Original Article

Frequency of Human Platelet Antigens −1 to −5 and −15 in Turkmen Blood Donors

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Abstract

Background: Due to the presence of platelet antigen polymorphisms, human platelet membrane glycoproteins can be identified as an alloantigen or autoantigen. The aim of this study was to determine the frequencies of human platelet antigens (HPAs)-1 to-5 and-15 in Turkmen blood donors and establish a panel of accredited HPAs negative donors as well as an HPA-typed platelet donor registry.

Materials and Methods: HPA-1 to-5 and-15 typing was performed by the polymerase chain reaction-sequence-specific primer techniques on 80 unrelated Turkmen donors who were referred to Aq-Qala Blood Transfusion Center in Golestan Province from September 2018 to October 2019.

Results: The frequencies of HPA phenotypes were determined as follows: HPA-1aa: 92.5%, HPA-1ab: 7.5%, HPA-2aa: 77.5%, HPA-2ab: 20.0%, HPA-2bb: 2.5%, HPA-3aa: 75.3%, HPA-3ab: 50%, HPA-3bb: 11.2%, HPA-4aa: 100%, HPA-5aa: 78.5%, HPA-5ab: 21.5%, HPA-15aa: 41.2%, HPA-15ab: 56.2% and HPA-15bb: 17.5%.

Conclusion: Determining the genotype of HPAs that play an important role in platelet refractory can improve the management of alloimmunization due to the incompatibility of HPAs between the recipients and donors. Therefore, the registration process for national platelet donors can help patients accelerate and improve the quality of transfused platelets.

Keywords: Blood donors, human platelet antigen, polymerase chain reaction with SSP, platelets

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Introduction

The plasma membrane of platelets is composed of a large number of glycoproteins and phospholipids containing antigenic sites; the highest number of known platelet antigens including HPA-1 is located on glycoprotein IIb/IIIa.[1] Due to the presence of platelet antigen polymorphisms, human platelet membrane glycoproteins can be identified as an alloantigen or autoantigen.

To date, a total of 41 HPAs have been described in the Immune Polymorphism Database (https://www.versiti.org/hpa).[2] Numerous types of HPAs are involved in different clinical conditions including posttransfusion purpura, platelet transfusion refractoriness (PTR), alloimmune thrombocytopenia, idiopathic thrombocytopenic purpura,[3] fetal alloimmune thrombocytopenia,[4] and susceptibility to hepatitis C virus infection.[5] Today, FNAIT is the leading cause of severe thrombocytopenia in living infants and accounts for approximately 40% of neonatal referrals to the intensive care unit.[6]

The alloimmunization against platelet antigens or HLA might be evolved and lead to immune PTR. Platelet donor registry with genotyping of HPAs polymorphisms can improve PTR treatment.

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Differences in the frequency of HPAs in different communities and ethnicities indicate variation in the pattern of frequency of HPAs, and this highlights the need to study the frequency of these antigens in different communities.

The aim of this study was to determine the frequencies of HPA −1 to −5 and −15 in Turkmen blood donors and establish a panel of accredited HPAs negative donors as well as an HPA-typed platelet donor registry. For this purpose, we investigated the distributions of HPA −1 to −5 and −15 in 80 unrelated Turkmen blood donors using a polymerase chain reaction with sequence-specific primers (PCR-SSP) method referring to Aq Qala Blood Transfusion Center in Golestan Province, Iran.

Materials and Methods

Study population

The present study was performed on 80 unrelated Turkmen blood donors who were referred to Aq Qala Blood Transfusion Center in Golestan Province from September 2018 to October 2019. All participants in the study were male with a mean age of 38.2 ± 7.7 years (range: 18–59 years). After receiving the informed consent of all participants, 6 ml of venous blood in the tube containing Ethylene Diamine Tetra Acetic Acid was taken. Exclusion criteria included individuals of non-Turkmen ethnicity.

DNA extraction and human platelet antigens polymorphism genotyping

Genomic DNA was extracted from the Ethylenediaminetetraacetic acid-anticoagulated whole blood samples using QIAamp DNA blood mini kit (Qiagen, Hilden, Germany) according to the manufacturer's specifications. Genotyping of the 6 HPA polymorphisms (HPA-1 T196C, HPA-2 T524C, HPA-3 T2622G, HPA-4 G526A, HPA-5 G1648A, and HPA-15 A2108C) was performed by PCR–SSP. Two sets of primers, each comprising an allele-specific and a common primer, were employed for the recognition of each allele [Table 1]. A pair of primers for the human growth hormone was included in the PCR of each allele, to serve as an internal control for the PCR. Negative control was carried out in all PCR runs. Furthermore, more than half of the SSP-PCR results were independently confirmed by the following confirmatory tests (RFLP, PCR sequencing, and Inotrain's exclusive panels). Furthermore, more than half of the SSP-PCR results were independently confirmed by the following confirmatory tests (RFLP, PCR sequencing, and Inotrain's exclusive panels).

