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ARTIGO DE REVISÃO

Trombocitopenia Aloimune Fetal/Neonatal: Uma Revisão Sistemática

Trombocitopenia Aloimune Fetal/Neonatal: Uma Revisão Sistemática

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RESUMO

Introdução: A trombocitopenia aloimune fetal e neonatal (FNAIT) é causada por incompatibilidade de antígenos plaquetários entre mãe e feto, que resulta em respostas imunológicas mediadas por anticorpos. O organismo da mãe produz anticorpos contra antígenos plaquetários fetais específicos. Esses anticorpos atravessam a placenta e ligam-se às plaquetas fetais, removendo-as da circulação através do sistema reticuloendotelial. OBJETIVO: Analisar, por meio de revisão sistemática, os casos de FNAIT para verificar a classe de antígenos plaquetários mais envolvida com a patologia e compreender as medidas preventivas adotadas durante a gestação. **Método:** Foi realizada uma busca de artigos científicos dos últimos 10 anos. A base de dados utilizada foi o PubMed, sendo os descritores "Thrombocytopenia, Neonatal Alloimmune" e "Clinical Laboratory Techniques" determinados pelo MesH (Medical Subject Headings). Foram aplicados critérios de inclusão com base na data de publicação, espécie e idioma. Os artigos considerados não relevantes e estudos do tipo "Relatos de Caso" foram excluídos. **Resultados:** Foram selecionados para fazer parte do escopo desta revisão 7 artigos, que apontaram as incompatibilidades envolvendo o HPA-1a como as principais responsáveis pelos quadros de FNAIT. Com relação aos tratamentos pré-natais oferecidos às gestantes, observou-se uma variabilidade nas estratégias terapêuticas, mas o emprego da imunoglobulina intravenosa associada ou não à corticosteróides têm se mostrado eficaz para a prevenção de hemorragia intracraniana e óbitos de fetos/neonatos. **Conclusão:** Os casos de FNAIT, apesar de raros, são graves e mostraram-se de difícil diagnóstico e manejo clínico. Portanto, há necessidade de desenvolvimento contínuo de novos estudos que auxiliem no diagnóstico e na padronização de métodos preventivos.

Palavras-chave: Trombocitopenia Neonatal Aloimune, Imunização, Hemorragias Intracranianas, Morte Fetal.

ABSTRACT

Introduction: Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is caused by the incompatibility of platelet antigens between mother and fetus, which results in antibody-mediated immune responses. The mother's organism produces antibodies against specific fetal platelet antigens. These antibodies cross the placenta and bind to fetal platelets, removing them from circulation through the reticuloendothelial system. **Objective:** To analyze, through systematic review, the cases of FNAIT to verify the platelet antigen class most involved with the pathology and to understand the preventive measures adopted during pregnancy. **Method**: A search for scientific articles from the last 10 years was performed. The database used was PubMed, with the descriptors "Thrombocytopenia, Neonatal Alloimmune" and "Clinical Laboratory Techniques" determined by the MesH (Medical Subject Headings). Inclusion criteria were applied based on the date of publication, species, and language. Non-relevant articles and "Case Reports" studies were excluded. RESULTS: Seven articles were selected to be part of the scope of this review, which pointed out the incompatibilities involving HPA-1a as the main responsible for FNAIT. Regarding the antenatal treatments offered to pregnant women, there was variability in therapeutic strategies, but the use of intravenous immunoglobulin associated or not with corticosteroids has been shown to be effective for preventing intracranial hemorrhage and fetal/neonatal deaths. **Conclusion:** Although rare, FNAIT cases are severe and difficult to diagnose and clinical management. Therefore, there is a need for continuous development of new studies that help in the diagnosis and standardization of preventive methods.

Keywords: Thrombocytopenia, Neonatal Alloimmune, Immunization, Intracranial Hemorrhages, Fetal Death.

INTRODUCTION

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is the most common cause of thrombocytopenia in neonates and affects between 1/800 and 1/1,000 live births, and the occurrence of severe cases (counting platelets <50,000/mm^3) caused by anti-HPA-1a antibodies is approximately ½,500 pregnancies[1-3].

FNAIT is caused by an incompatibility of platelet antigens between the mother and the fetus, which results in antibody-mediated immune responses. The mother's body produces antibodies of the immunoglobulin G (IgG) type against specific fetal platelet antigens that are of paternal origin. These produced antibodies cross the placental barrier and bind to fetal platelets, which will later be removed from the circulation by the reticuloendothelial system resulting in fetal thrombocytopenia[1,4].

