. Management of Rhesus Negative Mother

Contributed by

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Introduction

The guidelines are to provide recommendations to aid General Practitioners and Obstetricians in the management of **Rhesus Negative Mother**. This treatment could be initiated in a primary care setting or in centres with advanced facilities The objective of management is to make an early identification of Rh-negative mothers, prevent complications, and consequently to improve quality of life.

.1 Scope of the guideline

Rhesus (Rh) factor is a protein found on the red blood cells of most people. When a person does not have this factor they are called Rh-negative. When a Rh-negative mother is exposed to Rh-positive red blood cells she may produce antibodies in her blood (isoimmunization). This situation could arise during a pregnancy and in labour. These antibodies could cross to the fetus across the placenta and form complexes with the Rh positive fetal red cells. These affected RBC get destroyed by the reticulo-endothelial system of the fetus.

The numerous antigens on the surface of Rh positive red cells have been placed into groups. The Obstetricians are familiar with the Rhesus (Rh) group as it is the most clinically relevant and is still responsible for the largest proportion of hemolytic disease in the newborn. The Rhesus antigen constitutes at least three very similar transmembrane proteins called C, D, E. Although D is the most important antigen other Rh antibodies can provoke antibody formation such as anti E and anti C. However the non-Rh groups such as Kel, MNS and Kidd also have assumed increasing importance since they could produce red cell antibodies as well.

If Rhesus prophylaxis is not given:

- 1% chance of developing antibodies after the delivery of the first baby,
- 7% chance of developing antibodies after the delivery of the second baby,
- 17% chance of developing antibodies after the delivery of the third baby,
- 30% chance of developing antibodies after the delivery of the sixth baby.

Post-delivery immunoprophylaxis using anti-D Immunoglobulin (anti-D Ig) began in Sri Lanka several decades ago. Although the incidence of Rh-negative population has been estimated as 5%, no data is available with regard to Sri Lankan pregnant population.

.2 Pathogenesis

Angiogenesis in the fetus at about 3 weeks of in-utero life and Rh antigen has been identified in the red cell membrane as early as 38 days after conception and it prevails. [6]

After feto-maternal haemorrhage in a Rh-negative mother who is carrying Rh-positive fetus;

- The initial response to D antigen is slow sometimes taking as long as 6 months to develop.
- Re-exposure to the antigen produces a rapid immunological response which usually can be measured in days.

However;

- When the sensitized mother produces IgG anti-D antibodies they cross the placenta and coat D-positive fetal red cells, which are then destroyed in the fetal spleen (haemolysis).

- Mild to moderate haemolysis manifest as increased indirect bilirubin. It appears in the amniotic fluid.
- Severe haemolysis leads to increased red blood cell production by the spleen and liver of the fetus.
- Subsequently, hepatic circulatory obstruction (portal hypertension) with placental oedema, which interferes with placental perfusion and eventually ascites, develops in the fetus.
- Hepatomegaly, increased placental thickness, and polyhydramnios often precede the development of fetal heart failure.
- As liver damage progresses decreased albumin production results in the development of hydrops fetalis.
- ABO incompatibility reduces this risk.
- The reduced risk of Rh sensitisation with ABO incompatibility may result from the rapid clearance of incompatible red cells thus reducing the overall exposure to D antigen.

□.□ Management

Identification of Rh-negative mother pre & post conceptionally and education are important aspects in the management.

□.□.1 Management of post-partum mother (non sensitized)

□.□.1.1 Management in the delivery room (post-partum)

A.

i. Active management

During the active management feto-maternal transfusion is enhanced. However, considering the poor availability of the Rh negative blood and the potential for post partum haemorrhage active management of the third stage is not discourage.

-Let out cord blood after clamping baby side

ii. Cord blood should be taken for; **(Grade X)**

a) 2ml. of blood in a plain bottle for **blood group and Rhesus.**
b) 2ml. of blood in an EDTA bottle for **fetal Hb.**
c) 2ml. of blood in a plain bottle for **serum bilirubin level.**
d) 2 ml. of blood in a plain bottle for **direct coomb's test**.
e) 2ml. of blood in an EDTA bottle for **reticulocyte count**.

