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Dr. Chandrakant S Madkar
Professor, Dr. D.Y. Patil, Vidyapeeth, Pune, Maharashtra, India

Dr. Madhukar J Shinde
Associate Professor, Dr. D.Y. Patil, Vidyapeeth, Pune, Maharashtra, India

Dr. Hemant G Deshpande
HOD. Professor, Dr. D.Y. Patil, Vidyapeeth, Pune, Maharashtra, India

Dr. Swarna Gupta
Resident, Dr. D.Y. Patil, Vidyapeeth, Pune, Maharashtra, India

Dr. Apurva Singh
Resident, Dr. D.Y. Patil, Vidyapeeth, Pune, Maharashtra, India

Correspondence
Dr. Madhukar J Shinde
Associate Professor, Dr. D.Y. Patil, Vidyapeeth, Pune, Maharashtra, India

Comparative study of fetomaternal hemorrhage in normal vaginal delivery and lower segment casearean section

Dr. Chandrakant S Madkar, Dr. Madhukar J Shinde, Dr. Hemant G Deshpande, Dr. Swarna Gupta and Dr. Apurva Singh

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Abstract
Background: Fetomaternal Hemorrhage has got specific importance particularly in case of haemolytic disease of the new-born. It has got special impact in patients of RH incompatibility that is RH negative mother bearing RH positive foetus. It has played a significant role in reducing the morbidity and mortality in RH incompatibility cases in past. The base of this treatment is prophylactic Anti-D injection after delivery abortion and other conditions related with FMH. The dose of this Anti-D is dependent on the quantity of FMH which can be detected by using KB Test.
Material and Method: In our study we have done comparative study of FMH in cases of full term normal vaginal delivery in primigravida against lower segment caesarean section in primigravida. The sample size will be 60 in each group. The blood was collected after 2-4 hours following FTND and LSCS. The FMH was decided by KB Test and analysis of both groups was done to know whether there is significant difference.
Result: Our study showed that there is no significant difference in FMH in cases of FTND and LSCS.

Keywords: FMH- Fetomaternal haemorrhage HDFN-Haemolytic disease of foetus and new born KB Test-Kkeihauer-Betke test INJ. ANTI-D-Injection Anti-D

1. Introduction
The placenta is considered as an ephemeral organ which acts as the interface between mother and fetus [1]. It ensures the foetus a proper supply of nutrition and safeguards the surrounding against unfavourable conditions and also secures immunological environment [2].
Both the foetal and maternal blood is differentiated by a selectively permeable 'placental barrier [3]. However, some foetal red blood cells can disturb the placental barrier, permeate it and enters into the maternal circulation before or during the delivery posing a serious risk to the foetus resulting in foetal morbidity and mortality [4, 5]. This pathological condition is referred as fetomaternal haemorrhage (FMH).
FMH is defined as the loss of foetal blood cells into the maternal circulation [6]. It is estimated that less than 1ml of foetal blood is lost to the maternal circulation during normal labor in around 96% of deliveries [6, 7]. The loss of this small amount of blood may sensitise event and stimulate antibody production to the foetal red blood cells, resulting, in anaemia, jaundice complications like hydrops fetalis, neurological injury, stillbirth or neonatal death [4]. This has got more significance in case of Rh negative mother bearing Rh positive foetus in ABO compatible blood groups of mother and foetus.
However, small amount of FMH can occur in all trimesters of pregnancy through the microscopic breach in the maternal barrier which can cause sensitization before the delivery. About 3% of FMH has been observed in the first trimester, increasing to 12.1% in the second trimester, 45.5% in the final semester and 63.6% after delivery [8].
FMH is mainly concerned with the haemolytic disease of foetus and new-born (HDFN). The pathology of which is immunological sensitization in different blood groups. In practice A, B, AB and O are associated with the presence or absence of D antigen which is known by Rh positive and Rh-negative blood group respectively. There are other blood group antigens also like Lewis antigen, Kell antigen, Duffy antigen and MNS antigen, but they are rare and does not come in common practice [9].

The importance of the study of FMH lies in the fact that the incidence of Rh isoimmunisation and different complications related to it (HDFN) which was occurring before the invention of injection Anti-D (1960) has fallen dramatically due to the use of Anti-D prophlaxis [10]. The base of this Anti-D Prophylaxis is amount of FMH.

In our study we are studying the amount of FMH which occurs in normal delivery and lower segment caesarean patients and to counter check the prescribed fixed dose of Anti-D that is 300mcg. (This dose of anti D is calculated as 10 mcg per ml of FMH).

The present strategy of prophylactic dose given to Rh negative mother for prevention of allo-Isoimmuniation is 300mcg. While deciding this dose it has been assumed that the amount of FMH is maximum 30 ml of whole blood and the formulae is 10mcg/ml of whole blood.

