International Journal of Reproduction, Contraception, Obstetrics and Gynecology
Rashmi et al. Int J Reprod Contracept Obstet Gynecol. 2025 Jan;14(1):152-157
www.ijrcog.org
pISSN 2320-1770 | eISSN 2320-1789

DOI: https://dx.doi.org/10.18203/2320-1770.ijrcog20243942

Original Research Article

Study of feto-maternal haemorrhage during third trimester of pregnancy and immediate postpartum period in a secondary care hospital in India

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Received: 30 October 2024
Revised: 30 November 2024
Accepted: 02 December 2024

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ABSTRACT

Background: Feto maternal haemorrhage (FMH) & isoimmunisation leads to erythroblastosis fetalis. Post partum Rh anti-D immunoglobulin within 72 hours can significantly reduce the incidence of alloimmunization but about 1.8% women become immunised due to 'silent' FMH during third trimester of pregnancy. To find out the incidence & volume of FMH during third trimester of pregnancy and just after delivery. Secondary objective was to consider the justified dose of anti-D immune globulin required after delivery in Rh negative mothers.
Methods: After getting approval from institutional ethics committee, we conducted a prospective observational study considering the inclusion and exclusion criteria. We took three maternal blood samples from each mother- at 28, 32 weeks & within 6 hours of delivery for performing Kleihauer Betkes test to detect FMH and its amount.
Results: 19 mothers out 0f 100 in our study developed FMH. Out of 19 mothers, 16 (84.2%) mothers had <1 ml Volume of haemorrhage and 3 mothers (15.8%) had 1-3 ml Volume of haemorrhage.
We found FMH is statistically associated with maternal blood pressure, mode of placenta delivery & number of foetuses.
Conclusion: Though incidence of FMH is low in antepartum period than intrapartum & postpartum period and incidence of large FMH is significantly lower, it is indeed needed to test the volume of FMH in every Rh-negative pregnancy, so that antenatal prophylaxis can be given and no silent bleeds cause isoimmunisation. Routine testing of FMH and subsequent patient specific adjusted dose to prevent isoimmunization can be cost effective**.**

Keywords: Fetomaternal haemorrhage, Kleihauer Betke test, Pregnancy, Isoimmunization

INTRODUCTION

Alloimmune haemolytic diseases of the foetus and newborns (HDFN) is caused by maternal immunoglobulin (IgG) which gain access to the foetal circulation during gestation and results in destruction of foetal red cells. Maternal alloantibodies directed against the D antigen of the Rh blood group system causes most serious form of HDFN as D antigen has the highest immunogenicity. By giving appropriate dose of prophylactic anti-D (RhIG) at the appropriate time Rh D HDFN can be prevented. First exposure to Rh positive red cells in a Rh-negative woman occurs most commonly during labour or during pregnancy by feto maternal haemorrhage (FMH), when foetal Rh-positive cells are transferred trans placentally into mother's blood circulation due to leak in placental barrier. Other causes are-by mismatched transfusion with Rh positive blood, by vaccines containing human sera or from her mother (grandmother theory). After FMH, fetal RBC is destroyed in maternal circulation, leading to primary immune response & formation of short-lived IgM which cannot cross placenta. So alloimmunization during the first

pregnancy is unlikely. Subsequent FMH leads to IgG mediated secondary immune response which can cross placenta and cause destruction of fetal RBCs leading to erythroblastosis fetalis or HDFN. Post partum Rh anti-D immunoglobulin (it prevents the Rh-positive cells from reaching the reticulo endothelial system and thus sensitization) within 72 hours after delivery can significantly reduce the incidence of alloimmunization but in spite of prophylaxis, about 1.8% women become immunised due to 'silent' feto-maternal bleeds during pregnancy which mostly occurs during third trimester of pregnancy. Thus, these women can be protected by Rh anti-D immunoglobulin at 28 weeks of gestation.

