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Estudo retrospectivo descritivo transversal

ARTIGO ORIGINAL

Frequency of red blood cells antibodies in possible cases of hemolytic disease of the newborn laboratory evidenced at Hemopa

Frequência de anticorpos anti-eritrocitários em possíveis casos de doença hemolítica perinatal evidenciados laboratorialmente na Fundação Hemopa

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Summary **Purpose:** To describe the frequency of red blood cells antibodies possibly related to Hemolytic Disease of the Newborn cases reported laboratory evidenced at Fundação Centro de Hemoterapia e Hematologia do Pará. **Methods:** This is a descriptive, cross-sectional study, based on the analysis of immuno-hematological reports of newborns filed at the Immunohematology Laboratory of the Fundação Centro de Hemoterapia e Hematologia do Pará , released between January 2015 and December 2019. The obtained data are related to ABO/Rh(D) typing; Direct Antiglobulin test; Irregular Antibodies test; Eluate, which were transcribed to Microsoft Excel sheets for later descriptive analysis. The study was approved by the Research Ethics Committee of the Fundação Pública Estadual do Hospital das Clínicas Gaspar Vianna, under the legal opinion #3,435,869. **Results:** A total of 37 reports were analyzed, laboratory evidence showed 34 cases of positive Direct Antiglobulin Test, of these, 22 presented positive eluate test. The highest frequency of described cases was related to anti-D antibody (19/22; 86.4%), followed by anti-c (1/22; 4.5%) and clinically significant antibody associations (2/22; 9.1%). **Conclusion:** The most frequent alloantibody evidenced was anti-D, however, other red blood cells alloantibodies observed drew attention and were considered relevant scientific evidence regarding the subject.

Keywords: anemia, hemolytic; pregnancy, high-risk; erythrocytes.

Resumo **Objetivo:** Descrever a frequência de anticorpos anti-eritrocitários possivelmente envolvidos em casos de doença hemolítica do recém-nascido evidenciados laboratorialmente na Fundação Centro de Hemoterapia e Hematologia do Pará. **Métodos:** Trata-se de um estudo descritivo, a partir da análise de laudos imuno-hematológicos de recém-nascidos arquivados no Laboratório de Imuno-hematologia da Fundação Centro de Hemoterapia e Hematologia do Pará, cujos exames laboratoriais foram realizados no período de janeiro de 2015 a dezembro de 2019. Foram obtidos os dados referentes à Tipagem ABO/Rh(D); Teste de Antiglobulina Direta; Pesquisa de Anticorpos Irregulares; Eluato, nos quais foram transcritos para o programa *Microsoft Excel* para posterior análise descritiva. O estudo foi aprovado pelo Comitê de Ética em Pesquisa da Fundação Pública Estadual do Hospital das Clínicas Gaspar Vianna, sob o parecer de nº 3.435.869. **Resultados:** Um total de 37 laudos foram analisados, observou-se evidência laboratorial de 34 casos de Teste de Antiglobulina Direta positivo, destes, 22 apresentaram teste do eluato positivo. A maior frequência dos possíveis casos descritos foi relacionada ao anticorpo anti-D (19/22; 86,4%), seguido do anti-c (1/22; 4,5%) e associações de anticorpos clinicamente significantes (2/22; 9,1%). **Conclusão:** O anticorpo mais frequente evidenciado foi o anti-D, entretanto, chama-se atenção para outros aloanticorpos anti-eritrocitários observados, sendo relevante evidências científicas acerca da temática.

Descritores: anemia hemolítica; gravidez de alto risco; eritrócitos.

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INTRODUCTION

The Hemolytic Disease of the Newborn (HDN) is an immunological pathology caused by blood incompatibility between the mother and the fetus or newborn, that results in a destruction of the fetus/newborn red blood cells IgG maternal antibodies, they pass through the placenta and connect to the fetal red blood cells causing hemolysis, anemia, jaundice and perhaps other mild or severe clinical conditions[1].

The development of HDN occurs mostly when the mother does not have the D antigen in the surface of her red blood cells (categorized as negative RhD) and it is exposed to fetal positive RhD, inducing the development of alloantibodies by the mother's immune system (alloimmunization)[2], given that the D antigen even in low quantities is highly immunogenic and is able to induce the development of anti-D[3]. The previous maternal alloimmunization sensitizing obstetrical events during pregnancy, thus once the mother is sensitized there is the possibility of complications in future pregnancies[2].

