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Comparison Between HLA-B Allele Groups Among Peripheral Blood Stem Cell Donation Volunteers from Various Iranian Ethnicities

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Abstract

This research included 2064 individuals from various ethnic groups in Iran, all hematopoietic stem cell donors. The primary goal of this research was to establish an HLA database and assess genetic diversity across different ethnicities. DNA was extracted using the magnetic method, and HLA-typing was performed at a low-resolution level using the PCR-SSP method. The results were analyzed manually by independent experts as well as through software. HLA data from four ethnic groups, including Gilak (n=510), Lur (n=465), Kurdish (n=719), and Arab (n=370), were examined for associations between alleles and ethnicity. Allele frequencies were assessed through statistical methods to identify significant relationships, with the significance level set at 0.05. Additionally, standardized residuals were calculated to determine which ethnic groups exhibited allele frequencies that exceeded expected values based on assumptions of independence. Among HLA-B alleles, HLA-B*35 and HLA-B*51 were found to have the highest frequencies, while HLA-B*67, *78, *81, *82, and *83 were absent in this research. Significant differences were observed in 17 alleles across the ethnic groups for HLA-B, with $P< 0.05$. Standardized residuals exceeding a threshold of 2 indicated statistically significant deviations between observed and expected values at the 0.05 significance level. All analyses were conducted using R software. Determining HLA allele frequencies helps identify similarities and differences among ethnic groups. This information can assist in developing donor services strategies in Iran's diverse regions and establishing stem cell registries. In the future, this data may also contribute to clinical applications in transplantation, vaccine development, and infectious disease research.

Keywords: HLA, Hematopoietic Stem Cell; Donors; Ethnicity; Iran.

Introduction

Human Leukocyte Antigens (HLA) class I and II are cell surface glycoproteins encoded by genes located on the short arm of chromosome 6. The genomic region housing these genes is called the major

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histocompatibility complex (MHC) and is typically inherited as a haplotype. The HLA system exhibits the most extraordinary genetic diversity in humans. Specifically, HLA-A, HLA-B, and HLA-C genes encode the corresponding class I antigens A, B, and C, while HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DQA1, HLA-DQB1, HLA-DPA1, and HLA-DPB1 encode class II antigens (1). New HLA alleles are continuously being discovered, with over 38,000 (HLA) identified (2).

Population migration and genetic mixing are critical drivers in the evolution and diversification of polymorphic HLA molecules. This wide diversity of alleles is shaped by natural selection and historical demographic events like migrations and population bottlenecks, contributing to human genetic variability. The adaptive importance of HLA variability is underscored by its critical role in the immune response, particularly in pathogen recognition and antigen presentation to T cells (3).

Hematopoietic stem cell transplantation (HSCT) has emerged as a critical treatment for severe blood disorders, and its demand has surged over the last decade as a viable therapeutic option for many malignancies. To minimize graft-versus-host disease (GVHD), matching HLA molecules between donors and recipients across multiple loci is essential, as the degree of compatibility directly influences transplantation outcomes.

Stem cell registries are designed to provide HLA-matched unrelated volunteers for patients awaiting transplantation. However, finding a HLA-compatible hematopoietic stem cell donor is challenging due to the high level of HLA polymorphism. A registry's size and genetic diversity are critical factors in determining the likelihood of finding a suitable donor (4).

Certain diseases are associated with specific HLA antigens in the population, highlighting the importance of investigating HLA-disease associations (5, 6). This research aims to determine the type and frequency of HLA allelic groups in four Iranian ethnic groups to expand the HLA database for patients. Moreover, understanding the prevalence of HLA alleles and haplotypes across different ethnicities is valuable for genetic investigations and insights into population relationships (7-9). Additionally, HLA polymorphism is a valuable tool in anthropological investigations (10, 11).

While HLA-compatible siblings are the preferred donors for transplantation, due to the low likelihood of genetic similarity among siblings and the declining number of family members in many societies, more than 70% of patients require HLA-compatible non-relative donors from stem cell registries. The greater the number of hematopoietic stem cell donors, the higher the chances of finding a non-relative donor with identical HLA for a patient (12).

