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Original Article

Single nucleotide polymorphisms of innate immune receptors in patients with renal rejection

Razieh Abdolvahabi[1*], Abdolfatah Sarrafnejad[1], Mohsen Nafar[2], Aliakbar Amirzargar[3,4]

1. Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
2. Chronic Kidney Disease Research Center, Department of Nephrology, Shahid Labbafinejad Medical Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Department of immunology, School of medicine, Tehran University of Medical Sciences, Tehran, Iran
4. Molecular Immunology Research Center, Tehran University of Medical Sciences, Tehran, Iran

Abstract

Background: The innate immunity plays an important role in the host response to transplantation by Toll-like receptors and results in development of acute allograft rejection. The aim of this study was to evaluate the association of TLR2 and CD14 (co-receptor) gene polymorphisms with acute rejection in kidney transplant recipients.

Methods: The study was conducted in a population of 239 subjects consisting of 71 patients with acute rejection, 71 patients without acute rejection (SGF) and 97 Healthy Control (HC). The allele and genotype frequencies of TLR2 (R753Q, rs5743708) and CD14 (-159 C>T, rs2569190) polymorphisms were genotyped by Real-time PCR in the study groups.

Results: Genotype distribution of CD14 -159 polymorphism was significantly different in AR vs. SGF and HC. CD14 -159 TT genotype was more prevalent in rejection than SGF and HC (P<0.0001, P<0.007, respectively). Also Graft loss, defiened as need of dialysis after acute rejection, was occurred in 24 patients (33.8%) from AR group. The frequencies of three genotype in CD14 (TT, CT, CC) in rejection With Graft loss were 75.0%, 20.8% and 4.1% respectively, While 25.5%, 31.9% and 42.5% in rejection without Graft loss (P<0.0001 for TT vs. CT, CC). Many recipients with AR were involved with graft loss had CD14 -159 TT genotype, whereas only a few recipients without graft loss had TT genotype (p<0.0001).

Conclusion: Therefore, due to the importance of CD14 polymorphism (-159 C/T, rs2569190) in disease progression and also as a biomarker, could be considered as a crucial therapeutic target in early prognosis of acute rejection.

Keywords: Kidney transplantation, Acute rejection, SNP, TLR2, CD14

Introduction

Kidney transplantation is the best treatment for patients with end stage of renal disease (ESRD) which could affects innate immune system to promote acute rejection (Banas et al., 2010). Acute rejection with the occurrence rate of 15-25% is one of the major risk factors for development of chronic allograft nephropathy and reduces Graft survival (Goldfarb, Naiman., 2010). Our Previous studies on molecular immune mediated tissue destruction have revealed the profound effect of various genes and proteins on kidney acute rejection

*Corresponding author. Razieh Abdolvahabi, MSc
Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
Tel. 09191844492
Email: vahabi_razieh@yahoo.com

(Amirzargar et al., 2015, Nafar et al., 2015, Ahmadpoor et al., 2015, Ahmadpoor et al., 2014, Amirzargar et al., 2014).TLRs are a family of transmembrane receptors in innate immunity which recognize special ligands (Leventhal, Schroppel., 2012). It is established that TLR2 have an important role in kidney transplantation and renal diseases. TLR2 is cell surface receptor, expressed on tubular and epithelial renal cells (Jeong et al., 2008). TLR2 along with TLR1 or TLR6 detects gram positive bacteria (Snyder et al., 2012). CD14, a crucial coreceptor for signalling of TLR4 and TLR2, binds to LPS and interacts with TLR4-MD2 complex to initiate signalling cascade and lead to regulating expression of inflammatory cytockines (i.e. IL1, TNFα) (Bell et al., 2006). Many human and animal studies reported that ischemia-reperfusion injury after kidney or lung transplantation have significant role in development of allograft rejection by increasing expression of TLR2 mRNA from tubular cells (Jang, Rabb., 2009, Arslan et al., 2010). Previous Studies revealed that single nucleotide polymorphism in TLRs gene is associated with acute allograft rejection (Ferwerdaet al., 2008) and infectious disease (Nicolas, Schröder., 2005). Polymorphism in promotor region of CD14 gene (rs2569190) and next to the binding site for transcription factor SP1 (specificity protein1) affects TLRs signalling (Chatterjee et al., 2012). However, no mutant homozygous or heterozygous polymorphism of TLR2 were not observed in Asian population (Chinese or Korean) (Jang, 2009, Choi et al., 2006, Hang et al., 2004). Moreover, Eliuna et al (2010), found no significant association between recipients with TLR2 polymorphism and reduction of kidney rejection numbers (Brunialti et al., 2010). Palmer et al (2005), suggested that presence of SNP in TLR2 in donor and not in recipient is associated with decreased rate of acute rejection (Burch et al., 2005).

