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Minor Histocompatibility Antigen HA-1 Frequency and Acute GVHD in Some Hematopoietic Stem Sell Transplanted Iranian Patients

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Abstract

Background: It is suggested that HA-1 mismatching among hematopoietic stem cell transplanted recipients-donors is associated with acute graft-versus-host disease (aGVHD). So the aim of this study was to evaluate HA-1 frequency and examine the correlation between HA-1 disparity and GVHD patients who received transplantation from a HLA-identical sibling.

Samples and Methods: the DNA was extracted from 30 pairs of HLA-A2-positive Iranian recipients-donors with GVHD I-IV and 25 pairs without GVHD. All the patients received HSCT from HLA-identical siblings. HA-1 was detected by SSP-PCR method. The HA-1 typing was performed using SSP method (SSP Minor Histocompatibility Antigen primer sets).

Results: The frequency of HA-1R and HA-1H alleles in patients were 0.55 and 0.45 respectively, and showed no significant difference with these alleles frequency in donors 0.53 and 0.47 respectively ($p>0.05$). The HA-1 disparity was detected in 8 out of 55 donor/recipient pairs (14.5%). The aGVHD (grades I-IV) occurred in 6 (75%) out of 8 patients but in spite of higher incidence of it in the group of patients with HA-1 incompatibility, it was not statistically significant.

Conclusion: In spite of higher frequency of HA-1 disparity in GVHD+ group our data did not reflect a significant association between the HA-1 disparity and risk of acute GVHD.

Keywords: HA-1 disparity, GVHD, Hematopoietic Stem cell transplantation (HSCT), Iranian

Introduction

The minor histocompatibility antigens (mHags), as HA-1 are immunogenic alloantigen responsible for the graft-versus-host disease (GVHD) in HLA-identical Hematopoietic Stem Cell Transplanted (HSCT) siblings. The antigen has two known alleles, resulting in a single amino acid polymorphism. The HA-1H allele encodes Histidine, whereas the HA-1R allele encodes Arginine(1). The Graft-versus-host disease (GvHD) is main complication of allogeneic bone marrow transplantation. In HLA-identical bone marrow transplantation, the GVHD may be induced by disparities in minor histocompatibility antigens (mHags) between donor and recipient, mean while antigen will be present in the recipient and not in the donor (2). These complications may arise from disparities in minor histocompatibility antigens between donor and recipient, mean while antigen is

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present in recipient and not in donor. In such cases, T cells in transplanted donor marrow responds to minor histocompatibility antigens in recipient. For immune recognition, the HA-1, -2, -4 and -5 antigens must be presented to cytotoxic T cells by the major histocompatibility antigen HLA-A2. In this way they behave like to antigens recognized in an HLA-restricted fashion. The HA-1 antigen is present in 69 percent of normal people who express HLA-A2 (3). The Acute graft-versus-host-disease (aGVHD) is one of main complications after BMT, the incidence of severe grades, even in HLA-identical transplants, ranges from 20 to 50%, that depends on the disease and type of immunosuppressive prophylaxis. The mismatch of mHag-HA1 has been associated with an increased risk of aGVHD after the HLA-identical marrow transplantation and the emergence of donor-derived HA1 specific cytotoxic T lymphocytes (CTLs) in different cohorts of transplanted patients with the GVHD, suggests an immunodominant behavior of HA-1. This minor histocompatibility antigen is a nonapeptide encoded by the KIAA0223 gene, located on chromosome 19, which has two known alleles, named HA-1H and HA-1R, differing by a single amino acid polymorphism. The HA-1H peptide is recognized by HLA-A*0201-restricted cytotoxic T cells (CTLs), while the HA-1R peptide is not presented or is presented in very low amounts by HLA-A*0201 on cell surface, thus can't elicit a detectable immune response. Goulmy reported a mismatch of minor histocompatibility antigen HA-1 that could cause GVHD in adult recipients of allogeneic bone marrow from HLA-identical donors. However, the real influence of this mHag is still controversial and a paper reporting data on 613 HLA-A2 donor–recipient pairs showed no statistically significant association between HA-1 disparity and a higher incidence of aGVHD (4). So the aim of this study was to evaluate HA-1 gene frequency and it's relationship with the aGVHD in some Iranian patients who suffered different hematological disorders under going Heamatopoietic stem cell transplantation (HSCT).

Patients and Methods

Samples

A total of 55 HLA-A*0201 positive patients who underwent the HSCT from an HLA-identical sibling between 2003 and 2006 at Dr Shariati Hospital Hematology/Oncology/BMT Research Center (Tehran), with a minimal follow-up of 3 months, were studied. The DNA samples of 30 patients with the acute GVHD I-IV and 25 patients without it and their related siblings were analyzed for HA-1 allele frequency. HLA-A-B and -DR typing were performed in the center already. All patients received methotrexate and cyclosporine for GVHD prophylaxis.