Materials and Methods

1. Sample preparation, DNA extraction and HLA-typing by PCR-SSP method

Blood samples were collected from all stem cell donation volunteers at the Iranian Blood Transfusion Organization. Each volunteer provided a 10 mL volume of whole blood, with EDTA as the anticoagulant. The samples were stored at temperatures between 1 and 10°C before being transported to the laboratory. DNA extraction was performed using the magnetic bead method with the MagCore Automated Nucleic Acid DNA Extractor (Switzerland). The PCR-SSP (polymerase chain reaction with sequence-specific primers) method was used for HLA typing. This technique uses sequence-specific primers to target and amplify a particular HLA DNA sequence. It comprises multiple PCR reactions, each specific to an allele or group of alleles. After amplification, the alleles are detected through agarose gel electrophoresis. Since SSP primers target particular DNA sequences, the amplification product confirms the presence of the allele containing that sequence (1). In this method, a kit is used for each sample, and each HLA-ABDR determination kit contains 96 microtubes, with primers specific to the HLA sequences and internal control primers.

For this research, HLA-typing kits from Olerup (Sweden) were the primary kits used, with Innotrain (Germany) kits employed to resolve ambiguities. Each kit contained 24 reactions for HLA-A, 48 reactions for HLA-B, and 24 reactions for HLA-DRB1 allele groups. After PCR, the samples were electrophoresed on a 1.5% agarose gel, and two independent experts analyzed the resulting images. If the image quality was adequate, the bands were counted and interpreted manually and by software.

2. Statistical analysis

This investigation employed the chi-square test to examine the association between ethnicity and allele frequency within the population sample. When applicable, Fisher's exact test was utilized. Standardized residuals were computed to identify notable deviations between the observed and expected allele frequencies under the null hypothesis of independence. A standardized residual with an absolute value greater than 2 indicated a meaningful discrepancy at the 0.05 threshold. All statistical analyses were conducted using R software.

Results

1. HLA-Typing results in the studied population of four Iranian ethnicities

Performing low-resolution HLA-typing tests on the samples of different ethnicities of Gilak (n= 510), Lur (n= 465), Kurdish (n= 719), and Arab (n= 370) led to the determination of allelic groups in HLA-A, -B and -DRB1 gene loci in the studied populations.

2. Frequency of Alleles in the Studied Population

The frequencies of allelic groups related to the HLA-A, HLA-B, and HLA-DRB1 gene loci were identified. The highest allelic frequencies for HLA-A in the entire studied population were HLA-A*02 (19.6%), HLA-A*24 (15.2%), and HLA-A*03 (12.1%). For HLA-B, the most frequent alleles were HLA-B*35 (19%) and HLA-B*51 (14.2%) (Table 1). In terms of HLA-DRB1, the most frequent alleles were HLA-DRB1*11 (22.7%), HLA-DRB1*15 (11.7%), and HLA-DRB1*04 (11.1%). No alleles from the HLA-A*80 and HLA-B*67, *78, *81, *82, and *83 groups were detected in the studied population. Due to the volume of data, the allelic groups for HLA-B are exclusively presented in detail by ethnic group.

Table 1. Frequencies of allelic groups related to HLA-B in the entire studied population

HLA-B in the entire studied population					
HLA-B		Frequency	Percent	Valid Percent	CumulativePercent
Valid	07	187	4.5	4.5	4.5
	08	164	4.0	4.0	8.5
	13	106	2.6	2.6	11.1
	14	85	2.1	2.1	13.1
	15	127	3.1	3.1	16.2
	18	219	5.3	5.3	21.5
	27	93	2.3	2.3	23.8
	35	786	19.0	19.0	42.8
	37	57	1.4	1.4	44.2
	38	153	3.7	3.7	47.9
	39	48	1.2	1.2	49.1
	40	122	3.0	3.0	52.0
	41	162	3.9	3.9	55.9
	42	10	.2	.2	56.2
	44	201	4.9	4.9	61.0
	45	16	.4	.4	61.4
	46	6	.1	.1	61.6
	47	11	.3	.3	61.8
	48	2	.0	.0	61.9
	49	99	2.4	2.4	64.3
	50	200	4.8	4.8	69.1
	51	586	14.2	14.2	83.3
	52	272	6.6	6.6	89.9
	53	37	.9	.9	90.8
	54	6	.1	.1	91.0
	55	189	4.6	4.6	95.5
	56	11	.3	.3	95.8
	57	51	1.2	1.2	97.0
	58	93	2.3	2.3	99.3
	59	1	.0	.0	99.3
	73	27	.7	.7	100.0
	Total	4128	100.0	100.0	

HLA-B* 67, *78, *81, *82, *83 allele groups were not detected.

