analysis by expectation-maximization algorithm for PD-1.3 and PD-1.9 SNPs also showed no statistical differences between the two groups of the patients (Table 5). In addition, multivariable logistic regression analysis demonstrated that DGF was associated with graft loss in the AAR group (P = .02, Table 6).

Table 4. Alleles and Genotypes *PDCD1* and *PDCD1LG1* Gene in Kidney Allograft Recipients With Acute Allograft Rejection by rejection Type*

Genotype	Patients with Acute Rejection		*P*
	Cell-mediated (n = 53)	Antibody-mediated (n = 7)	
PDCD1			
+7146			
G>G	42 (79.2)	7 (100)	0.33
A>G	11 (20.7)	0	0.33
A>A	0	0	
G	95 (89.6)	14 (100)	0.35
A	11 (10.4)	0	
+7625			
C>C	49 (92.4)	5 (71.4)	0.14
C>T	4 (7.54)	2 (28.6)	0.14
T>T	0	0	
C	102 (96.2)	12 (85.7)	0.14
T	4 (3.8)	2 (14.3)	0.14
PDCD1LG1			
+8923			
A>A	14 (26.4)	3 (42.9)	0.39
A>C	38 (71.7)	4 (57.1)	0.41
C>C	1 (1.9)	0	1.00
A	66 (62.3)	10 (71.4)	0.70
C	40 (37.7)	4 (28.6)	

Table 3. Alleles and Genotypes *PDCD1* and *PDCD1LG1* Gene in Kidney Allograft Recipients and in Patients With Acute Allograft Rejection With and Without Delayed Graft Function*

Genotype	AAR Group (n = 61)	SGF Group (n = 61)	*P*	OR (95%CI)	AAR Group			
					DGF (n = 27)	No DGF (n = 43)	*P*	OR (95%CI)
PDCD1								
+7146								
G>G	24 (88.9)	78 (82.1)	.55	0.57 (0.12 to 2.34)	17 (94.4)	33 (76.8)	.14	0.19 (0.01 to 1.72)
A>G	3 (11.1)	17 (17.9)		1	1 (5.6)	10 (32.2)		1
A>A	0	0			0	0		…
G	51	173	.57	1.67 (0.44 to 7.48)	35	76	.17	4.61 (0.57 to 99.86)
A	3	17		1	1	10		1
+7625								
C>C	20 (74.1)	86 (90.5)	.04	0.30 (0.09 to 1.02)	14 (77.8)	41 (95.3)	.05	0.17 (0.02 to 1.27)
C>T	7 (25.9)	9 (9.5)		1	4 (22.2)	2 (4.6)		1
T>T	0	0		…	0	0		…
C	47	181	.05	0.33 (0.11 to 1.06)	32 (88.8)	84 (97.7)	.06	0.19 (0.02 to 1.30)
T	7	9			4 (11.1)	2 (2.3)		1
PDCD1LG1								
+8923								
A>A	9 (33.3)	28 (29.5)	.88	1.20 (0.43 to 3.25)	4 (22.2)	13 (30.2)	.74	0.66 (0.15 to 2.76)
A>C	17 (63)	66 (69.5)	.68	0.75 (0.28 to 2.01)	13 (72.2)	30 (69.8)	.90	1.13 (0.29 to 4.55)
C>C	1 (3.7)	1 (1)	.39	3.62 (0.00 to 137.90)	1 (5.6)	0	.29	…
A	35	122	.93	1.03 (0.52 to 2.03)	21 (58.3)	56 (65.1)	.61	0.75 (0.31 to 1.80)
C	19	68	> .99		15 (41.7)	30 (34.9)	> .99	1
+6777								
C>C	27 (100)	95 (100)			18 (100)	43 (100)		…

*AAR indicates acute allograft rejection; SGF, stable graft function; DGF, delayed graft function; OR, odds ratio; and CI, confidence interval.

Table 5. *PDCD1* Haplotypes in Kidney Allograft Recipients*

PD1.3-PD1.9 Haplotypes	AAR Group (n = 61)	SGF Group (n = 61)	OR (95% CI)	*P*
G-C, %	86.4	84.4	1.29 (0.48 to 3.55)	.61
A-C, %	8.7	7.4	1.00 (0.27 to 3.65)	> .99
G-T, %	4.6	8.2	0.58 (0.13 to 2.54)	.72

*AAR indicates acute allograft rejection; SGF, stable graft function; OR, odds ratio; and CI, confidence interval.

