Survey of platelet glycoprotein specific antibodies and HLA class 1 antibodies in a cross section of Nigerian multiparous women

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ABSTRACT

Aims: The frequencies of alloantibodies to platelet glycoproteins and HLA class 1 have not been reported in this part of the world. To determine the prevalence of these antibodies in Nigeria. Methods: The sera of 100 multiparous women were tested for antibodies to HPA and HLA class 1 antigens using GTI PakPlus solid phase ELISA Kit in a cross sectional study. Results: The prevalence of platelet glycoproteins specific antibodies was obtained as follows: GP IIB/IIIa (10%), GP Ia/IIa (48%), GP IV (8%) and GP Ib/IX (12%). Further analysis revealed the prevalence of each platelet antibody subgroup as follows: anti-HPA-1a,-3a,-4a (0%), anti-HPA-1b,-3a,-4a (10%), anti-HPA-5a (18%), anti-HPA-5b (30%). HLA class 1 antibody was prevalent in 22% cases. The production of these antibodies was parity dependent. Conclusions: There is a high prevalence of anti-HPA-5b and anti-HLA class 1 in our female population which can pose potential threat to our blood donor pool.

Keywords: Platelet glycoproteins, Platelet antibodies, HLA class 1 antibodies, Multiparous women, Nigeria

INTRODUCTION

Formation of antibodies to human platelet specific antigens (HPA) and human leucocyte antigens (HLA) class 1 are among the non-infectious risk of blood transfusion that has been identified [1]. These platelet-specific and HLA class 1 antigens are carried on the platelet membrane glycoproteins which occur as heterodimeric compounds i.e. each consisting of two different glycoprotein molecules [2]. Platelet glycoproteins are therefore often paired (e.g. Ia/IIa, IIb/IIIa or Ib/IX referring to the alpha and beta chains in each complex) [2]. The GP Ia/IIa and IIb/IIIa complexes are members of the broadly distributed family of adhesion molecules called integrins, which are essential for platelet adhesion and aggregation because they serve as receptors for ligands such as fibrinogen, von willebrand factor, collagen and fibronectin. GP Ib/IX is a leucine rich membrane glycoprotein and plays a major role in primary haemostasis [2-4]. GP IV (CD 36) is a membrane glycoprotein that is highly but variably expressed on platelets [5].
HLA and platelet specific antibodies do not occur naturally but are acquired through exposure to alloantigens through pregnancy and blood transfusion. These antibodies have been incriminated in several clinical conditions ranging from transfusion related acute lung injury (TRALI) caused by HLA class 1 antibodies to neonatal alloimmune thrombocytopenia (NAIT), platelet refractoriness (PR) and post transfusion purpura (PTP) caused by platelet specific antibodies [6-11].

The female population anywhere in the world forms part of the blood donor pool and majority of them are multiparous women. Although these women could be good long term donors, multiple pregnancies expose them to the risk of developing higher than usual titres of HPA and HLA antibodies to the foetal antigens of paternal origin [12, 13].

It is hypothesized that in a typical African setting where the cultural beliefs encourage multiple pregnancies, the proportion of these antibodies could be high among multiparous women thus rendering them “dangerous blood donors”.

There is no data in our country reporting the incidence or prevalence of these antibodies. This study was therefore aimed at determining the prevalence of HPA and HLA class 1 antibody among multiparous females and assesses the influence of age and parity on the production of these antibodies.

**MATERIALS AND METHODS**

**Subjects:** The study population consisted of 100 apparently healthy multiparous women recruited from the staff of the University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt. They all had given birth to at least two children and had no children in the one year prior to the study. The age range was from 26–59 years (mean 40.6±8.4 years). Their parity ranged from 2–11 with a mean of 4.0±1.9. Their parity status was grouped into two, those having less than or equal to four children constituted the highest percentage (72.0%) while those having more than or equal to five children constituted 28.0%. These subjects were selected randomly from February to June, 2009.

**Serological Studies:** All specimens were tested in duplicate and examined to detect HLA class 1 and platelet specific glycoprotein by a US–FDA licensed commercial solid-phase enzyme-linked immunosorbent assay kit, PakPlus (GTI; Waukesha, WI, USA). Testing performed was for polymorphic epitopes of the specific platelet antigens; including GP I Ib/IIa, GP Ia/IIa (HPA-5b/5b), GP Ia/IIa (HPA-5a/5a), GP IV, GP Ib/IX and isolated HLA class 1 antigen, immobilized in specific wells of the microplate.