Although most HDN cases are related to the Rh system, being D antigen the most responsible for the disease severeness[4], the ABO maternal-fetal incompatibility is the most common, however it is responsible for minority of HDN cases, once ABO antibodies are mostly of the IgM, and occasionally of the IgG, when they can cause hemolytic disease of a benign and rarely severe[3]. There are some reports indicating that multiple antibodies against antigens of the systems Kell, Duffy, MNS, Kidd, Diego and others may be related as a cause to mild/medium HDN[2,4].

Although there are studies showing that these antibodies might be connected to HDN, there is a necessity to look for a better understanding of this subject in the medical and obstetrical community, because it is a major consensus that this disease is only possible to occur on RhD negative pregnant women, making health professionals able to proceed with immune-hematological tests and analyze its results for diagnostic purposes in the perinatal context.

The study aims to describe the frequency of anti-erythrocyte antibodies possibly involved in cases of HDN evidenced in the Immunohematology Laboratory of the Centro de Hemoterapia e Hematologia do Pará (HEMOPA).

METHODS

Retrospective, cross-sectional, descriptive study coming by the analysis of the reports of immune-hematological examinations of newborns that were concluded and archived Immunohematology Laboratory of the HEMOPA computerized database, which is the coordenating blood center of the state of Pará.

From every medical record it was possible to gather the results of qualitative lab tests such as: blood type ABO/Rh(D); Direct Antiglobulin Test (DAT); Irregular Antibodies Test (IAT); Eluate. So, the results of the analyzed variables were transcribed to the data collection form (sequency cases number). The data were scanned to a *Microsoft Excel* (*Software Office* 365 for Mac Version 16.42) electronic sheet for further descriptive analysis, which was based on the calculation of absolute and relative frequencies of the studied variables.

The immune-hematological reports of the newborns added were released between January 2015 and December 2019, whose samples have been sent for immune-hematological analysis, due to pre-transfusion test disturbance; and that have been TAD positive, with free irregular maternal antibodies shown in the serum and/or recovered in eluate. It is worth mentioning that a few samples were added in the data analysis, when available.

The present study has been approved by the Research Ethics Committee of the Fundação Pública Estadual Hospital das Clínicas Gaspar Vianna (CAAE: 15417319.7.0000.0016), under the legal opinion #3,435,869.

RESULTS

From a total of 37 newborn (NB) exam results analyzed, there were 34 (91.9%) cases of positive DAT observed in laboratory, out of which 22 (64.7%) tested positives for IAT and/or in an eluate. Among the antibodies identified in the samples of positive DAT neonates, the isolated anti-D was the most frequent (44.1%). Table 1 describes the results of the antibody profile identified by IAT.

Table 1. Antibody profile and frequency among newborns with positive Direct Antiglobulin Test (n=34).

Antibody	n (%)
Rh System	
Anti-D	15 (44.1)
Anti-c	1 (3.0)
Kell System	
Anti-K	1 (3.0)
Associations	
Anti-D + Anti-C	4 (11.7)
Anti-E + Anti-c	1 (3.0)
Negative	8 (23.5)
Unperformed	4 (11.7)
Total	**34 (100)**

Source: Fundação Centro de Hemoterapia e Hematologia do Pará (2020).

Analyzing those 22 cases (64.7%), it was possible to identify the specificity of maternal antibodies in the IAT and/or in an eluate of the newborn, suggesting a possible case of HDN. Most cases were triggered by the isolated anti-D (19/22; 86.4%); followed by the isolated anti-c (1/22; 4.5%); and associations of anti-E + anti-c antibodies (1/22; 4.5%); anti-D + anti-C (1/22; 4.5%).

The ABO typing analysis of the NBs included in the study revealed that more than 50% of them had group O blood, followed by groups A and B. The positive RhD phenotype presented itself in most samples, however, one sample showed negative RhD. In the other 4 samples it was not possible to identify the ABO/RhD blood types (Table 2).

