Original Research

Place of HPA1a Antigen in Neonatal Thrombocytopenia to Obstetric and Pediatric Gynecology Hospital of Douala and Laquintinie Hospital of Douala

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ABSTRACT

Introduction: Platelet antigens called Human Platelet Antigen (HPA) present on the surface of platelets are involved in immunological conflicts sometimes leading to severe thrombocytopenia related to anti HPA1a antibody. The aim of this study was to detect platelet antibodies in newborns and the associated risk factors.

Material and Methods: We conducted a cross-sectional analytical study from 05 January to 30 June 2017 at the Laquintinie Hospital in Douala and the Gynaecological Obstetrics and Paediatrics Hospital in Douala, among newborns with thrombocytopenia and mothers in compliance with ethical considerations. Socio-demographic and clinical data were collected, blood was collected on EDTA tubes for newborns and on tubes without anticoagulant for mothers in order to determine the presence of anti HPA1a antibody. Statistical analysis was performed using SPSS version 22 software.

Results: A total of 35 newborns and 35 mothers were recruited in this study. The mean age of the newborns was 8.5 ± 7.2 days with a sex ratio of 1.7% in favour of boys. The prevalence of neonatal thrombocytopenia was 4.1% and the prevalence of HPA1a antigen in newborns and those whose mothers had been transfused at least once during or before pregnancy was 17.1%. Most of the newborns were born to primiparous mothers (57.1%) and 80.0% had premature and neonatal jaundice as reasons for hospitalization. Male newborns and those whose mothers had been transfused at least once during or before pregnancy had respectively 4 (OR = 3.53; p-value < 0.0001) and 14 (OR = 14; p-value = 0.0483) times more risk of having the anti-HPA1a antibody.

Conclusion: Our study has shown that the anti-HPA1a antibody is a risk factor for neonatal thrombocytopenia and is associated with maternal transfusion.

INTRODUCTION

Platelet antigens called Human Platelet antigen (HPA) on the surface of platelets are involved in immunological conflicts such as post-transfusion purpura, platelet transfusion refractory state and maternal-fetal alloimmunisation [1]. The seriousness of this condition lies essentially in the risk of intracranial haemorrhage in 20% of cases, half of which occur in utero; it is the cause of death in 10% to 15% of cases or of serious neurological sequelae in 15 to 30%[1]. The incidence of these thrombocytopenias is often estimated at 1 case per 800 to 1000 births and in 78-90% of cases the cause is anti-HPA1a antibody [2–4]. Although these
thrombocytopenias are rare, the first pregnancy is often affected in 50% of cases and the presence of anti-HPA1a antibodies increases the risk of recurrence in subsequent pregnancies [2]. Their early detection allows better management of neonatal thrombocytopenia due to alloimmunisation. From all the above, very little data are available in the world, in Africa and particularly in Cameroon on thrombocytopenia related to platelet alloimmunization in newborns due to the absence of systematic screening for these incompatibilities. The aim of this study is to analyse, through a prospective study, the proportion of platelet antibodies to anti-HPA1a in newborns presenting thrombocytopenia in our context.

MATERIAL AND METHODS

Design of the Study
This study was conducted in the city of Douala located in the coastal region of Cameroon. It was a prospective study conducted during the period from 05 January to 30 June 2017. The study population consisted of mothers and their newborns who were admitted or came to the outpatient clinic for primary haemostasis or haemolysis disorders recruited from the neonatology and maternity departments of the Gynaecological Obstetrics and Paediatrics Hospital of Douala and the Laquintinie Hospital of Douala. The biological analyses were carried out at the clinical biology laboratory unit of the Gynaecological-Obstetric and Paediatric Hospital in Douala for the analysis of the samples. All mothers whose newborns had thrombocytopenia and all thrombocytopenic newborns whose mothers gave free and informed consent were included; and all false thrombocytopenias were excluded. Sampling size was defined in convenience and the recruitment procedure was done in non-probability basis. All those who met the inclusion criteria were recruited. Sample quality was determined by consecutive exhaustive sampling.

Ethical Considerations
Prior to the implementation of the study, ethical approvals were obtained from the Institutional Committee of Ethics for Human Health Research of the University of Douala (Ref. CEI-UDo/720/01/2017/M). Finally, a research authorization was obtained from the officials of the Gynaecological Obstetric and Paediatric Hospital of Douala and the Laquintinie Hospital of Douala.

Laboratory Analysis
Pre-analytical Phase
After obtaining free and informed consent, the patients (mothers) completed a questionnaire based on socio-demographic characteristics (age, sex, birth weight, for newborns); (age, marital status, level of education, occupation for mothers); and clinical and biological characteristics (bleeding, purpura, history of thrombocytopenia,) then two samples were taken from the elbow.

