Good Perinatal Outcome of Rhesus Incompatibility in Multigravida without Anti-D Injection Therapy: A Rare Case Report

Abdillah Husada,1 Peby Maulina Lestari,1 Ziske Maritska,2 Dian Puspita Sari,3 M. Al Farisi Sutrisno,1 Bella Stevanny1

1Department of Obstetrics and Gynaecology, Faculty of Medicine, Sriwijaya University/Dr. Mohammad Hoesin General Hospital, Palembang
2Department of Biology, Faculty of Medicine, Sriwijaya University
3Department of Paediatrics, Faculty of Medicine, Sriwijaya University/Dr. Mohammad Hoesin General Hospital, Palembang

Correspondence: Abdillah Husada, Email: adillhusada26@gmail.com

Abstract
Introduction: Rhesus (Rh) incompatibility problem arises exclusively when an Rh-positive male impregnates an Rh-negative female, resulting in maternal Rh sensitization to produce anti-D antibodies that can bind and destroy Rh-positive erythrocytes of the fetus. Hemolytic disease of the neonate due to Rh incompatibility ranges from self-limited hemolytic anemia to severe hydrops fetalis. Rh incompatibility can be prevented by administering anti-D injection therapy containing Rh Intravenous Immunoglobulin (RhIVIG). We report a rare case of good perinatal outcome of rhesus incompatibility in multigravida without anti-D therapy injection due to weak D phenotype.

Case Illustration: A gravida 3, para 2 woman at 27 weeks gestation with Rh-negative blood type, who has not experienced any previous compatibility problems, came to our facility for routine antenatal care. The husband has an Rh-positive blood type with a Dd genotype (heterozygous), suggesting a 50% probability that the offspring will have an Rh-positive blood type. Laboratory results showed a negative Coombs test and weak D phenotype. The patient had never received an anti-D therapy injection in this pregnancy and her previous two pregnancies. None of her children developed hemolytic disease in the neonate. Ultrasonography showed a well-developed 27-week gestational age fetus with no major congenital disorders. The good perinatal outcomes of her children might be due to weak D phenotype. Pregnant women with weak D phenotype have fewer D antigens that can still result in Rh sensitization but not enough to cause serious complications to the fetus.

Conclusion: Rhesus incompatibility with weak D phenotype can have good perinatal outcomes without anti-D injection therapy. Administration of Anti-D injection remains a viable option to prevent subsequent Rh alloimmunization.

Key words: Anti-D injection therapy, Multigravida, Rhesus incompatibility, Rh immunoglobulin, Weak D phenotype

Inkompatibilitas Rhesus pada Multigravida dengan Luaran Perinatal Baik tanpa Terapi Injeksi Anti-D: Laporan Kasus

Abstrak
Pendahuluan: Masalah inkompatibilitas Rhesus (Rh) muncul secara eksklusif pada ayah Rh-positif dan ibu Rh-negatif, sehingga terjadi sensitisasi Rh ibu untuk menghasilkan antibodi anti-D yang dapat mengikat dan menghancurkan eritrosit janin yang Rh-positif. Penyakit hemolitik pada neonatus akibat kelidakkocokan Rh dapat berupa anemia hemolitik yang bisa sembuh sendiri hiinga hidrops fetalis berat. Inkompatibilitas Rh dapat dicegah dengan pemberian terapi injeksi anti-D yang mengandung Rh Intravenous Immunoglobulin (RhIVIG). Kami melaporkan kasus langka dengan hasil perinatal yang baik inkompatibilitas rhesus pada multigravida tanpa injeksi terapi anti-D akibat fenotip D yang lemah.

Ilustrasi Kasus: Seorang wanita gravida 3, para 2 pada usia kehamilan 27 minggu dengan golongan darah Rh-negatif, sehingga terjadi sensitisasi Rh ibu untuk menghasilkan antibodi anti-D yang dapat mengikat dan menghancurkan eritrosit janin yang Rh-positif. Penyakit hemolitik pada neonatus akibat kelidakkocokan Rh dapat berupa anemia hemolitik yang bisa sembuh sendiri hiinga hidrops fetalis berat. Inkompatibilitas Rh dapat dicegah dengan pemberian terapi injeksi anti-D yang mengandung Rh Intravenous Immunoglobulin (RhIVIG). Kami melaporkan kasus langka dengan hasil perinatal yang baik inkompatibilitas rhesus pada multigravida tanpa injeksi terapi anti-D akibat fenotip D yang lemah.