Subject’s serum was added to micro wells coated with platelet and HLA glycoprotein allowing antibody, if present to bind. Unbound antibodies were then washed away. An alkaline phosphate labelled anti-human globulin reagent (anti-IgG/A/M) was added to the wells and incubated. The unbound anti-IgG/A/M was washed away and the substrate P-Nitro-Phenyl phosphate (PNPP) was added. After 30 minutes incubation period, the reaction was stopped by a sodium hydroxide solution. The optical density of the colour that developed was measured in an ELISA reader at wavelength 405 or 410 nm. The results were calculated and interpreted based on the instructions provided by the manufacturer.

**Statistics:** The frequency and difference of antibody presence in age and parity were evaluated by the Pearson Chi-square test using statistical package for the social sciences (SPSS) (Version 17, Chicago, IL, USA). A p value of less than 0.05 was considered statistically significant.

**RESULTS**

Table 1 shows the age and parity of the one hundred multiparous women. Women of the age group 35–44 years constituted the majority (40%), followed by those in the 25–34 years age bracket (28%), then 45–54 years (26%) and 6% for 55 years and above. The proportion of the women who had less than or equal to four children were 72% while those with parity five and above constituted 28% of the study population.

| Characteristics | Percentage |
|-----------------|------------|
| Age groups      |            |
| 25–34           | 28.0%      |
| 35–44           | 40.0%      |
| 45–54           | 26.0%      |
| 55 and above    | 6.0%       |
| Parity          |            |
| ≤ 4             | 72.0%      |
| ≥ 5             | 28.0%      |

The prevalence of HLA class 1 antibody was 22% while the remaining 78% accounted for platelet glycoprotein specific antibody. The distribution of the platelet glycoprotein antibodies were as follows: anti-GP-Ib/IIa (10%), anti-GP-Ia/IIa (48%), anti-GP IV (8%) and anti-GP-Ib/IX (12%). These results are shown in table 2.

To further analyze the prevalence of each platelet antibody sub-group, the proportion is shown in table 3. Anti-HPA-Ia, -3a, -4a (0%), anti-HPA-Ib, -3b, -4a (10%), anti-HPA -5a (18%), anti-HPA-5b (30%) anti-GP Ib/IX (12%) and anti-GP IV (8%).

The influence of age and parity on the prevalence of the antibodies (HPA and HLA antibodies) is shown in table 4. Apart from anti-HPA-1a, -3a, -4a which did not occur in this study population, Fishers exact test analysis revealed that there is a strong association between parity, platelet and HLA class 1 antibody at various levels of significance. Age was selectively found to associate significantly with anti-HPA-5b (Fisher’s exact 5.679, p<0.05) and HLA class 1 antibodies. (Fishers exact 14.188, p<0.001)
Table 2: Production of HLA class 1 and platelet antibodies among the 100 multiparous women.

| Antibody | Percentage positive |
|----------|---------------------|
| HLA class 1 antibody only | 22% |
| Platelet specific antibodies only | 78% |
| GP IIb/IIia | 10% |
| GP Ia/IIa | 48% |
| GP IV | 8.0% |
| GP Ib/IX | 12.0% |
| HLA class 1 and platelet specific antibodies | |
| HLA + GP Ia/IIa | 44% |
| HLA + GP Ia/IIa + GP IV | 2.0% |
| HLA + GP Ia/IIa + GP Ib/IX | 8.0% |
| HLA + GP IIb/IIIa | 4.0% |
| HLA + GP IIb/IIIa + GP Ia/IIa | 2.0% |
| GP Ib/IX + GP IV | |

Table 3: Proportion of different antibodies among multiparous women producing platelet specific antibodies.

| Antibodies of platelets | Percentage positive |
|-------------------------|---------------------|
| All GP IIb/IIIa | 10.0% |
| Anti- HPA-1a,-3a,-4a | 0% |
| Anti-HPA-1b,-3b,-4a | 10.0% |
| All GP Ia/IIa | 48.0% |
| Anti-HPA 5a | 18.0% |
| Anti-HPA 5b | 30.0% |
| All GP Ib/IX | 12.0% |
| All GP IV | 8.0% |

Table 4: Influence of parity and age on the prevalence of platelet and HLA class 1 antibodies.