B. Test for the size of feto-maternal haemorrage

Studies have shown that 99.2 — 99.3% of women have a FMH less than 4 ml. at the time of delivery.

However, the following clinical circumstances are most likely to be associated with larger feto maternal haemorrhage (FMH).

- Traumatic deliveries including Caesarean Section,
- Manual removal of the placenta,
- Stillbirths and intrauterine deaths,
- Abdominal trauma during the third trimester,
- Twin pregnancies (at delivery),
- Unexplained hydrops fetalis.

International recommendations are as follows.

i. Kleihauer acid elusion test which detects fetal haemoglobin (HbF) is the test usually undertaken. **(GradeY)**

In some European countries (exceptions include the UK, France and Ireland), a standard postnatal dose of 1000-1500iu is used with no requirement for a routine Kleihauer test.[10] Unfortunately, this policy does not take account of the fact that up to 0.3% of women have a FMH greater than 15 ml. which will not be adequately covered by 1500iu of anti-D Ig.

ii. Flow cytometry offers an alternative technique for quantifying the size of FMH.[11]

It has a number of advantages in that results are more accurate and more reproducible than those from the Kleihauer test and that it detects RhD positive cells, making it particularly helpful in patients with high HbF levels. **(Grade Z)**

iii. The resetting technique is a relatively simple serological method, which offers another alternative for quantifying FMH of RhD positive red cells greater than 4 ml.

National recommendations

The recommended policy in Sri Lanka is to obtain an anticoagulant blood sample as soon as possible (within two hours) after the delivery and to undertake the Kleihauer screening test to identify women with a suspected larger FMH who need additional anti-D Ig.

Since this facility is available only in General and Teaching Hospitals, suspected larger FMH should be covered with an additional anti-D Ig dose. **(GradeY)**

C. Anti-D Ig preparations available in Sri Lanka (Human Immunoglobulin)

i. Rhophylac 300 micrograms/2ml. (1500iu) IM/IV
ii. Rhogam 300 micrograms/2ml. (1500iu) IM/IV
iii. Rhesogamma micrograms/2ml. (1500iu) IM/IV
iv. Rhesuman

D. Administration

Intramuscular anti-D Ig is best given to the deltoid muscle.

Injections to the gluteal region often reach the subcutaneous tissues and hence absorption may be delayed.

For successful immunoprophylaxis, every effort should be made to give anti-D Ig within 72 hours; best as soon as possible after the sensitizing event. **(Grade X)**

If it is not given before 72 hours, it should still be administered as a dose given within 9-10 days, which may provide some protection. **(Grade X)**

Women who are already sensitized should not be given anti-D Ig. **(Grade X)**

Women who have a weak expression of the RhD blood group (D^u) do not form anti-D and do not therefore require prophylaxis.

It should be noted that anti-D Ig does not protect against the development of other antibodies which can also cause hemolytic disease of the newborn.

National recommendations;
Intramuscular anti-D Ig should be given in the upper part of the deltoid muscle as soon as possible within 72 hours. However failure to do so, administering intramuscular anti-D Ig within 9-10 days is advisable. **(Grade X)**

□.□.2 Management of a mother following early pregnancy complications (Non-sensitized)

Prophylaxis following abortion, ectopic pregnancy, mole.

National recommendations;
All RhD negative women require anti-D Ig following abortion; 250 iu before 20 weeks' gestation and 500iu after 20 weeks. **(Grade X)**
A test for the size of FMH should be performed where available when anti-D Ig is given after 20 weeks. **(Grade Z)**

□.□.**2.1 Termination of pregnancy**: Anti-D Ig should be given to all RhD negative women having or suspected of a termination of pregnancy by surgical or medical methods, regardless of gestational age. **(Grade X)**
□.□.**2.2 Ectopic pregnancy**: Anti-D Ig 250iu should be given to all RhD negative women who have an ectopic pregnancy. **(Grade X)**

□.□.**2.□ Spontaneous miscarriage**: Anti-D Ig 250iu should be given to all RhD negative women who have a spontaneous complete or incomplete abortion after 12 weeks of pregnancy. There is evidence that significant FMH only occurs after curettage to remove products of conception but does not occur after complete spontaneous miscarriage.[12, 13] **(Grade X)**

If surgical evacuation is carried out for incomplete miscarriage Anti-D prophylactic is recommended.