Kesimpulan: Inkompatibilitas rhesus dengan fenotip D lemah dapat memberikan outcome perinatal yang baik tanpa terapi injeksi anti-D. Injeksi Anti-D tetap dapat diberikan untuk mencegah aloimunisasi Rh di kemudian hari.

Kata kunci: Fenotip D lemah, Immunoglobulin Rh, Inkompatibilitas rhesus, Multigravida, Terapi injeksi anti-D
Introduction

Blood types serve as antigenic and genetic markers with utility in investigating immune-hematologic complications within populations. The Rhesus (Rh) blood group system, following the ABO blood group, holds significant clinical relevance. Rh antigens, on the membrane surface of human red blood cells (RBCs), include the RhD antigen, akin to the A and B antigens. An individual’s Rh status is delineated by the presence or absence of the RhD antigen, classifying them as Rh-positive or Rh-negative, respectively. The prevalence of the RhD antigen exhibits regional disparities, with higher occurrence among Africans and lower incidence among Asians. Sensitization of a Rh-negative mother to the RhD antigen, resulting in anti-D antibody production (i.e., alloimmunization), poses risks of erythrocyte destruction in Rh-positive individuals, potentially leading to severe fetal or neonatal health complications, including mortality. Hemolytic disease of the neonate due to Rh incompatibility ranges from self-limited hemolytic anemia to severe hydrops fetalis. To mitigate these risks, the United States Preventive Services Task Force (USPSTF) strongly recommends blood typing and Rh(D) antibody screening during initial prenatal consultations for all pregnant women. Furthermore, the USPSTF recommends repeat antibody testing for all non-sensitized Rh-negative mothers at 24 to 28 weeks gestation unless the father possesses Rh-negative status. Rh incompatibility can be prevented by administering anti-D injection therapy containing Rh Intravenous Immunoglobulin (RhIVIG). Individuals with weak D phenotype express all epitopes of D at a low level. Weak D red cells have fewer D sites per cell than normal Rh D positive red cells, and might prevent alloimmunization in pregnant women with Rh incompatibility. We report a rare case of good perinatal outcome of rhesus incompatibility in multigravida without anti-D therapy injection due to the weak D phenotype of the fetus.

Case

A 34-year-old woman came to our facility for routine antenatal care. She has been identified as rhesus-negative since seven years ago, during the delivery of her second child, which was initially scheduled for cesarean section. The patient denied experiencing abdominal pain radiating to the waist, mucous blood discharge, or water discharge from the genitals. There was no history of abdominal massage, trauma, or post-coital activity, nor had she consumed herbal medicine. Notably, her husband tested positive for rhesus blood type, as did both of their children. During the physical examination, the patient exhibited normal consciousness, with a blood pressure of 120/80 mmHg, a pulse rate of 98 beats per minute, and a respiratory rate of 20 breaths per minute. Her body temperature was within the normal range, measuring 36.8°C, while her weight was recorded at 69 kg and her height at 153 cm. Evaluation of the patient’s conjunctiva and sclera revealed no indications of anemia or jaundice. Leopold’s examination revealed a fundus uteri height ranging between the xiphoid process and the umbilicus, measuring 21 cm, with the fetus positioned longitudinally, the back on the left side, and the head in the lower part of the uterus. Fetal heart sounds were distinctly audible at a rate of 145 beats per minute, and the estimated fetal weight was calculated to be 1240 grams. Laboratory analyses conducted yielded results indicating a hemoglobin concentration of 10.5 g/dL, a red blood cell count of 3.33 x 10⁶/mm³, a white blood cell count of 11.75 x 10³/mm³, a hematocrit level of 31%, and a platelet count of 387 x 10³/mm³. Additionally, the laboratory findings included a serum iron level of 122 ug/dL, a total iron binding capacity (TIBC) of 377 ug/
dL, a ferritin concentration of 49.30 ng/mL, a blood sugar serum (BSS) test result of 77 mg/dL, and a non-reactive triple elimination outcome.

