CAUSES AND PREVENTION OF RH IMMUNISATION

by

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Introduction

Erythroblastosis Foetalis or haemolytic disease of the new born is an important cause of perinatal mortality. Today a prophylactic approach based on blocking the mother's immunological mechanism seems to be the answer for ultimate conquest of Rh disease. The causes and the mechanism of Rh immunisation and the principles underlying the prophylactic treatment are discussed in this paper.

Rh Sensitisation and its Effects

The mechanism of Rh sensitisation is well understood now. Rh positive foetal red blood cells enter the maternal circulation via chorionic villi, and are carried to the reticulo-endothelial system where the response to the antigen in activated. Antibodies are usually detected about 4 months postpartum after the sensitising pregnancy (Fig. 1 A & B). In the subsequent pregnancy, the maternal Rh antibodies pass into the foetal circulation and combines with foetal red cell antigen to cause haemolysis. When haemolysis is considerably severe, haemolytic anaemia followed by cardiac failure occurs. Extramedullary erythropoiesis is the liver and fibrosis accompanying this lead to hepatocellular failure, increase in umbilical venous pressure, placental venous oedema and decrease of placental function.

Inability to form proteins due to hepatic dysfunction leads to hypoproteinemia.

Hydrops-foetalis is the result of congestive cardiac failure and hypoproteinemia. Progress of this condition combined with placental failure may cause intrauterine death of the foetus. Exchange transfusion to combat the hyperbilirubinaemia and anaemia is a big milestone which greatly reduced neonatal deaths and kernicterus. Amniocentesis and spectrophotometric examination of liquor to judge the intrauterine condition of the foetus was another big step to prevent intrauterine deaths by timely pre-term delivery. Intrauterine transfusion in severely affected babies is an additional tool to prevent intrauterine deaths, but has only about 40 to 60% success in highly specialised centres. At the present time, prophylactic treatment of Rh immunisa-

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tion with antigammaglobulin has been found to be safe and effective, promises to be the ultimate answer to this problem and could wipe out the disease within a generation.

Factors Causing Immunisation

(a) *Foetomaternal Haemorrhage*:

The presence of foetal cells in the maternal blood indicates foetomaternal transfusion and greater the foetomaternal haemorrhage the higher the risk of immunisation.

The extent of foetomaternal haemorrhage was studied in 113 women after normal deliveries and 231 women who had complications of pregnancy or labour or required operative interference. Venous blood samples were collected within 24 hours after delivery. An estimation of foetal cells and foetal blood in maternal circulation was made by microscopic method (Fig. 2) as per Zipursky *et al* (1959) and by alkali denaturation method (Miale, 1958). Table 1 shows the extent of foetomaternal transfusion in normal and complicated deliveries.

The findings indicate that there is greater transfusion of foetal cells into the maternal blood in cases who had caesarean section, manual removal of the placenta, toxaemia and accidental haemorrhage as compared to normal cases.

Sodi *et al* (1969) studied foetal haemoglobin in non-pregnant women in normal pregnancy and in abnormal pregnancy. Their results are as below:

Foetal Haemoglobin in Normal& Control Groups by Sodi et al

	No. of cases	Foetal haemoglobin	Mean & S.D.
(a) Non-pregnant	50	1.5 to 3.5	2.5 + 0.83
(b) 1st trimester	35	2.1 to 3.5	2.6 + 0.7
2nd trimester	35	2.1 to 5.0	3.5 + 1.13
3rd trimester	80	2.1 to 5.0	3.2 + 0.6
(c) Puerperium (follow-up)	50	1.7 to 4.3	2.4 + 0.65
Foetal Haemoglobin in Abnormal Pregnancy			
Pre eclampsia (Toxaemia)	7	2.2 to 4.0	3.48
Postmaturity	5	2.7 to 3.0	2.9
Anaemia in pregnancy	6	3.4 to 4.3	3.9
Rh. negative	3	2.5 to 5.0	4.2
Hydatidiform mole	2	3.0 to 3.2	3.1