Statistical analysis

Statistical analysis was performed with the SPSS version 24.0 software (SPSS Inc., Chicago, IL, USA). Deviation from Hardy-Weinberg equilibrium was analyzed by Pearson's χ^2 test. The genotyping results were sorted into three groups, corresponding to the three genotypes, homozygous aa, bb, and heterozygous ab.

Results

The genotype and allele frequencies of HPA −1 to −5 and −15 in 80 unrelated Turkmen blood donors with a mean age of 38.2 ± 7.7 years (range: 18–59 years) are shown in Table 2. All results were consistent with Hardy-Weinberg equilibrium except HPA −1, −4, and −15 [Table 3, $P < 0.05$]. The highest frequency of the "b" allele was ascertained in HPA-15, followed by HPA-3, where the a/b heterozygous form was 56.25% and 50% and the b/b homozygous form was 17.5% and 11.25%, respectively. The highest frequency of "a" allele was seen in HPA-4 and HPA-1, which were found only in the form of a/a homozygous in HPA-4 and the form of a/a homozygous and a/b heterozygous at 92.5% and 7.5% in HPA-1, respectively. Our results demonstrated that while the frequency of a/a homozygous genotype in HPA-1 (92.5%), HPA-2 (77.5%), HPA-5 (78.5%) were higher than those of a/b heterozygous and b/b homozygous genotypes, the frequency of a/b heterozygous genotypes in HPA-3 (50%) and HPA-15 (56.25%) were higher than both a/a and b/b homozygotes genotypes. In opposition to other HPAs, not only a/b heterozygous genotypes but also the b/b homozygous genotypes were not identified in HPA-4 and all individuals had the a/a homozygous genotypes. Furthermore, no b/b homozygous genotypes were found in HPA-1, HPA-4, and HPA-5.

The most frequent genotypes in the studied population with a frequency higher than 2% of the population are shown in Table 4. Thirteen genotypes were found with a frequency of more than 2% of the population, which is equivalent to 83.75% of the total population. The highest frequency was the genotype of "HPA-1aa, HPA-2aa, HPA-3ab, HPA-4aa, HPA-5aa, HPA-15ab," followed by "HPA-1aa, HPA-2aa, HPA-3aa, HPA-4aa, HPA-5aa, HPA-15ab" with 18.75% and 13.75% of the total population, respectively. After the aforementioned two genotypes, the "HPA-1aa, HPA-2aa, HPA-3ab, HPA-4aa, HPA-5aa, HPA-15aa" genotype has a frequency of 10%, indicating that the first three genotypes be responsible for approximately 42.5% of the total frequency of the Turkmen population genotypes. Figure 1 shows the electrophoresis of PCR products in a 2% agarose gel.

Discussion

By knowing that ethnicity might play a role in the pattern of HPAs genotype, we run this study which is the first report on the allelic abundance of HPAs in Iranian blood donors of Turkmen ethnicity. Evaluation of the allelic prevalence of HPAs showed that the highest homozygosity rates were related to HPA-4a/a and then HPA-5a/a with frequencies of 100% and 78.5%, respectively. Furthermore, the lowest homozygosity was observed for HPA-1b/b and HPA-4b/b with a frequency of 0%.

The highest heterozygosity was related to HPA3a/b with a frequency of 56.2% and the lowest heterozygosity was related to HPA2a/b with a frequency of 20%.

Table 1: Human platelet antigens -1—-5 and human platelet antigens-15 gene primers sequence for sequence-specific primers- polymerase chain reaction

