

Study of Feto-Maternal Haemorrhage in Later Part of Pregnancy With Special Reference to Rh Negative Mothers.

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Abstract: Haemolytic disease of foetus and newborn is most commonly the result of Rh incompatible pregnancies. This entity has been studied vastly and over decades and the prevention and treatment of Rh D alloimmunization is a true success story in obstetrics. Most of the prospective studies done on this subject is from developed countries and the problem still continues to burden the developing world. This study attempts to review and document feto-maternal haemorrhage during pregnancy and labour. The amount and timing of fetomaternal haemorrhage is documented and whether ante-natal prophylaxis is justified is also tried to be determined. We conducted this study on 102 pregnant patients and tested them for feto-maternal haemorrhage at 28 week, 32 weeks and post-partum by Kleihauer-Betke test.

Conclusion: Even low risk women, as selected in our study have been shown to have FMH both ante-natally and during delivery, therefore the prophylactic administration of immunoglobulin's at 28 weeks is justified.

Most of FMH calculated (<3 mL) could have been neutralized by lower doses which might have lower costs than administering 300 µg dose which is currently in practice in our country for affording mothers. Further investigation into the cost-effectiveness and scalability of patient-specific dosing of prophylactic anti-D appears warranted.

Keywords: Anti-D immunoglobulin, Feto-maternal haemorrhage, Hemolytic disease of foetus and newborn, Kleihauer-Betke's test, Rh negative.

Date of Submission: 08-08-2017

Date of acceptance: 23-08-2017

I. Introduction

Hydrops fetalis was recognized as early as 400BC by Hippocrates. The pathogenesis, treatment and prophylaxis of this disease have been elucidated over a span of 30-40 years. In 1930, the 4 conditions of hydrops foetalis, icterus gravis neonatorum, congenital anaemia and erythroblastosis foetalis were recognized as one clinical entity. In 1939, the new blood group Rhesus was discovered by Landsteiner and Wiener when they injected rabbits with blood of Rhesus monkey. Rabbits produced antibodies which agglutinated monkey's RBCs and 80% of human RBCs. These were named as "Rhesus" antibodies and thus the Rh blood group system was discovered. Alloimmune hemolytic diseases of the fetus and newborns (HDFN) results from the break down of red cells by maternal immunoglobulin (IgG) antibodies that enter the fetal circulation during gestation. The most serious form of HDFN is caused by maternal alloantibodies directed against the D antigen of the Rh blood group system due to the high immunogenicity of D antigen. RhD HDFN in Rh-negative women can be prevented if the appropriate dose of prophylactic anti-D (RhIG) is given at the appropriate time [1-6]. The incidence of Rh (D) negativity in India is about 5% [7,8]. Maternal isoimmunization occurs in approximately 1-2% of Rh (D) negative women [9,10]. Approximately 10% of isoimmunized women have a fetus affected by severe haemolytic anemia [11]. Rh isoimmunization is disastrous for a pregnant woman, and she often presents with repeated fresh stillbirths and recurrent pregnancy losses [12]. In a Rh-negative woman, first exposure to Rh positive red cells can occur-

- During pregnancy or during labour
- By mismatched transfusion with Rh positive blood.
- By vaccines containing human sera.
- During gestation or delivery when Rh positive cells are transferred transplacentally from her mother (grand mother theory)

Most commonly the first exposure occurs during pregnancy or delivery. A healthy placenta permits transfer of dissolved nutrients, gasses, and waste products between the mother and fetus while keeping the cellular components of the two circulations separate. Fetomaternal hemorrhage (FMH) occurs when the normal flow of blood within the placenta is disrupted and fetal whole blood is transferred into the maternal circulation. It is common for the placental filter to “leak” during normal pregnancy and delivery, resulting in transfer of small volumes of fetal whole blood into the maternal bloodstream

Feto maternal bleeding can occur throughout the pregnancy and also during delivery. Its immense clinical importance is highlighted by Rh isoimmunization. Sensitization is likely to occur after a primary stimulus of more than 0.5 ml of foetal blood and a booster dose of more than 0.1 ml. Numerous factors influence Rh isoimmunisation. Larger the amount of bleed, more is the chance of sensitization. Isoimmunisation is likely to be prevented by-

- ABO incompatibility of mother and fetus which destroys fetal RBCs earlier and thus they circulate for less time.
- Varying rates of occurrence of red cell antigens and their variable immunogenicity.
- Variability in maternal immune response.