Table 6. Predictive Values of Covariate for Graft Loss*

Variables	OR (95% CI)	*P*
Cytomegalovirus infection	1.774 (0.348 to 9.047)	.49
Age	1.045 (0.993 to 1.100)	.09
Sex	1.367 (0.320 to 5.841)	.67
Donor sources		.31
Cadaveric	2.405 (0.150 to 38.694)	.53
Living unrelated	0.855 (0.058 to 10.643)	.78
Delayed graft function	0.163 (0.035 to 0.774)	.02
PD-1.9	0.981 (0.155 to 6.189)	.98
PD-1.3	0.940 (0.231 to 3.820)	.93

*OR indicates odds ratio and CI, confidence interval.

DISCUSSION

In spite of modern immunosuppressant therapies that are effective in reducing the incidence of AAR, chronic allograft nephropathy and graft loss are still a major problem in solid organ transplantation.[26-28] Apart from nonimmunologic factors including thrombosis, graft failure has several significant immunological etiologies, in which T lymphocytes play a crucial role in almost all of these immune responses to allograft antigens.[23] Hence, molecular and structural investigations of the co-stimulatory molecules involved in regulation of T cell activity may have critical importance in evaluation of cellular alloimmunity and possible consequent therapeutic interventions. Several studies have shown that genetic variations in some of these co-stimulatory molecules such as *PDCD1*, *PDCD1LG*, *CTLA4*, *CD86*, and *ICOS* could be considered as genetic susceptibility risk factors for graft rejection although a little is known about clinical relevance of *PDCD1* and *PDCD1LG1* gene polymorphisms in kidney transplant patients.[1,4,20,29]

Genetic analysis of PD1 and PD1 ligand molecules in the kidney allograft recipients of this retrospective study demonstrated that all patients with AAR or with stable graft function had a higher frequency of PD-1.9 +7625 C > T genotype in comparison with healthy controls. This genotype was also more frequent among patients with SGF compared to healthy controls. In addition, all trnasplant patients showed higher frequencies of PD-1.9 +7625 T allele than healthy controls. Remarkably, among all patients, those with DGF during the first week after transplantation showed an increased frequency of PD-1.9 C > T genotype and T alleles in comparison with recipients without DGF. Also, a similar difference was found between AAR patients with DGF and those without DGF. A higher but marginally significant frequency of T allele for PD-1.9 +7625 position was also observed in patients with DGF as compared to those without DGF in the AAR group. A similar study by Karimi and colleagues,[21] but on liver transplant patients, demonstrated that PD-1.9 C > T and PD-1.3 A > G gene polymorphisms were not associated with AAR. We also observed no significant differences for these SNPs between the AAR and SGF groups of patients. The only study on kidney transplant patients by Ebadi and coworkers[4] showed a significant association between PD-1.1 A > A genotype and AAR episodes, which is different regarding the evaluated PD1 SNPs in our study. Thus, this comparison should be interpreted cautiously.

According to the key role of this inhibitory pathway in induction of regulatory responses, it is rational that selective stimulation of PD1-PD1 ligand pathway may play an important inhibitory role in regulating T cell activation and allograft rejection.[30] In other words, in the context of submaximal T cell co-stimulation or in the framework of CD28 or CD154 blockade, targeting PD1 or PD1 ligand using monoclonal antibodies can modulate T- and B-cell mediated responses and consequently block allograft rejection.[31]

It is speculative that any potential therapeutic intervention targeting PD1-PD1 ligand pathway could be more effective by clarification of precise mechanism of this pathway as an integral part of maintaining immune tolerance, which is not yet fully understood.[32] In this context, understanding the expression patterns, structure, and function of these molecules mainly influenced by genetic

variations could be very informative and somewhat predictive for outcome of alloimmune responses in transplant patients, in order to modulate T cell-mediated responses using more specifically and minimal dose of immunosuppressant.

Observation of the higher frequency of PD-1.9 +7625 C > T genotype in our patients compared to healthy controls could be indicative of probable altered contribution of PD1 molecule in PD1-PD1 ligand pathway due to nonsynonymous polymorphism (Val215Ala) and subsequently different effect of this pathway on T cell-mediated responses in kidney transplant patients. More importantly, we found higher significant frequencies of the same genotype in patients with DGF and expectedly in the AAR subgroup of patients with DGF.

