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TGF-β Codon 25 Polymorphism and the Risk of Graft-Versus-Host Disease after Allogenic Hematopoietic Stem Cell Transplantation

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ABSTRACT

Some of the genotypes of cytokines are associated with acute graft versus host disease after bone marrow transplantation. The purpose of the present investigation was to find out the possible association between transforming growth factor beta-1 (TGF-β1) codon 25 polymorphism (rs:1800471) and acute graft versus host disease (aGVHD) after bone marrow transplantation from the sibling with the similar HLA among the Iranian population.

In this retrospective case-control investigation, 172 subjects including 86 Iranian patients and their siblings with the similar HLA as donor/recipient pairs were recruited. All of the patients were diagnosed with one group of blood disorder consisting of Acute Myeloid Leukemia (AML)=40, Acute Lymphoblastic Leukemia (ALL)=25 and Chronic Myeloid Leukemia (CML)=21. PCR-SSP method was carried out to ascertain TGF- β1 codon 25 G/C polymorphism genotypes.

The frequency of TGF- β1 codon 25 GG, GC and CC genotypes among all cases were 77.3%, 21.5% and 1.2%, respectively. Recipients with the GG genotype developed severe aGVHD significantly more than those with CC or GC genotypes (Odds Ratio =12.133, *P*=0.015).

Genetic background of TGF-β1 may be involved in aGVHD development and/or severity in the patients who received Bone Marrow Transplantation (BMT) from their siblings with the similar HLA among the Iranian population.

Key words: Acute GVHD; BMT; Cytokine gene polymorphism; SNP; TGF-β1

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INTRODUCTION

Bone Marrow Transplantation (BMT) is one of the most important approaches for treatment of some

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malignant and non malignant disorders.[1] Unfortunately its application is restricted by acute graft-versus-host-disease (aGVHD), the most major adverse effect of allogenic BMT which it is observed among 30–80% of allogenic BMT from the sibling with the similar HLA and can be lethal in up to 50% of cases.[2,3]

Cytokines are important GVHD modulators and it has been shown that proinflamatory and immunoregulatory cytokines have inducing and suppressive effects on aGVHD development, respectively.[4,5] Previous studies have indicated a potential role for some cytokines polymorphisms in outcome of BMT from the sibling donor.[6-8] TGF-β1 is a pleiotropic cytokine with immunoregulatory characteristics that has been involved in the initiation of alloantigen specific tolerance.[9] The location of TGF- β1 is on chromosome 19q13.1–13.3 and eight polymorphic areas have been recognized in this gene. G915C (Arg 25 Pro) is one of the main TGF- β1 signal (leader) sequence polymorphism which results in an amino acid substitution at codon 25 (Arg→Pro) that changes protein structure and influences on TGF- β1 export.[10,11]

It has been suggested that the genetic structure of donors and recipients has a great influence on the success or failure of BMT,[5] and also, the results of our previous study showed the association of recipient TGF- β1 codon 10 genotype and aGVHD development,[12] therefore, the aim of this study was to examine the association of the TGF- β1 codon 25 polymorphism in both BMT donors and recipients with regard to the development and/or severity of aGVHD.

MATERIALS AND METHODS

Patients and Donors

Participants were patients suffered from hematological malignancies including AML, ALL, and CML who underwent allogenic BMT from their siblings with the similar HLA in the Hematology-Oncology and BMT Research Center, Shariati university teaching Hospital, Tehran, Iran, during 2002 and 2005. Before transplantation, informed consent forms were taken from each subject or their guardians and then venus blood were collected and stored at –20°C. All patients were monitored for six months in terms of BMT adverse effects particularly GVHD and their data were stored in Shariati Hospital computers and was published in another journal.[13] In 2008 we designed a retrospective case-control study to investigate the influences of TGF-β1 gene codon 25 polymorphism (rs: 1800471) on the incidence of aGVHD following allogenic BMT. Those recipients (and their respective donors) who developed aGVHD up to 100 days after transplantation were regarded as cases and those who did not were regarded as controls. Glucksberg criteria were used for GVHD grading as previously described.[14] According to the severity of aGVHD clinical manifestations, those patients who developed either aGVHD grade III or grade IV were categorized as severe aGVHD group.[15-16] Case and control matching were performed for all samples according to some of the previously established GVHD risk factors including: diagnosis, age, recipient-donor sex mismatch, type of transplantation (PBSC or BM) and serum Cytomegalovirus (CMV) status.[17-19] Busulphan (Bu) plus cyclophosphamide (CY) were used for the conditioning regimen and cyclosporine plus methotrexate (MTX) for GVHD prophylaxis as previously described.[20-22] Those recipients that did not match were excluded from this study and finally 172 samples consisting of 86 Iranian patients and their siblings with the similar HLA as donor/recipient pairs were collected for this study. This investigation was carried out in accordance with the Declaration of Helsinki and subsequent revisions and approved by the Ethics Committee at Tehran University of Medical Sciences.