The ultrasound examination (Figure 1) revealed that the fetus within the uterus was a viable singleton, presenting with a cephalic position. Biometric measurements of the fetus included a head circumference (HC) of 24.94 cm, biparietal diameter (BPD) of 6.89 cm, abdominal circumference (AC) of 22.34 cm, femur length (FL) of 5.05 cm, and estimated fetal weight (EFW) of 1023 grams. Furthermore, assessments of the middle cerebral artery index (Pi MCA) yielded a value of 1.59, the umbilical artery index (Pi Umb) recorded at 0.63, the single deepest vertical pocket (SDP) is measured at 3.64 cm, and the placenta is located in the anterior corpus. These findings align with the fetal age estimated to be 27 weeks based on biometric parameters.

Drawing upon the patient’s medical history, physical examination findings, additional investigations, and therapeutic interventions, the accurate diagnosis for the individual is determined to be a gravida 3, para 2, abortus 0, at 27 weeks of gestation, with Rhesus incompatibility and weak D phenotype, with a living singleton intrauterine fetus. The patient had never received an anti-D therapy injection in this pregnancy, as well as her previous two pregnancies. Subsequently, the patient received supplementation with multivitamins and was advised to attend a follow-up appointment in one week for the scheduled administration of Rho(D) Immune Globulin (Human) at a dosage of 300 μg. Subsequent monitoring revealed that the patient’s two children, aged 9 and 7 years, respectively, remained in good health without any manifestations of complications.

Figure 1 A-F Ultrasonography findings of the patient shows biometric measurements of the fetus corresponding to well-developed 27-weeks gestational age fetus with no major congenital disorders.
associated with Rhesus incompatibility with the patient.

Discussion

Rhesus (Rh) blood group system is regarded as the second most medically necessary blood group system after the ABO blood group. Rh antigens are located on the plasma membrane of erythrocytes in humans. The RhD antigen is considered a significant blood group antigen, similar to the A and B antigens found on the surfaces of red blood cells. The Rh-positive categorization refers to persons whose red blood cells have the RhD antigen, while those who do not have this antigen are classified as Rh-negative.1,2

The prevalence of the D antigen is higher in Africans and appears to be lower in Asians. Identifying the blood type of RhD-negative pregnant women enables the application of preventive measures to reduce hazards to the fetus. This situation becomes clinically significant when a woman who is Rh-negative becomes sensitized to the D antigen, resulting in the production of anti-D antibodies, which is known as alloimmunization. These antibodies have the ability to bind to and potentially cause the destruction of Rh-positive red blood cells. When an Rh-negative woman is exposed to Rh-positive fetal blood, her immune system recognizes it as an antigen and starts producing antibodies that target Rh antigens. These antibodies have the ability to cross the placenta and specifically attack fetal red blood cells, leading to serious health difficulties and potentially causing death for the fetus or baby. Alloimmunization, which leads to hemolytic illness in the fetus and infant, is one of the main causes of fetal loss and mortality in RhD-negative mothers.1,3

