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Indirect Coombs Test and Limitations in RH Incompatibility

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Abstract

Aim: RHD incompatibility occurs in the case of mother is RHD negative (-) when the father and the fetus have RHD positive (+) blood group. Our aim with our study is to determine RH incompatibility reliably and to emphasize the importance of detecting it with non-invasive methods as much as possible. In this way, unnecessary tests and possible risks for RHD(-) fetus will be eliminated. In this study, it is aimed to retrospectively evaluate the accuracy of the Indirect Coombs Test (ICT) and the necessity of RHogam application against the risk of RHD blood group incompatibility that may occur between the mother and the fetus.

Material and Methods: Age, weight, gestational week, previous pregnancies, complications, blood group type, ICT results, RHogam administration before and after birth of 40 pregnant women with RHD (-) blood group, who were followed up and delivered in Bruni University Medical Faculty Hospital, between the years 2019-2021 were retrospectively analyzed.

Results: In the study, in a sample of 40 people, 30% were RHD (-); The presence of 70% RHD (+) babies was detected. It was the first pregnancy of 17 of the pregnant women. Nine of 40 pregnant women had negative ICT results and 31 had positive. Unnecessary RHogam was implemented to 6 of these pregnant women, although the ICT result was negative. It was observed that the ICT test was negative in the next pregnancy of 17 of 23 pregnant women who had given birth before. This shows that the RH antibody in maternal blood is not at a detectable level.

Keywords: RH incompatibility; Maternal alloimmunization; RH blood group; Indirect Coombs Test

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Introduction

RHD incompatibility occurs in the case of mother is RHD negative when the father and the fetus have RHD positive blood group. RH incompatibility can cause severe clinical manifestations such as erythroblastosis fetalis and neonatal hemolytic disease [1].

Landsteiner and Wiener discovered that rabbit serum created against Rhesus monkeys in the 1940s agglutinated 85% of human serum samples and named this serum which agglutinating human serum as 'RH factor' or 'Coombs serum'. Agglutinated erythrocytes were defined as RH (+). After that, they defined RH blood groups and classified them with different names. Today, the RH antigen is called C, c, D, E, and e. Fisher and Race, on the other hand, stated that these antigens are in 3 different antigenic structures and in both gene alleles (DE/cde, CDe/Cde, Cde/cDe) [2,3].

Fetal RH antigens are detected on the 30th to 38th days of intrauterine from fertilization, so fetal erythrocytes may cause maternal alloimmunization after the 6th gestational week. Alloimmunization is an immunological reaction against foreign antigens that are not in one's own cells. The RH antigen is a type of lipoprotein found on the cell membrane. It regulates proton or cation pumps in the cell membrane by interacting with intramembrane ATP'ase. It provides cell membrane stability. The RH antigen is located on the short arm of chromosome 1. RH antigens lie on the inner part of the membrane, with other glycoprotein antigens such as Kell, Duffy, and extend towards the outer part of the membrane. This feature is an important point in terms of immune response [4,5].

About 45% of RH antigen positive people are homozygous for this antigen while the rest 55% are heterozygous. These ratios are especially important for the spouse of a RH (-) woman (paternal zygosity). If a RH (+) father has previously had RH (-) children, he is heterozygous and has a 50% chance of the fetus being RH (+) in the current pregnancy. However, if a RH (+) father does

not have a RH (-) child before, paternal zygosity determination is recommended. If the father is homozygous, the fetus will be born as RH (+). In paternal zygosity determination, fetal cells taken by amniocentesis are evaluated by PCR technique in terms of D antigen and fetal DNA is examined with PCR primer probes suitable for the father's RH structure [6].