It was found that feto-maternal haemorrhage, is most common during labour. Post partum Rh anti-D immunoglobulin significantly decreases the incidence of Rh isoimmunisation. Given within 72 hrs of delivery it prevents the Rh positive cells from reaching the reticulo endothelial system and thus sensitization. Despite this about 1.8% women become immunized even after post partum prophylaxis. These are mostly due to ‘silent’ fetomaternal bleeds during pregnancy. From various studies it was found that these ‘silent’ bleeds commonly occur in the third trimester. Thus these women can be protected by Rh. Anti-D immunoglobulin at 28 wks.

The amount of anti-D immunoglobulin given routinely can neutralize 15ml of Fetal RBCs, but the amount of feto maternal haemorrhage may be more or less than this amount. Kleihauer and Betke in 1957 developed a method to demonstrate fetal red cells in maternal circulation. It is standard method of detecting fetomaternal haemorrhage and the principle is, that, unlike adult haemoglobin, fetal haemoglobin is resistant to acid elution. Measuring the amount of fetomaternal bleed and thereby by giving adequate dose of immunoprophylaxis further improves the outcome of Rh negative women carrying Rh positive pregnancies. Clinical experience with Rhesus (Rh) disease and its post-icteric sequelae is limited among high-income countries because of nearly over four decades of effective prevention care. Following a worldwide study, it has been concluded that Rh hemolytic disease is a significant public health problem resulting in stillbirths and neonatal deaths, and is a major cause of severe hyperbilirubinemia with its sequelae, kernicterus and bilirubin-induced neurologic dysfunction. Knowing that effective Rh-disease prophylaxis depends on maternal blood-type screening, healthcare afforded to the high-risk mothers needs to be free of bottlenecks and coupled with unfettered access to effective Rh-immunoglobulin. This study attempts to review and document feto-maternal haemorrhage during pregnancy and labour.

The amount and timing of fetomaternal haemorrhage is documented and whether ante-natal prophylaxis is justified is also tried to be determined.

II. Aims And Objectives

Primary Objective

To study the incidence and document feto-maternal haemorrhage and its amount during later half of pregnancy.

Secondary Objectives To calculate the average amount of anti-D immune globulin required for post-partum prophylaxis in Rh negative mothers. To justify antenatal prophylaxis and quantify its dose and appropriate time.

III. Materials And Methods

Pregnant women presenting during early pregnancy to the antenatal clinic who fulfilled all inclusion criteria were selected for the study

Inclusion criteria:

Since fetomaternal haemorrhage is a clinical entity which can occur in all pregnancies, irrespective of mother’s blood group. The cases were picked up randomly irrespective of their gravidity, parity and blood groups.

Exclusion criteria

- 1) Any history of abdominal trauma
- 2) History of antepartum haemorrhage
- 3) External cephalic version
- 4) Prenatal diagnostic investigations such as chorionic villus sampling and amniocentesis.
- 5) Patients with known haemoglobinopathy
- 6) Already sensitized Rh negative mothers.

This was a prospective observational study conducted on 102 antenatal mothers and they were followed up until after delivery. Each case was asked for a detailed history of her menstrual cycles, obstetrical career and for any complaints during her present pregnancy. All routine investigations including ABO grouping and Rh typing, complete blood picture and urine examination were performed.

3 samples of maternal blood were drawn in each case

- At 28 weeks of gestation
- At 32 weeks
- Within 6 hours of delivery

Following delivery notes were made

- a. Mode of delivery : normal vaginal, forceps or caesarean section
- b. Onset of labour: spontaneous or induced.
- c. Delivery of placenta: spontaneous or manual removal
- d. Fetal outcome

To detect fetomaternalhaemorrhage KleihauerBetkes test was performed on all samples and the amount of fetomaternal haemorrhage if any was determined. The principle of this test is that the acid buffer removes the adult haemoglobin or haemoglobin A, because it is more soluble than the haemoglobin F of fetal red cells. The fetal cells containing haemoglobin F appear darker in the background of the pale and unstained maternal red cells (the ghost cells, devoid of haemoglobin). Samples were examined within 6 hrs. of collection.

