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Frequency of Human Platelet Antigens (HPA-2/3/5) Polymorphism in Iranians Evaluated by RFLP-PCR

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Abstract

Background: Knowing the human platelet antigens (HPA) and genes frequency in different populations is important not only for population studies but also for clinical transfusion practice. HPA genes frequency in Iran is not evaluated, so we investigated the HPA-2, HPA-3, and HPA-5 frequency and their polymorphism.

Materials and Methods: DNA from 120 Iranians (99 Iranian blood donors and 21 patients with platelet refractoriness) was isolated from peripheral blood mononuclear leucocytes and tested by restricted fragment length polymorphism analysis (RFLP).

Results: The frequency of HPA phenotypes was determined as follows: (in donors) HPA-2a/2a:75.4%, HPA-2a/2b:24.8%, HPA-3a/3a:8.1%, HPA-3a/3b:70.7%, HPA-3b/3b:21.2%, HPA-5a/5a:99 % and HPA-5a/5b:1%; (in patients) HPA-2a/2a:66.7%, HPA-2a/2b:33.3%, HPA-3a/3a:23.8%, HPA-3a/3b: 47.6%, HPA-3b/3b:28.6%, HPA-5a/5a:90.5%, and HPA-5a/5b: 9.5%. Frequency of genotypes evaluated by RFLP were as below: HPA-2a=0.87, HPA-2b=0.13, HPA-3a =0.28, HPA-3b=0.72, HPA-5a=0.99, and HPA-5b=0.01 in both donors and patients.

Conclusion: HPA gene frequencies observed in Iranians were in the range obtained in previous studies but not exactly equal to other populations. It might be due to their relations with other populations. There was not any significant difference between two groups in this study.

Keywords: Blood donor, Human platelet antigens (HPA), Polymerase chain reaction, Restricted fragment length polymorphism analysis, Iran.

Introduction

The plasma membrane of platelets is composed of many glycoproteins (GP) and phospholipids. The major platelet glycoproteins are GPIIb-IIIa, GPIa-IIa, and GPIb-IX.[1] Several distinct platelet specific alloantigen systems have been described.[2] Twenty four HPAs have been identified serologically, and the molecular basis of 22 of these is determined (table 1).[3] The frequency of HPAs vary among different populations.[4]

A single base pair substitution leads to a single amino acid difference in the respective glycoprotein (GP). Twelve HPAs are grouped into six biallelic systems (HPA-1a/1b, 2a/2b, 3a/3b, 4a/4b, 5a/5b, and 15a/15b). The higher frequency allele is designated as "a" (such as HPA-1a) and the lower frequency allele "b" (such as HPA-1b). Human platelet alloantigens (HPA) are involved in different conditions such as neonatal alloimmune thrombocytopenia (NAIT), posttransfusion purpura (PTP), platelet transfusion refractoriness, passive alloimmune thrombocytopenia, idiopathic thrombocytopenic purpura (ITP), drug-induced immune thrombocytopenia (DITP), and transplantation-associated alloimmune thrombocytopenia (3,6). Recently, it has been suggested that HCV infection can induce autoimmunity in platelets, and may be carried by some receptors on platelets. There is an association between this infection and HPA-5b allele.[2]

Historically, platelet typing was performed by phenotyping using reference antisera of human origin. Since development of molecular biology

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techniques, phenotyping has been abandoned by most laboratories. Currently, most laboratories perform DNA extraction from whole blood and genotyping by polymerase chain reaction-sequence-specific primers (PCR-SSP) using two primer pairs (one specific allele primer and a common amplifying primer). The PCR-SSP is the most common genotyping method. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) is the oldest genotyping method and is used to solve interpretation problems. After amplification of DNA fragments and specific enzymatic cleavage, electrophoresis of fragments produces a characteristic banding pattern. During recent years, an increasing number of new methods have been developed for analysis of polymorphisms, based on new technologies such as chips or microarrays.[1,3]

Human platelet antigen (HPAs) frequencies vary between different populations. Knowing HPA frequency in different populations is important and necessary as HPA genotyping is essential in diagnosis and treatment of several conditions.[3] Platelet transfusion is widely used in Iran but no report on HPA prevalence has previously been published in Iranian population. As the frequency of human platelet antigen (HPA) systems vary between different ethnic groups,[7] the purpose of this study was to determine HPA-2/3/5 gene frequency in a group of Iranian population by PCR-RFLP.