HA-1 genotyping

HA-1 genotyping was performed by using SSP Minor Histocompatibility Antigen Primer Sets (One Lambda, Inc). the DNA was prepared from 5 ml of whole blood via using DNA extraction kit (Roche). In this assay, 1µl samples containing 100 ng DNA were used in conjunction with 7µl primer set D-mix (containeing given amount of dNTPs and Reaction Buffer) which was added to 2µl of primer set PCR reaction and 0.5 µl at 5 units/ µl of Taq polymerase (Roche). The mixture was subjected to : 1 Cycle : 96 ^{0c} 130s and 63 ^{0c} 60s, 9 Cycle : 96 ^{0c} 10s and 63 ^{0c} 60s, 20 Cycle : 96 ^{0c} 10s and 59 ^{0c} 50s and 72 ^{0c} 30s and ended in 4 ^{0c} in a Roche Thermaocycler using allele-specific 5' primers for the HA-1H (Histidine, 345ACACT349) and the HA-1R allele (Argenine, 345ACACT349) and 3' primers for the HA-1H allele(500CTGCA505) and HA-1R allele (500TTGCG 504). 10µl of PCR product was analyzed by electrophoresis in 2.5% agarose (Sigma). Amplification with primers gave a 190 bp fragment (Fig 1). The recipient HA-1 disparity was defined as presence of HA-1H in recipient but not in donor.

Statistical Analysis

The genotype frequencies were determined by direct counting. Hardy-Weinberg Equilibrium used to calculate HPA gene frequency. Statistical analysis was carried out using the SPSS software. Non Parametric Mann Whitney test was used to compare the distributions of organ stages and overall grades of GVHD for patients with the HA-1 disparity and those without it. The statistical significance of differences between groups was calculated using X2, with *P* values less than 0.05 regarded as statistically significant.

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Results

16 (29.1%) out of patients were female and 39 (70.9%) of patients were male while 24 (36.6%) out of donors were female and 31 (56.4%) of them were male. Patients' mean age was 27 ± 12 (ranged 4 – 51) years (7 patients<16 y).

21 (38.2%) of patients suffered the AML, 11ones (20%) had ALL, 11 ones of them had CML, 5 ones (9.1%) of them suffered Aplastic Anemia, 6 (10.9%) patients had Thalessemia major and 1 one (1.8 %) suffered Burkitt's Lymphoma. 10 (33.3%), 8 (26.7%), 9 (20%), 3(10%) out of patients developed the GVHD-I –IV, respectively.

Our data showed: HA-1 genes frequencies were compatible with the Hardy-Weinberg equilibrium. The HA-1R and HA-1H genes frequencies in all 110 recipients and donors were 0.54 and 0.46 respectively. The Results of HA-1 typing and the GVHD status have been shown in table 1. HA-1R and HA-1H genes frequenies in recipients were 0.55 and 0.45 and in donors were 0.53 and 0.47 respectively (no significant difference was detected P= 0.69).The HA-1 phenotypes have been shown in tables 2-4.

Recipient HA-1 incompatibility was detected in 8 of 55 donor/recipient pairs (14.5%) . Four (50%) of 8 patients with the HA-1 disparity developed the aGVHD grades II-IV meanwhile 15 (62.5%) of the 24 patients were without HA-1 disparity had GVHD , showing no statistical significance (P>0.05). 2 of patients with HA-1 disparity, showed developed the GVHDI and 2 of patients with it did not develop the GVHD at all. Using the Mann Whitney analysis, The recipient HA-1 disparity was not significantly associated with an increased probability of GVHD grades I-IV or severity(P= 0.98). The severity of skin, gut and liver involvement was similar in 2 groups. We did not find any significant difference about the HA-1 disparity, while evaluating the GVHD incidence among different sibling pairs phenotype combinations . There was no difference in the HA-1 disparity between aGVHD+ and aGVHD– groups (P=0.21) .