Table 2. Frequency of ABO/Rh(D) typing among newborns samples

ABO typing	n (%)	Rh typing	n (%)
O	13 (59.1)	D+	17 (77.3)
A	4 (18.2)		
B	1 (4.5)	D-	1 (4.5)
AB	0 (0.0)		
Undefined	4 (18.2)	Undefined	4 (18.2)
Total	**22 (100)**	**Total**	**22 (100)**

Source: Fundação Centro de Hemoterapia e Hematologia do Pará (2020).

Regarding the maternal samples that were available for the ABO/RhD blood typing analysis, 2 samples were identified for group O blood; 2 for group A; 1 type B blood; and 1 of AB phenotype (Table 3). As for the RhD typing, most maternal samples were RhD negative (Table 3).

Table 3. Frequency of ABO/Rh (D) typing among maternal samples

ABO	n (%)	Rh	n (%)
O	2 (33.3)	D+	2 (33.3)
A	2 (33.3)		
B	1 (16.7)	D-	4 (66.7)
AB	1 (16.7)		
Undefined	0 (0.0)	Undefined	0 (0.0)
Total	**6 (100)**	**Total**	**6 (100)**

DISCUSSION

It was described the profile of antibodies related to 22 possible cases of HDN, showing that 64.7% of newborns show maternal antibodies in their red-blood cells. The largest number of cases was associated with anti-D with a frequency of 86.4%, as evidenced by the eluate test, which is justified by the fact that the D antigen is highly immunogenic, and it is the most associated to triggering the disease[3]. Scientifical studies confirm that anti-D is still the most frequent and is the main cause of HDN, being responsible for 80% to 90% of cases[5,6].

The results of IAT and eluate tests revealed that newborns with anti-c alone (4.5%) and the same frequency associated to anti-E. Chatziantoniou et al[7] showed anti-c alone as the cause of severe clinical conditions in 10% of the total number of severely affected newborns, which highlights the clinical significance of this antibody as a cause of severe HDN. Additionally, the reported case by Yoda et al[8] reveals a newborn affected by anti-c with anti-E, it needed phototherapy and blood transfusion, once laboratorial signs of hemolytic anemia were observed, also high levels of bilirubin in the blood, due to immunogenicity of these antibodies.

In this study, it was found 11.7% cases which IAT revealed maternal antibodies of anti-D with anti-C, meanwhile, in the eluate test, in 13.6% cases the identified was anti-D isolated, showing that 3 (13.6%) newborns have not shown antigen C in its erythrocital membrane, revealing possible HDN by anti-D. It should be noted that anti-C has the potential to cause HDN. As observed in the present study, an association of anti-D + anti-C antibodies in the serum and recovered in eluate of a newborn (4.5%), pointing to HDN. Peteers et al[9] the clinical importance of these antibodies demonstrating significant hyperbilirubinemia due to high presence of anti-D + anti-C, needed intrauterine blood transfusion.

It was observed maternal anti-K (3.0%) in the serum of a newborn, however, the eluate did not show reaction, making impossible to assume HDN by anti-K, because the newborn probably had negative K phenotype, once this is antigen that is less frequent in the population. If the newborn had the K antigen on the erythrocyte membrane, it was expected its detection on eluate, attached to the newborn red-blood cells and possibly leading to HDN, since this antibody can trigger severe cases of the disease[10,11].

Among the positive DAT cases, in the present study, in 12 of them it was not possible to evidence or exclude the suspicion of the disease. In these cases, the IAT was not performed (33.3%), or even, IAT did not show reactivity (66.7%), which can be justified by the possibility of complement fraction and/or the antibodies being attached to the newborn's erythrocytes. In these cases, the eluate test was negative (50%) or not performed (50%). This negative results in the eluate may have occurred due to low immunoglobulin levels that may be below the sensitivity threshold of the test, making it impossible to detect the antibody[3,12].

Reche et al.[12] analyzed newborns with a clinical picture of HDN from a Brazilian Hemoclinic, confirming that the ABO system was the most predominant in 75% of HDN cases. Nevertheless, among the samples that could be analyzed in this study, there were no maternal-fetal ABO incompatibility, probably due to the newborns not needing blood transfusion by a possible involvement of HDN, showed by discrepancies in pre-transfusion tests which resulted in forwarding the sample for immune-hematological studies at the Immunohematology Laboratory of the HEMOPA.