Volume of fetomaternalhaemorrhage is given by the formula

Volume of FMH in ml =

$$\frac{\text{maternal blood volume} \times \text{maternal haematocrit} \times \% \text{ of fetal cells by K - B test}}{\text{fetal haematocrit}}$$

Here maternal blood volume was taken as 5000ml, fetal haematocrit 50% and maternal haematocrit 35%. The results were tabulated and statistically analysed.

IV. Results and Analysis

Table – 1: FMH in antepartum and post partum period.

Time of examination	No. of cases	No. of cases with FMH	Percentage
Antepartum	102	5	4.90%
Post partum	102	14	13.73%

Out of 102 cases 4.90% (5) had FMH antepartum and 13.73% (14) had FMH during delivery which was detected in the post-partum samples. The 95% confidence interval for antepartum FMH ranges from 2.11% to 10.96% and that of postpartum FMH ranges from 8.36% to 21.73% .

Table – 2: FMH in relation to period of gestation.

Gestational age	No. of cases	No. of cases with FMH	Percentage
28 wks	102	2	1.96%
32 wks	102	3	2.94%

1.96% cases had FMH at 28 wks and 2.94% had FMH at 32 wks.

Table – 3: Post-partum detection of FMH with relation to the mode of delivery

Mode of delivery	No. of cases	No. of cases having post partum FHH	Percentage
NVD	41	5	12.19%
Forceps	14	2	14.28%
LSCS	47	7	14.89%

Incidence of FMH intra partum which is detected in the post-partum blood samples was highest in those undergoing LSCS (14.89%) followed by those delivered by forceps (14.28%) and was least in those confined normally 12.19%. Chi square test gives value of P as 0.932 (>0.05 , not significant).

Table – 4: FMH with relation to onset of labour

Onset of labour	No. of cases	No. of cases with FMH post partum	Percentage
Spontaneous	48	7	14.58%
Induced	31	7	22.58%

The incidence of FMH in patients having spontaneous onset of labour was 14.58% whereas the incidence was much higher in those patients in whom labour was induced 22.58%. Fisher's Exact Probability Test gives value of P as 0.558 (>0.05 , not significant).

Table – 5: Post-partum detection of FMH with relation to mode of delivery of placenta

Mode of delivery of placenta	No. of cases	No. of cases with FMH post partum	Percentage
Spontaneous	85	8	9.41%
Manual	17	6	35.29%

Manual removal of placenta lead to increased incidence of FMH (35.29%) compared to spontaneous separation (9.41%).Fisher's Exact Probability Test was used to calculate value of P which came out to be 0.0321(<0.05,significant).

Table – 6: Volume of FMH

Volume of FMH	Total no. of cases with FMH	No. of cases having FMH according to volume	Percentage
Upto 1 ml	19	16	84.21%
1 – 3 ml	19	03	15.79%
>3ml	19	00	0

The volume of FMH was found to be less than 1 ml in 84.21%, 1-3 ml in 15.79% and none of the cases had FMH of >3ml.

V. Discussion

V.1: Testing for feto maternal bleeds

Blood samples are obtained during antepartum period, after delivery and following any event suspected to cause a feto maternal bleed. Various tests are available for detecting FMH.

Rosetting Test: an initial, qualitative test

Principle – Rh-positive fetal cells coated with anti-D forms rosettes with Rh-positive indicator cells, making them distinguishable.

Kleihauer –Betke Test: A quantitative test-a monolayer blood film is stained.

Principle – acid buffer remove the adult haemoglobin, adult RBC appears pale, cells containing haemoglobin F stains darkly and can be distinguished[13]

Problems causing false results.