The amount of anti-D immunoglobulin given routinely can neutralize about 15 ml of fetal RBC. But amount of FMH may be more or less than this amount. In 1957, Kleihauer, Betke and Braun introduced Kleihauer technique (acid elution test) to demonstrate foetal RBC in maternal circulation based on the principal that foetal haemoglobin is resistant to acid elution unlike adult haemoglobin.[1] Measuring the amount of FMH followed by adequate dose of prophylaxis can improve the outcomes of Rh-negative women carrying Rh positive pregnancies.

In India incidence of Rh (D) negative population is significant (5%).[2] Considering this, our study aimed to try to document the timing & amount of FMH in Rh (D) negative mothers by Kleihauer technique so that timing and doses of prophylaxis can be justified.

Our primary objective was to find out the incidence of feto-maternal haemorrhage along with its amount during third trimester of pregnancy and just after delivery. Secondary objective was to consider the justified dose of anti-D immune globulin required after delivery in Rh negative mothers.

METHODS

Study design

After getting approval from institutional ethics committee of IPGME&R, Kolkata on 19/07/2021(memo number IPGME&R/IEC/2021/337), we conducted a prospective observational study from 1st August 2021 to 28th February 2022 at Department of Obstetrics & Gynaecology, M. R. Bangur hospital, Tollygunge, Kolkata-700033, West Bengal, India on all pregnant women attending the antenatal outdoor and delivered in M.R. Bangur hospital, over the stipulated period, after considering the inclusion and exclusion criteria.

Sample size

Sample size calculated using the formula N =zα/22 π (1-π)/ L2 where π = 0.186, Z α/2= 1.645 for 90% confidence interval level, L= 0.1, margin of error on either side (assuming incidence of FMH is 18.6% as described by Nazaneen S, et al.[3] The sample size came out to be 41. We included 100 cases of pregnant women based upon the inclusion and exclusion criteria during the study period. Their sampling strategy was purposive in nature.

Inclusion criteria

As FMH can occur in any pregnancy, we considered all mothers irrespective of their parity, gravida and blood groups except those having exclusion criteria.

Exclusion criteria

We excluded mothers having history of antepartum haemorrhage, any history of abdominal blunt trauma, mothers who undergone external cephalic version, history of prenatal invasive diagnostic investigations like chorionic villous sampling or amniocentesis, known case of haemoglobinopathies and already sensitized Rh negative mothers.

We picked up study subjects from antenatal clinic by inclusion and exclusion criteria. After detailed history taking, examinations and basic investigations we followed up the mothers until after delivery. We took three maternal blood samples from each mother- at 28 weeks, at 32 weeks and within 6 hours of delivery for performing Kleihauer Betkes test at our department to detect any FMH and its amount.

We examined the blood sample within 6 hours of collection. The acid buffer was used to remove or elute the HbA (adult haemoglobin) which is more soluble than the HbF (fetal haemoglobin). So, the foetal RBC having HbF looked darker in the background of the pale maternal RBC (looks like the ghost cells, without haemoglobin). Number of foetal RBC per 6000 maternal ghost cells was calculated.

Volume of FMH in millilitre was determined by the formula (Maternal blood volume×maternal haematocrit×% of foetal cells by Kleihauer Betke test)/Foetal haematocrit where maternal blood volume was taken 5 litre, foetal haematocrit 50% and maternal haematocrit 35%.

Statistical analysis

For statistical analysis, data were entered into a Microsoft excel spreadsheet and then analysed by SPSS (version 27.0; SPSS Inc., Chicago, IL, USA) and Graph Pad Prism version 5. P value≤0.05 was considered for statistically significant.

RESULTS

In our study, Table 1 shows distribution of mother according to age, parity, maternal blood pressure, singleton/ multiple pregnancy, mode of delivery, mode of placenta delivery. We found 19 mothers out 0f 100 in our study who developed feto maternal haemorrhage (FMH).

Distribution in relation to period of gestation described in Table 2. Out of 19 mothers, 16 (84.2%) mothers had <1 ml volume of haemorrhage and 3 mothers (15.8%) had 1-3 ml volume of haemorrhage. In our study, we found FMH is statistically associated with maternal blood pressure, mode of placenta delivery & number of foetuses as described in Table 3.

