Is There any Greater Possibility in Finding HLA-identical Unrelated Hematopoietic Stem Cell Donors among Thalassemia Families for Transplantation of Thalassemia Patients?

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Background: Thalassemia is probably the most common single gene disorder causing a major public health problem in the world. Currently, allogenic hematopoietic stem cell transplantation (HSCT) is the only curative therapy for thalassemia. One major limitation of HSCT is the lack of HLA-identical sibling donors, so attention has turned to finding phenotypically matched unrelated donors.
Patients and methods: From 1991 to 2002, 182 thalassemia patients referred to our center for HSCT. Donor selection was based on HLA class I and class II histocompatibility matching. The results of the serologically HLA class I typing of 549 subjects (patients and their families) and HLA class II typing of 182 patients were compared with HLA class I and II antigens of 100 healthy Iranians normal people. The comparisons between these two groups were tested in univariate analysis, using the Pearson chi-squared statistics.
Results: In comparing, thalassemic families (549 subjects) and healthy Iranians (100 subjects) for HLA class I antigens, significant differences for 11 antigens, including A9 (p= 0.029), A11 (p= 0.01), A19 (p= 0.000), B16 (p= 0.000), B17 (p= 0.029), B27 (p= 0.003), B41(p= 0.000), C2 (p= 0.015), C3(p= 0.012), C4 (p= 0.004), C7 (p= 0.000) were found. For HLA class II antigens, we found that only HLA-DR7 was significantly different (p= 0.002) between 182 thalassemia patients and the healthy Iranian normal group.
Conclusion: In this study, we found that thalassemia families showed significant differences, compared to the healthy Iranian group in several HLA antigens. Comparing HLA polymorphism and finding enough similarity in thalassemia families in the countries, located in the thalassemia belt, may provide benefits for establishing a common HLA bank of thalassemia families.

Key words: HLA, Stem cell, Thalassemia, Transplantation.

Introduction:
At present, Hematopoietic stem cell transplantation (HSCT) is the only curative therapy for fatal types of thalassemia.[1,4] However, a major problem in HSCT is finding HLA-identical donors.[5-8] Since only 30-35% of potential transplant candidates have HLA-identical siblings, attention has turned to alternative donor transplants including those using a partially mismatched related donor[9]or phenotypically matched unrelated donor.[10] The search for an unrelated donor from general HLA registry banks is costly, time-consuming and has been attained for less than half of the patients who seek an alternative donor. During the last two decades, conventional therapy has improved the prognosis of thalassemia. However, despite such improvements, it still remains a progressive disease with therapy-related complications (such as hepatitis, liver fibrosis, cardiac disease) progressing over time. Conventional treatment may postpone but not eliminate these complications with the associated morbidity and mortality. In contrast to this, HSCT can prevent or delay progression of the aforementioned complications. Approximately 70% of thalassemia patients lack a related matched donor. For those patients who lack HLA-identical sibling donors, alternative sources of hematopoietic stem cell must be sought. It is well known that thalassemia is more frequent in many developing societies, where the mortality from this disease remains extremely high and few patients survive to adulthood because it is not possible to give conventional treatments due to inadequate transfusion services and costly chelating treatments.[7]
In this article, we sought to investigate differences in the frequency of HLA-antigen polymorphism between Iranian thalassemia families and the healthy population. The answer to this question may help in finding HLA matched unrelated HSC donors for transplantation of major beta-thalassemia patients.

Methods:
From 1991 to 2002, 182 beta thalassemia patients referred to our center for HSCT. In order to find HLA-identical donors, all patients and their first-degree family members were referred to the Iranian Blood Transfusion Center or the Immunogenetics Laboratory of Tehran University of Medical Sciences for HLA class I and II typing in microcytotoxicity NIH method.
The results involving 549 HLA class I antigens (patients and their first-degree family members) and 364 HLA class II antigens (patients and their HLA-identical family members) were compared with the results of the HLA class I and II typing of 100 healthy Iranians (done by Nikbin, et al)[11]. In order to compare the frequencies of different HLA-antigens, we

calculated the splits of each antigen in one group. Rare antigens were omitted from our study and a Chi-square test was used to compare the frequencies of each antigen in both the healthy and thalassemic populations. P values less than 0.05 were considered significant.