This association of PD-1.9 +7625 C > T genotype and T allele with occurrence of DGF also suggests that single amino acid change in PD1 structure can affect the function of this molecule and subsequently the status of T cell responses. As the most cases with DGF were in the AAR group, higher frequency of this genotype and allele among this group of patients may indicate the altered function of PD1-PD1 ligand inhibitory pathway in regulation of T cells alloimmunity. Nonetheless, several other inhibitory molecules such as CTLA-4 and ICOS, not been tested in this study, are involved in regulating the dynamic balance between effector T cells and regulatory T cells in any immune responses.[1,21,24]

Some studies on transplant patients have shown that protective role of regulatory T cells is induced through PD1-PD1 ligand activation. Accordingly, it has been demonstrated that PD1 mRNA levels may act as an informative biomarker for AAR and corresponding outcome after renal transplantation so that, increased levels PD1 as a negative co stimulatory molecule counters the effect of alloimmune responses during AAR episodes. Thus, decreasing or lack of PD1 function can lead to allograft dysfunction and even graft failure.[32] Utilizing this effective biomarker at DNA, mRNA, or protein levels besides other immune markers, such as antibodies to donor's antigens (mainly HLA), T cell activation marker (sCD30), regulatory T cells profiles, and mediators in peripheral blood, allows the clinicians to extend the potential benefits of tapering immunosuppressant to an upgrading number of patients with predictable outcome.[32-36] However, as we did not analyse the PD1 at mRNA or protein level in these patients, our data should be interpreted cautiously with respect to the association between *PDCD1* gene polymorphism and clinical outcome of kidney allografts.

CONCLUSIONS

To our knowledge, this is the first report on correlation between occurrence of DGF and presence of PD-1 +7625 C > T genotype in kidney transplant patients with biopsy-proven AAR. Our results indicate that potentially functional genetic variation in *PDCD1* can influence the outcome of kidney transplantation. Further investigations with larger sample sizes are needed to determine whether this amino acid change alters the protein structure and affects its function and more importantly to find the clinical relevance of this genetic variation with kidney allograft outcome.

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CONFLICT OF INTEREST

None declared.

REFERENCES

1. Krichen H. Evaluation of CTLA-4, CD28 and CD86 Genes Polymorphisms in acute renal allograft rejection among Tunisian Patients. In: Kidney Transplantation New Perspectives. 2011. p. 111-26.
2. Lessan-Pezeshki M, Amirzargar AA, Golabi N, et al. Prediction of Rejection in Renal Transplantation by Immune Parameters. Iran J Immunol. 2005;2:87.
3. Nankivell BJ, Alexander SI. Rejection of the kidney allograft. N Engl J Med. 2010;363:1451-62.
4. Ebadi P, Karimi MH, Tahoori MT, et al. Polymorphisms of the programmed cell death 1 and ICOS genes and kidney transplant rejection in Iranian patients. Afr J Microbiol Res. 2012;6:6918-23.
5. Liu X, Hu LH, Li YR, Chen FH, Ning Y, Yao QF. Programmed cell death 1 gene polymorphisms is associated with ankylosing spondylitis in Chinese Han population. Rheumatol Int. 2011;31:209-13.
6. Qian BP, Wang XQ, Qiu Y, Jiang H, Ji ML, Jiang J. An exon polymorphism of programmed cell death 1 gene is associated with both the susceptibility and thoracolumbar kyphosis severity of ankylosing spondylitis in a Chinese

Han population. J Orthop Sci. 2013;18:514-8.