The following conditions must be met for RH alloimmunization [6]:

I. Fetus must be RH (+), mother RH (-),

II. RH (+) erythrocytes must be in the maternal circulation in sufficient quantity to create an adequate immune response (fetomaternal hemorrhage),

III. The mother should be able to give an immune response to the erythrocyte cells carrying the D antigen, which can generate anti-D serum. Approximately 16% of RH (-) women who are compatible with ABO blood group become susceptible in their first pregnancies. 2% of these cases during their first pregnancy; 7%, within 6 months of birth; 7% of them develop an immune response in their second pregnancy. RH sensitization rate is around 1% to 2% in RH alloimmune cases with ABO incompatibility. In ABO incompatibility, intravascular hemolysis (C3 complement activation) and erythrocytes carrying the D antigen undergo hemolysis before they can reach the fetal Reticuloendothelial System (RES).

At first contact In RH alloimmunization, (fetomaternal hemorrhage), maternal primary immune response is weak. Antibody is formed in the IgM structure (19S Ig, 900,000 kD) and does not cross the placenta. Secondary immune response, on the other hand, occurs more rapidly and with a small amount of red blood cells. Antibodies are formed in the IgG structure (7S Ig, 160,000 kD: IgG1, IgG2, IgG3). These antibodies cross the placenta and cause fetal hemolysis. Ig G and especially Ig G3 antibodies formed by the RH (-) mother, especially after the secondary response, pass through the placenta and adhere to erythrocytes with fetal D + antigens, causing them to agglutinate and hemolysis. The resulting hemolysis causes fetal anemia and activation of fetal RES. Both medullary and extramedullary (spleen, liver) hematopoiesis increases due to tissue hypoxia due to anemia and acidosis. Due to the hyperdynamic blood cycle, the heart shows signs of loading after a while and cardiomegaly develops. In the first stages of hemolysis, the resulting acidosis is compensated by an increase in the amount of 2,3 diphosphoglycerate. As a result, hepatosplenomegaly and portal hypertension develop. Decreased hepatic protein synthesis and decreased intravascular colloidal pressure cause fetal acidity, scalp edema, hydrothorax, placentomegaly and immune hydrops fetalis. Heme products produced by erythrocyte destruction also cause polyhydramnios by creating an osmotic effect and also by increasing the increased blood cycle and glomerular filtration rate. After a while, placentomegaly causes a decrease in uteroplacental blood flow [5,7].

To prevent RH incompatibility, administering 300 micrograms (mcg) of anti-D as a prophylactic dose to RHD-negative pregnant women may prevent alloimmunization at exposures up to 30 ml of RH-positive fetal blood or 15 ml of fetal erythrocytes. Widespread implementation of Anti-D to RH-negative patients in the first 72 h after birth reduced RH alloimmunization by 80% to 90%. Since asymptomatic fetomaternal hemorrhage causes prenatal alloimmunization in 2% of the cases in the risk group, this rate was reduced below 0.2% with the routine antenatal anti-D implementation at 28 weeks of gestation. There are also guidelines saying that if the newborn blood group is positive after delivery, after routine implementation of 300 mcg ant-D at 28 weeks of gestation, the dose is repeated [8].

The Indirect Coombs Test (ICT) is a screening test for RH antibodies in the blood. It helps to determine whether antibodies against the RH antigen of the baby are formed in the maternal blood. A person with RH antigen (D, E, etc.) on their erythrocytes is considered to be RH (+), and those who do not have is considered to be RH (-). Antibodies develop in the RH (-) mother exposed to the blood cells of the RH (+) fetus. To prevent this, ICT should be performed in the RH (-) mother early in pregnancy (at 28. week). A negative ICT indicates that the mother is not alloimmunized. A negative ICT does not give us any information about the fetal blood group, and 300 mcg ant-D is routinely implemented to mothers with negative ICT at 28. week of pregnancy, assuming that fetal blood group is positive [9].

The fact that the technique used in our study is at the level of the systems found in the infrastructure of all hospitals shows that it is a very applicable method. Recent studies have focused on the exclusion of false positivity and negativity.