Results:

Table 1 shows the frequency of HLA class I and II antigens in 182 transplanted thalassemia patients and 100 healthy Iranians. It is evident that there are significant differences ($p<0.05$) in the frequency of the seven HLA class I antigens including A11, B16, B17, B27, B41, C4 and C7 and one class II antigen which is DR7.

Table1: Comparison of the frequency of HLA class I and II antigens between thalassemia patients and healthy Iranians.

Antigen name	Thalassemia Positive panel (No=182)	%	Normal Positive panel	%	p.valae
A1	35	19.2	23	23	N.S
A2	57	31.3	33	33	N.S
A3	38	20.9	17	17	N.S
A9	63	34.6	24	24	N.S
A10	20	11	16	16	N.S
A11	34	18.7	28	28	0.005
A19	23	12.6	26	26	N.S
A28	16	8.8	11	11	N.S
B5	57	31.3	32	32	N.S
B7	13	7.1	5	5	N.S
B8	15	8.2	7	7	N.S
B12	15	8.2	10	10	N.S
B13	11	6	8	8	N.S
B14	9	4.9	8	8	N.S
B15	3	1.6	5	5	N.S
B16	7	3.8	13	13	0.004
B17	19	10.4	3	3	0.026
B18	19	10.4	10	10	N.S
B21	21	11.5	12	12	N.S
B22	15	8.2	7	7	N.S
B27	6	3.3	11	11	0.009
B35	65	35.7	32	32	N.S
B40	7	3.8	6	6	N.S
B41	1	0.5	8	8	0.001
B4	15	8.2	66	66	0.000
B6	18	9.9	74	74	0.000
C1	9	4.9	6	6	N.S
C2	8	4.4	10	10	N.S
C3	5	2.7	8	8	N.S
C4	44	24.2	38	38	0.017
C5	1	0.5	2	2	N.S
C7	6	3.3	22	22	0.000
DR1	22	14.7	13	13	N.S
DR2	51	34	28	28	N.S
DR3	32	21.3	17	17	N.S
DR4	22	14.7	24	24	N.S
DR5	55	36.7	45	45	N.S
DR6	19	12.7	9	9	N.S
DR7	15	10	21	21	0.002
DR8	4	2.7	2	2	N.S
DR9	6	4	2	2	N.S
DR10	4	2.7	1	1	N.S
DR52	101	67.3	78	78	N.S
DR53	54	36	42	42	N.S
DQ1	91	60	63	63	N.S
DQ2	45	30	30	30	N.S
DQ3	66	44	56	56	N.S

In table 2, frequencies of HLA class I antigens of 549 members of thalassemia families have been compared to 100 healthy Iranians.

Table2: Comparison of the frequency of HLA class I antigens between thalassemia family members and healthy Iranians.

Antigen name	Thalassemia(549) Positive	%	Normal Iran(100) Positive	%	p.value
A1	101	18.4	23	23	-
A2	183	33.3	33	33	-
A3	113	20.6	17	17	-
A9	195	35.5	24	24	0.029
A23	6	-	1	-	-
A24	148	-	22	-	-
A10	55	10	16	16	-
A25	0	-	1	-	-
A26	46	-	15	-	-
A11	97	17.7	28	28	0.01
A19	62	11.3	26	26	0.000
A28	43	7.8	11	11	-
B5	189	34.4	32	32	-
B7	38	6.9	5	5	-
B8	36	6.6	7	7	-
B12	50	9.1	10	10	-
B13	31	5.6	8	8	-
B14	26	4.7	8	8	-
B15	14	2.6	5	5	-
B16	20	3.6	13	13	0.000
B17	53	9.7	3	3	0.029
B18	59	10.7	10	10	-
B21	60	10.9	12	12	-
B22	43	7.8	7	7	-
B27	19	3.5	11	11	0.003
B35	181	33	32	32	-
B40	22	4	6	6	-
B41	2	0.4	8	8	0.000
BW4	50	9.1	66	66	0.000
BW6	64	11.7	74	74	0.000
CW1	29	5.3	6	6	-
CW2	23	4.2	10	10	0.015
CW3	14	2.6	8	8	0.012
CW4	131	23.9	38	38	0.004
CW5	4	0.7	2	2	-
CW7	15	2.7	22	22	0.000