TABLE 1

Foetomaternal Haemorrhage in Normal and Complicated Labours

	No. of cases	Foetal cells per H.P. field	Foetal Hb.% in maternal blood
1. Normal labour	113	2.5	1.5
2. Breach deliveries	12	2.8	1.6
3. Forceps deliveries	92	2.4	1.8
4. Twins	9	3.4	2.0
5. Manual removal of placenta	11	5.7	5.0
6. Lower segment caesarean section	96	4.0	3.3
7. Accidental haemorrhage	7	12.6	6.0
8. Toxaemia	4	9.0	4.9

(b) *ABO Incompatability*: Another finding was that 63% of women who had ABO compatability, had presence of foetal cells in maternal blood while among women having ABO incompatability with their baby, only 14% had presence of foetal cells in maternal blood.

Parikh *et al* (1971) have also observed a higher incidence of foetal cells in ABO compatable cases. Lewis *et al* (1961) Rucknagel (1962), Bomberg *et al* (1957), Finn *et al* (1963), Fraser *et al* (1964), Cohen *et al* (1967), Taneja *et al* (1969), Deshpande & Sharma (1971) also had similar findings.

(c) *Amniocentesis*: In a separate project, 240 women and amniocentesis to study liquor amnii. In these cases maternal blood was collected within two hours of amniocentesis to study the presence of foetal cells. In only 6 cases foetal cells were detected out of these 240 and all these six cases had blood or blood stained liquor tapped. The risk of foetomaternal haemorrhage and further sensitisation seems small compared to its advantage in the treatment of selected Rh immunised cases.

(d) *Past Obstetric History*: Past-obstetric history and its relation to immunisation was studied in 1326 Rh negative cases. Of these 106 were immunised, whereas 1220 were not immunised.

Of the immunised cases, 10 were due to mismatched Rh positive transfusion in some hospitals. This is a very sure way of sensitisation leading to extremely poor foetal prognosis. Nine out of these 10 cases had antibody titre of 1: 512 and 1 had a titre of 1: 128. Of the ten babies only 4 could be salvaged by timely induction and exchange transfusions and other s[illegible] were premature stillbirths.

Of the 96 remaining immunised cases, 81 cases had complications during pregnancy and labour as follows: 13 had severe toxaemia, 16 gave history of abortion, 5 had caesarean section, 7 had manual removal of placenta, 21 had antepartum haemorrhage and 19 had postpartum haemorrhage. Thus, 84% immunised women gave history of complications, whereas among 1220 non-immunised cases only 6% gave history of complications during pregnancy and labour.

Krishna *et al* (1967) have shown similar findings in their study at N.W. Maternity Hospital.

Discussion

The presence of foetal cells in maternal circulation has been detected as early as 12th week of pregnancy and the extent of this micro-transfusion increases with the advance of pregnancy due to the growth of placental vascular bed, increase in pressure gradient and progressive thinning of the barrier between foetal capillaries and maternal sinuses, but by far the greatest danger of foetal cell transfusion is during labour. Many workers such as Winhoffer *et al* (1962), Zipursky *et al* (1959, 1963), Queenan (1967) have studied the extent of foeto-maternal haemorrhage after various abnormalities of pregnancy and labour. Conditions such as toxaemia, diabetes, chronic hypertension, hydramnios, anaemia, folic acid deficiency where the integrity of the placental vascular bed is disturbed, contribute to increased foetomaternal haemorrhage. Premature placental separation, manual removal of placenta, caesarean section, difficult forceps and trauma to maternal tissues, such as cervical and vaginal tears, can cause entry of foetal cells into the maternal blood. Instrumental evacuation of an incomplete abortion, or a therapeutic abortion would disrupt the maternal sinuses. Entry of foetal cells is more likely when uterus is

relaxed, as the sinuses are then open. Atonic postpartum haemorrhage and curettage under general anaesthesia are therefore hazardous. Foetomaternal transfusion in caesarean section can be due to manual shearing of the placenta, the absorption of foetal cells from blood spilled in the peritoneal cavity and the effect of anaesthesia.