□.□.**2.4 Threatened miscarriage**: Anti-D Ig 250iu should be given to all RhD negative women with a threatened miscarriage after 12 weeks of pregnancy. **(Grade X)**

Where bleeding continues intermittently after 12 weeks' gestation, anti-D Ig should be given at 6-weekly intervals till the bleeding ceases. Evidence that women are sensitized after uterine bleeding in the first 12 weeks of pregnancy where the fetus is viable and the pregnancy continues is scant[14], though there are very rare examples.[15] However, it may be prudent to administer anti-D Ig where bleeding is heavy or repeated or where there is associated abdominal pain particularly if these events occur as gestation approaches 12 weeks. The period of gestation should be confirmed by ultrasound.

□.□.□ Management of non-sensitized mother-antepartum

□.□.□.**1** Check the blood group of husband of a Rh negative pregnant mother. It is recommended that this should be done after discussing with her.

□.□.□.**2** Anti-D Ig should be given to all non-sensitized RhD negative women after the following potentially sensitizing events during pregnancy;

- Ante-partum haemorrage,
- External cephalic version of the fetus,
- Closed abdominal injury,
- Intrauterine death,
- Invasive procedures for prenatal diagnosis (amniocentesis, chorion villus sampling, fetal blood sampling).

National Recommendation;
A dose of 250 iu is recommended for prophylaxis for these sensitizing events up to 20 weeks of pregnancy. **(Grade X)**
For all events after 20 weeks, a minimum dose of 500iu anti-D Ig should be given followed by a test to identify FMH where facilities are available. **(Grade X)**
If found to be greater than 4 ml. red cells, additional anti-D Ig should be given accordingly. **(Grade Y)**

□.□.□.□ At or around 28 and 34 weeks to prevent sensitisation due to silent FMH routine ante-natal prophylaxis is carried out in developed countries although the incidence of sensitisation is 1%.

National Recommendation;
Although advisable to carry out this prophylaxis, to recommend it as a national recommendation is difficult due to financial constraints. **(Grade Y)**

□.□.4 Management of transfusions of Rhesus (RhD) positive blood components

□.□.4.1 RhD positive platelet transfusion to RhD negative mother:

If an appropriate product is not available during an emergency, it may be necessary to use RhD positive platelets. In these circumstances, RhD prophylaxis should be given against possible Rh alloimmunisation by red cells contaminating the platelet product.

250iu (50mcg) anti-D Ig should be given following every three units of platelets. Patients who have marked thrombocytopenia anti-D Ig should be given subcutaneously to avoid the possibility of haematoma following intramuscular injection.

□.□.4.2 Inadvertent transfusion of RhD positive blood:
When less than 15 ml. of RhD positive blood has been transfused accidentally to a Rh-negative woman 500 iu of anti-D Ig should be given.
When more than 15ml have been transfused, it is preferable to use the larger anti-D Ig IM preparation (2500iu or5000iu).
The dose should be calculated on the basis that 500 iu of Anti-D Ig will suppress immunization due to transfusion of 4 mls of RhD positive red blood cells.

When more than 2 units of Rh positive blood have been transfused, consideration should be given to undertaking an exchange transfusion to reduce the load of RhD positive red blood cells in the circulation. In this situation, the patient should be counselled regarding the implications of both non-intervention (for future pregnancies) and of the treatment, including any hazards from receiving donated blood, the exchange procedure itself and of larger

doses of anti-D Ig. In such situation discussion with the experts of the National Blood Transfusion Service is recommended. **(Grade X)**

Passive anti-D Ig given in large doses may be detectable for up to 6 months or more and tests for immune anti-D may not be conclusive for 9-12 months.

□.□.5 Management of sensitized antenatal mother

□.□.**5.1** In Rh-negative mother unexpected antibody (Rh antibody & atypical antibody) levels should be checked at booking visit, 28, 32, and 36 weeks of period of gestation. If positive result is found close follow up is necessary.

In general the principles used in the management of Rh-negative sensitized patient and the management of the patient with atypical blood antibodies do not differ.

However, the management of the kell-sensitized pregnancy may require more intensive surveillance, since maternal titres and amniotic fluid bilirubin level do not necessarily correlate with disease severity. May need marrow suppression.