Table 1: Distribution of mother according to age, parity, maternal blood pressure, number of fetus, mode of delivery, mode of placenta delivery.

	Number of mother (n=100)	% of mother (n=100)
Distribution of mother according to age		
Less than 20 years	10	10
21- 30 years	65	65
31-40 years	21	21
More than 40 years	4	4
Distribution of mother according to parity		
Primipara	56	56
Multipara	44	44
Distribution of mother according to maternal blood pressure		
Hypertensive	14	14
Normotensive	86	86
Distribution of mother according to number of fetus		
Singleton pregnancy	83	83
Multiple pregnancy	17	17
Distribution of mother according to mode of delivery		
Caesarean section	46	46
Instrumental delivery (forceps/ventouse)	13	13
Normal vaginal delivery	41	41
Distribution of mother according to mode of placenta delivery		
Manual removal of placenta	24	24
Spontaneous expulsion/ placenta delivery by controlled cord traction (CCT)	76	76

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

Period of gestation	Number of mothers developed FMH, detected by Kleihauer Betkes test (n=100)	%
At 28 weeks	2	2
At 32 weeks	3	3
Immediate post-partum	14	14

Table 3: Association of FMH with maternal age, parity, maternal blood pressure, number of fetus, mode of delivery & mode of placenta delivery.

	Mothers who did not develop FMH	Mothers who developed FMH	Total
Age less than 20 years	6	4	10
Row%	60	40	100
Col %	7.4	21.1	10.0
Age between 20-30 years	57	8	65
Row%	87.7	12.3	100.0
Col %	70.4	42.1	65.0
Age between 31-40 years	16	5	21
Row%	76.2	23.8	100.0
Col %	19.8	26.3	21.0
Age more than 40 years	2	2	4
Row%	50.0	50.0	100.0

Continued.

	Mothers who did not develop FMH	Mothers who developed FMH	Total
Col %	2.5	10.5	4.0
Total	81	19	100
Row %	81.0	19.0	100.0
Col %	100.0	100.0	100.0
Multi para	37	7	44
Row %	84.1	15.9	100.0
Col %	45.7	36.8	44.0
Primi para	44	12	56
Row %	78.6	21.4	100.0
Col %	54.3	63.2	56.0
Total	81	19	100
Row %	81.0	19.0	100.0
Col %	100.0	100.0	100.0
Hypertensive	4	10	14
Row %	28.6	71.4	100.0
Col %	4.9	52.6	14.0
Normotensive	77	9	86
Row %	89.5	10.5	100.0
Col %	95.1	47.4	86.0
Total	81	19	100
Row %	81.0	19.0	100.0
Col %	100.0	100.0	100.0
Multiple pregnancy	5	12	17
Row %	29.4	70.6	100.0
Col %	6.2	63.2	17.0
Singleton pregnancy	76	7	83
Row %	91.6	8.4	100.0
Col %	93.8	36.8	83.0
Total	81	19	100
Row %	81.0	19.0	100.0
Col %	100.0	100.0	100.0
Caesarean section	36	10	46
Row %	78.3	21.7	100.0
Col %	44.4	52.6	46.0
Instrumental delivery	10	3	13
Row %	76.9	23.1	100.0
Col %	12.3	15.8	13.0
Normal vaginal delivery	35	6	41
Row %	85.4	14.6	100.0
Col %	43.2	31.6	41.0
Total	81	19	100
Row %	81.0	19.0	100.0
Col %	100.0	100.0	100.0
Manual removal of placenta	9	15	24
Row %	37.5	62.5	100.0
Col %	11.1	78.9	24.0
Placental expulsion spontaneous or by CCT	72	4	76
Row %	94.7	5.3	100.0
Col %	88.9	21.1	76.0
Total	81	19	100
Row %	81.0	19.0	100.0
Col %	100.0	100.0	100.0