To date, the impact of TLRs and CD14 polymorphism on kidney transplantation outcome, such as acute rejection episodes has not been conclusively elucidated. In the present study, we evaluated possible association between SNPs in TLR2 and CD14 genes with AR in comparison with SGF group and HC.

Matherials and Methods

Clinical data collection

In the present Study a total of 142 kidney allograft recipients (2008 - 2013) 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 AR (AR group, n=71) and having clinically stable graft

function (SGF) without any previous episode during the 5 years of follow-up (SGF group, n=71). In this study 97 age and sex matched subjects as healthy control were also enrolled. 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.

Extraction of genomic DNA

For each subject, 5ml of peripheral blood was collected in tube containing EDTA (ethylene diamide-tetraacetic acid) and then Genomic DNA was extracted using Qiagen DNA extraction kits. After solving DNA in TE buffer, concentration of each sample (ng/ul) was calculated by UV spectrophotometer in which to evaluate purity of extracted DNA.

Determination of TLR2/CD14 polymorphisms

Genetic polymorphism in TLR2 (R753Q, rs5743708), and CD14 (-159 C/T, rs2569190) genes were analysed by the ABI7300 sequence Detection system using Taq-man genotyping PCR. We evaluated the frequency of genotypes and haplotypes of these polymorphisms in AR, SGF and HC. SNP Taq-man genotyping assay were designed by primer and probe Express software (Applied Biosystems, USA). The minor groove-binding (MGB) protein was located in the structure of Taq-man probe that increases thermo stability of interaction target DNA-probe and decreases fluorescence background. Fluorescein detector (VIC/FAM) was labeled at 5´end of probe for wild-type or mutant alleles and 3´end probe was labeled with non-fluoresent quencher (NFQ) signal. Cycles of amplification in Real time PCR was performed in 3 stage including: 95°C for 10 minutes followed by 45 cycles at 5°C for 15 sec and 60°C for 1 min. To ensure quality of the test, 10% of the samples were randomly replicated for SNPs in TLR2, TLR4 and CD14 genes.

Statistical analysis

The continuous variables were reported as meanand 95% confidence interval and categorical variables were reported as counts and percentage. Frequencies of genotypes and alleles in AR, SGF and HC were analyzed by Chi-square or Fisher's exact test, where appropriate. Multiple Logistic regression analysis was performed to compare the effect of risk factors on allograft rejection. The odds ratio (OR) and adjusted OR were calculated with 95% confidence intervals (CI) and a Pvalue of less than 0.05 was considered as significant.

Results

Demographic characteristics of studied subjects

According to demographic characteristics analaysis, age and gender in three studied

groups were not revealed to be significantly different. Also there were no significant differences for the risk factors in AR and SGF groups completely shown in Table 1. However, ratio of DGF (14.0% vs. 2.8%) and CMV (22.5% vs. 7.0%) infectionas shown in Table 1. Also, Graft loss occurred in 24 (33.8%) out of 71 patients with acute rejection.