1. Faulty technique.
2. Some fetal cells containing haemoglobin. A will be missed.
3. Genetic haemoglobinopathies

Flow cytometry: A quantitative test, can analyze large number of cells with objectivity and reproducibility[14].It is reportedly a more sensitive test than Kleihauer – Betkes Test.A prospective study conducted in Ethiopia calculated FMH by the two methods (Kleihauer- Betkes and flow cytometry) have good correlation; $r = 0.828$ ($p = 0.000$) for categorized and $r = 0.897$ ($p = 0.000$) for continuous values and the agreement between the FCM and KBT was moderate with kappa (κ) value of 0.53 ($p = 0.000$)[15].

V.2: Incidence of FMH in ante-partum and intra partum period

In the present study feto maternal haemorrhage was detected in 4.90% of cases during ante-partum period and in 13.73% of cases during post-partum period. The incidence of feto maternal haemorrhage in the ante partum period has been reported to be ranging from 4% to 45.5% (Bowman, Pollock, 1986)[16]. A study mentioned that the incidence of third trimester FMH is about 28.9%.It was concluded from a study that transplacental FMH during third trimester is around 7% [17]. If the delivery is normal and the placenta is handled carefully then about 20% women will have transplacentalhaemorrhage. The incidence of FMH observed in the current study was less as compared to other studies. This is probably because high risk cases for developing FMH (abdominal trauma, external cephalic version, ante partum haemorrhage etc.) were excluded from the study. To detect very small volume of haemorrhage, a very large number of fields need to be examined and a more sensitive method than K-B test needs to be employed.

V.3:Incidence of FMH with relation to period of gestationIn our study, FMH was detected in 2 cases (1.96%) at 28 wks and in 3 cases (2.94%) at 32 wks.Other studies have documented FMH to occur in 3% of pregnancies in the first trimester,12% in the second trimester, 45% in the third trimester, and 64 to 100% after delivery [1,18].

V.4: Incidence of FMH in relation to maternal age

Most of the case studies belonged to the age group 20-40 years. The incidence of ante partum FMH in the age group 21-30 yrs is (2.99%) and in 31-40 yrs is (9.52%), the incidence of intra partum FMH is higher and 14.29% in 31-40 yrs and 8.96 in 21-30 years respectively. This may be due to increased number of operative deliveries in the 31-40 years age group. The number of cases examined in the extremes of reproductive age group (less than 20 and more than 40 years) are too less to make a definite conclusion. V.5: Incidence of FMH in relation to parity Antepartum FMH in primi (5.26%) are comparable to the incidence of antepartum FMH in multi para patients (4.44%). But there is higher incidence of post partum FMH in primipara (15.79%) as compared to multipara (11.11%). This may be due to the higher incidence of operative deliveries in primi patients but the difference is not statistically significant.

V.6: Incidence of FMH in relation to number of fetuses

The incidence of FMH in singleton pregnancies was 4.35% and 10% during antepartum and intra partum period respectively. In comparison in the 10 cases of multiple pregnancies studied, FMH was observed in 10% antenatally and in 40% following delivery. So from this study multiple pregnancy is a risk factor for feto maternal haemorrhage but it is not statistically significant. Salim[19] in his study reported no significant difference between the incidence of large FMH between singleton (6.7%) and multiple pregnancies (5.2%). Another recent study has reported the incidence of fetal-to-maternal transfusion and its severe form was significantly higher in twin pregnancies (7/21 cases and 5/21 cases respectively, 33.3% and 23.8%) than in singleton pregnancies (22.5%, and 5.9%, $P < 0.001$) [20].

V.7: Incidence of FMH with relation to mode of delivery

Our study shows highest number of transplacental haemorrhage in those undergoing caesarean section 14.89%. In cases having forceps and normal vaginal delivery the FMH was 14.28% and 12.19% respectively.

The difference is insignificant statistically.

Study by Devey figures FMH in LSCS 46.55% and normal delivery 40.9% [21]. But another study in 1969 failed to find any positive co-relation with various mode of delivery and feto maternal bleeding. A recent study from Czech Republic concluded that delivery by caesarean section presented a higher risk of incidence of FMH of more than 2.5 mL (odds ratio, 2.2; $p = 0.004$) when compared with normal vaginal delivery. It did not, however, present a significant risk factor for the incidence of excessive volumes of FMH of more than 5 mL [22]. So, different studies shows different incidences. Though the incidences are different, this study shows a higher incidence in caesarean section as compared to normal vaginal delivery.