Table 2. Comparison between the frequency distribution of allelic groups in the HLA-B gene locus in 4 ethnic groups of Iran: Lur, Gilak, Arab and Kurd

Name	Ethnic	Allele	N (%)	Standardized Residuals	P-value
	Lur	Yes	36(3.87)	-0.18	
HLA-B*08		No	894(96.13)	0.18	
	Gilak	Yes	24(2.35)	-3.05	
		No	996(97.65)	3.05	
					<0.001
	Arab	Yes	49(6.62)	4.07	
		No	691(93.38)	-4.07	
	Kurd	Yes	55(3.82)	-0.36	
		No	1383(96.18)	0.36	
HLA-B*14	Lur	Yes	16(1.72)	-0.83	
		No	914(98.28)	0.83	
	Gilak	Yes	14(1.37)	-1.78	
		No	1006(98.63)	1.78	
					0.001
	Arab	Yes	29(3.92)	3.93	
		No	711(96.08)	-3.93	
	Kurd	Yes	26(1.81)	-0.83	
		No	1412(98.19)	0.83	
	Lur	Yes	23(2.47)	-1.21	
HLA-B*15		No	907(97.53)	1.21	
	Gilak	Yes	28(2.75)	-0.71	
		No	992(97.25)	0.71	
					<0.001
	Arab	Yes	45(6.08)	5.22	
		No	695(93.92)	-5.22	
	Kurd	Yes	31(2.16)	-2.5	
		No	1407(97.84)	2.5	
	Lur	Yes	29(3.12)	2.02	
HLA-B*27		No	901(96.88)	-2.02	
	Gilak	Yes	14(1.37)	-2.18	
		No	1006(98.63)	2.18	
					0.018
	Arab	Yes	11(1.49)	-1.55	
		No	729(98.51)	1.55	
	Kurd	Yes	39(2.71)	1.45	
		No	1399(97.29)	-1.45	
HLA-B*35	Lur	Yes	188(20.22)	1.04	
		No	742(79.78)	-1.04	
	Gilak	Yes	232(22.75)	3.47	
		No	788(77.25)	-3.47	
					<0.001
	Arab	Yes	84(11.35)	-5.88	
		No	656(88.65)	5.88	
	Kurd	Yes	282(19.61)	0.68	
		No	1156(80.39)	-0.68	

3. Frequencies of HLA-B Alleles in Different Ethnic Groups

The HLA-B allele frequencies among the studied ethnic groups were also evaluated. As shown in Table 2, 31 allelic groups for HLA-B were compared, and significant differences (P-value < 0.05) were found in 17 of these allelic groups (Table 2).

It should be noted that the results for HLA-B*07, HLA-B*13, HLA-B*18, HLA-B*40, HLA-B*44, HLA-B*47, HLA-B*48, HLA-B*49, HLA-B*54, HLA-B*56, HLA-B*57, HLA-B*73, HLA-B*46, and HLA-B*59 were not statistically significant between the ethnic groups.

Table 2. Continued

Name	Ethnic	Allele	N (%)	Standardized Residuals	P-value
HLA-B*37	Lur	Yes	5(0.54)	-2.5	
		No	925(99.46)	2.5	
	Gilak	Yes	25(2.45)	3.38	
		No	995(97.55)	-3.38	
					0.001
	Arab	Yes	4(0.54)	-2.16	
		No	736(99.46)	2.16	
	Kurd	Yes	23(1.60)	0.88	
		No	1415(98.40)	-0.88	
HLA-B*38	Lur	Yes	33(3.55)	-0.29	
		No	897(96.45)	0.29	
	Gilak	Yes	30(2.94)	-1.49	
		No	990(97.06)	1.49	
					0.016
	Arab	Yes	42(5.68)	3.13	
		No	698(94.32)	3.13	
	Kurd	Yes	48(3.34)	-0.92	
		No	1390(96.66)	0.92	
HLA-B*39	Lur	Yes	12(1.29)	0.41	
		No	918(98.71)	-0.41	
	Gilak	Yes	20(1.96)	2.74	
		No	1000(98.04)	-2.74	
					0.0257
	Arab	Yes	5(0.68)	-1.36	
		No	735(99.32)	1.36	
	Kurd	Yes	11(0.76)	-1.74	
		No	1427(99.24)	1.74	
	Lur	Yes	37(3.98)	0.1	
		No	893(96.02)	-0.1	
	Gilak	Yes	50(4.90)	1.85	
HLA-B*41		No	970(95.10)	-1.85	<0.001
	Arab	Yes	41(5.54)	2.5	
		No	699(94.46)	-2.5	
	Kurd	Yes	34(2.36)	-3.77	
		No	1404(97.64)	3.77	
	Lur	Yes	1(0.11)	-0.95	
HLA-B*42		No	929(99.89)	0.95	
	Gilak	Yes	1(0.10)	-1.08	
		No	1019(99.90)	1.08	
					0.018
	Arab	Yes	6(0.81)	3.47	
		No	734(99.19)	-3.47	
	Kurd	Yes	2(0.14)	-0.99	
		No	1436(99.86)	0.99	