Importance

A positive ICT indicates that there is blood incompatibility between the mother and the baby and that the mother is alloimmunized. In cases where ICT is negative, if the baby's blood group has not been determined by paternal RH zygosity, it cannot be predicted. Even if the result is not homozygous when paternal zygosity is performed, fetal blood group cannot be predicted until birth. In this case, RHogam is administered between 28 to 34 weeks of pregnancy. If the ICT test is negative at week 28, an anti-D Ig (RHIg) injection is given to the mother in order to clear the fetal RH-positive erythrocytes in the mother's bloodstream and prevent the mother from producing more RH antibodies, since the risk of fetomaternal bleeding increases at this week and in case of bleeding with fetomaternal. At birth, the RH group of the baby is determined and if 12 weeks have passed after the dose at the 28th week or if the amount of fetomaternal hemorrhage is thought to be very high at birth; If the baby is RH positive, the mother is given 1 more dose of RHogam within 72 h to protect the fetus in the next pregnancy. If the baby is RH (-) there is no need for another RHIg injection to the mother [6].

Methodology

The study is a retrospective study. The study was initiated with the approval of the Clinical Research Ethics Committee with the decision number 2015-KAEK-43-19-28. In this study, the suitability of RHogam applications before and after birth was examined by considering two independent groups with positive and negative ICT.

Age, weight, gestational week, previous pregnancies, complications, baby's blood type, ICT results, RHogam administration before and after birth of pregnant women with RH (-) blood group, who were followed up and delivered in Bruni University Medical Faculty Hospital, were retrospectively analyzed. Between the years 2019-2021, 40 pregnant women who met the inclusion criteria were included in the study (Table 1).

According to the indirect Coombs test results, the relationship between the two groups with and without RHogam was statistically analyzed with the Mann-Whitney U Test ($p<0.05$).

Results

Demographic data of pregnant women were evaluated. The mean age of the pregnant women included in the study was 30.1 (23-39), their mean weight was 72.4 (54-112), and their mean gestational week was 13.1 (10-21) (Table 2).

In the study, in a sample of 40 people, 30% (12/40) were RHD negative; The presence of 70% (28/40) RHD positive babies was detected. Although 12 (30%) of 40 pregnant women were RHD negative, 3 (25%) pregnancies were implemented RHogam before delivery, although it was not necessary ($p<0.05$) (Table 3).

It was the first pregnancy of 17 of the pregnant women. These pregnant women have no known history of miscarriage or abortion. Nine (22.5%) of 40 pregnant women had negative ICT results and 31 (77.5%) had positive. Unnecessary RHogam was implemented to 6 (35%) of these pregnant women, although the ICT result was negative ($p<0.0001$) (Table 4).

It was observed that the ICT test was negative in the next pregnancy of 17 (73%) of 23 pregnant women who had given birth before. This shows that the RH antibody in maternal blood is not at a detectable level.

Discussion

With our study, a retrospective study was conducted on the ICT and limitations in RH incompatibility. The Indirect Coombs Test (ICT) is a screening test for RH antibodies in the blood. It helps to determine whether antibodies against the RH antigen of the baby are formed in the maternal blood. A positive ICT indicates that there is blood incompatibility between the mother and the baby and that the mother is alloimmunized [7].

Fetal erythrocytes enter the mother's circulation through the placenta for reasons such as abortion, childbirth or invasive methods. It creates antibodies in the mother against RH (+) antigens that come from the fetus and are not found in the mother. With this antibody response, fetal erythrocytes are destroyed. This problem may not occur in the first pregnancy because the first response that occurs is the IgM response. The problem may not occur because IgM does not cross the placenta. But in the second pregnancy, an IgG response occurs. This response is both much shorter and more effective [8].

Ig G and especially Ig G3 antibodies, pass through the placenta and adhere to erythrocytes with fetal D + antigens, causing them to agglutinate and hemolysis. The resulting hemolysis causes fetal anemia and activation of fetal RES. Both medullary and extramedullary (spleen, liver) hematopoiesis increases due to tissue hypoxia due to anemia and acidosis. Due to the hyperdynamic blood cycle, the heart shows signs of loading after a while and cardiomegaly develops. In the first stages of hemolysis, the resulting acidosis is compensated by an increase in the amount of 2,3 diphosphoglycerate [9]. As a result, hepatosplenomegaly and portal hypertension develop. Decreased hepatic protein synthesis and decreased intravascular colloidal pressure cause fetal acidity, scalp edema, hydrothorax, placentomegaly and immune hydrops fetalis. Heme products produced by erythrocyte destruction also cause polyhydramnios by creating an osmotic effect and also by increasing the increased blood cycle and glomerular filtration rate. After a while, placentomegaly causes a decrease in uteroplacental blood flow. For these reasons, RH incompatibility causes fetal and neonatal morbidity and mortality [10].