Materials and Methods

After obtaining a written consent form, blood samples were collected from 120 Iranians (99 healthy blood donors in Tehran blood center and 21 patients with platelet refractoriness in Arad hospital) and stored in EDTA tubes. Corrected count increment (CCI) was measured as a criterion for defining platelet refractoriness. A CCI above 7500 u/L indicates an adequate response to platelet transfusion.[8]

CCI= (Post-transfusion platelet count – Pretransfusion platelet count) * Body area (m2)/ Number of transfused platelets (multiples of 10^{11})

DNA was extracted from 5mL of whole blood using the DNA extraction kit (Qiagen). Primers for HPA-2, HPA-3, HPA-5 were made and ingternal control was performed in Iranian blood transfusion organization (IBTO) laboratory. Sequences of specific primers are shown in table 2. PCR was performed as described by Metcalfe in NIBSC,[9] with some modifications in our laboratory. Two

Table 1. Human platelet antigens- 1-5 [5]

System	Antigen	Original names	Glycoprotein	CD marker
HPA-1	HPA-1a	Zw , Pl	GPIIIa	CD61
	HPA-1b	Zw , Pl		
HPA-2	HPA-2a	Ko	GPIb	CD42b
	HPA-2b	Ko , Sib		
HPA-3	HPA-3a	Bak , Lek	GPIIb	CD41
	HPA-3b	Bak		
HPA-4	HPA-4a	Yuk , Pen	GPIIIa	CD61
	HPA-4b	Yuk ,Pen		
HPA-5	HPA-5a	Br , Zav	GPIa	CD49b
	HPA-5b	Br , Zav, Hc		

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µL of DNA (about 100µg/ml) was subjected to amplification using Corbett research thermal cycler. The final reaction mixture contained 0.07 µL Taq polymerase (Roche,1000Units), 1 µL 10x reaction buffer, 0.2 µL dNTP (Roche, each dNTP 2.5mM) in 5µL primer mixture buffer, and 1.8µL nuclease free distilled water in addition to DNA (total volume =10.07µl). PCR procedure was performed under the following conditions: 1 cycle, 96 oC for 60 s; 5 cycles, 96oC for 25 s, 68oC for 45 s, 72oC for 30 s; 20 cycles, 96oC for 25 s, 61oC for 45 s, 72oC for 30s; 15 cycles, 96oC for 25 s, 51oC for 1 minute, 72oC for 2 minutes; and 1 cycle, 4oC for 3 minutes. Restriction enzymes were purchased from New England Biolabs (SfaNI and Fok-1) and Fermentase (MNl 1). Polymerase chain reaction (PCR) products were incubated overnight with SfaNI for HPA-2 and Fok I for HPA-3 at 37oC, and with MNl1 for HPA-5 at 55oC. RFLP master mixtures are summarized in table 3. Digested products were analyzed by 3% high resolution agarose lectrophoresis (Sigma, Biotools (and stained with ethidium bromide. Several samples were characterized by Metcalfe laboratory in the UK to validate our method and results. Observed genotype frequencies were estimated by direct counting. The data were tested using Hardy-Weinberg equilibrium. Chi-square and Z tests were used to compare HPA gene frequencies

Table 2. The sequence of the HPAs primers

Sequences	Length (mer)	Antigen	System
5`GCCCCCAGGGCTCCTGAC3`	18	2a	
5`GCCCCCAGGGCTCCTGAT 3`	18	2b	**HPA-2**
5` TCAGCATTGTCCTGCAGCCA 3`	20	common	
5` TGGACTGGGGGCTGCCCAT 3`	19	3a	
5` TGGACTGGGGGCTGCCCAG 3`	19	3b	**HPA-3**
5` TCCATGTTCACTTGAAGTGCT 3`	21	common	
5`AGAGTCTACCTGTTTACTATCAAAG 3`	25	5a	
5` AGAGTCTACCTGTTTACTATCAAAA 3`	25	5b	**HPA-5**
5` CTCTCATGGAAAATGGCAGTACA 3`	23	common	

Table 3. RFLP Master Mix

Enzyme	PCR Product	BSA	Distillated Water	Buffer	System
SfaNI-(0.8 µl)	13.2 µl	_	4 µl	2 µl (10X NEBUFFER)	**HPA-2**
BseGI(FokI)-(0.8 µl)	13.2 µl	_	4µl	2 µl (10X with BSA BUFFER)	**HPA-3**
MNl1-(0.8 µl)	13 µl	0.2 µl	4 µl	2 µl (10X NEBUFFER)	**HPA-5**

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between the patient and control groups, and with other populations.

Results

Samples were collected from 120 Iranians (20% female and 80% male). Mean age of the participants was 40.1±10 years (range: 20 to 70 years). The frequency of HPA phenotypes and genes in both groups are shown in table 4. There was no significant difference in gene frequency between two groups. There is no significant deviation from the Hardy–Weinberg equilibrium for HPA-2 and HPA-5, but observed frequencies for HPA3a/b were not as expected. There is no relationship between refractoriness and HPA gene in the patients group.

Discussion

The Allele gene frequency of HPA-2/3/5 obtained from 120 unrelated Iranians are shown in comparison with other populations in table 5. HPA-2/-5 a and b allele frequencies are in the ranges obtained in previous studies but HPA-3a and 3b seem to be different.