Alloimmunization occurs for one or more human platelet antigens (HPA). These antigens are located on platelet glycoproteins, and most of which are located on the transmembrane glycoprotein GPIIb/IIIa. It was found that the antibodies involved with FNAIT are related to six types of biallelic antigens (HPA-1, HPA-2, HPA-3, HPA-4, HPA-5, and HPA-15). The different HPA alleles vary according to ethnic groups, with the antibody against HPA-1a being the most prevalent in Caucasians, responsible for 70 to 85% of cases of FNAIT in this population[5].

Babies who develop FNAIT have thrombocytopenia, petechiae, and bruises. In addition, about 10% of cases may progress to intracranial hemorrhage (ICH), the most serious complication that can lead to death or neurological disability in approximately 90% of cases[6,7].

As there is no routine screening for all pregnant women, there is no way to predict the occurrence of FNAIT today. Therefore, to reduce cases of complications for ICH, the intervention method is the treatment of pregnancies subsequent to a previous pregnancy identified with FNAIT. However, only 13% of cases are diagnosed using this obstetric history as a predictor. In prenatal examinations, preventive measures are adopted for high-risk pregnancies, such as weekly infusion of intravenous immunoglobulin (IVIG) to the mother, associated or not with corticosteroids, and intrauterine platelet transfusion. Initially, a fetal blood sample is taken for platelet count and then, if necessary, intrauterine transfusion of compatible platelets is performed. However, these measures are associated with premature births and abortions in 1 to 2% of the procedures[2,7].

Given the severity of FNAIT and the challenge for its diagnosis even in the prenatal period, the aim of this systematic review was to analyze, from a laboratory and therapeutic perspective, the cases of fetal and neonatal alloimmune thrombocytopenia to check the class of platelet antigens most involved in the cases and understand the preventive measures adopted during the gestational period.

METHODS

Research strategies

A search for scientific articles from the last 10 years was performed, until August 31, 2019. The database used was PubMed, with the descriptors "Thrombocytopenia, Neonatal Alloimmune" and "Clinical Laboratory Techniques" determined by MesH (Medical Subject Headings). Using the advanced search tool through the Boolean operators "OR" and "AND" 763 articles were found. Then, some inclusion criteria were applied based on the date of publication, species, and language. Articles considered not relevant and "Case Reports" studies were excluded (Chart 1).

RESULTS

763 scientific articles were identified involving alloimmune neonatal/fetal thrombocytopenia and clinical laboratory techniques. However, after applying the previously established inclusion criteria, 227 studies remained for reading the titles. Excluding those considered not relevant, 79 articles were chosen to read the abstracts and "Case Report" studies were excluded. Finally, 7 articles were selected to be part of the scope of this systematic review. Figure 1 shows the flowchart of the article selection process.

In general, the studies analyzed involved different approaches, such as: I) retrospective analysis of cases of FNAIT or alloimmunization by HPA-1a; II) follow-up studies of women who received prenatal treatments in their pregnancies, as they already had children with ICH; and III) comparisons between previous pregnancies and subsequent pregnancies of the same women who had children with FNAIT (Table 1).

As has already been described in the literature, all studies in the scope of this systematic review pointed out the incompatibilities involving the HPA-1a antigen as the main responsible for the FNAIT presentations, with prevalence ranging from 85 to 95% of cases. The authors demonstrated the importance of a more in-depth assessment of FNAIT or ICH history in previous pregnancies in order to adopt preventive measures for subsequent pregnancies. Regarding the prenatal treatments offered to pregnant women, it was possible to observe variability in the therapeutic strategies adopted by hospitals or therapy centers, but, despite this heterogeneity, the use of IVIG associated or not with corticosteroids has been shown to be effective for the prevention of ICH and deaths of fetuses or neonates.

DISCUSSION

The results showed the importance of preventive methods in the prenatal period for pregnant women who had a history of ICH

Table 1 . Inclusion criteria and exclusion criteria.

Inclusion criteria	
Type	· Humans.
Idiom	- English, Portuguese and Spanish.
Date of publication	· 10 years (2009 to 2019).
Exclusion Criteria	
Types of article	· Case reports.
Text availability	· Incomplete texts (abstracts only).
Variables analyzed	
· Diagnosis of fetal/neonatal alloimmune thrombocytopenia.	
· Forms of prevention.	
· Treatment protocols.	