***Table 1**: Result of HA-1 typing and GVHD statues in 55 HLA-A2 positive donors-recipients*

Result of typing (Donors/Recipients)	GVHD+	No GVHD
+/+	11(36.7%)	22(88%)
-/-	5(16.6%)	1(4%)
+/-	8(26.7%)	-
-/+*	6(20%)	2(8%)
Total	30(100%)	25(100%)

+ : means presence of HA-1H allele, * HA-1 Disparity

***Table 2**: Distribution of HA-1 phenotypes in all individuals tested*

HA-1 fenotypes	Patients	Number (%) Donors	Total
H/H	9(16.3%)	11(20%)	20(18.1%)
H/R	32(58.2%)	30(54.5%)	62(56.4%)
R/R	14(25.5%)	14(25.5%)	28(25.5%)
Total	55(100%)	55(100%)	110(100%)

***Table 3**: Distribution of HA-1 phenotypes in GVHD+ group*

HA-1 fenotypes	Patients	Number (%) Donors	Total
H/H	2(6.7%)	3(10%)	5(8.4%)
H/R	15(50%)	16(53.3%)	31(51.6%)
R/R	13(43.3%)	11(36.7%)	24(40%)
Total	30(100%)	30(100%)	60(100%)

***Table 4**: Distribution of HA-1 phenotypes GVHD- group*

HA-1 fenotypes	Patients	Number (%) Donors	Total
H/H	7(28%)	8(32%)	15(30%)
H/R	17(68%)	14(56%)	31(62%)
R/R	1(4%)	3(12%)	4(8%)
Total	25(100%)	25(100%)	50(100%)

Discussion

We found the HA-1 disparity as 14.5% between donors and recipients in which 6 ones were in the GVHD+ group and 2 of HA-1 disparity were in the GVHD- group. In spite of higher frequency of HA-1 disparity in the GVHD+ group our data did not reflect any significant association between the HA-1 disparity and risk of acute GVHD.

The HA-1R gene, like other studies showed a higher frequency than the HA-1H genes frequency in our study but these two allele frequencies were significantly different from values reported by Kotzampasaki (1) and Nesci (4) but is similar to values reported by Tseng (5).

The Graft-versus-host disease (GVHD) is a main complication after haematopoietic stem cells transplantation (HSCT) and the acute forms

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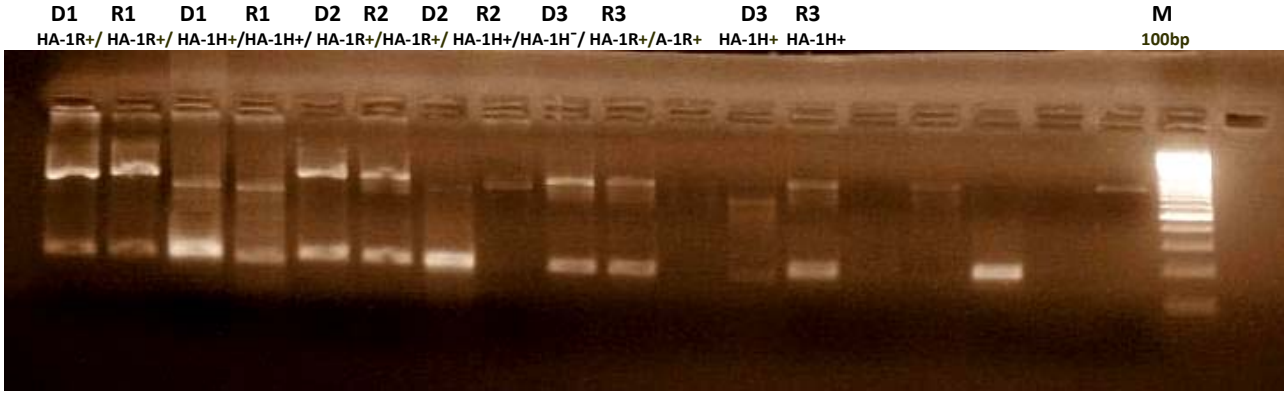
(aGVHD) occur in 20–40% of cases even after donor and recipient HLA matching, apparently because of donor/ recipient minor histocompatibility antigen (mHA) mismatch and cytokine polymorphisms (6). The HA-1 locus encodes two alleles (i.e., the HA-1H and the HA-1R allele). HLA-A* 0201 presents the immunogenic HA-1 peptide. Mismatch of the HA-1H allele is associated with the severe aGVHD grades II to IV after HLA identical HSCT (7). Goulmy et al (3) studied 148 leukemic recipients of bone marrow and their sibling donors, who were genotypically HLA identical then they reported that mismatch of minor histocompatibility antigen HA-1 could cause GVHD in adult recipients of allogeneic bone marrow from HLA-identical donors and suggested that prospective HA-1 typing may improve donor selection and identifying recipients who are at high risk for the GVHD. Different studies found a trend towards a higher risk of aGVHD grades II–IV in recipients mismatched for HA-1. Tseng (5) studied HA-1 disparity for 237 HLA-A2-positive white patients who received a marrow or peripheral blood stem cell transplant from an HLA-identical sibling at the Fred Hutchinson Cancer Research Center between 1981 and 1996. Recipient HA-1 disparity was detected in 36 of 237 donor/recipient pairs (15.2%). Twenty-two (64.7%) of 36 patients with HA-1 disparity developed grades II-IV acute GVHD, compared with 86 (42.8%) of the 201 patients without HA-1 disparity. The recipient HA-1 disparity was significantly associated with an increased probability of grades II-IV GVHD (*P* =. 02). They found that HA-1disparity was associated with increased severity of the GVHD in the skin and gut but not the liver. Gallardo et al (8) performed a study on 215 HLA-A2-positive patients who received an HLA-identical sibling SCT, in order to determine differences in acute and chronic GVHD incidence on the basis of presence or absence of the HA-1 antigen mismatch they of the detected 34(15.8%) patient-donor pairs mismatched for HA-1 antigen. Grades II-IV acute GVHD occurred in 51.6% of HA-1-mismatched pairs compared with 37.1% of the non-mismatched pairs which showed statistical significance (P= 0.035). The incidence of HA-1 disparity in our study(14.5%) was similar to what reported by Tseng(7), Gallardo(8), and the Nesci(4). But we could not confirm any relationship between HA-1 disparity and GVHD ; some other studies also failed to confirm a statistically significant association with development of GVHD. These different reports may reflect differences in methods used for sample selection, or more effective GVHD prophylaxis or small sample size.