Gene	Primer	TM	Sequence
HPA-1	1a	61.5	5' TCA CAG CGA GGT GAG GCC A 3'
	1b	62.5	5' TCA CAG CGA GGT GAG GCC G 3'
	Common	56.1	5' GGA GGT AGA GAG TCG CCA TAG 3'
HPA-2	2a	63.8	5' GCC CCC AGG GCT CCT GAC 3'
	2b	62.3	5' GCC CCC AGG GCT CCT GAT 3'
	Common	59.5	5' TCA GCA TTG TCC TGC AGC CA 3'
HPA-3	3a	64.2	5' GGG GGA GGG GCT GGG GA 3'
	3b	65.7	5' GGG GGA GGG GCT GGG GC 3'
	Common	54.6	5' GGC CCT GGG ACT GTG AAT G3'
HPA-4	4a	62.3	5' CTG GCC ACC CAG ATG CG 3'
	4b	61	5' CTG GCC ACC CAG ATG CA 3'
	Common	59.8	5' GGT AGA AAG GAG CTA TAG TTT GGC 3'
HPA-5	5a	53.5	5' AGA GTC TAC CTG TTT ACT ATC AAA G 3'
	5b	54.2	5' AGA GTC TAC CTG TTT ACT ATC AAA A 3'
	Common	51.8	5' CTC TCA TGG AAA ATG GCA GTA CA 3'
HPA-15	15a	51.7	5' TTC AAA TTC TTG GTA AAT CCT CG 3'
	15b	51.6	5' TTC AAA TTC TTG GTA AAT CCT CT 3'
	Common	20.3	5' ATG AAC CTT ATG ATG ACC TAT TC 3'
HGH	Forward	57.8	5' GCC TTC CCA ACC ATT CCC TTA 3'
	Reverse	54	5' TCA CGG ATT TCT GTT GTG TTT 3'

HPA: Human platelet antigens, HGH: Human growth hormone, TM: Melting temperature

Table 2: The genotype and allele frequencies human platelet antigens -1—-5 and -15 in 80 unrelated Turkmen blood donors

HPA systems	Genotype (%)			Allele (%)	
	a/a	a/b	b/b	a	b
HPA-1	92.5	7.5	0	0.96	0.04
HPA-2	77.5	20	2.5	0.88	0.12
HPA-3	75.3	50	11.2	0.64	0.36
HPA-4	100	0	0	1	0
HPA-5	78.5	21.5	0	0.9	0.1
HPA-15	41.2	56.2	17.5	0.54	0.46

HPA: Human platelet antigens

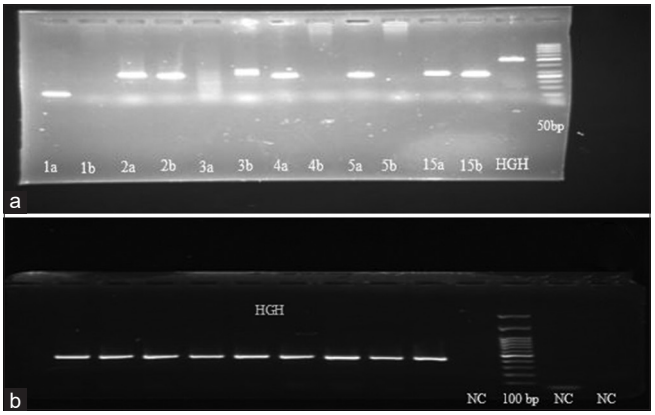

Figure 1: Polymerase chain reaction -SSP detection of HPAs systems and HGH. (a) Example of genotyping an individual for HPA-1 to-5 and-15. The HPA genotype reads as 1aa, 2ab, 3bb, 4aa, 5aa, 15ab. (b) HGH band; NC: Negative control, HGH: Human growth hormone, HPAs: Human platelet antigens

The allelic and genotypic abundance of platelet antigens −1, −4, and −15 did not match the expected frequency according to the Hardy–Weinberg equation, which is probably due to the small sample size.

The genetic distribution of HPAs genotypes in the Iranian general population was reported by *Shaiegan et al*.[3,7] The frequency of HPAs alleles in various populations in Iran is shown in Table 5. Comparison of the obtained results illustrated that there was no significant difference in the distribution of the frequency of HPAs in Turkmen donors and the Iranian general population without ethnicities taking into consideration ($P < 0.05$).

In addition, while the frequency of distribution of HPA-3 in blood donors and hematopoietic stem cell recipient/donor pairs were significantly different from other populations, the distribution of the frequency of HPA-1, HPA-4, HPA-5, and HPA-15 was almost the same in all populations, and no noticeable difference was observed. However, differences in the frequency of distribution of genes in HPA −2, −3, and −5 systems were observed in the present study and what was previously studied among blood donors in Iran. The allelic frequencies of HPA-1a and-1b in the present study were 96% and 4%, respectively, which has not shown a statistically significant difference with the allelic frequency of HPA-1 in donors.[7] The allelic frequency of HPA-2 in the present study was significantly different from what was observed in blood donors. The allelic frequency of HPA-2a in Iranian donors was reported at about 54%, which has shown a marked increase

Table 3: The evaluation of the distribution of gene frequencies of human platelet antigens -1—-5 and -15 by hardy-weinberg equilibrium test in Turkmen blood donors