Table 1 Baseline clinical characteristics of study subjects

Study group	AR	SGF	P
	n. (%)	n. (%)	
N	71	71	
Male:Female (% male)	46:25 (64.7%)	41:30 (57.7%)	NS[a]
Donor type			
Cadavor	20 (28.1)	17 (23.9)	NS
Living related	3 (4.2)	2 (2.8)	
Living unrelated	48 (67.6)	52 (73.2)	
Graft loss or Dialysis	24 (33.8)		
DGF	10 (14.0)	2 (2.8)	0.000[b]
Viral Infection			
CMV	16 (22.5)	5 (7.0)	0.053[b]
BK	0 (0)	3 (4.2)	NS
EBV	0 (0)	2 (2.8)	NS
HbsAg	0 (0)	2 (2.8)	NS

NS; not significant, DGF; Delayed Graft Function.
[a] T-test, [b] Pearson's X2 test

Table 2 Distribution of genotype and allele frequencies for TLR2 and CD14 SNP in AR, SGF and HC group

SNP ID Genotype		AR vs. SGF n (%) OR (CI 95%) P	AR vs. HC n (%) OR (CI 95%) P	SGF vs. HC n (%) OR (CI 95%) P
Rs5743708 (TLR2)	GG	68 (95.7) vs. 71 (100) 0.32 (0.006 - 4.17) 0.619	68 (95.7) vs. 95 (97.9) 0.47 (0.03 - 4.30) 0.711	71 (100) vs. 95 (97.9) Undefined 0.378
	AG	3 (4.2) vs. 0 (0) undefined 0.119	3 (4.2) vs. 2 (2.0) 2.08 (0.23 - 25.61) 0.711	0 (0) vs. 2 (2.0) 0.67 (0.01 - 13.2) >0.999
	G	139 vs. 142 0.33 (0.006 - 4.16) 0.622	139 vs. 192 0.48 (0.04 - 4.28) 0.713	142 vs. 192 undefined 0.381
	A	3 vs. 0	3 vs. 2	0 (0) vs. 2
Rs2569190 (CD14)	CC	21 (29.5) vs. 36 (50.7) 0.40 (0.20 - 0.81) 0.010	21 (29.5) vs. 33 (34.0) 0.81 (0.42 - 1.57) 0.542	36 (50.7) vs. 33 (34.0) 1.99 (1.06 - 3.73) 0.030
	CT	20 (25.3) vs. 31 (43.6) 0.50 (0.25 - 1.01) 0.054	20 (25.3) vs. 42 (43.2) 0.51 (0.26 - 0.98) 0.045	31 (43.6) vs. 42 (43.2) 1.01 (0.54 - 1.88) 0.963
	TT	30 (37.9) vs. 4 (5.6) 12.26 (4.02 - 37.31) < 0.0001	30 (37.9) vs. 22 (22.6) 2.49 (1.27 - 4.87) 0.007	4 (5.6) vs. 22 (22.6) 0.20 (0.06 - 0.62) 0.003
	C	62 vs. 103 0.29 (0.17 - 0.48) < 0.0001	62 vs. 108 0.61 (0.39 - 0.95) 0.030	103 vs. 108 2.10 (1.32 - 3.34) 0.002
	T	80 vs. 39	80 vs. 86	39 vs. 86

SGF, Stable Graft Function; HC, Healthy Control

Association of TLR2 and CD14 SNPs with Acute rejection

The genotype frequency ofrs2569190 C/T differed significantly between AR, SGF and HC. The odds ratio for TT over CC genotype in AR compared with SGF group was 12.26 (95% CI, 4.02-37.31; P<0.0001) (Table 2). Therefore, recipients carrying CD14 TT genotype had a higher risk of acute rejection than those with heterozygous or Wild-Type genotype (Fisher's exact test, P<0.05). In contrast to CD14, no significant differences were observed in TLR2 SNPs between AR, SGF and HC (Table 2).