- One in a 2ml paediatric tube with EDTA anticoagulant for newborns to screen for anti-HPA1a antibodies
- The other in a dry tube without anticoagulants for the mother to identify the anti-HPA1a antibody

Analytical Phase: This was Done in Three Steps
Blood Count.
This count was carried out on the SYSMEX XN 1000 (automatic blood count machines) which uses the principle of flow cytometry and enabled us to identify RBCs, WBCs and platelets, to measure BH, to calculate and measure the haematocrit, the VGM and the erythrocyte constants, and to establish an approximate leucocyte formula. The parameter that interested us in this case was the platelet count, which had to be less than 150G/L.

The Making of the Blood Smear
Carried out on a slide and stained with May-Grunwald-Giemsa (MGG) stain, it is the cytological study of the figurative elements of blood. In our study, we checked for the presence of platelet aggregates and giant platelets or not in order to confirm thrombocytopenia.

Detection of HPA1a Antibody
Detection of HPA1a antibody was performed using the MAIPA (Monoclonal Antibody Specific Immobilisation of Platelet Antigen) technique which is a qualitative test used to screen for antiplatelet antibodies in serum or plasma (MAIPA Indirect) and/or to detect antibodies bound to a patient’s platelets (MAIPA Direct). A positive MAIPA Indirect or MAIPA Direct test result requires subsequent identification of antibody specificity using the same method.

The principle of the test is based on the capture of a platelet antigen using mouse monoclonal antibodies to human platelet membrane glycoproteins and the analysis of the binding of human antibodies to this antigen by an enzyme-linked immunosorbent assay (ELISA).

Throughout our study we performed the direct MAIPA test in newborns with a platelet count of less than 150G/L with absence of platelet aggregates on the smear slide in order to detect platelet-bound antibodies in the latter. Antibody identification was done with sera from mothers with positive newborn screening.
Reading and Interpretation of Results

Validation and Interpretation of Screening Tests:
The validation of the screening test depended on the optical density (OD) values of the control samples. After subtraction of the blank OD value:
✓ The OD value for the negative control had to be less than 0.1
✓ The OD value for the positive controls should be greater than 0.5

Interpretation of the tests is relatively simple,
With an OD cut-off value of 0.2 (after subtraction of the blank value):
✓ OD values above 0.2 were considered positive
✓ OD values below 0.2 were considered negative

All positive screening test results were confirmed by identification testing in the mothers of each corresponding new-born.

Table 1. Birth Weight by Gender and Pregnancy Type

<table>
<thead>
<tr>
<th>Birth weight</th>
<th>Workforce</th>
<th>Single fetal</th>
<th>Twinned</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>&lt; 2500 g</td>
<td>28</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>2500 - 4000 g</td>
<td>7</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>&gt; 4000 g</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>11</td>
<td>18</td>
</tr>
</tbody>
</table>

Fig. 1. Chronological Age Distribution of Newborns

Statistical Analysis

The data were entered into an Excel sheet (Microsoft Office 2007) and subsequently analysed with SPSS 22 for Windows (SPSS, Inc., Chicago, IL, USA). The variables were presented as percentages (95% confidence intervals) in tables and graphs (pie charts and histograms). Fisher’s exact test (bivariate statistics) and chi-square test of homogeneity (univariate statistics) were used to compare proportions. Logistic regression (univariate model) was used to identify factors associated with the presence of HPA1a antibody. This test was used to quantify the association between the dependent variable (presence of HPA1a antibody) and the independent variables through the calculation of the Odds Ratio (OR) and its 95% confidence interval. The significance threshold was set at p-value < 0.05.
Table 2. Factors Associated with HPA1a Antibody