Statistically, it can cause significant error because in some patient the dose can be inadequate and the whole purpose of giving Anti-D injection goes in vain. On the contrary, in some patient the FMH can be significantly less than 30 ml, where excessive dose can be wasted and it has got its own disadvantages of additional risks that are related to plasma derived products [11]. The other thing which require consideration is the cost of Anti-D injection which is quite high (300mcg for 2500/-) which plays a significant role in developing and under developing countries like India. So, we can minimise this by estimating the exact dose of Anti-D by calculating FMH level using KB TEST. For example, if 5ml of FMH occurs then we need to give 50IU of Anti-D which costs for 500 Rs/- approx. instead of 2500/- by doing this we can give justice to prophylactic treatment and also economically challenged people and that's the main purpose of our study.

That's why screening of all women at the time of delivery for FMH calculation should be done to know the exact dose of Anti-D. This we have conducted with the help of KEIHAUBER BETKE TEST (KB). However other methods are also available for estimation of FMH like Rosette test, flowcytometry, Shepard method etc.

The Rosette test is one of the only qualitative screening tests approved by FDA in the USA. Though it has got advantage of low cost, simple process, and easy availability, this method is unreliable in case where the mother or foetus has a variant of Anti-D called the weak D [12, 13] and also it does not estimate the amount of FMH. So, not much useful in cases where the exact estimation of FMH is required.

The most recent developed flowcytometry has been preferred choice of test as it is precise and gives fast result due to its ability to count great number of cells in short period [14] but it has also got disadvantages that include expensive machinery and technique and easy availability.

The KB test has been considered as the gold standard test for FMH assessment due to its rapidity [11]. KBT was successful in detecting FMH of the samples studied and considered as the suitable screening test for even today [6, 15-19]. It is observed to be the most sensitive with the sensitivity of 0.1% [20]. This test is inexpensive [16] and does not require any high-tech apparatus to conduct the test like flowcytometry that is the reason for selecting KB Test in our study.

This test measures the amount of foetal haemoglobin transferred from, foetus to its mother's blood stream [21, 22]. It takes advantage of the differential resistance of foetal haemoglobin to acid. A standard blood smear is prepared from the mother's blood, and exposed to an acid bath. This removes adult haemoglobin, but not foetal haemoglobin, from the red blood cells.

2. Material and Method

2.1 Material

Study was conducted on the patient having full term normal vaginal delivery and lower uterine segment caesarean section in Dr. D.Y Patil Medical College and Hospital, Pimpri, Pune.

Institute ethics committee clearance obtained.

Type of study-Randomised prospective study

Period of study-October 2018 to March 2019

Sample size- 60 normal vaginal deliver y and 60 lower segment caesarean section.

Place of study- Dr. D. Y. Patil Medical College, Pimpri, Pune

Source of data- PNC ward of Dr. D.Y. Patil Medical College

Inclusion Criteria

1. The patients who have given consent for the inclusion in the study
2. Age group -20 to 35
3. Primigravidae
4. Singleton pregnancy
5. Full term normal vaginal delivery
6. Full term lower segment caesarean section

Exclusion Criteria

1. Patient not willing to participate in the study
2. Age group less than 20 year and above 35 year
3. Patients having complications during delivery like APH AND PPH
4. Multiple pregnancies
5. Mother with O positive blood group and foetus with A, B and AB blood group because ABO incompatibility protects from RH incompatibility
6. Conditions with high HBF which will give wrong results e.g. Sickle cell anaemia and thalassemia.

2.2 Procedure of Collection of Material

As mentioned above the study was conducted after ethical committee permission. The patients detailed history and clinical examination was done to know their eligibility for inclusion in the study. Informed consent was taken of the patient after discussing with her about the study.

All the data was recorded over the proforma specially designed for this study.

After full term normal vaginal delivery and lower segment caesarean section the sample of mother's blood was collected in the EDTA bulb and oxalate bulb up to 2-4 hrs. The cord blood of the baby was collected immediately after delivery of baby and before separation of placenta. The blood was sent to the laboratory immediately for doing following tests-

For specific collection and storage, we are using guidelines of sure take diagnostic associative, in (CLS417, Clinical Haematology II)

1. Estimation of FMH by KB TEST
2. Confirmation of blood group of mother
3. Blood group and Rh status of baby

As far as possible this collection of samples was done during day time when our laboratories are working. If we require to collect some samples in night the sample was stored in fridge between 2-8 degree centigrade and was sent to the laboratory the next day.

The method which we have selected for the estimation of FMH is Kleihauer Test considering all the pros and cons of all different test that are available.

A thin blood smear is prepared from maternal haemoglobin. The

smear is stained by erythrocin to stain foetal red cells. Maternal cells appear as ghost against the stained foetal red cells. Under the light microscope the number if the foetal cells per 100000 ghost cells are counted.

For calculation of FMH we also used the routine method given in the textbook that is number of foetal red blood cells for 50 low power field. For ex if there are 80 foetal red blood cells in 50 low power field the FMH will be 50*80/100 that is 4ml.

The data is analysed to know variability of FMH in our sample and average FMH is calculated. We also studied the significance difference with respect to FMH in two groups that is first group of full-term vaginal delivery and lower segment caesarean section. We also took the review of the routine dose versus calculated dose as per standard formulae of 10mcg/ml of whole blood to understand the adequacy, inadequacy and overdosing of the injection Anti-D.