In the present study, we could not find any homozygosity for b alleles of HPA-2b/b and HPA-5b/b. Sun et al also reported absence of HPA b/b form for HPA-1/2/5/6 in 148 random unrelated blood donors.[10] Absence of homozygosity for some other alleles was reported in other studies.[15,19]

Our data showed that HPA-3b gene frequency (0.72) is more than HPA-3a (0.28). Kalkarni et al and Shih et al reported higher frequency of homozygote b forms of HPA-3 and HPA-15 in Indians and Filipinos, respectively.[20,21] The frequency of HPA-3a and -3b were reported to be from 0.50 to 0.61 and 0.38 to 0.50, respectively.[15]

Statistical tests showed our results were different from Korean people (HPA-2, P< 0.05) and Dutch people (HPA–2/5, P< 0.05).

In Parsi people who migrated from Iran to India

Table 4. Frequency distribution Hman platelet antigen (HPA) genotyping and Allele gene in donor and patients

HPA System	Genotyping & Allele Frequency	Both Groups N=120	Donors N=99	patients N=21
HPA -2	2a/a	74%	75.5%	66.7%
	2a/b	26%	24.5%	33.3%
	2a	0.87	0.88	0.83
	2b	0.13	0.12	0.17
HPA -3	3a/a	10.8%	8.1%	23.8%
	3a/b	66.7%	70.7%	47.6%
	3b/b	22.5%	22.5%	28.6%
	3a	0.28	0.26	0.38
	3b	0.72	0.74	0.62
HPA -5	5a/a	98.3%	99%	90.5%
	5a/b	1.7%	1%	9.5%
	5a	0.99	0.995	0.98
	5b	0.01	0.005	0.02

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Table 4. Frequency distribution of HPAs in different population

Populations (sample size)	HPA-2 alleles		HPA-3 alleles		HPA-5 alleles		Reference
	2a	2b	3a	3b	5a	5b	
Dutch (N=98)	0.93	0.07	0.55	0.45	0.90	010	1993;Simsek *et al.*(11)
Finns (N=200)	0.91	0.09	0.59	0.41	0.95	0.05	1995;Kekomaki *et al.*
Austrian (N=900)	0.92	0.08	0.61	0.39	0.89	0.11	1995;Holensteiner *et al.*
African Amazon (N=100)	0.82	0.18	0.63	0.37	0.79	–	*1995;Kim et al*
Japanese (N=331)	–	–	–	–	–	–	1996;Tanaka *et al.* (12)
Korean (N=200)	0.92	0.08	0.55	0.45	0.98	0.02	1998;Seo *et al.* (13)
Spanish (N=500)	0.90	0.10	0.65	0.35	0.88	0.12	1998;Muñiz-Diaz *et al.*
Amazon Indian (N=95)	0.96	0.04	0.71	0.29	0.96	0.04	*2000;Chiba et al (14)*
Berber (N=110)	0.82	0.18	0.68	0.32	0.86	0.14	*2002;Ferrer et al (15)*
Croatian (N=279)	–	–	–	–	–	–	2006;Tomicic et al (16)
Bahrainian (N=194)	0.77	0.23	0.57	0.43	0.86	0.13	2007;Al-Subaie et al (17)
Algerian (N=485)	0.85	0.15	0.58	0.42	0.91	0.09	2010;Brouk et al (18)
Iranians (N=120)	0.87	0.13	0.28	0.72	0.99	0.01	***Present study***

almost 13 centuries ago, HPA-2a/2a, HPA3b/3b, HPA-5a/5a, and HPA-5a/5b were seen in 100%, 100%, 81%, and 19%, respectively.[19] But in our study HPA-2a/2a, HPA-3b/3b, HPA-5a/5a, and HPA-5a/5b were found in 74%, 22.5%, 98.3%, and 1.7%, which is significantly different from the previous study ($P< 0.05$). This difference may be due to inbred community of Parsis in India.

The investigation of gene and genotype frequencies of human platelet alloantigens is important not only for population and genetic studies, but also for clinical trawnsfusion practice where HPA-typed platelets may be required for alloimmunized patients and the most probable alloantigens and antibodies involved in this phenomenon, especially antibodies against human platelet antigens e.g. HPA-1a, 3a, 4a, 5a, and Gova, could be defined.[7,15,22]

Conclusion

Some HPA gene frequencies observed in Iranian blood donors were different from the values reported in other populations. It might be due to their ethnic relations with other populations. We did not find the refractoriness reason in the patient group and could not find any significant difference between our two study groups. These data may suggest the reason of platelet refractoriness in the patient group is not only related to HPA antigen mismatching and antibodies. This study is the first report of the allele frequencies of HPAs (HPA-2, -3, and -5) in an Iranian group using the PCR-RFLP method. These results could be useful in future for population and genetic studies and clinical applications. More investigations are recommended about HPA-genotyping in different tribes of country or different diseases.

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