Ascertaining the Genotypes of TGF-β1 Codon 25

Genomic DNA extraction was performed using standard salting out method.[23] Cytokine genotyping was achieved by polymerase chain reaction with sequence specific primers (PCR-SSP) assay. Allele specific, internal control primers and PCR conditions were as earlier publications.[24,25] Following genotyping process, 10 samples were selected randomly and sequenced for verification.

Statistical Methods

Statistical analysis was carried out by SPSS statistical package, version 13.5 for windows. Genotype and allele frequencies of TGF-β1 (codon 25) were compared using Chi-square and Fisher's exact test. The Odds Ratio was calculated for each genotype and allele. Chi-square was used to evaluate the effect of Sex mismatch, type of transplantation, underlying disease and serum CMV status on aGVHD severity. Also independent sample T- test was used to compare

age in two groups in terms of aGVHD severity. *P*-value less than 0.05 was regarded as significant.

RESULTS

Population Study and GVHD Risk Factors

The median age (Interquartile range) of donors was 22.5 (14) years and of recipients was 23 (15) years. The male/female ratio of donors was 53/33, and of recipients was 49/37. Out of 86 recipients, 43 patients (50%) showed some degrees of aGVHD: 11 patients (25.6%) developed aGVHD grade I, 17 patients (39.4%) developed aGVHD grade II, 12 patients (27.8%) developed aGVHD grade III and 3 patients (6.8%) developed aGVHD grade IV. 8 patients (9.3%) died before 100 days as a result of aGVHD complications. Table 1 shows diagnosis, age, recipient-donor sex mismatch, transplantation type and serum CMV status in aGVHD positive group and control group. No statistically significant differences were found between these two groups with respect to the mentioned criteria. Statistical analysis of TGF-β1 codon 25 genotypes also showed no significant deviations from the Hardy-Weinberg equilibrium (data not shown).

Genotype Distribution and Allele Frequencies of the TGF-β1 Codon 25 Polymorphism in the aGVHD Positive Group and Control Group

The frequency of TGF-β1 codon 25 GG, GC and CC genotypes among all subjects were 77.3%, 21.5% and 1.2% respectively. As shown in Table 2, the distribution of TGF-β1 codon 25 G/C Polymorphism genotype was not differed significantly among the cases and controls in either recipients or donors.

Our findings also showed that there was no significant difference among cases and controls in terms of distribution of TGF-β1 codon 25 alleles either in recipients or donors (Table 3).

Table 1. Case and control matching criteria

aGVHD risk factors	Case n(%)	Control n(%)	P-value
Diagnosis			
AML*	20 (46.5)	20 (46.5)	
ALL**	12 (27.9)	13 (30.2)	0.957
CML**	11 (25.6)	10 (23.3)	
Age (year)#			
Recipient	23 (15) #	21 (14) #	0.837
Donor	22.5 (14) #	23 (14) #	0.683
Transplantation type			
PBSC##	41 (95.3)	40 (93)	1.000
BM###	2 (4.7)	3 (7)	
Sex Mismatch	22 (51.2)	21 (48.8)	1.000
Serum CMV status	7 (16.3)	7 (16.3)	1.000

* AML- Acute Myeloid Leukemia; ** ALL – Acute Lymphoblastic Leukemia; *** CML – Chronic Myeloid Leukemia; # Median (Interquartile Range); ## PBSC – Peripheral Blood Stem Cells; ### BM – Bone Marrow.

Table 2. Association between TGF-β1 codon 25 (Arg/Pro) polymorphism and aGVHD development

Genotype	Recipient			Donor		
	Case n (%)	Control n (%)	P-value	Case n (%)	Control n (%)	P-value
GG	29 (45.3)	35 (54.7)		35 (50.7)	34 (49.3)	
GC	13 (61.9)	8 (38.1)	0.252	7 (43.8)	9 (56.2)	0.531
CC	1 (100)	0 (0)		1 (100)	0 (0)	

Table 3. Association between recipient and donor allele distribution and aGVHD development

Genotype	Recipient			P	Donor			P
	Case n(%)	Control n(%)	Odds Ratio (CI 95%)		Case n(%)	Control n(%)	Odds Ratio (CI 95%)	
			0.494				0.494	
GG or GC	42 (49.4)	43 (50.6)	(0.398-0.613)	1.000	42 (49.4)	43 (50.6)	(0.398-0.613)	1.000
CC	1 (100)	0 (0)			1 (100)	0 (0)		
			0.473				1.158	
CC or GC	14 (63.6)	8 (36.4)	(0.174-1.285)	0.216	8 (47.1)	9 (52.9)	(0.400-3.352)	1.000
GG	29 (45.3)	35 (54.7)			35 (50.7)	34 (49.3)		

We also checked if there was any relationship between severity of aGVHD with allelic or genotypic distributions of TGF- β1 codon 25 and found no significant associations among donor TGF- β1 codon 25 genotype and allele distribution with severity of aGVHD in case and control groups. In contrast, we found a significant association between TGF-β1 codon 25 GG genotype and severe aGVHD in recipients (Odds Ratio: 12.133, CI: 1.399 – 105.255, P= 0.015) (Table 4).