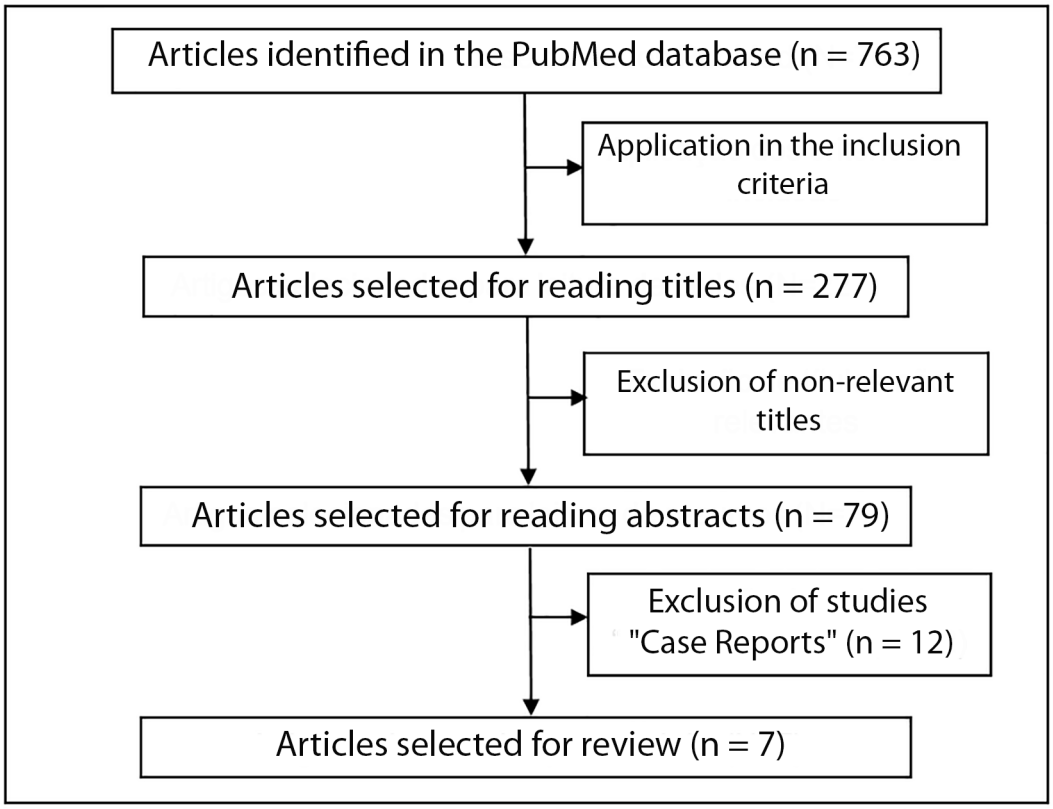

Figure 1 . Flowchart of the article selection process**.**

or FNAIT in previous pregnancies, despite the therapeutic regimens involving IVIG and corticosteroids vary between studies.

Efficacy of therapies in the prenatal period for preventing ICH or deaths in fetuses/neonates

Studies have shown the importance of therapies with intravenous immunoglobulin, associated or not with corticosteroids and intrauterine platelet transfusions, to minimize the occurrence of hemorrhagic complications, such as ICH, which can result in deaths of fetuses/neonates.

This can be demonstrated in the study by Cook et al. (2012)[6], in which 16 women were treated with IVIG 1 g/kg/week, and three of whom received an association with corticosteroids (prednisolone), four neonates received platelet transfusions and five received IVIG after birth and there was no ICH or death in these cases. A similar approach was performed in the study by Giers et al. (2010)[9] where pregnant women were also treated with IVIG 1 g/kg/week, but, before delivery, the fetuses received intrauterine platelet transfusion: there were no hemorrhagic complications and no deaths, despite not showing a consistent increase in blood count fetal platelets.

Tabela 1 . Sumário dos estudos e seus resultados principais sobre antígenos plaquetários e métodos de prevenção no período pré-natal.