Impact of CD14 SNP on renal function

Graft loss, defined as need of dialysis after acute rejection, was occurred in 24patients (33.8%) from AR group in which 18 (75.0%), 5 (20.8%), 1 (4.1%) patients had respectively TT, CT, CC genotype (Table 3). However, none of the studied polymorphisms, had no association with DGF or rate of viral infection (data not shown).

Table 3 Association of CD14 genotypes (rs2569190) with Graft loss

Rs2569190 (CD14)	AR		OR (CI 95 %)	P
	With Graft loss n (%)	WithoutGraft loss n (%)		
CC	1 (4.1)	20 (42.5)	0.05 (0.007-0.47)	0.001
CT	5 (20.8)	15 (31.9)	0.56 (0.17-1.79)	0.326
TT	18 (75.0)	12 (25.5)	8.75 (2.81-27.16)	< 0.0001
C	7	55	0.12 (0.04-0.29)	< 0.0001
T	41	39		

Discussion

Acute rejection is one of the major risk factors to develop chronic allograft nephropathy. Kidney transplantation could activate innate immune system by TLRs and result in promoting acute allograft rejection (Banas et al., 2010).

CD14 is expressed on the surface of monocytes as a crucial co-receptor in TLR2 signalling pathway and binds to LPS from gram negative bacteria and initiates immunity against pathogens (Chatterjee et al., 2012). Numerous studies identified mechanisms on how CD14 (SNP) in promoter region could have an influence on allograft rejection (Chatterjee et al., 2012, Hu et al., 2012). Expression of sCD14 and production of inflammatory cytokines from DCs were increased in recipients carrying T allele. Activated DCs stimulated CCL2/CCL5 chemokines; finally recruited $CD4^+$/$CD8^+$ Tcells by suppressing of Treg cells responses (Hu et al., 2012). In this study, it was shown that CD14 polymorphism (rs2569190) was associated with acute rejection and graft loss after kidney

transplantation. Many recipients with AR were involved with graft loss had CD14 -159 TT genotype, Whereas only a few recipients without graft loss had TT genotype (p=0.0001) and also the frequency of T vs. C allele was shown a significant difference. Therefore, this is reason that why renal transplant recipients carrying CD14 -159 TT polymorphism are at higher risk of acute graft rejection.Also Our studies confirmed worked carried out by Palmer et al (2007) where it was found An earlier onset of acute rejection, worse post transplant graft survival in patients with the CD14 -159 TT genotype (Klimecki et al., 2007).

Also, low prevalence of TLR2 genotypes in Asian population is major cause to why no significant data was found (Hang et al., 2004, Brunialti et al., 2010). This suggests that geographic and racial differences could influence on the rate of acute rejection that shown any genotypes of TLR2 alleles in Chinese and Korean races. Also, Krichen H (2013) found, there was no significant effect of TLR2 polymorphism on graft survival during 6-years; However, a significant association of a polymorphism in the TLR3gene (F412L), TLR9 C/T with the increase of acute rejection and DGF. Therefore difference polymorphisms of TLRs have difference effects on expression of inflammatory cytokines. So that TLR3, TLR9 along with TLR2 increased from renal cells after kidney transplant and maybe change graft outcome post-transplant (Dhaouadi et al., 2013). Palmer etal (2005) revealed that TLR2 polymorphisms in the donors' kidney and not the recipients, contribute to improvement of renal transplantation outcomes (Burch et al., 2005).

Conclusion

Conflicting data about polymorphism in TLR genes and their association with renal rejection indicate the need for the more rigorous studies to be carried out in order to recognize the exact function of TLRs on alloreactive responses in kidney transplantation. Renal transplant recipients carrying CD14 -159 TT polymorphism have significantly higher risk of acute rejection than patients with heterozygous or wild-type genotypes. This finding suggests the probable role of CD14 in the pathogenesis of acute rejection.

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