The RhD protein expresses the D antigen, while the RhCE protein carries either C or c antigens (involving the second extracellular loop) and E or e antigens (involving the fourth extracellular loop) on the same protein. Figure 3 illustrates an instance of a hybrid gene that partially codes for the D^VI variant. The figure depicts ten exons encoding the RHD gene represented by white boxes, while the ten exons of RHCE are depicted by red boxes. Amino acid alterations associated with
common antigens are denoted using a single-letter designation and the corresponding protein position. For instance, the E+ red blood cell (RBC) phenotype arises from the substitution of alanine (A) at amino acid position 226 with proline (P), encoded within exon 5 of RHCE. The ct versus C+ phenotype is linked to alterations encoded by the RHD gene (white box). The overlapping exon 2 of RHD and RHCE contributes to expressing G(G+) antigens on both RhCe and RhD proteins. Most Rh-negative (D-negative) phenotypes result from the deletion of the RHD gene. An example of a gene rearrangement between RHD and RHCE leading to a partial D phenotype is depicted, along with the emergence of a novel Rh antigen, BARC. Maternal sensitization in Rh-negative mothers occurs when they are exposed to RhD antigens, which usually occurs when the mother carries a fetus that is Rh-positive or comes into contact with Rh-positive blood. Nevertheless, suppose the RhD antigen is encountered during the first pregnancy, the negative consequences of Rh incompatibility typically do not affect the early stages of pregnancy, as the fetus is usually delivered before developing anti-D antibodies. After being made aware of a certain condition, future pregnancies are at risk of a condition called hemolytic disease of the newborn (HDN) due to Rh incompatibility if the fetus has Rh-positive traits.

In general, the first pregnancy in Rh-D-mediated disease typically exhibits no visible effects because IgM, being a large pentamer, cannot traverse the placental barrier. However, in subsequent pregnancies, exposure to even small amounts, as little as 0.03 mL, of Rh-positive cells can trigger the production of anti-D IgG immunoglobulins. These antibodies can freely cross the placenta and attach to fetal red blood cells containing D-surface antigens. Consequently, the fetal reticuloendothelial system identifies and eliminates these antibody-coated cells.

Figure 3 Diagram of RHD and RHCE genes shows the changes associated with common antigen polymorphisms, haplotypes (R0, R1, etc.), and an example of a hybrid gene encoding partial DVI. Reproduced with permission from Avent et al.\textsuperscript{6}
releasing significant quantities of bilirubin into the fetal circulation. Throughout the antenatal period, maternal conjugating enzymes work to eliminate excess bilirubin. However, following birth, neonates may develop jaundice or kernicterus, along with severe hemolytic anemia, due to the premature inadequacy of glucuronyltransferase enzyme activity.\(^7\)

Once red blood cell alloimmunization occurs, IgG antibodies are released into the maternal bloodstream. This results in antigen-antibody complexes when Rh-positive fetal red blood cells attach to these antibodies. This intricate mechanism initiates a series of reactions leading to the breakdown of the red blood cells, finally ending in alloimmune-induced hemolytic anemia. When red blood cells deteriorate, they create bilirubin, which causes neonatal jaundice. Hemolytic disease of the fetus and newborn (HDFN) continues to be a substantial problem that can occur during pregnancy.\(^2\) Furthermore, in cases where the mother possesses RhD-negative blood while the fetus carries RhD-positive blood, there is a risk of maternal antibody formation upon exposure to fetal antigens, known as RhD sensitization. This irreversible process may induce immunological memory in the mother, influencing future pregnancies.\(^8\) In such cases, the presence of Rhesus incompatibility may trigger an alloimmunization process in the red blood cells, potentially culminating in the destruction of the Rh-positive erythrocytes of the fetus. Consequently, complications such as anemia may occur in the fetus, representing one of the most common manifestations. Furthermore, the risk and severity of the sensitization response are increased with each subsequent pregnancy involving an Rh-positive fetus. In our case, considering that the patient is currently in her third pregnancy, wherein the risk and severity of sensitization response may increase, the potential complications could also intensify accordingly.

The amount of transplacental hemorrhage, the strength of the mother’s immunological response, and the contemporaneous ABO incompatibility are some of the variables that affect maternal sensitization. It is observed in around 17% of pregnant women after exposure to 1 mL of Rh-positive cells, and this percentage increases to 70% after exposure to 250 mL of rhesus-positive cells. If Rh-positive fetal red blood cells are released into the maternal circulation, which is typically caused by the rupture of the embryonic chorion that separates the fetal and maternal circulations, the immune system of an Rh-negative woman recognizes these cells as foreign and initiates a primary immune response by producing IgM antibodies initially.\(^7\)

Figure 4  Schematic of a Rh-negative mother and homozygous (left) and heterozygous (right) Rh-positive father.

