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Detection of fetomaternal haemorrhage using the microcolumn agglutination test**

Wykrywanie przecieku płodowo-matczynego przy użyciu aglutynacyjnego testu mikrokolumnowego

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Keywords

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Słowa kluczowe

przeciek płodowo-matczyny, technika mikrokolumnowa, profilaktyka anty-D, choroba hemolityczna płodu/noworodka

Summary

Introduction. Quantitative evaluation of fetomaternal haemorrhage is important for the selection of the dose of anti-D Ig in the prevention of haemolytic disease of the fetus/newborn. It is usually performed using the microscopic test and flow cytometry.

Aim. The aim of this study was to implement and evaluate column agglutination method ID-FMH Screening-Test (DiaMed, Switzerland) for the detection of RhD positive fetal RBCs in the blood of RhD negative mothers.

Material and methods. Blood samples of donors: 10 RhD positive and 12 RhD negative used to prepare 54 mixtures: 0.1%; 0.2%; 0.4% and > 0.4% (0.5-1.0%) and 14 commercial sets of mixtures: 0% (negative control); 0.1%; 0.2%; 0.4%.

Results. In 54% of the samples without the RhD positive RBCs non-specific reactions were obtained, which falsely suggested the presence of 0.1% or 0.2% of such cells. In 50% of the samples with the concentration of 0.4% RhD positive blood cells and in all the samples containing > 0.4% of such cell reactions were as expected.

Conclusions. The agglutination test for the presence of fetal RhD positive RBCs in the mother's blood may be a screening test for the detection of a large fetomaternal haemorrhage (> 0.4% means > 9 ml RBCs). In such cases, in order to apply an appropriate anti-D Ig dose further tests are necessary using flow cytometry.

Streszczenie

Wstęp. Ilościowa ocena przecieku płodowo-matczynego krwinek czerwonych ma istotne znaczenie w doborze dawki Ig anty-D w profilaktyce choroby hemolitycznej płodu/noworodka. Wykonuje się ją zazwyczaj za pomocą testu mikroskopowego oraz z zastosowaniem cytometrii przepływowej.

Cel pracy. Celem badań było wdrożenie i ocena aglutynacyjnego testu kolumnowego ID-FMH Screening-Test (DiaMed, Szwajcaria) do wykrywania krwinek RhD dodatnich płodu w krwi RhD ujemnej matki.

Materiał i metody. Próbki krwi dawców: 10 RhD dodatnich i 12 RhD ujemnych, z których sporządzono 54 mieszaniny o procentowości: 0,1%; 0,2%; 0,4% i > 0,4% (0,5-1,0%) oraz 14 firmowych zestawów: 0% (kontrola ujemna); 0,1%; 0,2%; 0,4% (łącznie 110 próbek).

Wyniki. W 54% próbek niezawierających krwinek RhD dodatnich uzyskano reakcje nieswoiste, które fałszywie sugerowały obecność 0,1% lub 0,2% takich krwinek. W 50% próbek o stężeniu 0,4% krwinek RhD dodatnich i we wszystkich próbkach zawierających > 0,4% takich krwinek reakcje były zgodne z oczekiwanymi.

Wnioski. Aglutynacyjny test na obecność krwinek płodowych RhD dodatnich w krwi matki może być testem przesiewowym do wykrywania dużego przecieku płodowo-matczynego (> 0,4%, czyli > 9 ml krwinek). W takich przypadkach, w celu zastosowania odpowiedniej dawki Ig anty-D konieczne jest dalsze badanie z użyciem cytometrii przepływowej.

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INTRODUCTION

Antibodies that cause haemolytic disease of the fetus and newborn (HDFN) are IgG antibodies made in response to exposure of fetal red blood cells (RBCs) during pregnancy and delivery. The most common antibodies found to cause HDFN are directed against D, c, K, C, E, Fy^a antigens on the fetal RBCs. There are the following severe symptoms of HDFN: anaemia, hepatic dysfunction, hydrops fetalis, heart failure, brain damage by the unconjugated bilirubin crossing the blood--brain barrier and even death. In such cases, intrauterine transfusions are needed to save the fetus (1-4). Mothers can be alloimmunised to RBC antigens because of spontaneous haemorrhage of fetal RBCs into the maternal circulation. The volumes of fetomaternal haemorrhage (FMH) are usually small, about 0.5 ml of erythrocytes. In 1% of mothers FMH is bigger than 3 ml and in 0.3% bigger than 15 ml. Number of HDFN cases due to anti-D has been reduced since specific immunoprophylaxis was introduced in the '70s. Anti-D immunoglobulin (Ig) neutralizes RhD positive fetal RBCs in the circulation of mother and protects her against alloimmunization (4-8). The postpartum standard dose of anti-D Ig in Poland is 150 μg, which can neutralize 7.5 ml of fetal RhD positive RBCs. In other countries, anti-D Ig doses vary from 100 to 300 μg. Despite of the use of immunoprophylaxis, from 1 to 2% women produce allo-anti-D. In some countries (for example United Kingdom, Ireland), all RhD negative mothers are tested after labour for detection and quantification of FMH and if it is necessary, the dose of anti-D Ig is increased. Microscopic or flow cytometry tests usually base on detection of the cells containing fetal haemoglobin (HbF) between cells with adult haemoglobin (HbA). If a test for the detection of RhD antigen is used, it is applicable only when the mother is RhD negative and the fetus is RhD positive. Results from flow cytometry tests with anti-HbF or anti-RhD have very good sensitivity and specificity but these methods require special expensive equipment (9-16). Gel agglutination technique to screen the FMH could be used in many serological laboratories, where serological gel microcolumn tests for grouping, crossmatching and antibody screening (DiaMed, Switzerland) are routinely performed. It is semiquantitative method based on the consumption of anti-D antibodies by RhD positive fetal RBCs, indirectly measured by the use of RhD positive indicator RBCs. When fetal RhD positive RBCs are absent or their percentage is smaller than 0.1%, the reaction with indicator cells is strong (4+), but when in mother's blood sample there is > 0.4% of fetal cells, all anti-D bind to fetal antigens and reaction with indicator cells is negative (fig. 1).