Genotypes	Blood donors (n=100)		
	EN	ON	*P*
HPA-1			
aa	70.7	92.5	0.00
ab	51.1	7.5	
bb	9.2	0	
HPA-3			
aa	78.5	77.5	0.529
ab	17.9	20	
bb	1.0	2.5	
HPA-3			
aa	78.7	75.3	0.201
ab	43.2	50	
bb	5.9	11.2	
HPA-4			
aa	100	89.9	0.00
ab	0	201	
bb	0	1.1	
HPA-5			
aa	79.7	78.5	0.484
ab	19.2	21.5	
bb	1.2	0	
HPA-15			
aa	41.2	49.3	0.00
ab	56.2	40.0	
bb	17.5	8.1	

HPA: Human platelet antigens, EN: Expected number, ON: Observed number

Table 4: The most frequent genotypes and haplotypes of human platelet antigens -1—-5 and -15 in Turkmen blood donors

Genotype	*n* (%)
1aa2aa3ab4aa5aa15ab	15 (18.75)
1aa2aa3aa4aa5aa15ab	11 (13.75)
1aa2aa3ab4aa5aa15aa	8 (10)
1aa2aa3bb4aa5aa15aa	5 (6.25)
1aa2ab3ab4aa5aa15ab	5 (6.25)
1aa2aa3aa4aa5ab15aa	5 (6.25)
1aa2aa3aa4aa5aa15aa	5 (6.25)
1aa2ab3aa4aa5aa15ab	3 (3.75)
1aa2ab3ab4aa5ab15aa	2 (2.5)
1aa2ab3aa4aa5aa15aa	2 (2.5)
1aa2aa3ab4aa5ab15ab	2 (2.5)
1aa2aa3aa4aa5ab15ab	2 (2.5)
1ab2aa3ab4aa5aa15aa	2 (2.5)

to 88% in the Turkmen ethnic group. The allelic frequencies of HPA-3a and-3b in the present study were 64% and 36%, respectively, which was significantly different from the frequency of this HPA in Iranian donors.[7] More abundance of HPA-3a allele was observed in Turkmen ethnicity compared to HPA-3b allele. The allelic frequency of HPA-5b in the present study was 10%, which is higher than the frequency reported in Iranian blood donors (1%), which indicates a significant difference in the frequency distribution of this HPA in the Turkmen ethnic group compared to the Iranian donors. Furthermore, the allelic frequency of HPA-15a in Iranian donors (47%) was lower than the reported frequency in Turkmen ethnicity (54%).

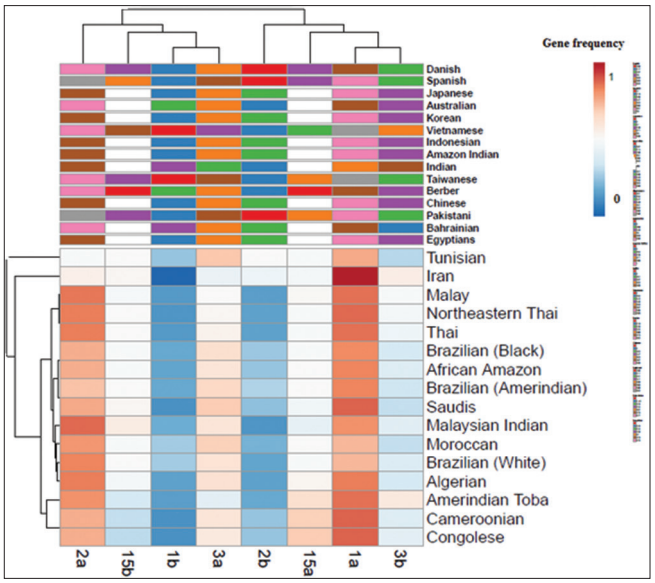

Figure 2: Heat-map showing gene frequencies of human platelet antigens −1 to −5 and −15 together with hierarchical clustering of populations based on similarity of human platelet antigens genotype distribution

To elucidate whether the frequency distribution of HPAs in Turkmen ethnicity differs from other populations, a comparison of the distribution of HPAs genotype in the present study in unrelated Turkmen blood donors with previous studies on different continents was performed which is shown in Table 6. Analysis of selected studies elucidated that among HPA − 1, −2, −3, −5, and − 15, the highest level of difference is seen in the frequency of HPA-1 with 69% in the Turkmen ethnic with other countries. Our results showed that in comparison with the Turkmen population, the frequency distributions of HPA-3, HPA-5, and HPA-2 were approximately 58%, 44%, and 41% different from other populations, respectively.