Nesci (4), examined 94 thalassemic recipients of unmodified bone marrow, and their genotypically HLA-identical donors. The HA-1H allele frequency was 0.343, while the HA-1R allele frequency was 0.657. Recipient HA-1 incompatibility was detected in 15 of the 94 donor/recipient pairs (15.9%). Five (33.3%) of 15 patients with HA-1 disparity developed the aGVHD grades II-IV, compared with 14 (17.7%) of 79 patients without HA-1 disparity. This higher rate of aGVHD in HA-1 mismatched group was not statistically significant. Kogler et al (9) analyzed 115 CB recipients and their unrelated CB grafts for genotype associated with TNF-(TNFd3/d3) and IL-10 (IL-10, 11–16) and for disparities in major and three minor histocompatibility antigens, HY, HA-1, and CD31 codon 125. They observed HA-1 mismatch in seven patient/donor pairs that six HA-1 mismatched patient developed aGVHD grades 0 to 1, and one HA-1 mismated patients developed aGVHD grades II to IV and Negligible number of HA-I mismateches within this study didin't more statistical interpretation carryon, this reason might be responsible not to confirm any corelationship between aGVHD occurrence and HA-1 disparity. Katagiri et al. (10) analyzed the alleles HA-1 and four adhesion molecules for 106 patients transplanted with HLA-identical stem cell grafts and investigated association of mismatches as correlates the graft-versus-host disease (GVHD). They reported that the frequency of acute GVHD did not differ regardless to incompatibilities.

HY is an HLA-A2 restricted minor histocompatibility antigen (11). The immune system of females is capable to recognize and react against this male-specific minor histocompatibility antigen (mHA), HY (12). The H-Y genes have an X-chromosome homolog with similarity of 91-99% at amino acid level. Males develop tolerance to these self-antigens, but female T cells are capable to recognizing peptides derived from H-Y proteins following transplantation into male recipients (13). While analyzing the HA-1 disparity in donor - recipient gender combination (e.g., male recipients

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***Figure 1**: Representative gel electrophoretogram showing results of HA-1 PCR for 3 donor-recipient (D=Donor , R=recipient pairs) , Interpretation : Donor 1: HA-1R/H, Recipient 1: HA-1 R/H , Donor 2 : HA-1R/ H , Recipient 2: HA-1R/R , Donor 3 : HA-1R/H , Recipient3 : HA-1 R/H*



and female donors in 4 pairs) we observed a significant difference in comparison with other gender combinations of donor – recipient(e.g. ; male-male, female-female, male-female) (Z test, P= 0.048).

Results of previous studies had suggested that reduction in risk of GVHD from typing and matching for a single mHA is likely to be low, but substantial benefit could come from typing and matching for multiple mHA.[19] Additional studies will be needed to assess the effects of HA-1 disparity on other important endpoints such as chronic GVHD, leukemia relapse, and survival (7). Heinemann (14) findings indicated that impact of mHag disparity on aGVHD development in HSCT from HLA-matched sibling seems to for low classic aGVHD risk factors. It seems, other elements of the immunogenic potency of the HA-1 antigen could also be considered for example, a response by cytotoxic-T-cell precursors that are specific for a minor histocompatibility antigen requires helper T cells(3)..

In conclusion, in spite of higher frequency of HA-1 disparity in GVHD+ group our data did not reflect significant association between HA-1 disparity and risk of acute GVHD .Numbers of HA-1 mismatches within this study might be very slight to allow further statistical interpretation so studies with larger patient numbers and detection of several minor histocompatibility antigens are suggested to substantiate a clinically relevant effect of multiple mHag disparities between donors and recipients.

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