3. Observation and Results

In this study total number of 120 cases of delivery was selected. They were divided into 2 groups each group having sample size of 60 as mentioned in material and method. The first group consisted of primigravidae who has under gone full term normal vaginal delivery and second group included primigravidae undergone uncomplicated lower segment caesarean section. The blood of mother and new-born were sent to the laboratory for the estimation of blood group and FMH. To maintain the uniformity of the sample with respect to the gravidity only primigravidae were included in the study.

Table 3.1 Shows the age wise distribution of both groups.

Age	Normal Vaginal Delivery	Lower Segment Caesarean Section	Total
Less Than 2o	13	10	23
21-25	27	35	62
26-30	15	12	27
31-35	05	03	08
Total	60	60	120

Chi –square =4.08

P=0.25

The table shows both the groups were comparable.

Table 3.2 Showing amount of FMH in both groups

FMH	Normal Vaginal Delivery	Lower Segment Ceaserean Section
NO FMH	06	04
UPTO 4ML	18	20
>40ML-10ML	20	22
>10ML-30ML	14	12
> 30ML	02	02
TOTAL	60	60
MEAN	7.7588	7.798
SD	7.911	7.947

Test Applied= Wilcoxan signed rank test P value=0.60 (not significant)

This table shows that the range of FMH was variable. In the first group there was no FMH in 6 patients as oppose to 4 patients in second group. It was up to 4ml in 18 patients of first group as compare to 20 patients in second group. 14 patients in first group were having FMH more than 10ml to 30ml as oppose to 12 in second group. Only 2 patients in both the groups showed FMH more than 30ml.

This shows the range of amount of FMH is same in both groups that is normal delivery and lower segment caesarean calculated by statistical test of significance.

Table 3.3 Showing the dose of Anti-D required by the patient depending upon FMH

Fmh Level	Normal Vaginal Delivery	Anti-D (Dose In Mcg)	Lower Segment Caesarean Section	Anti-D (Dose In Mcg)
Negative	06	60	04	40
Less Than 4ml	18	180	20	200
4ml-10ml	20	200	22	220
10-30ml	14	140	12	120
More Than 30ml	02	200	02	200
Total	60	-	60	-

The above table shows that the dose required of Anti-D by calculating the FMH level using the KB TEST was quite variable. The first group shows the range of Anti-D from 60mcg to 200 mcg and the second group shows from 40 mcg to 200mcg with 2 people from each group showing FMH level more than 30ml and requiring dose more than 300mcg which is given routinely.

Interesting to note that out of 60 patients in the first group 38 patients were having dose requirement of 100 mcg only as oppose to 300 mcg which is given routinely. Whereas in the second group out of 60 patients 42 patients dose requirement was 100 mcg as oppose to 300mcg which is prescribed routinely. Anti-D injection is quite costly costing about 2500 rupees for 300 mcg. It means that in about more than 60% patient this dose can be reduced to one third in both the groups giving significant reduction in the financial burden of the patients which is important in under developing and developing countries like India.

Only two patients from each group shows requirement of Anti-D more than 300mcg which is prescribed routinely. So, in these patients this routine dose seems to be inadequate hampering the purpose of prevention of isoimmunisation.

4. Discussion

In our study we calculated the amount of FMH in primigravidae having full term normal vaginal delivery in first group and lower segment caesarean section in second group with the sample size of 60 each group.

The table shows the age wise distribution of number of patients in each group the statistical test shows that both the groups were comparable giving value of p=0.25 which is not significant.

The second table shows the amount of FMH range in 2 groups

which were analysed by 5 categories starting from no FMH to FMH more than 30ml. This analysis has shown that there was no significant difference in FMH in both the groups that is first group of full term normal vaginal delivery and second group of full-term lower segment caesarean.

The third table shows the amount of Anti–D required in both groups tis we did for the purpose of knowing financial burden on the patients and weather it can make any difference if the dose is given according to the formulae 10 mcg per ml as oppose to routine 300mcg. Interestingly it showed that this financial burden can be reduced if dose is given by calculation of FMH level by KB TEST. As seen from the table it is obvious that more than 60% patients required only one third dose of Anti-D because they were having FMH up to 10ml only which shows that it is having cost effectiveness value if the dose is given by calculation. Also 2 patients were having FMH more than 30ml required more dose than the prescribed dose routinely and these patients should has been missed if KB TEST would not have been done. All this shows that instead of giving 300mcg the calculated dose should be given with the assessment of FMH by KB TEST which will serve the purpose if reducing financial burden on society and giving appropriate protection from iso-immunisation in the patients who are having more FMH (as seen in 2 patients of each group).

More studies should be undertaken for confirmation of our findings and conclusion

5. Conclusion

1. FMH is most important factor in HDFN which depends upon amount.
2. The prescribed dose if Anti-D seems to be adequate, however, in some patients it can be more or less if calculated on the basis of FMH.
3. The Anti-D injection is costly, so, if it is given on the basis of FMH, the economic burden of the patient can be reduced which is more important in our country like India.

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