Alloantibodies to glycoprotein Ia/IIa (anti-HPA-5a and -5b) and IIb/IIIa (anti-HPA1a, -3a and -4a) in Nigerian parous women

Z. A. JEREMIAH*, J. E. OBURU†, O. ERHABOR* and F. I. BUSERI*

Haematology and Blood Transfusion Science Unit, Department of Medical Laboratory Sciences, College of Health Sciences, Niger Delta University; and †Department of Haematology and Blood Transfusion, University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria

Accepted: 27 July 2010

ABSTRACT

Human platelet antibodies are often implicated in conditions such as neonatal alloimmune thrombocytopenia (NAIT), idiopathic thrombocytopenic purpura (ITP) and platelet refractoriness; however, the frequency of such alloantibodies has not been reported in Nigeria and West Africa. A cross section of apparently healthy adult female staff at a tertiary health facility in the Niger Delta, Nigeria, was screened for alloantibodies to human platelet antigens (HPA) using the GTI PakPlus qualitative solid-phase enzyme-linked immunosorbent assay (ELISA) method. Among the 100 women screened, no anti-glycoprotein IIb/IIIa (anti-HPA-Ia,-3a and -4a) antibodies were detected; however, prevalence of anti-glycoprotein Ia/IIa (anti-HPA-5b) was 30% and pf anti-glycoprotein Ia/IIa (anti-HPA-5a) was 18%. Parity had a significant influence on the development to HPA antibodies (Fisher's Exact test: 11.683, $P<0.05$; 13.577, $P<0.01$). Platelet count did not have an influence on the development of antibodies ($P>0.05$). Clearly, there is need to initiate platelet serology in this setting and also a need to educate women about the risk associated with frequent pregnancies. Furthermore, caution should be exercised when recruiting parous women as blood donors

KEY WORDS: Blood platelets.
Isoantibodies.
Pregnancy.

Introduction

Glycoprotein IIb/IIIa is an integrin receptor of the α-IIbβ3 complex found on the platelet surface. It is also a receptor for fibrinogen and aids platelet activation. The complex is formed via calcium-dependent association of GPIIb and GPIIIa, a necessary step in normal platelet aggregation and endothelial adherence.[1,2] Antibodies to GPIIb/IIIa have been linked to most cases of chronic idiopathic thrombocytopenic purpura (ITP) and also to many cases of drug-induced immune thrombocytopenia.[3]

The glycoprotein GPIa/IIa (HPA-5b) is an integrin of α2β1, which is distributed widely on different cell types and can mediate adhesion to collagen.[4] The HPA-5 system has been recognised as the second most frequent cause of neonatal alloimmune thrombocytopenia (NAIT).[5] Anti-HPa-5 has been implicated in approximately 20% of serologically confirmed cases of NAIT as well as post-transfusion purpura.

In the Nigerian setting, cultural beliefs encourage frequent pregnancy and it is hypothesised in this study that this could lead to a high prevalence of anti-HPA antibodies. This may contribute to a high incidence of NAIT, platelet refractoriness and other conditions associated with platelet antibodies. There is little information on anti-HPA antibodies among parous women in Nigeria and West Africa, and this study also aims to determine the frequency of some HPA antibodies and their risk factors among this female population.

Materials and methods

The study population comprised adult women who had given birth to at least two children and had not been pregnant for at least a year prior to the study. This was confirmed by use of a one-step pregnancy test (Global one-step rapid hCG test, USA). They were volunteers recruited from among the staff of the University of Port Harcourt Teaching Hospital (UPTH). Age range was 26–59 years (mean: 40.6 years), while number of pregnancies ranged from two to 11. The study received institutional ethical approval from the Department of Medical Laboratory Sciences, Rivers State University of Science and Technology, Port Harcourt, Nigeria. Written informed consent was obtained from each participant.

Venous blood (4 mL) was collected from each participant into ethylenediaminetetraacetic acid (EDTA) for platelet counts. Serum was obtained from a clotted blood sample in a plain tube for platelet serology studies.