The allelic frequency of HPA-1a in this study is similar to the frequency reported in Central Asian,[8] East and Southeast Asian countries[9] such as China (100%), Thailand (98.5%), Malaysia (97.5), Japan (99. 8%), and the Philippines (98%). On the other hand, both HPA-4a and -4b alleles have been reported in some Southeast Asian countries, such as Japan.

The frequency of the HPA-5a allele in the present study (90%) was quite similar to previous studies in Pakistan[5] and did not differ significantly with the frequency distribution of this allele in Southeast Asian countries such as Malaysia.[9]

The frequency of the HPA-15 system in the present study was different from the frequency reported in previous studies in Iran[7] as well as Saudi Arabia.[2]

Table 5: The frequency of human platelet antigens -1–-5 and -15 alleles in various populations in Iran

Population	15b	15a	5b	5a	4b	4a	3b	3a	2b	2a	1b	1a	*n*
Hepatitis C	0.57	0.43	0	1	0	1	0.44	0.56	0.47	0.53	0.8	0.92	71
Donor and HSC recipient/donor pairs	0.53	0.47	0.1	0.99	0	1	0.57	0.43	0.46	0.54	0.2	0.98	210
Diverse ethnicities	0.54	0.46	0.9	0.91	0	1	0.41	0.59	0.12	0.88	0.1	0.9	300
Turkmen donor	0.46	0.54	0.1	0.9	0	1	36	0.64	0.12	0.88	0.4	0.96	80

HSC: Hematopoietic stem cell

Table 6: Gene frequencies of human platelet antigens -1–-5 and -15 in various populations

Population	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	15a	15b
Vietnamese[11]	0.98	0.01	0.95	0.04	0.48	0.51	1	0	0.97	0.02	0.47	0.52
Indian[12]	0.92	0.07	0.99	0	0.01	0.99	0.99	0	0.95	0.04	-	-
Taiwanese[13]	0.99	0	0.96	0.04	0.57	0.42	0.99	0	0.98	0.01	0.53	0.46
Han Chinese[14]	0.99	0	0.95	0.04	0.59	0.4	0.99	0	0.98	0.01	0.53	0.46
Pakistani[5]	0.88	0.11	0.92	0.08	0.69	0.31	1	0	0.9	0.1	0.59	0.41
Bahrainian[15]	0.76	0.24	0.77	0.23	0.57	0. 43	0.93	0.07	0.86	0.13	-	-
Egyptians[16]	0.76	0.23	0.75	0.24	0.7	0.29	1	0	0.72	0.27	-	-
Thai[8]	0.98	0.01	0.95	0.04	0.56	0.44	1	0	0.96	0.03	0.49	0.5
Saudis[2]	0.8	0.2	0.71	0.29	0.65	0.35	0.99	0.01	0.8	0.2	0.47	0.52
Iran (this study)	0.96	0.04	0.88	0.12	0.64	0.36	1	0	0.90	0.10	0.54	0.46
Malay[9]	0.97	0.02	0.96	0.03	0.5	0.49	0.99	0	0.95	0.05	0.51	0.48
Malaysian Chinese[9]	1	0	0.96	0.0.3	0.57	0.42	0.99	0	0.98	0.01	0.49	0.5
Malaysian Indian[9]	0.88	0.11	0.96	0.04	0.62	0.38	0.99	0	0.94	0.06	0.4	0.59
Argentinean[17]	0.87	0.12	0.87	0.12	0.61	0.38	1	0	0.92	0.07	0.51	0.48
Amerindian Toba[17]	1	0	0.94	0.05	0.38	0.61	1	0	1	0	0.68	0.31
Algerian[18]	0.83	0.16	0.83	0.16	0.62	0.37	1	0	0.83	0.15	0.53	0.47

To investigate the similarity of the HPAs frequency distribution in the present study and other studies, hierarchical population clustering is shown in Figure 2. This diagram shows that the Iranian ancestors are more like the Indo-Aryan ancestors.

Considering that the frequency of HPAs polymorphisms regardless of the ethnicity has been studied in Iran so far,[3,5,7,10] the existence of such differences in the frequency of platelet antigen systems in the present study (Turkmen ethnicity) with other studies in the country can be attributed to racial differences that need further investigation in this regard.

Conclusion

Determining the genotype of platelet antigens might play an important role in PTR management, thus can improve the outcome of patients with alloimmunization due to the incompatibility of HPAs between the receptor and the donor. Therefore, the registration process for national platelet donors (based on demographic information and the specific genotype of each ethnicity) can help patients accelerate and improve the quality of transfused platelets.

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Conflicts of interest

There are no conflicts of interest.

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