AUTHOR (YEAR)	SAMPLE	METHOD/INTERVENTION	RESULTS
Bussel *et al.* (2010)[8]	33 women (37 pregnancies	33 women (37 pregnancies) who have had children with ICH classified in groups: extreme high risk (EHR) (ICH <28 weeks), very high risk (VHR) (ICH between 28 and 36 weeks) and high risk (HR) (perinatal ICH). Mothers treated with IVIG with or without prednisone.	*95% (35/37) HPA-1a, 1 HPA-3a and another unknown. *5 cases of ICH (3 due to therapy failure). *8 EHR pregnancies: 1 case of ICH (the therapy changed and there were no more cases). *17 VHR pregnancies: 2 ICH cases (not due to thrombocytopenia). *12 HR pregnancies: 2 ICH cases (change in therapy)..
Giers *et al.* (2010)[9]	29 pregnancies	Retrospective analysis of the follow-up of fetuses with FNAIT from women who received treatment with immunoglobulin G. Fetal blood samples were taken before infusions.	* 25/29 anti-HPA-1a, 2 anti-HPA-3a and 2 anti-HPA-5b. *Without ICH and bleeding. *No fetal/neonatal losses. *Maternal and fetal IgG increased in women receiving IVIG, but without a consistent increase in fetal platelets and without significant changes in maternal or fetal anti-HPA levels.
Knight *et al.* (2011)[10]	173 cases	National (UK) descriptive population-based study to estimate incidence of FNAIT. The cases were identified between October 2006 and September 2008 through three different sources.	* 30% recognized in early pregnancy. *81% anti-HPA-1a, 7% anti-HPA-5b, 5% anti-HPA-1a and anti-HPA-5b and 7% others. *15% with ICH. *Unknown cases at the beginning: increased risk of bleeding and ICH. *116 newborns followed for 1 year: no deaths and disabilities known at the beginning and 2 deaths and 7 disabilities unknown.
Cook, Qiu e Dickinson (2012)[6]	20 cases (13 women)	Retrospective analysis of FNAIT pregnancies was performed in a hospital in Australia between 2001 and 2011. The diagnosis was based on the neonate's platelet count <50,000/mm^3 and the presence of maternal alloantibodies against HPA.	*17/20 anti-HPA-1a and 3/20 anti-HPA-5b. *3 cases of ICH. *4 untreated cases and 16 treated with IVIG (3 also treated with corticosteroids). *4 neonates received platelet transfusions and 5 received IVIG (there was no death or ICH in either case).
Sainio *et al.* (2013)[11]	84 index pregnancies and 45 subsequent pregnancies (129 pregnancies). subsequentes (129 gestações).	Analysis of cases of alloimmunization by HPA-1a referred to the Finnish Red Cross Blood Service, between 1986 and 2010. Of the 45 subsequent pregnancies, 34 received prenatal treatment (intrauterine platelet transfusion or IVIG).	* Index: 5/84 intrauterine deaths (2 from ICH). 8 ICH cases in live births. Levels of anti-HPA-1a and neonatal platelets: no significant difference (except in cases of ICH and cutaneous hemorrhage). *Subsequent: 2 ICH cases. High levels of anti-HPA-1a in the indices decreased in subsequent ones. Strong anti-HPA-1a correlation and fetal platelets.
Tiller *et al.* (2016)[12]	45 subsequent pregnancies	Comparison: pregnancies of alloimmunized women HPA-1a from one study (index pregnancies) with subsequent pregnancies of the same women (subsequent pregnancies). Maternal anti-HPA-1a levels quantified in three stages: 22 and 34 weeks of gestation and 6 weeks after delivery. No woman received IVIG therapy.	* 1 case of ICH index pregnancies; with no subsequent cases. *18% of neonatal platelets in the subsequent pregnancies went up to category, 52% unchanged and 30% worse. *Platelets that remained low: higher anti-HPA-1a. *Platelets that changed to moderate or normal: anti-HPA-1a dropped. * Comparison of immunization during pregnancy or postpartum: platelets without a difference.
Lakkaraja *et al.* (2016)[13]	99 women (104 fetuses)	Groups: IVIG 2 g/kg/week or IVIG 1 g/kg/week with prednisone. Initiation of therapy: 20 weeks without a fetal sample (32 weeks only). No response: IVIG 2 g/kg/week and prednisone (staggered). Divided fetuses/neonates: plaques ≥50,000/mm^3; platelets <50,000/mm^3; and no blood sample.	*93 HPA-1a (3 também HPA-5a), 7 HPA-5a isolado, 1 HPA-11b e 1 desconhecido. *3/104 neonatos com ICH. *19 fetos com amostragem de sangue em 32 semanas: plaquetas <50.000/mm^3 (apesar da terapia). *13 destes as mães receberam terapia escalonada e 85% (11/13) atingiram a contagem de plaquetas de 50.000/mm^3.

Legenda: FNAIT (Trombocitopenia aloimune fetal e neonata)l; ICH (Hemorragia intracraniana); IgG(Imunoglobulina G); IVIG(Imunoglobulina intravenosa).