The GTI PakPlus kit (GTI Diagnostics, Waukesha, USA) was used for the detection of platelet antibodies, and was performed according to the manufacturer's instructions. Briefly, 50 µL control or test serum (1 in 2 dilution using specimen diluent solution) was added to duplicate wells in a

Correspondence to: Dr. Zaccheaus A. Jeremiah
Haematology and Blood Transfusion Science Unit, Department of Medical Laboratory Sciences College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria
Email: zacjerry@yahoo.com / drzajeremiah@yahoo.com

Table 1. Demographic characteristics of the 100 study participants.

Characteristics	Frequency (%)
Age group (years)	
25–34	28.0
35–44	40.0
45–54	26.0
≥55	6.0
Parity	
2	18.0
3	28.0
4	26.0
5	12.0
6	8.0
8	4.0
9	2.0
11	2.0
Ethnicity	
Ijaw	32.0
Ikwere/Etche	26.0
Ekpey/Ogba	16.0
Igbo	14.0
Yoruba	6.0
Akwa-Ibom	6.0

micro test plate and incubated for 40 min at 37°C. The plate was washed (x3) with 225 µL wash solution per well and 50 µL alkaline phosphatase-conjugated, affinity purified goat antibody to human immunoglobulin (1 in 100 dilution) was added to each well. After incubation for 40 min at 37°C and three additional washes, 100 µL ρ-nitrophenyl phosphate solution (1 in 100 dilution in enzyme substrate buffer) was added and the mixture was incubated in the dark at room temperature (18–22°C). The reaction was stopped after 30 min by the addition of 100 µL ELISA stop solution, and the absorbance of each well was measured at 405 nm in an ELISA plate reader (STAT FAX 2100, Awareness Technology, USA). Test wells producing an absorbance (*A*) reading equal to or greater than twice the mean *A* of the negative control wells were regarded as positive. Whenever the *A* reading of either of the duplicate test wells exceeded 20% of the mean *A* of the two wells then the test was considered invalid and was repeated.

Platelet counts were performed using the ICSH-approved procedure using 1% ammonium oxalate reagent.

Table 2. Frequency of antiglycoprotein and antiplatelet antibodies.

Antiglycoproteins	Anti-HPA antibodies	Positive (%)
GPIIb/IIIa	Anti-HPA-1a	
	Anti-HPA-3a	0 (0.0)
	Anti-HPA-4a	
GPIa/IIa	Anti-HPA-5b	30 (30.0)
	Anti-HPA-5a	18 (18.0)

Statistical analysis

Statistical analysis was performed using a statistical software package (SPSS version 12.0 for Windows, Chicago, USA). Results were expressed as mean (±SD) and ANOVA for descriptive statistics. Non-descriptive statistics were expresses as a frequency and Fisher's Exact Test was used to test significance. $P \leq 0.05$ was regarded as significant.

Results

Table 1 shows the demographic characteristics of the study participants. Frequencies of anti-HPA antibodies are shown in Table 2. Mean age, parity and platelet count results are shown in Table 3. Age and ethnicity were not found to exert any influence on the prevalence of antiplatelet antibodies. (Tables 4 and 5) while parity was found to exert a significant influence on the prevalence of anti-HPA-5b (Fisher's Exact Test: 11.638, $P<0.05$) and anti-HPA-5a (Fisher's Exact Test: 13.577, $P<0.01$). No significant relationship was established between platelet count and antiplatelet antibodies ($P>0.05$)

Discussion

This study investigated the frequency of alloantibodies to HPA antigens in Nigerian women with a history of frequent pregnancies. The main findings of the study include: i) a high prevalence of anti-HPA-5b; ii) the significant influence of the number of previous pregnancies on the occurrence of anti-HPA-5b and anti-HPA-5a; and iii) the complete absence of anti-HPA-1a on the GPIIb/IIIa complex.

These findings are consistent with studies of maternal HPA alloimmunisation in populations in Tunisia, Austria and the United States.[6,7–11] In these countries, anti-HPA-5b had the highest prevalence, whereas the presence of anti-HPA-1a

Table 3. Mean age, parity and platelet count.

	Mean (SD)	Minimum	Maximum
Age (years)	40.64 (8.4)	25	59
Parity	4.0 (1.9)	2	11
Platelet count (x10⁹/L)	204.6 (35.6)	129	294

Table 4. Influence of age on the prevalence of antiplatelet antibodies.