AIM

The aim of this study was to implement and evaluate column agglutination method ID-FMH Screening-Test (DiaMed, Switzerland) for the detection of RhD positive fetal RBCs in the blood of RhD negative mothers.

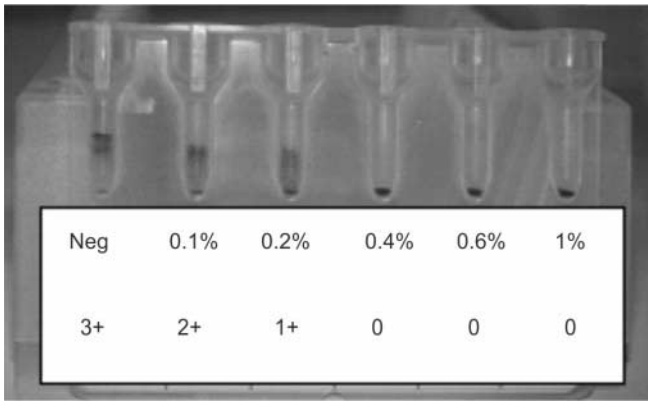

Fig. 1. Example of the results for FMH Screening-Test

MATERIAL AND METHODS

Fifty four artificial mixtures imitating FMH: 0.1%, 0.2%, 0.4% and > 0.4% (0.5-1.0%) were prepared with RBCs from 10 RhD positive and 12 RhD negative blood donors. The same 12 blood samples were tested as the negative control. Furthermore 14 panels of ID-FMH standard cells (DiaMed, Switzerland) were evaluated. Each of them contained RhD negative RBCs, and the following mixtures: RhD negative with 0.4%, 0.2% and 0.1% of RhD positive RBCs. Blood samples, standard cells and lyophilised anti-D were prepared according to the procedure. Briefly, 50 μl of anti-D reagent was incubated for 60 min, with continuous mixing, with 250 μl of tested RBCs. Next, the cells were centrifuged and 50 μl of supernatant was tested against 50 μl of indicator cells on a gel card with anti-IgG antibodies; incubation for 15 min at 37°C. The card was then centrifuged, and the results were interpreted by comparison with the pattern observed with standard RBCs.

RESULTS

Figure 1 shows reactions of agglutination obtained from different mixtures imitating FMH and their concordance with expected pattern of reactions is presented in table 1. Only for RhD positive RBCs concentrations bigger than 0.4% the results were the same as expected. In 54% of RhD negative blood samples, false, nonspecific positive reactions were observed, which falsely indicated the presence of 0.1% or 0.2% of RhD positive RBCs.

DISCUSSION

Numerous laboratories use gel agglutination technique for routine serological investigation of blood recipients, pregnant women and patients with allo- and autoantibodies. It is the main reason to introduce this method to screen the FMH in blood samples from RhD negative mothers whose newborns are RhD positive. It seems important, especially that the microscopic Kleihauer-Betke test and flow cytometry tests are rarely performed in our country (15, 16). Some authors obtained satisfactory results using the FMH gel test, but there are only few such publications (17-19). Our studies revealed that the test is suitable for detection of

Table 1. Association between expected and observed reactions in negative control samples and in blood mixtures imitating FMH

Concentration of RhD+ RBCs (%)	Number of tested mixtures	Strength of agglutination				Results as expected
		0-0.5+	1-1.5+	2-2.5+	3-4+	
0	26	0	2	12	12	46%
0.1	18	3	6	4	5	22%
0.2	17	5	3	7	2	18%
0.4	20	10	7	3	0	50%
> 0.4	29	29	0	0	0	100%

*The number of expected results is highlighted in grey

a high concentration of RhD positive RBCs. This concentration has to be converted into RBCs volume using special calculation. It is necessary because anti-D Ig doses refer to the RBCs volume (20). Concentration of 0.4% RhD positive RBCs corresponds to their volume of about 9 ml. In such case, two doses of Ig anti-D 150 μg have to be injected. Results > 0.4% should be considered for further FMH evaluation, because 3 or more doses may be necessary. Furthermore, results from mixtures containing 0.1% and 0.2% of RhD positive RBCs in some cases were similar to this with 0.4%. For this reason, all final agglutination results close to zero should be verified by the flow cytometry test. In addition, it should be noted that the test procedure is not simple. In 2007, a new particle gel immunoassay (PaGIA) for determination of FMH (FMH-PaGIA) was described, but it was the only report about its advantage. Superparamagnetic particles were coated with monoclonal anti-D and mixed with blood samples from D-negative pregnant women. The particles were isolated using a magnetic particle concentrator and then placed into the reaction chamber of a gel card. Particles agglutinated on top or dispersed through the gel matrix indicated the presence of D positive cells (19). The critical level of RhD positive RBCs was > 0.3%, and it was better than > 0.4%. The disadvantage of this test was the necessity of the use a devise for generating of a magnetic field.

CONCLUSIONS

Semiquantitative gel agglutination test for quantification of fetomaternal haemorrhage with the current procedure is not easy and is time-consuming, which are its disadvantages. It may be a screening test for the detection of a large fetomaternal haemorrhage. In such cases, in order to apply an appropriate anti-D Ig dose, further tests using flow cytometry are necessary.

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