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ORIGINAL RESEARCH

Frequency of anti-glycoprotein Ia/IIa (anti-HPA-5b,-5a) and anti-glycoprotein IIb/IIIa (anti-HPA-1a,-3a,-4a) alloantibodies in multiparous women of African descent

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Background: Human platelet antibodies are often implicated in some disease conditions, such as neonatal alloimmune thrombocytopenia (NAIT), idiopathic thrombocytopenic purpura (ITP) and platelet refractoriness. The frequencies of these alloantibodies have not been reported in Nigeria and West Africa.

Methods: Screening for allontibodies to human platelet antigens (HPA) was undertaken using the GTI PakPlus® qualitative solid phase ELISA reagent. Platelet count was done using the ICSH approved procedure using 1% ammonium oxalate reagent.

Study design: A cross-section of apparently healthy adult Nigerian multiparous non-pregnant women, who were staff of a tertiary health facility in the Niger Delta, Nigeria, were screened for alloantibodies to human platelet antigens.

Results: Of the one hundred (100) women screened, the prevalence of anti-glycoprotein IIb/IIIa (anti-HPA-Ia,-3a,-4a) was zero percent (0%), anti-glycoprotein Ia/IIa (anti-HPA-5b) accounted for 30% of results, while anti-glycoprotein Ia/IIa (anti-HPA-5a) was 18%. Parity was found to exert significant influence on the development to HPA antibodies (Fisher's Exact Test = 11.683, $P < 0.05$; 13.577, $P < 0.01$). The platelet count of the women did not appear to exert any influence on the development of the antibodies ($P > 0.05$).

Conclusion: This study has observed a high prevalence of anti-HPA-5b in our sample population. The prevalence of alloantibodies to HPA antigens was found to associate strongly with parity. These results indicate that there is a need to initiate platelet serology in our tertiary health institutions, as well as educate our women on the risk associated with frequent pregnancies, and ensure that adequate caution is taken when recruiting multiparous women as blood donors.

Keywords: Nigeria, multiparous, blood donor, platelet serology, platelet count

Introduction

Glycoprotein (GP) IIb/IIIa is an integrin receptor of the alpha IIbβ3 complex which is found on the platelet surface, is also a receptor for fibrinogen, and aids in platelet activation. The complex is formed via calcium-dependent association of GPIIb and GPIIIa, a required 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, and also to many cases of drug induced immune thrombocytopenia.[3]

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The glycoprotein, GPIa/IIa (HPA-5b) is an intergrin of alfa 2 beta 1 (α_2 β1). It is widely distributed on different cell types and can mediate adhesion to collagen.[4] Recently, the HPA-5 system has been recognized as the second most frequent cause of neonatal alloimmnune thrombocytopenia.[5] Anti-HPa-5 has been implicated in approximately 20% of serologically confirmed cases of neonatal alloimmune thrombocytopenia, as well as post-transfusion purpura. In our setting, cultural beliefs encourage frequent pregnancies, and it is hypothesized in this study that this frequent pregnancy, resulting into high multiparity, could lead to high prevalence of anti-HPA antibodies. This may equally contribute to high incidence of neonatal alloimmune thrombocytopenia, platelet refractoriness, and other conditions associated with platelet antibodies. There is a paucity of information on anti-HPA antibodies among multiparous women in Nigeria and West Africa. This study was done to determine the frequency of some HPA antibodies and their risk factors among our female population.

Materials and methods

Subjects

The study population consisted of adult women who had given birth to at least two children and had stopped for a period of at least one year prior to inclusion into the study. They were confirmed nonpregnant at the time of the study by the pregnancy test using a one step hCG test (Global One Step Rapid hCG test; Global Inc, FL). They were volunteers recruited from among the staff of University of Port Harcourt Teaching Hospital (UPTH) Port Harcourt. Their ages ranged from 26–59 years with a mean age of 40.6 yrs, while parity status ranged from 2–11. 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 received from each participant before 4 mL of blood was collected from them.

Methods

The GTI PakPlus® reagent kit bought directly from GTI Diagnostics, (Waukesha, WI), was used for the detection of the platelet antibodies. The GTI PakPlus test was performed using 50 µL of control or test serum, diluted 1:2 with specimen diluents solution, and then added to duplicate wells in a micro test plate and incubated for 40 mins at 37°C. The plate was washed three times with 300 µL of wash solution per well and 50 µL of an alkaline phosphates-conjugate, after which affinity purified goat antibody to human immunoglobulin (1:100) dilution was added to each well. After incubation for 40 mins at 37°C, and three additional washes, 100 µL of p-nitrophenyl phosphate solution diluted 1:100 in the enzyme substrate buffer was added, and the mixture incubated in the dark at room temperature (18 to 22°C). The reaction was stopped after 30 minutes by the addition of 100 µL of ELISA stop solution, and the absorbance of each well at a wavelength of 405 nm was measured in an ELISA plate reader (STAT FAX 2100, Awareness Technology, Palm City, FL). Test wells having optical densities (ODs) equal to or greater than twice the mean OD of the negative control wells were regarded as positive. Whenever the OD reading of either of the duplicate test wells exceeded 20% of the mean OD of the two wells, the test of that serum was considered invalid and was repeated. Platelet count was assessed using the ICSH approved procedure using 1% ammonium oxalate reagent.

Statistical analysis

All statistical analyses were performed with statistical software (SPSS version 12.0 for Windows; SPSS Inc., Chicago, IL, USA) Results were expressed as mean and ± standard deviation for descriptive statistics. Nondescriptive statistics were expressed as frequency, and Fisher's exact test was used for a test of significance. Results of the statistical tests were deemed significant if the two-tailed probability (*P* value) was ≤ less than or equal to 0.05.

