STUDY THE FREQUENCY OF HUMAN PLATELET ANTIGEN-1 IN EGYPTIAN THROMBOCYTOPENIC PATIENTS WITH HEMATOLOGIC DISORDERS

Wafaa Ahmed EL-Neanaey, Dalia Ahmed Nafea*, Salma AlaaEl-din Imbaby, Amany Samy Abdel-latif Abd l-Magid

Department of Clinical and Chemical Pathology, Department of Internal Medicine*, Faculty of medicine, Alexandria university

Introduction

Platelets are active cells, which assist in hemostasis and blood coagulation. Their glycocalyx contains glycoproteins (GP) molecules through which platelet bind to other cells to perform their functions. The GPs (GPIa, GPIb, GPIIb and GPIIIa,) are produced by substitutions of single amino acid. Human platelet antigens (HPAs) are polymorphic amino acid sequences found on these GPs GPIIIa is considered the most polymorphic glycoprotein and bears HPA-1, HPA-4, and HPA-6. HPA-1 is defined as the most important antigenic system. HPA-1a or HPA-1b antigens are due to the existence of leucine or proline at position 33 of the GPIIIa where the "a" represents the common allele and the "b" represents the rare allele. HPA are associated with alloimmunization as they are the targets for platelet antibodies that lead to platelet destruction in post-transfusion refractoriness (PTR) and purpura (PTP) after platelet transfusion. Patients who are treated for hematological diseases associated with thrombocytopenia are highly vulnerable to platelet refractoriness when treated by prophylactic concentrated platelet transfusions to prevent bleeding. Consequently, accurate donor compatibility for platelet transfusions is very essential.

Aim of the work

Our study aimed at evaluating the allele &genotype frequency of HPA-1 among Egyptian healthy donors & thrombocytopenic patients with hematological disorders & to investigate the potential association between specific antigens and risk for alloimmunization and refractoriness to platelet transfusions.

Patients and Methods

This study was carried out on 50 thrombocytopenic patients with hematological disorders with platelet count < 20,000 µL indicated for random donor platelet transfusion and 50 random Egyptian blood donors. Cases and donors were subjected to history taking, clinical examination and routine investigations including (CBC, Blood group phenotyping and Virology: HBV, HCV, HIV). Detection of HPA-1 genotypes was done using PCR–restriction fragment length polymorphism (RFLP-PCR) using restriction enzyme MspI. Investigations: 1. Peripheral blood samples were obtained 2. According to the manufacturer's protocol, total DNA was purified by using the QIAamp DNA Blood Mini Kit, (QIAGEN, Germany). 3. DNA amplification using PCR technique was first performed using Thermo Scientific DreamTaq Green master.A PCR product of 193 base pairs (bp) was generated. 4. The amplified PCR products were digested with MspI enzyme with 10X Fast Digest Green Buffer (Thermo Scientific) at 37ºC for 5 minutes. 5. The digested products were applied to gel electrophoresis in a 2% agarose gel (125V for 35 minutes. 6. Genotypes were determined as follows: HPA-1 aa genotype [193base pair (bp)], bb (161 bp), and ab (193, 161 bp).

Results

Genotype and Allele frequencies among hematological patients and blood donors The Allele and Genotype frequencies obtained for Human Platelet Antigen-1 and the Hardy–Weinberg p-values in hematological patients and blood donors are shown in Table 10. The frequencies of hematological patients were 77% and 23 % for 1a and 1b respectively. While, frequencies of blood donors were 84% and 16 % for 1a and 1b respectively. The frequency for homozygous genotype HPA-1a/a (58%) was higher than the heterozygous genotype HPA-1a/b (38%) and only two with HPA-1b/b (4%) in hematological patients. In blood donors, the frequency of homozygous genotype HPA-1a/a (72%) was higher than the heterozygous genotype HPA-1a/b (24%) and only two with HPA-1b/b (4%). Frequencies for alleles 1a and 1b were 80.5% and 19.5 % respectively in both patient and donors as Egyptian population. The frequency of homozygous genotype 1a/a (65%) was higher than those of heterozygous genotype 1a/b (31%) and only four with 1b/b (4%) in both patient and donors as Egyptian population.

Table (1):Comparative analysis of Allele and Genotype Frequencies among hematological patients and blood donors.

	Hematological patients& Blood donors (n = 100)		Hematological patients (n = 50)		Blood donors (n = 50)			
Genotypes	**No.**	**%**	**No.**	**%**	**No.**	**%**	χ^2	**p**
aa	65	65	29	58	36	72	2.154	0.142
ab	31	31	19	38	12	24	2.291	0.130
bb	4	4	2	4	2	4	0.0	^{FE}p =1.0
	$^{HW}\chi^2$		0.265		0.574		χ^2	p
	p		0.606		0.449			
Allele	**No.**	**%**	**No.**	**%**	**No.**	**%**	1.561	0.212
a	161	80.5	77	77	84	84		
b	39	19.5	23	23	16	16		

The probability of incompatibility for aa and bb genotypes of HPA-1 between hematological patients and blood donors. Table (2) show the mismatch probabilities among homozygous hematological patients (aa and bb) considering the blood donors genotype frequencies. The probability of incompatibility for ab genotype patients is considered minimal or zero as both alleles are present. The probability of incompatibility for aa genotype patients using the formula (IN aa= F ab+ F bb) was 28%and probability of incompatibility for bb genotype of hematological patients using the formula (IN bb =F ab+ F aa) was 96% considering the frequencies of donor genotypes, Fab = blood donors ab genotype frequency, F aa = blood donors aa genotype frequency, F bb = blood donors bb genotype frequency, IN aa = aa hematological patients probability of incompatibility, IN bb = bb hematological patients probability of incompatibility as shown by Bianchi et al. (2012)

Table (2) :The probability of incompatibility for aa and bb genotypes of HPA-1 between hematological patients and blood donors.

and blood	Blood donors (n = 50)	
	No.	**%**
Genotype		
aa	36	72.0
ab	12	24.0
bb	2	4.0

Mismatch in aa patients	Confidence Interval 95%		Mismatch in bb patients	Confidence Interval 95%	
IN aa= F ab+ F bb	**LL**	**UL**	**IN bb =F ab+ F aa**	**LL**	**UL**
28% (0.28)	**0.15**	**0.41**	**96% (0.96)**	**0.90**	**1.02**

F ab = frequency for the ab genotype of blood donors; F aa = frequency for the aa genotype of blood donors; F bb = frequency for the bb genotype of blood donors; IN aa = probability of incompatibility in aa of hematological patient; IN bb = probability of incompatibility in bb hematological patients. LL=Lower Limit, UL=Upper Limit

Conclusion

An approximately, HPA-1b allele high frequency among Egyptian population may denote high risk of alloimmunization. Platelet genotyping *would be extremely helpful* for investigation of allo-immune thrombocytopenia and for delivering HPA matched platelet concentrates.