Rh-type maternal-fetal incompatibility occurs exclusively when an Rh-positive male impregnates an Rh-negative female. Considering that Rh-positive fathers can have either DD or Dd genotypes, two possible mating combinations have varying risks, as indicated in Figure 4. In cases when the father has any genotype, if he has the Rh-positive blood type and the mother has the Rh-negative blood type, it is widely accepted that there may be compatibility problems, which would require necessary measures to be taken. In our case, the father has an Rh-positive blood type with a Dd genotype (heterozygous), suggesting a 50% probability...
that the offspring will have an Rh-positive blood type.

Rh incompatibility is based on an individual’s Rh status. The USPSTF states that testing for Rh(D) antibodies and blood group is highly advised for all expectant mothers during their initial prenatal visit. In addition, the USPSTF recommends that non-sensitized Rh-negative mothers undergo repeat antibody testing between 24 and 28 weeks of gestation unless the father is also Rh-negative. Antibody testing should be conducted during the process of delivering a baby.2

Following the testing procedure, a variety of outcomes emerge. If the mother’s Rh status is positive, there is no risk of alloimmunization for the fetus, regardless of Rh type. In contrast, antibody screening can assess the potential for alloimmunization in Rh-negative mothers. If Rh-negative mothers test positive for antibodies, confirmation tests such as the Coombs test are required to guide further therapy and surveillance during pregnancy. Conversely, if Rh-negative moms test negative for antibodies, a paternal Rh test may be performed. When the father’s Rh status is negative, there is no chance of alloimmunization or difficulties caused by Rh incompatibility. However, if the father’s Rh status is positive, the fetus has a 50% chance of inheriting Rh-positive erythrocytes, increasing the risk of Rh incompatibility.2

The weak D antigen is a rare phenotype characterized by a weak expression of the D antigen on red blood cells. Standard D antigen testing will show a reaction with certain anti-D substances but not with others, particularly when subjected to a 37°C incubation or an instantaneous spin. Weak D RBCs possess the D antigen; however, their quantity is lower than that of regular Rh D-positive red blood cells. Weak D red cells are characterized by expressing all D epitopes at a low level. Individuals with the weak D phenotype are unable to produce anti-D antibodies. Red blood cells with the weak D phenotype should generally be considered Rh D positive for most transfusion purposes. Weak D red cells have fewer D sites per cell than normal Rh D positive red cells.5,9

There are two variations of the D antigen known as Weak D and partial D. These variations occur due to the varying expression of the D antigen, which is influenced by a wide range of RhD alleles. Weak D and partial D lead to quantitative and qualitative alterations in the Rh protein, as stated in the reference. Weak D types 1, 2, 3, 4.0, 4.1, and 5 can be considered Rh D positive and can receive transfusions of Rh-positive blood. Nevertheless, it is important to consider weak D types 4.2–11 and 15 as Rh D negative and administer Rh-negative blood during transfusion. Partial D can elicit targeted antibody synthesis. Therefore, it is important to classify partial D as RhD negative in these circumstances. The molecular assays required for determining weak D types were inaccessible at our facility.5 In our case, the patient had never received an anti-D therapy injection in this pregnancy as well as her previous two pregnancies. None of her children developed hemolytic disease in the neonate. Ultrasonography showed a well-developed 27 weeks gestational age fetus with no major congenital defect. The good perinatal outcomes of her children might be due to weak D phenotype. Pregnant women with weak D phenotype have fewer D antigens that can still result in Rh sensitization but not enough to cause serious complications to the fetus.5

Preventing Rh incompatibility is achievable by administering RhoGAM, making prevention the optimal treatment strategy. The treatment approach for an already affected infant varies based on the severity of the condition. RhoGAM, a specialized immune globulin, is currently employed to prevent Rh incompatibility in Rh-negative mothers. If the biological father
of the baby has Rh-positive blood or if his blood type is not known, the mother will be receiving a RhoGAM injection during the second trimester. A second injection will be administered to the mother shortly after delivery if the infant is identified to be Rh-positive. This intervention effectively averts the development of antibodies against Rh-positive blood.\(^\text{10}\)