	Anti-HPA-1a Anti-HPA-3a Anti-HPA-4a	Anti-HPA-5b	Anti-HPA-5a
Age group (years)	Pos(%)	Pos(%)	Pos(%)
25–34	0 (0.0)	8 (28.6)	2 (7.1)
35–44	0 (0.0)	6 (15.0)	8 (20.0)
45–54	0 (0.0)	14 (53.8)	8 (30.0)
≥55	0 (0.0)	2 (33.3)	0 (0.0)
Fisher's Exact Test	–	5.679	2.729
P value	–	0.117	0.386

$P \leq 0.05$ regarded as significant.

Table 5. Influence of ethnicity on the prevalence of antiplatelet antibodies.

	Anti-HPA-1a Anti-HPA-3a Anti-HPA-4a	Anti-HPA-5b	Anti-HPA-5a
Ethnicity (n=100)	Pos (%)	Pos (%)	Pos (%)
Ijaw	0 (0.0)	12 (37.5)	4 (125)
Ikwerre/Etche	0 (0.0)	8 (30.8)	8 (30.8)
Ekpeye/Ogba	0 (0.0)	0 (0.0)	0 (0.0)
Igbo	0 (0.0)	4 (28.6)	4 (28.6)
Yoruba	0 (0.0)	4 (66.7)	0 (0.0)
Akwa-Ibom	0 (0.0)	2 (33.30)	2 (33.30)
Fisher's Exact Test	–	6.246	5.006
P value	–	0.251	0.357

$P \leq 0.05$ regarded as significant.

Table 6. Influence of parity on the prevalence of antiplatelet antibodies.

	Anti-HPA-1a Anti-HPA-3a Anti-HPA-4a	Anti-HPA-5b	Anti-HPA-5a
Parity	Pos (%)	Pos (%)	Pos (%)
2	0 (0.0)	0 (0.0)	2 (11.1)
3	0 (0.0)	6 (21.4)	0 (0.0)
4	0 (0.0)	8 (308)	6 (23.1)
5	0 (0.0)	9 (50.0)	2 (16.7)
6	0 (0.0)	4 (50.0)	4 (50.0)
8	0 (0.0)	2 (50.0)	0 (0.0)
9	0 (0.0)	2 (100.0)	2 (100.0)
11	0 (0.0)	2 (100.0)	2 (100.0)
Fisher's Exact Test	–	11.683	13.577
P value	–	0.05	0.01

$P \leq 0.05$ regarded as significant.

Table 7. Relationship between platelet count and antiplatelet antibodies

		Platelet count x 10^9/L		
	Mean	Mean square	F value	*P* value
GPIIb/IIIa				
Anti-HPA-1a				
Anti-HPA-3a	None detected			
Anti-HPA-4a				
GP Ia/IIa				
Anti-HPA-5b	Pos (217.33) Neg (199.26)	3430.86	2.805	0.1
Anti-HPA-5a	Pos (222.67) Neg (200.73)	3550.83	2.909	0.095

$P \leq 0.05$ regarded as significant.

was rare in Caucasians, occurring in one to two cases among 500–1000 pregnant women.[12–14] Thus, the absence of anti-HPA-1a in the present study could be due to the relatively small sample size. Taken together, the results of this study show that the pattern of maternal platelet alloimmunisation among Nigerian women is similar to that in other parts of the world.

One of the most serious consequences of the high prevalence of anti-HPA-5b is the possibility of NAIT. Anti-HPA-5 antibodies are known to be less immunopathogenic than anti-HPA-1a antibodies, and most cases of passive thrombocytopenia reported in the literature follow transfusion of blood products containing anti-HPA-1a antibodies.[15,16] However, a case of transient and moderately severe thrombocytopenia caused by passive transfusion of plasma containing anti-HPA-5b antibodies has been reported.[17] Therefore, it appears that most cases of NAIT among Caucasians are caused by anti-HPA-1a, the frequency of which was low in the African population studied here.