| Parity Status | HPA-1a/3a/4a | HPA-1b/3b/4a | HPA-5b | HPA-5a | GP Ib/IX | GP IV | HLA |
|---------------|--------------|--------------|---------|---------|----------|--------|------|
| ≤ 4           | Pos.         | Pos.         | Pos.    | Pos.    | Pos.     | Pos.   | Pos. |
| ≥ 5           | 0            | 4.0          | 14.0    | 8.0     | 8.0      | 2.0    | 10.0 |
| Fisher’s exact Age groups (Yrs) | 0    | 6.0          | 19.0    | 10      | 4.0      | 4.0    | 12.0 |
| 25-34         | 0            | 2.0          | 8.0     | 2.0     | 4.0      | 0      | 6.0  |
| 35-54         | 0            | 4.0          | 6.0     | 8.0     | 2.0      | 2.0    | 0    |
| 45-54         | 0            | 4.0          | 14.0    | 8.0     | 4.0      | 4.0    | 1.0  |
| 55 and above  | 0            | 0            | 2.0     | 0       | 2.0      | 0      | 2.0  |
| Fisher’s exact Age groups (Yrs) | 0.971 (ns) | 5.679*       | 2.729 (ns) | 3.083 (ns) | 2.788 (ns) | 14.188*** |

* = Significant p<0.05; ** = Significant at p<0.01; *** = Significant at p<0.001; ns = Not significant; Pos.=Positive

DISCUSSION

This study describes the prevalence of antibodies to human platelet antigens and human leucocytes antigens (HLA) class 1 in a cross section of Nigerian multiparous women. The main findings of this study are 1) the high prevalence of anti-HPA-5b and HLA class 1 antibodies; 2) the zero prevalence of anti-HPA-1a,-3a,-4a; 3) the significant influence of the number of previous pregnancies on the occurrence of both HPA and HLA class 1 antibodies.

The findings of the high prevalence of anti-HPA-5a and anti-HPA-5b are consistent with observation of maternal HPA alloimmunization in other populations like Tunisia, Austria and United States [14-18]. In these countries, anti-HPA-5b was also shown to be of highest prevalence whereas the detection of anti-HPA-1a is a rare event, in Caucasians occurring in 1–2 cases among 500–1000 pregnant women [19, 20]. Thus the zero prevalence of anti-HPA-1a,-3a,-4a in this study could be due to the relatively small size of the study population.

Second to anti HPA-5b is the prevalence of anti-HLA-class 1 antibodies (22.0%). This high prevalence is comparable with 20.7% prevalence rate obtained from maternal sera of pregnant women in Hyderabad, India [21] and Korea [24].

The most serious consequence of the presence of platelet antibodies and HLA class 1 antibodies are the possibilities of the occurrence of clinical disorders known as neonatal alloimmune thrombocytopenia (NAIT), platelet refractoriness (PR), post transfusion purpura (PTP) caused by anti-HPA antibodies and
transfusion related acute lung injury (TRALI) caused by anti-HLA class 1 antibodies. Anti HLA class 1 antibodies have also been implicated in some case of NAIT [8, 22–26].

Another aspect of the relevance of this study is that women are part of the blood donor pool and in our African setting where the cultural beliefs encourage frequent pregnancies, many of our female blood donors may actually be “dangerous blood donors” due to the presence of circulating HPA and HLA antibodies as observed in this study. More so, the presence of these antibodies showed a very significant association with parity. It follows therefore that the women with more frequent pregnancies carries higher potential to make both platelet and HLA antibodies. Since all the participants have stopped child bearing for the past one year prior to inclusion in this study, it means that the antibodies once developed can persist in their circulation for a long time.

In conclusion, this study has identified a high prevalence of HPA and HLA class 1 in the Nigerian female population. There is need to screen our female blood donors who have previous histories of frequent pregnancies in order to ensure safety in our blood supply to transfusion recipients.

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Author Contributions
Zaccheaus Awortu Jeremiah – Substantially contributed to conception and design, acquisition of data , drafting the article, revising it critically for important intellectual content and final approval of the version to be published

Justina Egwuma Oburu – Contributed to conception and design, analysis and interpretation of data and final approval of the version to be published

Guarantor
The corresponding author is the guarantor of submission.

Conflict of Interest
Authors declare no conflict of interest.

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