Association of age with FMH found to be non-significant: Chi-square value=7.5704; p-value = 0.0558. Association of Parity with FMH was not statistically significant: p=0.4849; Chi-square value: 0.4878; Odds Ratio: 1.4416 (0.5149, 4.0363). Association of Blood Pressure with FMH was statistically significant: p<0.0001; Chi-square value: 29.0755; Odds Ratio: 0.0468 (0.0121, 0.1803). Association of number of foetuses with FMH was statistically significant: p<0.0001; Chi-square value: 35.4188; Odds Ratio: 0.0384 (0.0105, 0.1407). Association of mode of delivery with FMH was not statistically significant: p=0.6465; Chi-square value: 0.8724. Association of mode of placenta delivery with FMH was statistically significant: p<0.0001; Chi-square value: 38.827

DISCUSSION

In our study FMH was detected in 2% cases and 3% cases at 28 weeks and 32 weeks of gestation respectively &14% cases in post-partum period in contrary to findings described by other authors. Bowman JM et al, suggested 75% of all pregnancies have FMH of various degrees.[4] Renaer M reported some degree of FMH in 15%-31% of pregnancies but bleed amount less than 0.1 ml in 1.5%-6% cases.[5] One of the probable reasons for low incidence of FMH in our study may be the exclusion of high-risk cases for developing FMH (abdominal trauma, external cephalic version, ante-partum haemorrhage, etc.). A very large number of fields need to be examined with use of a more sensitive method than the K-B test to detect a very small amount of haemorrhage.

No significant association between FMH & maternal age or parity found in our study, similar to the finding reported by Adeniji AO et al and de Wit H et al.[6,7] We found significant association for FMH with maternal hypertension, number of foetuses and mode of placenta delivery but we could not establish any significant association with mode of baby delivery. Though Akorsu EE et al in 2019 could not find any identifiable patient-specific factors in associated with occurrence of FMH.[8]

Salim R et al also found no difference between vaginal and caesarean deliveries in relation to FMH.[9] But Marek Lubusky et a, reported 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.[10] David M et al in 2004 reported twin pregnancy is the only independent risk factor for severe fetal-to-maternal transfusion which is similar to our study result.[11]

In our study, volume of FMH was less than 1 ml in 84.2% of cases and 15.8% cases had FMH between 1-3 ml. In none of the case FMH exceeded 3 ml. This is similar to findings reported by Augustson BM et al, who concluded 90.4% of the women had an FMH volume of 1.0 ml or less and 98.5% had a volume of less than 2.5 ml, only 0.4% of cases had an FMH volume of 6.0 ml or greater.[12] Zero incidence of large FMH in our study may be due to exclusion of high-risk cases for developing large FMH (abdominal trauma, ante- partum haemorrhage, etc.)

So, antenatal prophylaxis is needed for this kind of silent bleeds. It will help in reducing neonatal mortality and morbidity in subsequent pregnancies. From current study, it is difficult to calculate the exact amount required for ante-partum prophylaxis as number of cases included are less. For calculation of accurate dose of anti-D for antenatal prophylaxis, an extensive study with more sensitive method needs to be done.

CONCLUSION

Antepartum and postpartum immunization can prevent poor obstetric outcome in Rh-negative women with feto-maternal haemorrhage. In this study, the incidence of feto-maternal haemorrhage detected by KB test was found to be lower as compared to other studies as it is a relatively crude method for FMH detection, detecting small volume of haemorrhage requires examination of very large number of fields, exclusion of 'at risk' mothers for FMH from this study.

Though incidence of FMH is low in antepartum period than intrapartum & postpartum period and incidence of large FMH is significantly lower, it is indeed needed to test the volume of FMH in every Rh-negative pregnancy, so that antenatal prophylaxis can be given and no silent bleeds cause isoimmunisation. The dose of anti-D given routinely in our country is 300 micrograms which can neutralise 30 ml of fetal blood. Routine testing of FMH and subsequent patient specific adjusted dose to prevent isoimmunization can be cost effective in our country. There is need of developing optimized testing and assessing dosing protocol.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Rashmi, Ghosal A, Akhtar S, Bera TK, Dasgupta A. Study of feto-maternal haemorrhage during third trimester of pregnancy and immediate postpartum period in a secondary care hospital in India. Int J Reprod Contracept Obstet Gynecol 2025;14:152-7.