The evaluation of positive antibody screen should include identification of the antibody and its titre. (The ideal would be to check the antibody levels instead of titers). This could be done at Central Blood Transfusion Service Bank, Narahenpita.

□.□.**5.2 Identification of antibodies. All Rh-negative mothers should be referred to a specialist antenatal clinic.** **(Grade X)**

Antibody status of all Rh-negative mothers is better evaluated by the National Blood Transfusion Service.

- A titre of more than 1:4 is considered sensitized.
- The method used should be stated, as the titre will vary according to the method used in the respective laboratory.
- An albumin titre of 1:16 is equal to an indirect antiglobulin test (IAT) titre of 1:32 to 1: 128

□.□.5.□ **Already sensitized due to previous event.(no prior severely affected pregnancy)**

- If the partner is Rh-negative (or negative for the atypical antigen) then, no further test is necessary.
- IAT titres of ≤ 1:32 or less are managed noninvasively with repeat antibody titres every 2-4 weeks.
- IAT titres of ≥ 1: 64 – amniocentesis to be done at intervals of 2 to 3 weeks.
- IAT titres of > 1:32 with pregnancies at greater than 27 weeks – ideally are monitored with serial amniocentecis. However, this facility is available in certain centres only. **(Grade Z)**
- If the father is heterozygous (Dd) or his blood is unavailable then, amniocentesis may be used to determine the fetal Rh (or atypical antigen) status if the IAT titre is > 1:32 or albumin titre> 1:16

Scope for future development

- Fetal DNA testing is available for:
  - RhD, RhE, Rhc, RhC and Kell.
  - (Send 5.0 ml. of fluid in a unbreakable sterile plastic conical-bottom centrifuge tube. **Do not freeze**)
  - For RhE, Rhe, RhC, Kell, and Cellano (k) the parents' DNA should be tested concurrently (Send 5.0 ml. of blood in a lavender-topped tube on each parent. **Do not freeze**)
- If the fetus is antigen negative then no further testing is necessary.
- If the fetus is antigen positive then the pregnancy is followed with serial titres and ultrasound as long as titres remain below the "Critical" value.

□.□.5.4 First sensitized pregnancy/previously affected pregnancy

For patients with a previously affected pregnancy, the timing of the initial procedure is determined by past clinical history. It is usually performed at least 4-8 weeks earlier than the prior gestational age at which significant morbidity occurred in previous pregnancy.

□.□.5.5 In women with extremely high titres (≥ 256), at less than 28 weeks, where the fetus does not demonstrate hydrops, and there is a documented history of fetal death due to hydrops, intravenous immune serum globulin (IVIG) might be offered. The dose is 400mg./kg. per day for 5 days, with repeat infusions every 15 to 21 days. Specific contraindications to intravenous immunoglobulin use include a previous episode of intravenous immunoglobulin-induced anaphylaxis (rare) and selective IgA deficiency.

□.□.5.6 Surveillance

i. Doppler ultrasonography

Doppler ultrasonography of the middle cerebral artery has also been used to identify fetuses at risk for moderate to severe haemolytic disease. **(GradeY)**

Expected Peak Velocity of Systolic Blood Flow through MCA

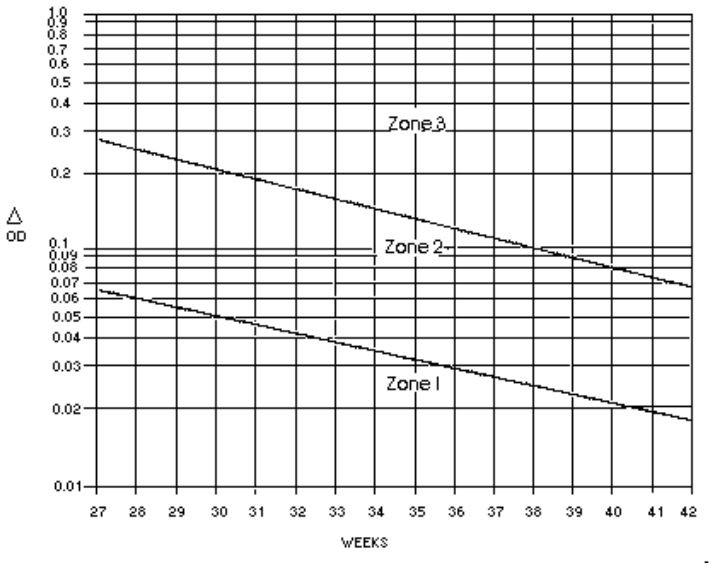
The middle cerebral artery is examined close to its origin in the internal carotid artery. The angle of the ultrasound beam and the direction of the blood flow should be zero degrees. The risk of anaemia is highest in fetuses with a pre-transfusion peak systolic velocity of 2.5 times the median or higher.