Results

Table 1 shows the demographic characteristics of the study participants. A majority of the women were between

Table 1 Demographic characteristics of the 100 participants

Characteristics	Frequency (%)
Age groups (years)	
25–34	28.0
35–44	40.0
45–54	26.0
55–above	6.0
Parity	
<5	72.0
5–11	28.0
Ethnicity	
Ijaw	32.0
Ikwerre/Etche	26.0
Ekpey/Ogba	16.0
Igbo	14.0
Yoruba	6.0
Akwa-Ibom	6.0

Table 2 Frequencies of antiglycoproteins and antiplatelet antibodies 100 participants

Anti-glycoproteins	Anti-HPA antibodies	No. of Positives (%)
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)

35–44 years (40%). Women with parity 3 (28%) were mostly highly represented in the study, with the Ijaw ethnic group dominating the study population (32%), closely followed by Ikwerre/Etch (26%). The frequencies of anti-HPA antibodies are as shown in table 2. The anti-GP IIb/IIIa (anti-HPA- Ia, -3a, 4a) was absent in the study population (zero prevalence). The highest prevalence rate of platelet alloantibody in this study was anti-GP Ia/IIa (anti-HPA- 5b), with a prevalence rate of 30%, while anti-HPA-5a was measured at 18%. The mean (SD) age of the study participants was 40.64 (8.4) years, parity 4.0 ± 1.9, and platelet count 204.6 ± 35.6 (Table 3) Age and ethnicity were not found to exert any influence on the prevalence of anti-platelet antibodies (Tables 4 and 5). 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 ($P > 0.05$). Table 6 shows the influence of parity on the prevalence of the platelet antibodies. It was observed that parity has an influence on the occurrence of anti-HPA-5b and 5a at $P < 0.05$ and $P < 0.01$ respectively. Platelet count did not show any influence on the development of antibodies, as shown in Table 7.

Discussion

This study describes the frequency of alloantibodies to HPA antigens in Nigerian women with a history of frequent pregnancies. The study is unique as there are no reports with comparable information from Central and West Africa so far.

Table 3 Mean values of the age, parity and platelet count

Parameters	Mean (+SD)	Minimum	Maximum
Age (years)	40.64 (8.4)	25	59
Parity	4.0 (1.9)	2	11
Platelet count ($\times 10^9$/L)	204.6 (35.6)	129	294

Table 4 Influence of age on the prevalence of antiplatelet antibodies

Parameters	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 and above	0 (0.0)	2 (33.3)	0 (0.0)
Fisher's exact test	–	5.679	2.729
P value	–	0.117^{ns}	0.386^{ns}

Abbreviation: ns, not significant.

The main findings of this study are (1) the high prevalence of anti-HPA-5b; (2) the significant influence of the number of previous pregnancies on the occurrence of anti-HPA-5b and anti-HPA-5a; and (3) the complete absence of anti-HPA-1a and other anti-HPA's on GP IIb/IIIa complex. These findings are consistent with observation of maternal HPA alloimmunization in other populations. (eg, Tunisia, Austria and United States).[6,7–11] In these countries, anti-HPA-5b was also shown to be the highest prevalence, whereas the detection of anti-HPA-1a is a rare event in Caucasians, occurring in only 1–2 cases among 500–1000 pregnant women.[12–14] Thus, the zero prevalence of anti-HPA-1a in this study could be due to the relatively small sample size of the study population. Taken altogether, this study shows that the pattern of maternal platelet alloimmunization among Nigerian women is similar to maternal alloimmunization in other parts of the world.

One of the most serious consequences of a high prevalence of the anti-HPA-5b antibody is the possibility of the

Table 5 Influence of ethnicity on the prevalence of antiplatelet antibodies

Parameters	Anti-HPA-1a Anti-HPA-3a Anti-HPA-4a	Anti-HPA-5b	Anti-HPA-5a
Ethnicity	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
Exact-significance (*P*)	–	0.251^{ns}	0.357^{ns}

Abbreviation: ns, not significant.

Table 6 Influence of parity on the prevalence of antiplatelet antibodies

Parameters	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 (30.8)	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**

Notes: *Significant at $P < 0.05$. **Significant at $P < 0.01$.

occurrence of the clinical disorder known as neonatal alloimmune thrombocytopenia (NAIT).

Anti-HPA-5 antibodies are known to be less immunopathegenic than anti-HPA-1a antibodies; indeed most cases of passive thrombocytopenia reported in the literature are secondary to the 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 also been reported.[17] It thus appears that most cases of NAIT among Caucasians are caused by anti-HPA-1a, which contrastingly is much reduced in our African population.

Table 7 Relationship between platelet count and antiplatelet antibodies

Parameters		Platelet count × 10^9/L		
Platelet glycoprotein (antiplatelet antibodies)	**Mean**	**Mean square**	**F value**	***P* value**
GPIIb/IIIa				
Anti-HPA-1a				
Anti-HPA-3a	No positive			
Anti-HPA-4a	detected			
GP Ia/IIa				
Anti-HPA-5b		3430.86	2.805	0.100^{ns}
	Pos-217.33			
	Neg-199.26			
Anti-HPA-5a		3550.83	2.909	0.095^{ns}
	Pos-222.67			
	Neg-200.73			

Abbreviation: ns, not significant.

Even though a case of NAIT has not been reported in Nigeria, it is possible that some cases of mild to moderate neonatal thrombocytopenia may have been caused by anti-HPA-5b and anti-HPA-5a without being noticed; probably due to a lack of facility for platelet serology in our hospitals. This calls for an urgent need to implement effective platelet serology practices in our hospitals, as well as a need to exercise caution when recruiting multiparous women as blood donors.

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