Despite the fact that NAIT has not been reported in Nigeria, it is possible that some cases of mild to moderate neonatal thrombocytopenia caused by anti-HPA-5b and -5a have occurred but have not been identified, perhaps due to lack of platelet serology in local hospitals. This issue needs to be addressed and caution should be exercised when recruiting parous women as blood donors. □

The authors thank GTI Diagnostics for support towards the provision of GTI PakPlus reagent kits, and the female staff of UPTH who volunteered to take part in this study.

References

1 Hato T, Ginsberg MH, Shattil SJ. Integrin alpha 2 beta 3. In: Michelson AD ed. *Platelet*. San Diego: Academic Press, 2002: 105–7.
2 Zou Z, Chen H, Schmaier AA, Hynes RO, Kahn ML. Structure-function analysis reveals discrete beta3 integrin inside-out and outside-in signaling pathways in platelets. *Blood* 2007; **109**: 3284–90.
3 Aster RH, Newman PJ. HPA-1a/b (PIA1/A2,Zwa/b): the odyssey of an alloantigen system. *Immunohematology* 2007; **23**: 2–8.
4 Barnes MJ, Knight CG, Farndale RW. The collagen-platelet interaction. *Curr Opin Hematol* 1998; **5**: 314–6.
5 Kaplan C, Morel-Kopp MC, Kroll H *et al.* HPA-5b (Br[a]) neonatal alloimmune thrombocytopenia: clinical and immunological analysis of 39 cases. *Hematology* 1991; **78**: 425–7.
6 Christie DJ. Pulkrabek S, Putnam JL, Slatkoff ML, Pischel KD. Posttransfusion purpura due to an alloantibody reactive with glycoprotein Ia/IIa (anti-HPA-5b). *Blood* 1991; **77**: 2785–9.
7 Ranzer S, Auerbach L, Cechova E *et al*. Maternal alloimmunization against fetal platelet antigens: a prospective study. *Br J Haematol* 1995; **90**: 655–60.
8 Kiefel V, Konig C, Kroll H, Santoso S. Platelet alloantibodies in transfused patients. *Transfusion* 2001; **41**: 766–70.
9 Kjeldsen-Kragh J, Killie MK, Tomter G *et al*. A screening and intervention program aimed to reduce mortality and morbidity associated with neonatal thrombocytopenia. *Blood* 2007; **110**: 833–9.
10 Bussel JB, Zabusky MR, Berkowitz RL, McFarland JG. Fetal alloimmune thrombocytopenia. *N Engl J Med* 1997; **337**: 22–6.

11 Yinon Y, Spira M, Solomon O *et al*. Antenatal noninvasive treatment of patients at risk for alloimmune thrombocytopenia without a history of intracranial hemorrhage. *Am J Obstet Gynecol* 2006; **195**: 1153–5.
12 Kjeldsen-Kragh J, Kim M, Killie MK, Husebelek A, Freedman HJ, Semple JW. In HPA 1a- immunized women the decrease in anti-HPA-1a antibody level during pregnancy is not associated with anti-idiotypic antibodies. *Haematologica* 2009: **94**: 441-3.
13 Killie MR, Husebelek A, Kjeldsen-Kragh J, Skogen B. A prospective study of maternal anti-HPA-1a antibody level as a potential predictor of alloimmune thrombocytopenia in the newborn. *Haematologia* 2008; **93**: 870–7.
14 Schnaidt M, Wernet D. Platelet-specific antibodies in female blood donors after pregnancy. *Transfus Med* 2000; **10**: 77–80.
15 Scott EP, Moilan-Bergeland J, Dalmasso AP. Posttransfusion thrombocytopenia associated with passive transfusion of a platelet-specific antibody. *Transfusion* 1988; **28**: 73–6.
16 Boehlen F, Bulla O, Michel M, Reber G, de Moerloose P. HPA-genotyping and antiplatelet antibodies in female blood donors. *Hematol J* 2003; **4**: 441–4.
17 Warkentin TE, Smith JW, Hayward CP, Ali AM, Kelton JG. Thrombocytopenia caused by passive transfusion of anti-glycoprotein Ia/IIa alloantibody (anti-HPA-5b). *Blood* 1992; **79**: 2480–4.