In the absence of alloimmunization and while carrying a Rh-positive fetus, Rh immunoglobulin, also known as RhIVIG, is only prophylactically administered to Rh-negative women or those who have experienced fetomaternal hemorrhage or abortion. Its primary purpose is to prevent sensitization, which involves coating fetal red blood cells with surface D antigens with antibodies. These antigen-antibody complexes traverse the placenta before the maternal immune system is activated. RhIVIG has a brief half-life of three months and is typically administered once between the 28th and 32nd weeks of pregnancy, as well as postpartum within 72 hours of delivery. The standard dosage is 300 mcg (1500 IU) for every 30 mL of fetal whole blood exposed to the maternal circulation. However, dosage adjustments may be warranted based on the extent of bleeding, determined by estimating fetal red blood cells in the maternal circulation using the Kleihauer-Betke acid elution test, given that hemoglobin F exhibits resistance to acid elution. In cases of abortion before 13 weeks, a mini-dose of 50 mcg (250 IU) is recommended, while the full standard dose is administered in cases of miscarriage. Approximately seventeen percent of RhD-negative women who give birth to RhD-positive fetuses will develop alloimmunization if the RhIVIG vaccine is not administered correctly. Nevertheless, the use of RhIVIG prophylaxis has substantially reduced the overall likelihood of Rh immunization from 13.2% to 0.2%. Hence, by strictly following recommendations on customized RhIVIG determinations and the regular administration of additional RhIVIG during non-spontaneous labor and/or problematic or lengthy third-stage labor, the occurrence of RhD immunization can be further reduced.\(^\text{7,8,11,12}\)

Even though 0.6% to 1.0% of people are thought to have RBCs that express a serologic weak D phenotype, there is no accepted procedure for treating Rh immunoprophylaxis in women who have this phenotype.\(^\text{13}\) The American College of Obstetricians and Gynecologists’ most recent practice guideline, published in 1999, said that women with a serologic weak D phenotype should be considered RhD-positive and should not receive RhIG.\(^\text{14}\) A weak D test is “unnecessary” for patients, including pregnant women, according to the most recent edition of the AABB Standards for Blood Banks and Transfusion Services (2014). Still, it is necessary for RBCs from a fetus or newborn of a RhD-negative mother to determine whether the mother should be a candidate for RhIG.\(^\text{15}\) Consequently, if a pregnant woman has inherited a serologic weak D phenotype and undergoes RhD typing in a facility that does not conduct a weak D test, she will be reported as RhD-negative and eligible for RhIG. No studies have shown that RhIG can shield individuals with weak D variants—linked to the development of anti-D antibodies—from RhD alloimmunization caused by D+ RBCs through transfusion or pregnancy.\(^\text{26}\) But given the extremely low chance of a negative reaction to a RhIVIG injection and the potential advantage of avoiding Rh alloimmunization, it is advised that women with these weak D types be allowed to potentially benefit from RhIVIG.\(^\text{13,16}\) Therefore, in our case, we still recommend the anti-D injection therapy. This is an exciting case of Rh incompatibility with good perinatal outcome without anti-D injection therapy due to weak D phenotype. To the best of our knowledge,
this is the first case report describing this rare phenomenon. The limitations of this study include a lack of data regarding the postnatal condition of the current pregnancy and also the RhD status of her previous two children.

**Conclusion**

Rh-negative mothers with a history of sensitization are at high risk of developing alloimmunization and hemolytic disease in their fetuses and newborns. Rhesus incompatibility in pregnant women with weak D phenotype can have good perinatal outcomes without anti-D injection therapy. Administration of Anti-D injection remains a viable option to prevent subsequent Rh alloimmunization.

**Acknowledgement**

We gratefully acknowledge the unwavering support and guidance provided by the Department of Obstetrics and Gynaecology, Department of Paediatrics, and Department of Biology, Faculty of Medicine, Sriwijaya University and Dr. Mohammad Hoesin General Hospital, Palembang.

**Conflict of Interest**

The authors declare no conflict of interest regarding the manuscript.

**References**