It is worth mentioning that the absence of ABO incompatibility, probably, favored maternal alloimmunization by anti-D (86.4%) and by other alloantibodies from other blood group systems (13.6%), acknowledging reduced risk of non-ABO alloimmunization, since anti-A or anti-B antibodies act as protection against the formation of irregular antibodies, due to the quick destruction of sensitized erythrocytes in the maternal circulation through immune response[13].

Studies in Brazil have shown that 93.2% of neonates with laboratory evidence of positive DAT and positive IAT are Rh(D) positive, in agreement with what was revealed in the study of 77.3% with

positive phenotype that, according to scientific literature, have a higher risk of maternal-fetal Rh(D) incompatibility[13]. Differently from what is reported in the literature, in the present study, a Rh(D) negative neonate was found with laboratory evidence of positive DAT and anti-D identified in the eluate, where it can be justified by the occurrence of blocked D phenomenon, in which conventional routine laboratory tests fail to detect the Rh(D) phenotype, due to possible case of Rh(D) variant, classifying the individual as Rh(D) negative, which reflects the risk of formation of the anti-D due to the presence of the D antigen[3].

In agreement with this finding, Jain et al.[14] showed a newborn with a transfusion indication, whose pre-transfusion laboratory tests by conventional methods sorted it as Rh(D) negative. However, the DAT was strongly positive, IAT was positive for anti-D and yet from the eluate test it was identified anti-D antibodies in the neonatal erythrocytes, suggesting that the newborn showed blocked D phenomenon and specific laboratory features of HDN.

In the present study, the data obtained referring to maternal ABO/Rh(D) blood typing showed women from all existing blood groups (A, B, AB and O), with the highest frequency in groups A and O (33.3%) each, followed by B and AB (16.7%) each, which corroborates the study by Siqueira et al.[15] carried out in a Brazilian obstetric population, which presented the most frequent blood type A (50%), followed by O (30%), B (15%) and AB (10%).

The maternal samples 66.7% had a negative Rh(D) phenotype, which was already expected, considering that the most frequent antibody as a possible cause of HDN was anti-D, since most newborns showed positive Rh(D) phenotype. Nevertheless, there is a need to draw attention to 33.3% of Rh(D) positive women alloimmunized by antibodies other than anti-D, leading to laboratory variations in newborns. According to scientific literature, Rh(D) negative women have higher risk of maternal-fetal Rh(D) incompatibility[12], but there is a possibility of Rh(D) positive women to produce clinically significant non-anti-D alloantibodies[16,17].

It is worth mentioning that the present study has limitations, once there is no clinical evidence of HDN in collected data, as these were collected from a hemotherapy and transfusion service being only results of immune-hematological tests performed in the Immunohematology Laboratory of the HEMOPA, due to variations on pre-transfusion tests. Thus, it is not possible to claim HDN in the cases described, however, the study shows variables related to the triggering of the disease, since blood transfusion was requested due to hematological discrepancies in the newborn, as well as the antibody profiles found in the study were like those described in the scientific literature, as potentially causing HDN.

Due to the development of the study being done in a blood bank, the amount of maternal samples was considered small when compared to other studies, the laboratory results of maternal ABO/Rh(D) typing were analyzed along with the newborns ones, when available, to compliment the data analysis, acknowledging the relevance of this data for the assessment of possible risk of maternal alloimmunization, and consequently HDN.

In Brazil, HDN still affects about 5 out of 1000 pregnancies[18]. Therefore, the results observed in this study, considering yet the studies described in scientific literature, highlight the importance of disseminating knowledge about the antigens and red blood cells antibodies related to the disease, specially, to medical and obstetrical community, since this subject is still limited to Hemotherapy and Hematology field.

CONCLUSION

The most frequent antibody found was anti-D, and others red blood cells antibodies such observed, it is very important to describe all antigens and antibodies possibly related to HDN, prioritizing

pregnant women with clinical history of risk for alloimmunization, with the intent to stablish adequate support for high-risk pregnant women, detecting possible process of alloimmunization by red blood cells antibodies potentially involved in HDN.

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