<table>
<thead>
<tr>
<th>Variables</th>
<th>Modalities</th>
<th>Effective</th>
<th>HPA1 (+)</th>
<th>OR (IC95%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Female</td>
<td>13</td>
<td>1 (7,7%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>22</td>
<td>5 (22,7%)</td>
<td>3,53 (1,36 - 34,19)</td>
<td>&lt; 0,0001</td>
</tr>
<tr>
<td>Age of babies (days)</td>
<td>[0 – 7]</td>
<td>18</td>
<td>3 (16,7%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>[7 – 14]</td>
<td>4</td>
<td>0 (0,0%)</td>
<td>2,50 (0,00 - 4,20)</td>
<td>0,9749</td>
</tr>
<tr>
<td></td>
<td>[14 – 21]</td>
<td>4</td>
<td>2 (50,0%)</td>
<td>5,00 (0,49 - 50,84)</td>
<td>0,1738</td>
</tr>
<tr>
<td></td>
<td>[21 – 28]</td>
<td>9</td>
<td>1 (11,1%)</td>
<td>0,63 (0,06 - 7,03)</td>
<td>0,7035</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>no</td>
<td>32</td>
<td>4 (12,5%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>yes</td>
<td>3</td>
<td>2 (66,7%)</td>
<td>14,0 (1,02 - 192,18)</td>
<td>0,0483</td>
</tr>
<tr>
<td>Blood type</td>
<td>A</td>
<td>10</td>
<td>1 (10,0%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>8</td>
<td>1 (12,5%)</td>
<td>1,29 (0,07 - 24,39)</td>
<td>0,8671</td>
</tr>
<tr>
<td></td>
<td>O</td>
<td>16</td>
<td>4 (25,0%)</td>
<td>3,0 (0,28 - 31,64)</td>
<td>0,3607</td>
</tr>
<tr>
<td>Gestational age</td>
<td>Premature</td>
<td>10</td>
<td>2 (20,0%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>15</td>
<td>3 (20,0%)</td>
<td>1,0 (0,14 - 7,39)</td>
<td>1,0000</td>
</tr>
<tr>
<td></td>
<td>Born at term</td>
<td>10</td>
<td>1 (10,0%)</td>
<td>0,44 (0,03 - 5,88)</td>
<td>0,5383</td>
</tr>
<tr>
<td>Type of thrombocytopenia</td>
<td>Slight</td>
<td>10</td>
<td>2 (20,0%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>14</td>
<td>1 (7,1%)</td>
<td>0,31 (0,02 - 3,96)</td>
<td>0,3663</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>9</td>
<td>3 (33,3%)</td>
<td>2,0 (0,25 - 15,99)</td>
<td>0,5134</td>
</tr>
</tbody>
</table>

OR = Odds ratio; CI95% = 95% confidence interval; P-value < 0.05 were considered significant; OR < 1 (protective factor); OR = 1 (neutral factor); OR > 1 (Risk factor).
RESULTS

1. Socio-demographic, Clinical and Biological Characteristics of the Study Population

During this study, we registered 1134 newborns of whom 855 had a blood count and only 35 after elimination of false thrombocytopenia were retained with the 35 corresponding mothers. The most represented age range of newborns was, between 0 and 7 days (51.4%). The average age of the babies was 8.5 ± 7.2 days, with extreme values of 1 and 28 days.

Most of the newborns were not born at full term, 25 beyond 37 weeks of amenorrhea, corresponding to 71.4% of the study population, very premature babies and medium premature babies representing 28.6% of the total study population. The average gestational age was 34.1 ± 3.9 weeks of amenorrhea with the extreme values of 28 and 41 weeks. Table 1 shows that most of the newborns had a weight of less than 2500g and were from mono-fetal pregnancies. The majority of thrombocytopenia’s found during our study were moderate thrombocytopenia’s represented here at 40.0% and very few have very severe thrombocytopenia’s (less than 20,000 G/L) (Fig.1).

2. Prevalence of HPA1a Antibody

Of the 35 new-borns with thrombocytopenia, 6 was tested positive for the anti-HPA1a antibody, giving a prevalence of 6/35 or 17.1%.

3. Risk Factors Associated with HPA1a

Table 2 shows that male and transfused babies were almost 4 times (OR = 3.53; P-value < 0.0001) and 14 times (OR = 14; p-value = 0.0483) more likely to be HPA1 positive compared to female and non-transfused babies respectively.

DISCUSSION

Our study focused on the role of the anti-HPA1a antibody in neonatal thrombocytopenia at the Laquintinie Hospital and the Gynaecological Obstetrics and Paediatrics Hospital in Douala. 1,134 new-borns were registered during the study, of which 855 had a blood count and only 35 was diagnosed to have thrombocytopenia representing 4.1. The most represented age group was that between 0 and 7 days, 51.4% of cases, early thrombocytopenia predominated. 22 male new-born’s, 62.9%, 13 female new-borns, 37.1%, with a sex ratio of 1.7, these results are comparable to those reported in 2008 in Tunisia by Gueririk Mohammed El Amine who, working on neonatal thrombocytopenia, who found the same age group at 88.82% and the predominance of male represented at 61% against 39% for the female [5].

The majority of the new-borns were premature (71.4%), of which 28.6% were very premature and 42.8% were medium premature. Moreover, 80.0% of the babies had a birth weight of less than 2500g. These results differ from those obtained by Djoupomb in Cameroon in 2007, which was 77.38% of new-borns at term with a weight between 2500g and 4000g [6]. This difference could be related to the maternal history or to the fact that the new-borns in our study were all thrombocytopenic at the base.