Discussion

This research focused on HLA-A, HLA-B, and HLA-DRB1 loci polymorphism among four Iranian ethnic groups: Lur, Gilak, Kurd, and Arab. The study identified the frequencies of HLA allelic groups and observed significant variations in the frequency of specific HLA-B alleles across the ethnicities (Table 2). When an important relationship was detected (P-value < 0.05), it indicated an association between the allele and the ethnicity. Further analysis using standardized residuals helped determine whether a specific ethnic group

Table 2. Continued

Name	Ethnic	Allele	N (%)	Standardized Residuals	P-value
HLA-B*45	Lur	Yes	3(0.32)	-0.36	
		No	927(99.68)	0.36	
	Gilak	Yes	2(0.2)	-1.13	
		No	1018(99.8)	1.13	
					0.022
	Arab	Yes	8(1.08)	3.35	
		No	732(98.92)	-3.35	
	Kurd	Yes	3(0.21)	-1.35	
		No	1435(99.79)	1.35	
HLA-B*50	Lur	Yes	34(3.66)	-1.92	
		No	896(96.34)	1.92	
	Gilak	Yes	29(2.84)	-3.43	
		No	991(97.16)	3.43	
					<0.001
	Arab	Yes	57(7.7)	4	
		No	683(92.3)	-4	
	Kurd	Yes	80(5.56)	1.57	
		No	1358(94.44)	-1.57	
HLA-B*51	Lur	Yes	170(18.28)	4.05	
		No	760(81.72)	-4.05	
	Gilak	Yes	95(9.31)	-5.15	
		No	925(90.69)	5.15	
					<0.001
	Arab	Yes	69(9.32)	-4.19	
		No	671(90.68)	4.19	
	Kurd	Yes	252(17.52)	4.48	
		No	1186(82.48)	-4.48	
HLA-B*52	Lur	Yes	50(5.38)	-1.69	
		No	880(94.62)	1.69	
	Gilak	Yes	87(8.53)	2.88	
		No	933(91.47)	-2.88	
					0.029
	Arab	Yes	46(6.22)	-0.45	
		No	694(93.78)	0.45	
	Kurd	Yes	89(6.19)	-0.76	
		No	1349(93.81)	0.76	
HLA-B*53	Lur	Yes	3(0.32)	-2.11	
		No	927(99.68)	2.11	
	Gilak	Yes	8(0.78)	-0.44	
		No	1012(99.22)	0.44	
					0.021
	Arab	Yes	13(1.76)	2.74	
		No	727(98.24)	-2.74	
	Kurd	Yes	13(0.9)	0.04	
		No	1423(99.1)	-0.04	

exhibited a higher or lower allele frequency. Under the independence assumption, absolute values exceeding 2 for standardized residuals indicated significant differences between observed and expected frequencies. Positive residuals indicate that the observed frequency exceeded the expected value, whereas adverse residuals suggest the observed frequency was lower than anticipated.

The HLA-B*67, B*78, B*81, B*82, and B*83 allelic groups were not observed in the overall population

Table 2. Continued

Name	Ethnic	Allele	N (%)	Standardized Residuals	P-value
HLA-B*55	Lur	Yes	42(4.52)	-0.1	
		No	888(95.48)	0.1	
	Gilak	Yes	82(8.04)	6.09	
		No	938(91.96)	-6.09	<0.001
	Arab	Yes	9(1.22)	-4.83	
		No	731(98.78)	4.83	
	Kurd	Yes	56(3.89)	-1.54	
		No	1382(96.11)	1.54	
	Lur	Yes	14(1.51)	-1.75	
HLA-B*58		No	916(98.49)	1.75	
	Gilak	Yes	32(3.14)	2.19	
		No	988(96.86)	-2.19	
					0.014
	Arab	Yes	23(3.11)	1.73	
		No	717(96.89)	-1.73	
	Kurd	Yes	24(1.67)	-1.85	
		No	1414(98.33)	1.85	

studied. The most common alleles for HLA-B were HLA-B*35 (19%) and HLA-B*51 (14.2%). Research conducted by Shaigan in 2011, which examined the allelic frequencies of HLA-A, HLA-B, and HLA-DRB1 in 244 individuals from the Fars ethnic group in Iran, reported results that aligned closely with the findings in the general population (13).