ii. Serial amniocentesis

Fetuses affected by haemolytic disease secreate abnormally high levels of bilirubin into the amniotic fluid. The amount of bilirubin can be quantitated by spectrophotometrically measuring absorbance at the 450nm wavelength in a specimen of amniotic fluid that has been shielded from light. Alternatively, percutaneous umbilical blood sampling (PUBS) may be used to determine all blood parameters directly.

If amniocentesis is used to monitor the fetus, the results (delta 450) are plotted on a "Liley" curve.

The Liley curve



The Liley curve is divided into tree zones.

- A result in Zone I indicates mild or no disease. Fetuses in zone I are usually followed with amniocentesis every 3 weeks.
- A result in zone II indicates intermediate disease. Fetuses in low zone II are usually followed by amniocentesis every 1-2 week.
- A result above the middle of zone II may require transfusion or delivery.

Patients with results in zone I or zone II can be allowed to proceed to term, at which point labour should be induced.

In most cases, patients in the middle of zone II can progress to 36-38 weeks of gestation. Depending on gestational age, patients in zone III should be delivered or should receive intrauterine fetal transfusion.

Serial determination of delta optical density at 450 nm and PUBS are the most common methods for the evaluation of fetal states.

□.□.5.6 Intravascular Fetal transfusion

Procedure

- The abdomen is aseptically prepared.
- A 20-guage, 5-inch spinal needle is then guided into the umbilical vein at the placental insertion under ultrasound guidance.
- Fetal blood is aspirated for immediate haematocrit, CBC, blood group and Rh factor.
- Prior to transfusion pancuronium bromide may be administered as an IV bolus.
- Transfusion is performed using group O Rh-negative, CMV-negative, washed irradiated packed cells, cross-matched against maternal blood.
- The donor blood is infused at 3-5 ml./ min.
- Fetal blood is aspirated at the conclusion of transfusion to determine final fetal haematocrit.

.4 Summary

The recommended policy of Sri Lanka is to obtain an anticoagulated blood sample as soon as possible (within two hours) after the delivery and to undertake the Kleihauer test to identify women with a suspected larger FMH who need additional anti –D Ig. Since this facility is available only in certain hospitals, suspected larger FMH should be covered with an anti-D Ig mega dose.

Intramuscular anti-D Ig should be given into the deltoid muscle as soon as possible within 72 hours. However in cases of failure it is advisable to administer within 9-10 days.

Some RhD negative women require anti-D Ig following abortion; 250iu before 20 weeks' gestation and 500iu thereafter. A test for the size of FMH should be performed if available when anti-D Ig is given after 20 weeks.

After suspected termination of pregnancy: Anti-D Ig should be given to all non-sterilized RhD-negative women having a termination of pregnancy, whether by surgical or medical methods, regardless of gestational age.

Ectopic pregnancy: Anti-D Ig 250iu should be given to all non-sensitized RhD-negative women who have an ectopic pregnancy.

Spontaneous miscarriage: Anti-D Ig should be given to all non-sensitized RhD-negative women who have incomplete abortion after 12 weeks of pregnancy. Threatened miscarriage: Anti-D Ig 250iu should be given to all non-sterilized RhD negative women with a threatened miscarriage after 12 weeks of pregnancy. Where bleeding continues intermittently after 12 weeks gestation, anti-D Ig should be given at 6-weekly intervals. A dose of 250iu is recommended for prophylaxis following sensitizing events up to 20 weeks of pregnancy. For all events after 20 weeks, at least 500iu anti-D Ig should be given followed by a test

to identify FMH greater than 4 ml. red cells; additional anti-D Ig should be given as required.

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