7. Shadmehri AA, Nicknam MH, Shokrgozar MA, et al. Assessment of PD-1 gene variation in patients with multiple sclerosis. Tehran Univ Med J. 2010;68: 87-93.
8. Hayashi M, Kouki T, Takasu N, Sunagawa S, Komiya I. Association of an A/C single nucleotide polymorphism in programmed cell death-ligand 1 gene with Graves' disease in Japanese patients. Eur J Endocrinol. 2008;158:817-22.
9. Butte MJ, Keir ME, Phamduy TB, Sharpe AH, Freeman GJ. Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses. Immunity. 2007;27:111-22.
10. Mitchell AL, Cordell HJ, Soemedi R, et al. Programmed death ligand 1 (PD-L1) gene variants contribute to autoimmune Addison's disease and Graves' disease susceptibility. J Clin Endocrinol Metab. 2009;94:5139-45.
11. Lee SH, Lee YA, Woo DH, et al. Association of the programmed cell death 1 (PDCD1) gene polymorphism with ankylosing spondylitis in the Korean population. Arthritis Res Ther. 2006;8:R163.
12. Prokunina L, Castillejo-Lopez C, Oberg F, et al. A regulatory polymorphism in PDCD1 is associated with susceptibility to systemic lupus erythematosus in humans. Nat Genet. 2002;32:666-9.
13. Ferreiros-Vidal I, Gomez-Reino JJ, Barros F, et al. Association of PDCD1 with susceptibility to systemic lupus erythematosus: evidence of population-specific effects. Arthritis Rheum. 2004;50:2590-7.
14. Ni R, Ihara K, Miyako K, et al. PD-1 gene haplotype is associated with the development of type 1 diabetes mellitus in Japanese children. Hum Genet. 2007;121:223-32.
15. Nielsen C, Hansen D, Husby S, Jacobsen BB, Lillevang ST. Association of a putative regulatory polymorphism in the PD-1 gene with susceptibility to type 1 diabetes. Tissue Antigens. 2003;62:492-7.
16. Soleimanifar N, Amirzargar AA, Mahmoudi M, et al. Study of programmed cell death 1 (PDCD1) gene polymorphims in Iranian patients with ankylosing spondylitis. Inflammation. 2011;34:707-12.
17. Yang J, Popoola J, Khandwala S, et al. Critical role of donor tissue expression of programmed death ligand-1 in regulating cardiac allograft rejection and vasculopathy. Circulation. 2008;117:660-9.
18. Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. Annu Rev Immunol. 2005;23:515-48.
19. del Rio ML, Buhler L, Gibbons C, Tian J, Rodriguez-Barbosa JI. PD-1/PD-L1, PD-1/PD-L2, and other co-inhibitory signaling pathways in transplantation. Transpl Int. 2008;21:1015-28.
20. Cornell LD, Smith RN, Colvin RB. Kidney transplantation: mechanisms of rejection and acceptance. Annu Rev Pathol. 2008;3:189-220.
21. Karimi MH, Motazedian M, Abedi F, Yaghobi R, Geramizadeh B, Nikeghbalian S. Association of genetic variation in co-stimulatory molecule genes with outcome of liver transplant in Iranian patients. Gene. 2012;504:127-32.
22. Solez K, Colvin RB, Racusen LC, et al. Banff 07 classification of renal allograft pathology: updates and future directions. Am J Transplant. 2008;8:753-60.
23. Haimila K, Turpeinen H, Alakulppi NS, Kyllonen LE, Salmela KT, Partanen J. Association of genetic variation in inducible costimulator gene with outcome of kidney transplantation. Transplantation. 2009;87:393-6.
24. Kusztal M, Koscielska-Kasprzak K, Drulis-Fajdasz D, et al. The influence of CTLA-4 gene polymorphism on long-term kidney allograft function in Caucasian recipients. Transpl Immunol. 2010;23:121-4.
25. Team RC. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2013.
26. Pascual J, Perez-Saez MJ, Mir M, Crespo M. Chronic renal allograft injury: early detection, accurate diagnosis and management. Transplant Rev (Orlando). 2012;26:280-90.
27. Hassan R, Gheith O. Chronic antibody-mediated rejection: review of literature. Iran J Kidney Dis. 2014;8:93-103.
28. Ganji MR, Broumand B. Acute cellular rejection. Iran J Kidney Dis. 2007;1:54-6.
29. Krichen H, Sfar I, Bardi R, et al. CD86 +1057G > A polymorphism and susceptibility to acute kidney allograft rejection. Iran J Kidney Dis. 2011;5:187-93.
30. Rothstein DM, Sayegh MH. T-cell costimulatory pathways in allograft rejection and tolerance. Immunol Rev. 2003;196:85-108.
31. Kitazawa Y, Fujino M, Wang Q, et al. Involvement of the programmed death-1/programmed death-1 ligand pathway in CD4+CD25+ regulatory T-cell activity to suppress alloimmune responses. Transplantation. 2007;83:774-82.
32. Gao W, Demirci G, Strom TB, Li XC. Stimulating PD-1-negative signals concurrent with blocking CD154 co-stimulation induces long-term islet allograft survival. Transplantation. 2003;76:994-9.
33. Wang YW, Wang Z, Shi BY. Programmed death 1 mRNA in peripheral blood as biomarker of acute renal allograft rejection. Chin Med J (Engl). 2011;124:674-8.
34. Amirzargar MA, Amirzargar A, Basiri A, et al. Early post-transplant immune monitoring can predict long-term kidney graft survival: soluble CD30 levels, anti-HLA antibodies and IgA-anti-Fab autoantibodies. Hum Immunol. 2014;75:47-58.
35. Solgi G, Furst D, Mytilineos J, Pourmand G, Amirzargar AA. Clinical relevance of pre and post-transplant immune markers in kidney allograft recipients: anti-HLA and MICA antibodies and serum levels of sCD30 and sMICA. Transpl Immunol. 2012;26:81-7.
36. Nikoueinejad H, Amirzargar A, Sarrafnejad A, et al. Dynamic changes of regulatory T cell and dendritic cell subsets in stable kidney transplant patients: a prospective analysis. Iran J Kidney Dis. 2014;8:130-8.

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