Abedini and colleagues performed a systematic review and meta-analysis of HLA allele frequencies in Iranian populations. Their reported frequencies were also similar to those of this research. For instance, the two most common alleles, HLA-B*35 and HLA-B*51, were observed at 19% and 14.2%, respectively, closely matching Abedini's 18% and 14% findings. However, differences were noted for rare alleles such as HLA-B*42 and HLA-B*45, which were absent (0%) in Abedini's research but appeared at 0.2% and 0.4% in this research. These discrepancies could be attributed to the larger sample size in this research (14).

An exciting aspect of this investigation was the variation in allele frequencies among different ethnic groups compared to the overall population. For instance, in the general population, HLA-B*35 had a frequency of 19%, and HLA-B*52 had a frequency of 14.2% (Table 1). However, in the Arab population, these frequencies were lower, with HLA-B*35 at 11.35% and HLA-B*52 at 9.32% (Table 2). Conversely, specific alleles, like HLA-B*15, had a higher frequency in the Arab population (6.08%) compared to the total population (3%). Similar trends were observed in other Arab populations. For instance, HLA-B*35 was highly prevalent among Palestinians (20.3%) and Lebanese-Armenians (19.8%). In contrast, HLA-B*35 was found at varying frequencies in Iraqi Kurds (15.6%), Omanis (15.3%), Jordanians (14.9%), and Arab Emiratis (11.1%). The second most frequent allele, HLA-B*51, was also common among Saudis (19.3%), Omanis (17.5%), and Arab Emiratis (15.6%) (15).

In 2017, Shaheswar and colleagues conducted genomic research analyzing the distribution of HLA-A and HLA-B alleles in the Lak population of Lorestan Province. This research aimed to compare these findings with data from the broader Iranian population. Their results showed significant parallels with the Lur population in this research. For example, the frequency of HLA-B*35 was 20.22% among Lurs, compared to 24% among the Lak population ($P< 0.05$). Similarly, HLA-B*51 was found at 18.28% among Lurs and 16% in the Lak population ($P< 0.05$). Both investigations showed strong agreement in allele frequencies for common alleles; however, differences were observed in the less common alleles. For instance, HLA-B*48 and HLA-B*55 were found at 0.11% and 4.52%, respectively, compared to 1% for both alleles in Shaheswar's research (P-value < 0.05). These variations may result from the distinct subpopulations studied. Shaheswar's research focused exclusively on the Lak population, while this research included the Lur population. Furthermore, Shaheswar's research had a sample size of 100 individuals, while this research involved 465 participants (16).

Suarez-Trujillo and colleagues researched the

prevalence of HLA alleles in Saqqez-Baneh Kurds in Iran. Their findings reported frequencies for common alleles such as HLA-B*35:01 (21.67%), HLA-B*51:01 (9.17%), HLA-B*18:01 (5.83%), and HLA-B*50:01 (5.83%). This research yielded similar results to Suarez-Trujillo's research; however, there was a notable difference in the frequency of HLA-B*51 (P-value < 0.05). In the Kurdish population of this study, HLA-B*51 was recorded at 17.52%, compared to 9.17% in Suarez-Trujillo's research. This discrepancy may be attributed to the distinct Kurdish subpopulations examined in the two investigations. While Suarez-Trujillo's research focused solely on Saqqez-Baneh Kurds, this research included Kurds from various regions of Iran (17,18) .

It is noteworthy that Hajighasemi and his team discovered prevalent HLA Class I and II alleles within the Iranian population through an immunoinformatic analysis of the database (www.allelefrequencies.net). This information could prove to be significant for vaccine development, organ transplantation, and donor matching. According to their research, the most frequently observed HLA-B alleles were HLA-B*35 (13%), B*51 (12%), and B*53 (13%). The findings of this study were consistent with Hajighasemi research (19).

Conclusion

Analyzing the distribution of HLA alleles across various ethnic populations reveals commonalities and distinctions within allelic groups. This analysis is critical in improving the efficiency of donor center programs in different regions and optimizing stem cell donation registries. The findings from this research can potentially drive groundbreaking clinical progress in fields like transplantation, vaccine creation, uncovering the genetic factors influencing drug responses, and combating infectious diseases.

Conflict of interest

No conflicts of interest were disclosed by the authors.

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