Here, in addition to the previously mentioned seven antigens, four other antigens (A9, A19, C2 and C3) have significant differences in their frequencies. In the case of the A9 antigen, although there is a significant difference in its frequency between thalassemia families and the normal population, the antigen subgroup A24 is much more frequent than A23 in both populations. The same is true for the A10 antigen, in which the majority of both populations belong to the A26 subgroup.

The frequencies of public antigens DR52 and DR53 in both thalassemia and normal populations were similar and DR52 was more frequent than DR53. DQ locus antigens showed a similar pattern of frequency in both populations and DQ1 was more frequent than DQ2.
As shown in Table 3, there is no significant difference in ABO blood groups.

Table 3: The frequency of ABO blood group in thalassemia family and healthy Iranians.

ABO & Rh	% Normal Iran	% Thalassemia
A	31.5	26.9
B	24	22.4
AB	6.5	4.4
O	37.5	43.2
Rh^+	89.6	91.4

Discussion:
In the study done in 1991, Nikbin, et al showed a significant increase in the frequency of HLA- B7 and B17 and a decrease in the frequency of HLA- A10, B27, B51, C2 and C4 in 77 thalassemia patients compared to 100 healthy Iranians.[12]
In a study one on 85 major beta thalassemia patients in New york, the frequency of HLA-B35 was shown to be significantly higher in thalassemia family members.[13]
Another study found that HLA-A2 and B46 are significantly more frequent in central Thailand thalassemia patients and the frequency of HLA-B13 is significantly more in patients from northern Thailand.[14]
In China, a study on patients with hemoglobin-H disease (a kind of alpha thalassemia) revealed that HLA-A11 was significantly less frequent in their patients than the normal Chinese population.[15] Two more studies from China suggest that HLA-DQB1*06 allele is associated with pathogenesis of[16] and susceptibility to[17] the major beta-thalassemia.
Our results showed that in HLA class I, A9 and B17 were significantly more frequent in thalassemia family members compared to the healthy Iranians normal population (similar to the results of Nikbin et al). Also the frequencies of HLA-A11, A19, B16, B41, B27, C2, C4 and C7 were significantly less in thalassemia patients in comparison to healthy Iranians, which was to some extent similar to the previous studies (A11 in the Chinese study and A19, B16, B41, B27, C2 and C4 in the study of Nikbin et al).
One study from Italy (Contu et al) was carried out on 479 families, each with a proband affected by homozygous beta-thalassemia, who were typed for HLA antigens. They did not find any significant differences in HLA allele, haplotypes and genotypes between affected and healthy children.
In most of the previous researches done on thalassemia patients, a lot of focus was devoted to association of MHC antigens with thalassemia disease. For this reason, different alleles have been reported from various populations. In this study, we considered the thalassemia families an ethnic group and found that there are several HLA antigens that, by statistical analysis, show significant differences between thalassemia and normal populations.
One study from Italy reported that the availability of HLA-matched siblings and parents for thalssemia patients in 129 Italian families were 33 % and 8.5 %, respectively. This finding is more than the expected ratio 25:50:25 and shows the higher chance of finding HLA-identical donors for thalssemia patients in their families.[19] One possible explanation for this increased chance can be limited HLA polymorphism in thalassemia families.
It may be logical to compare HLA polymorphism in thalassemia families of different countries, especially those living in the thalassemia belt. Finding enough similarity may provide the opportunity to establish a common cord blood and/or HLA bank for thalassemia families in these countries.

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