In the study by Lakkaraja et al. (2016)[13], whose aim was to assess whether the empirical therapy followed by scheduling would be effective and would allow omission of fetal blood collection, two treatment groups were created: I) IVIG 2 g/kg/week divided into two infusions in the week and II) IVIG 1 g/kg/week with 0.5 mg/kg/day prednisone, as empirical therapies initiated without fetal sampling, being performed only in the 32nd week of pregnancy. In this protocol, those who did not respond to therapy in fetal sampling, had an increase to IVIG 2 g/kg/week and 0.5 mg/kg/day prednisone (staggered therapy). Therefore, after fetal blood collection, 19/104 fetuses had platelets <50,000/mm^3 and 13 received staggered therapy: 11 of these were born with platelets > 50,000/mm^3 and 3/104 neonates suffered postnatal ICH. Then, these authors concluded that the increase in fetal platelet count occurred in almost all cases and that these two forms of initial treatment followed by escalation are reasonably safe and effective.

Bussel et al. (2010)[8], who studied the prevention of ICH through prenatal therapy with pregnant women from two consecutive studies, classified women into risk groups and different forms of therapy adopted, with adjustments in dosages being made: for example, a

pregnant woman from the high-risk group was treated with IVIG 1 g/kg/week and prednisone 1 mg/kg/day but, after losing the baby, the next pregnant women in this group were treated with IVIG 2 g/kg/week divided into two doses. The pregnancies in the first study in the very high-risk group received IVIG 1 g/kg/week and in the second IVIG study 1 g/kg/week or 2 g/kg/week. Finally, the pregnancies of the high-risk group in the first study received IVIG 1 g/kg/week and prednisone 1 mg/kg/day or IVIG 1 g/kg/week only and, in the second study, IVIG 1 g/kg/week or IVIG 2 g/kg/week.

Through the analysis of these studies taken as an example, it is noticed that different methods of prevention of hemorrhagic complications are adopted that can evolve to fetal/neonate losses, with variations in intravenous immunoglobulin dosages as well as the corticosteroid used (prednisone or prednisolone) and its dosages.

Laboratory techniques for FNAIT diagnosis

Regarding the techniques used by the authors to identify and quantify antibodies against HPA, Giers et al. (2010)[9], Sainio et al. (2013) 11 and Tiller et al. (2016)[12] used the immobilization test for platelet antigens by monoclonal antibodies (MAIPA).

MAIPA is an assay that uses a donor platelet panel with known genotyping and specific monoclonal antibodies against the glycoproteins that contain the target antigens, that is, when using a monoclonal antibody against glycoprotein IIbIIIa, the presence of antibodies against specific antigens of this complex is assessed, such as HPA-1 and HPA-3. As it is a totally "in-house" technique, it requires good standardization through the use of internal and external controls. In addition, it is a technically demanding test, requiring up to 8 hours to be performed and requires a high level of technical knowledge to interpret its results. For this reason, other methods for detecting these antibodies have been developed[14,15].

In addition to this, other techniques are also used, such as platelet immunofluorescence tests (PIFT) and enzyme-linked immunosorbent assay (ELISA), based on the use of purified platelet membrane glycoproteins. However, as MAIPA is the most sensitive and a specific technique for detecting alloantibodies against HPA, it has been considered the gold standard method for the diagnosis of FNAIT[16,17].

Fetal sampling and its complications

Authors of two studies pointed out complications associated with the procedure for collecting fetal blood samples. For example, Bussel et al. (2010)[8] reported that this may have been the cause of premature delivery at 24 weeks of gestation. In addition, after fetal blood collection, there was an occurrence of fetal tachycardia and eight occurrences of bradycardia, and in three of these cases an emergency cesarean section was necessary, in which one was due to hemorrhage at the needle perforation site. In the study by Lakkaraja et al. (2016)[13], which even aimed to assess whether fetal blood collection can be omitted through empirical therapy, of eighty women who underwent the procedure, 12.5%[10] suffered complications, such as premature birth or rupture of membranes in six cases and, in the remaining four cases, a cesarean section was performed due to fetal bradycardia. Finally, these authors concluded that the initial treatment protocols followed by empirical scheduling of therapy is reasonably safe, effective in increasing fetal platelets and allows the omission of fetal blood collection.

CONCLUSION

The present review shows that the majority of cases of mother/fetus incompatibility of platelet antigens involved the anti-HPA-1a class, as it has been described in the literature. In addition, the study demonstrates a variability in the therapeutic strategies adopted for prevention in high-risk pregnancies, but, despite this, the use of IVIG associated or not with corticosteroids proved to be effective for the prevention of ICH and deaths of fetuses or neonates.

FNAIT cases, although rare, are serious and have proved to be difficult to diagnose and manage clinically. Therefore, there is a need for continuous development of new studies that assist in the diagnosis of the disease and the standardization of effective preventive methods during the prenatal period.

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