V.8: Incidence of FMH in relation to onset of labour

In the present study FMH was observed in 6 cases (14.58%) with spontaneous labour onset. For induction prostaglandin E₂ and oxytocin were used, and incidence in this group was 22.58%. One study in 1985 found higher incidence of FMH in oxytocin induced labour 43.3% as compared to 20.3% in spontaneous labour. They explained that oxytocin induced contraction are much stronger and cause transplacental passage of fetal cells. Another study in 1988 could not relate oxytocin infusion to an increased risk of FMH. This study, shows a much higher incidence of FMH due to induction of labour (22.58%). This can also be related to the fact that induced labour leads to a greater number of emergency LSCS.

V.9: Incidence with relation to delivery of placenta

We found that with normal separation of placenta FMH was documented in 8 (9.41%) cases but, observed in 6 (35.29%) cases with manual removal of placenta. This difference in incidence is found to be statistically significant. One study in 1985 showed 45.8% FMH with manual removal of placenta. This study shows a much higher incidence of FMH in manual removal compared to normal delivery of placenta.

V.10: Incidence with relation to volume of FMH

The present study shows the volume of FMH in 84.21% cases is less than 1 ml and in 15.79% cases between 1 to 3 ml. In none of the cases haemorrhage exceeds 3 ml.

Our results are consistent with the result revealed by a large study conducted by Augustson et al. which concluded that 90.4% (4651/5148) of the women had FMH volume of 1.0 mL or less of Rh D-positive red cells, and 98.5% (5072/5148) had a volume of less than 2.5 mL. Only 0.4% of the cases had an FMH volume of 6.0 mL or greater (range, 6.0–92.4 mL) [23]. But the incidence of large FMH in our study is zero. This is probably due to the exclusion of high risk cases from our study.

Antenatal prophylaxis is definitely needed to ensure no silent bleeds cause isoimmunisation. This in turn will help reduced the neonatal mortality, morbidity and financial burden of neonatal care in subsequent pregnancies. In a study from Pakistan despite prenatal and post natal prophylaxis, risk of sensitization with D antigen in D negative women was high at 2.2% [24]. This indicates the need for testing for volumes of FMH so as to detect the cases of large bleeds and treat them accordingly. A study from USA states that although the occurrence of large antenatal fetomaternal hemorrhage is fortunately rare, it likely remains underreported and

underrecognized. A national registry should be created to advance our learning across institutions by reviewing the clinical presentations of fetomaternal hemorrhage, the variety of fetal heart rate tracings observed, the management strategies undertaken, and the outcomes achieved[25].

VI. Conclusion

FMH in an Rh-ve woman can cause poor obstetric outcome. But it is a clinical entity which can be prevented by clinicians' anticipation, vigil and timely immunization. The incidence of FMH detected by Kleihauer-Betkes test has been lower in our study as compared to other studies which might be due to the following drawbacks

- For detection of small volume of haemorrhage, very large number of fields need to be scanned. Ideally blood should be examined as early as possible after collection. RBC's may be destroyed on keeping for a long time.
- Kleihauer-Betkes' test is relatively crude method of detecting FMH.
- The 'at risk' mothers for FMH were excluded from this study.

The incidence of intra partum FMH is greater than ante partum haemorrhage. In the low risk pregnancies, FMH is rare before the 3rd trimester. In our study the only risk factor to contribute statistically significantly to FMH is found to be manual removal of placenta and should be avoided in Rh negative mothers. Even low risk women, as selected in our study have been shown to have FMH both ante-natally and during and after delivery, therefore the prophylactic administration of immunoglobulin's is justified. Most of FMH calculated (<3 mL) could have been neutralized by lower doses which might have lower costs than administering 300 µg dose which is currently in practice in our country for affording mothers. Further investigation into the cost- effectiveness and scalability of patient- specific dosing of prophylactic anti-D appears warranted.

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*Nazaneen S. "Study of Feto-Maternal Haemorrhage in Later Part of Pregnancy With Special Reference to Rh Negative Mothers." *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)* 16.8 (2017): 83-89