In Table 3, the implementation of RHogam to the mothers of RH (-) fetuses was examined. As can be seen, 12 out of 40 RH (-) pregnant women have RH (-) babies. This proves that prenatal RHogam performed without ICT screening test is unnecessary for 3 out of 12 pregnant women. As a result, in our study, 25% of mothers with RH (-) fetuses who were not at risk of blood incompatibility were implemented unnecessary Rhogam [11].

In Table 4, pregnant women were examined according to the application of ICT and RHogam. Although ICT gave negative results, unnecessary RHogam was implemented to 6 pregnant women before delivery. This shows that 15% of pregnant women do not actually need RHogam and are exposed to unnecessary RHIg [12].

It is seen that the ICT test is negative in the next pregnancy of 17 of 23 pregnant women who have given birth before. This indicates that there is no detectable level of RH antibody in maternal blood. As a result, it shows that 73% of the mothers who did not have their first pregnancy received adequate health care after the previous birth, and that antigen transmission due to bleeding between the mother and the baby is not serious. This indicates the success of the physician and his/her teammates [13].

Routine ICT is applied at the 28th week before the RHogam implementation is performed to the sample group we discussed in the study. If the ICT result is negative and there is no risk of complications, RHogam is not implemented. However, in cases which; the hospital where the routine follow-up was performed and the hospital where the delivery was performed were different, it was observed that routine RHogam implementation was performed to the pregnant women, even though the ICT result was negative [14]. Accurate pregnancy follow-up cannot be done due to the fact that pregnant women do not go to regular check-ups or apply to different physicians. Therefore, unnecessary RHogam can be implemented without evaluating the ICT result. Although ICT is routinely used in

Table 1: Inclusion and exclusion criteria.

Inclusion Criteria	Exclusion Criteria
Pregnancy confirmed by beta HCG test	No pregnancy
Having RH-D (-) blood group	Having RH-D (+) blood group
Age between 18-45	Less than age of 18, more than 45
Having defined blood disease	Having defined blood disease

Table 2: Demographic data of pregnant women.

Variance (n=40)	Mean Value ± (SD)	Interval
Age (year)	30.1 ± 3.7	23-39
Weight (kg)	72.4 ± 14.3	54-112
Pregnancy Week	13.1 ± 1.2	10-21

Table 3: Unnecessary RHogam implementation even though the fetus is RHD negative.

Fatus RHD Negative	Prenatal RHogam Implemented	Prenatal RHogam Not-Implemented
12	3*	9

* Mann-Whitney U Test, $p<0.05$

Table 4: RHogam Implementation According to ICT Result.

ICT Result	RHogam Implemented	RHogam Not-Implemented
Positive	1	-
Negative	6*	3

* Mann-Whitney U Test, $p<0.0001$

RH incompatibility, it can give erroneous results from time to time [15].

For this reason, pregnant women who underwent ICT with the suspicion of blood incompatibility are followed more frequently than other pregnant women. This situation can cause both anxiety, fear, and stress in pregnant women and also financial losses. Avoiding unnecessary further examinations will also have positive financial effects, since every procedure performed on the mother and fetus also brings a financial burden. With our study, the importance of minimizing the risks that the fetus and mother may face, reducing false positivity, popularizing the use of non-invasive techniques that can be easily applied to all pregnant women and providing a high detection rate were emphasized.

Conclusion

Evaluation of only pregnant women of childbearing age with RH incompatibility limits the sample size for the study. It is recommended to evaluate various complication parameters by adding them to the studies in a larger population.

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