Another significant factor of Rh pathogenesis is based on Levin's theory that if the mother has existing circulatory antibodies directed against baby's red cells e.g. anti-A in group O Rh negative mother with a positive baby, the initial immunisation is less likely as the A positive foetal cells are removed from maternal circulation by anti-A antibodies of the mother. In fact this partial protection given by the antibodies provided the clue to researchers to try anti-D antibodies to protect the mother.

Rh sensitised 6 months after delivery of Rh positive baby
Rh sensitised at the time of delivery of next Rh positive baby

Anti-Rh Gammaglobulin: The use of anti-Rh gammaglobulin is the most important prophylactic treatment of Rh immunisation. However, during pregnancy and labour, Rh negative women must be given greater care and attention to avoid factors contributing to immunisation mentioned above.

Mechanism of Action: The immunological response of Rh negative women to the foetal Rh positive cells is triggered when foetal cells in the maternal circulation increase. Such an increase is most likely to occur at delivery and postpartum. The antibodies produced as a result of this immunological response are usually detected about 4 months postpartum. This immunological response can be prevented by injecting anti-Rh gammaglobulin within 72 hours after delivery (Fig. 3 A & B). This destroys the foetal erythrocytes and blocks antigen sites and thus prevents active immunisation.

Anti-Rh gammaglobulin is not useful in mothers already immunised.

Review of Trials:

The work done by Pollock (1969) and Freda (1969) in U.S.A. and Finn and Clark (1969) in Britain have now shown the practical usefulness of the prophylaxis. The effect of Anti-Rh gammaglobulin in suppressing the antibody formation was initially tried in Rh negative male volunteers injected with Rh positive cells. Subsequently, clinical trials were conducted on women. Combined results of trials conducted by these workers are as follows as quoted by Bowe (1969).

	Treated	Untreated
Rh sensitised 6 months after delivery of Rh positive baby	3 out of 1452	127 out of 1678
Rh sensitised at the time of delivery of next Rh positive baby	1 out of 233	40 out of 313

Some of the failures may be due to unrecognised massive foetal bleeds when an increased dose of anti-Rh gammaglobulin could have been effective. In certain instances, the lag period before the injection may be critical i.e. if the newborn has a disease such as hereditary spherocytosis, where red cells are prematurely destroyed in maternal circulation, sensitisation may have started before injecting Rh gammaglobulin. Besides these, there are few other serological hypothesis for failure. The authors have observed only 29 Rh negative women who were administered anti-Rh gammaglobulin. The period after delivery varied from 4 to 18 months and none of these women were found to be immunised.

Criteria for Selection of Cases for Anti-Rh Gammaglobulin

For total prophylaxis, the injection should be given to all Rh-negative women after delivery of Rh positive baby or after an abortion. As anti Rh gammaglobulin is available in restricted quantities and is yet very expensive, it may be necessary for institutions to select deserving cases. Women who have suffered from antenatal complications such as toxaemia and those who underwent operative interference such as dilatation and curettage, manual removal of placenta, caesarean section or had antepartum or postpartum haemorrhage should be given the protection particularly. For third para or more, tubal sterilisation rather than gammaglobulin is recommended. The mother who has ABO compatability with her baby, has a greater risk of immunisation and must therefore be considered more deserving for treatment.

Foetal cell count in maternal blood after delivery may give an indication about the absence or significant presence of foetal cells in maternal circulation. Selection by this criteria is not absolutely safe and not recommended by most workers.

Summary

The causes and mechanism of Rh immunisation are discussed. Foetomaternal haemorrhage studies and past obstetric history indicate that abnormal obstetric procedures and obstetric complications lead to greater possibility of foetomaternal transfusion and consequent immunisation.

The action of anti-Rh gammaglobulin in prevention of immunisation is explained and a brief review of trials with this prophylactic treatment is presented.

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See Figs. on Art Paper I-II