There were no significant differences among age, Sex mismatch, type of transplantation, underlying disease and serum CMV status with severity of aGVHD (Table 4).

DISCUSSION

Recent investigations have shown that the genetic structure of recipients and donors can greatly influence on the success or failure of HSCT.[7,26,27] In our previous study, we showed that the recipient genotype of TGF-β1 codon10 polymorphism may influence on aGVHD development after allogenic BMT in Iranian population.[12] A relatively recent data revealed that the recipient TGF-β1 codon 25 genotype associated with severe aGVHD in American population.[28] As the genotype frequency of TGF-β1 codon 25 polymorphism differs between Iranian and American normal populations [28,29], it seems logical to consider whether there is any relationship between this polymorphism and aGVHD development or severity in Iranian allogenic BMT population or not?

Case and control matching were performed for all samples according to some of the previously established GVHD risk factors including: diagnosis, age, recipient-donor sex mismatch, type of transplantation (PBSC or BM) and serum CMV status.

The genotype frequencies observed in this study were different from that of Americans allogenic BMT [28] and Iranian normal population as well.[29]

In this study, the Argenin (Arg)/Prolin (Pro) polymorphism at codon 25 of TGF-β1 in recipients was associated with the development of severe aGVHD. We found that recipients with the GG genotype developed severe aGVHD significantly more than those with CC or GC genotype. In other words, it seems that Arg allele (CC or GC genotype) at codon 25 may have some effects on attenuating the aGVHD complications in Iranian BMT recipients. This result is to some extent in consistent with the report of Leffell et al.[28] To assess the effect of other factors on severity of a GVHD, we tested the association of aGVHD severity with diagnosis, age, recipient-donor sex mismatch, type of transplantation (PBSC or BM) and serum CMV status.

Table 4. Association between recipient and donor allele distribution and aGVHD severity

Genotype	Recipient			P	Donor			P
	Mild n(%)	Severe n(%)	Odds Ratio (CI 95%)		Mild n(%)	Severe n(%)	Odds Ratio (CI 95%)	
			0.643				0.643	
GG or GC	27 (64.3)	15 (35.7)	(0.513-0.805)	1.000	27 (64.3)	15 (35.7)	(0.513-0.805)	1.000
CC	1 (100)	0 (0)			1 (100)	0 (0)		
			12.133				4.667	
CC or GC	13 (92.9)	1 (7.1)	**(1.339-105.255)**	**0.015**	7 (87.5)	1 (12.5)	(0.516-42.189)	0.226
GG	15 (51.7)	14 (48.3)			21 (58.3)	15 (41.7)		

We did not find any significant difference among distribution of mentioned factors with aGVHD severity in cases with GG genotype as compared to cases with the CC or GC genotypes.

We did not find any association among this polymorphism genotypes or allele frequencies with aGVHD development. Although this is consistent with Leffell *et al*. data, but it can be due, at least in part, to the quite small sample size or heterogeneity of our patients. Further studies with larger sample size and lower heterogeneity, preferably in each disease group separately, are required to verify this point.

The mechanism by which this polymorphism may have protective effect on aGVHD development or severity is not entirely known. However, recent studies demonstrated that TGF- β1 in the presence of IL-10 provoked alloantigen-specific tolerance ex vivo, resulting in protection from GVHD [5]. Animal studies also confirmed that TGF- β1 and IL-10 act synergistically in avoiding aGVHD subsequent to allogenic SCT [9]. TGF-β1, therefore, is considered to be a negative regulator of aGVHD due to its anti-inflammatory and immunosuppressive effects [5].

The major drawback of this study was the small sample size and its heterogeneity.

As determination of individual risk of aGVHD may offer the chance of personalized immunosuppressive therapy, it is proposed that the association between this gene SNPs combined by other gene SNPs to be considered in larger cohorts with little heterogeneity in different populations and the results confirmed by Transmission Disequilibrium Test (TDT) and functional studies.

In conclusion, the current study suggests that Iranian allogenic BMT recipients having GG genotype at codon25 of TGF-β1 may be more prone to develop severe aGVHD than those having CC or GC genotype. Therefore, genetic background of TGF-β1 may influence the severity of aGVHD development. Further investigations are needed to clarify the effect of the polymorphisms of TGF-β1 on aGVHD development and/or severity following allogenic BMT.

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