The prevalence of neonatal thrombocytopenia obtained in our study was 4.1% with 5.7% of cases of very severe thrombocytopenia 25.7% of severe thrombocytopenia 40.0% of moderate thrombocytopenia and 28.6% of mild thrombocytopenia. These data are different from those obtained in Morocco in 2008 by Abdelkarim Belefkh and Mohammad Al Ghamdi, i.e., 2.00% and 23.3% respectively. This discrepancy could be related to the variability of the study environment.

The majority of new-borns were born in primiparous pregnancies at 51.4% with a significant difference and a p-value of less than 0.0001. This result is different from that observed in Benin in 2005 by Anani et al. who found 24.8% [7]. Neonatal jaundice and prematurity were the main reasons for hospitalization at 80.0% and 50.0% respectively. Prematurity and neonatal jaundice could therefore be factors associated with neonatal thrombocytopenia and could be explained as stated in the literature by the immaturity of platelet function and hepatitis during the neonatal period.

Maternal-fetal incompatibility in the platelet system (HPA) can lead to neonatal alloimmune thrombocytopenia (NIAT). Thus, we obtained 17.14% of anti-HPA1a antibodies in our study population. These antibodies were systematically found in the mothers of children with alloantibodies. Zainab et al. in 2016 in Morocco had found a risk frequency of alloimmunization of mothers of 20.63% against the HPA1a antigen; however, different and variable data were reported in 2008 by Agnes Mechoulain in France, in 2007 by Kjeldsen-Kragh et al. in Norway and in 2005 by Anani et al. in Benin with frequencies of 95%, 10.6% and 79.2% of mothers with anti-HPA1a alloantibodies respectively [2,7–9]. This discrepancy could be explained by the variability and diversity of the population, the population size and the duration of the study.

However, in Caucasians, the HPA-1 system is most involved in neonatal alloimmune thrombocytopenia (NIAT) while in Japanese, HPA-2b is responsible for ineffective platelet transfusions and HPA-4b is involved in NIAT. The main risk factors associated with the presence of the antibody were gender and blood transfusion. Our study showed that male new-borns and those whose mothers had been transfused were almost 4
times (OR = 3.53; P-value < 0.0001) and 14 times (OR = 14; P-value = 0.0483) more likely to be HPA1a antibody positive compared to female new-borns and those whose mothers had not been transfused. This could be explained by the fact that our study population was predominantly primiparous and had received blood transfusions, and that transfusion compatibility is often limited in Africa and does not involve testing for platelet markers. In our study 3 of the new-borns who tested positive for HPA1a antibody were from mothers who had been transfused at least once during or before pregnancy and would probably have become immune to the platelet allo antigens they had received from the various transfusions.

CONCLUSION

The frequency of neonatal thrombocytopenia was 4.1%, the presence of anti-HPA1a antibodies in 17.1% of thrombocytopenic subjects, the predominance of males among thrombocytopenic subjects with anti-HPA1a antibodies, the role of blood transfusion in the development of anti-HPA1a antibodies, and the fact that anti-HPA1a antibodies play a significant role in neonatal thrombocytopenia, so that immunological neonatal thrombocytopenia is also a reality in our working environment. We need to take up the assessment of the state of the art of anti-platelet immunization in a more comprehensive way.

Declarations

Ethics approval and consent to participate
The National Ethics Committee for Human Health Research (n°2020/18/082/CE/CNERSH/SP) approved the experimental protocols and methods used in this study. The study was performed for the Revised Helsinki Declaration (1989). All methods were carried out by relevant guidelines and regulations. Informed consent was obtained from all individual participants included in the study. The objectives of the study were explained to each participant and their parents/guardians, and written informed consent was obtained prior to their inclusion in the study.

What is known about this topic

- The incidence of these thrombocytopenias is often estimated at 1 case per 800 to 1000 births and in 78-90% of cases the cause is anti-HPA1a antibody.
- Platelet antigens called Human Platelet Antigen (HPA) are involved in immune conflicts that can lead to severe thrombopenia linked to the anti-HPA1a antibody.

What this study adds

- This study found a 4.1% incidence of neonatal thrombocytopenia.
- Anti-HPA1a antibodies were present in 17.1% of patients with thrombopenia.
- The newborns were mostly from primary mothers at 57.1% and had premature pregnancy and neonatal icter at 80.0% for hospitalization reasons.

Availability of data and materials
The data will be available upon reasonable request to the corresponding author.

Competing interests
The authors declare no competing interests.

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Author’s contributions
All authors contributed to the design and execution of the study, participated in article drafting and critical revision, and read and approved the final version of the manuscript.

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CONFLICT OF INTEREST

There is no conflict of interest in this research.

REFERENCES


