TRANSPLACENTAL PASSAGE OF FOETAL ERYTHROCYTES IN PREGNANCY

by

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Introduction

To understand the immunologic relationship between mother and foetus knowledge of the transplacental passage of erythrocytes into the maternal circulation is essential. Levine, in 1943, pointed out that foeto-maternal ABO compatibility is much higher in Rh immunized mothers than that in the general population. ABO incompatibility between mother and foetus protects the mother against Rh immunisation to a greater extent. Stern *et al.*, in 1961, showed that the risk of Rh immunisation is lower in subjects who are injected Rh positive red cells followed by an injection of anti-Rh serum. Estimation of volume of foetal blood in maternal circulation can indicate which Rh negative mothers could be given anti-Rh immunoglobulin to prevent Rh immunisation.

Acid-elution method of demonstrating foetal erythrocytes in maternal blood smears is the best available technique. Examination of blood films from the mother after treating them with an acid medium can identify foetal cells. This depends on the resistance of foetal haemoglobin in foetal red cells to acid elution compared with adult haemoglobin in maternal red cells. Foeto-maternal haemorrhage is more towards the end of pregnancy (Mollison, 1967).

The present work was started by detecting foetal cells in maternal blood smears by this technique. Next, an attempt was made to assess the approximate volume of foeto-maternal bleeding. Thereafter, correlation of factors such as ABO status of mother and foetus, time of collecting the samples after delivery, complications of labour, etc., on the incidence of foetal haemorrhage into maternal circulation was studied. Blood films from voluntary blood donors were examined to find out any foetal cells.

Materials and Methods

The acid-elution technique of Kleihauer and Betke modified by Shepherd *et al* (1962) was used to detect foetal cells. The blood films were prepared by diluting the blood with equal volume of saline, dried and fixed in 80% ethanol for five minutes and rinsed thoroughly in distilled water for five minutes. The slides were immersed for four minutes in citric acid-sodium phosphate buffer, pH 3.3, which was kept at 37°C for 30 minutes just be-

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fore use and rinsed thoroughly with distilled water for ten minutes. They were dried and then kept for three minutes in Mayer's haematoxylin and rinsed with tap water to blue the nuclei. Then they were counterstained with 0.1% erythrosin B for four minutes, washed thoroughly in distilled water and dried. They were examined under high power (X 240) throughout this investigation. In each slide 1000 erythrocytes were counted with corresponding number of foetal cells. An artificial mixture of adult and foetal cells was treated similarly as a positive control.

Blood films were made from mixtures of cord blood and adult blood in different proportions. These films were subjected to acid-elution as above. The findings of foetal cells in such smears were correlated with the standard mixtures.

The case material was obtained from the hospitalized women at Bai Motlibai Hospital. It consisted of 192 women who were studied within 24 hours after delivery irrespective of any selection. The time of collection after delivery was noted. The corresponding cord blood samples were also collected. Three millilitres of blood was collected in an oxalate bulb. Maternal blood samples were investigated for presence of foetal cells by acid-elution, ABO and Rh grouping and iso-antibody titre. Cord blood samples were tested for ABO and Rh grouping.

ABO blood grouping was carried out by standard saline tube technique while Rh (D) grouping was done by modified tube technique. Antibody titration was carried out by double dilution method.

Blood films from 100 voluntary donors were examined for foetal cells after acid-elution.

Results

The average and range of five experiments carried out for assessment of the

TABLE I

Approximate volume of foetal blood in maternal circulation based on estimate of foetal cells/maternal cells assuming normal blood volume of mother and foetus.

Group	1 : 1000	2 : 1000	3 : 1000	4 : 1000	5 : 1000	6 : 1000	7 : 1000	12 : 1000	15-23 : 1000	More than 23 : 1000
Unselected	9	4	5	4	7	3	Nil	1	1	Nil
ABO—compatible	6	4	5	4	7	2	Nil	1	1	Nil
ABO—incompatible	3	Nil	Nil	Nil	Nil	1	Nil	Nil	Nil	Nil
Calculated foetal blood loss (ml.)	..	..	upto 0.4 ml.	..	0.4 ml. to 2.5 ml.	..	2.5 ml. to 10 m.l			More than 10 ml.
Classification			mild		intermediate		severe			massive

approximate volume of foetal blood in maternal circulation is indicated in figure 1. From the findings of the number of

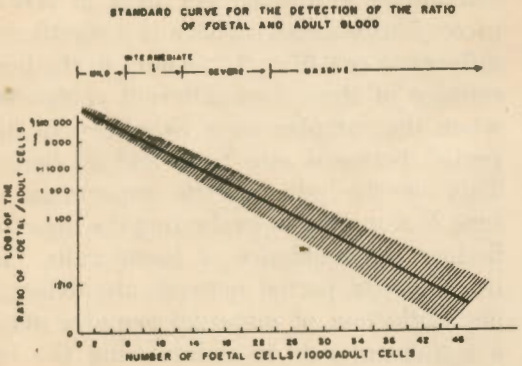

Fig. 1

foetal cells, the amount of foetal blood was calculated on the basis of assuming four litres as maternal blood volume and half litre as foetal blood. This quantitative data of foetal blood was utilized to classify the different categories of foetal haemorrhage such as mild, intermediate, severe and massive. Figure 2 gives the incidence of foetal cells in the samples. The same samples are classified into ABO compatible and ABO incompatible depending on ABO status of mother and foetus. Incidence in both these groups is calculated separately. The incidence of foetal blood is significantly higher in ABO compatible cases than in ABO incompatible cases ($p\ 0.05 > 1 > 0.02$). The period between delivery and collection of maternal samples and the respective incidence of foetal cells is shown in figure 3. Table I shows the different gradations of foetal blood loss. Incidence of ABO compatible cases is further classified on the basis of obstetric interference in Table II. Blood films from 98 voluntary blood donors did not show any foetal cells. Two persons showed 1/1000 and 2/1000 foetal cells.

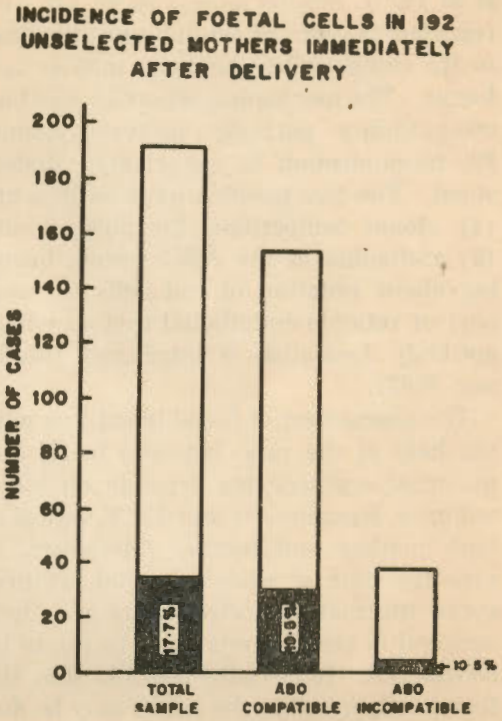

Fig. 2

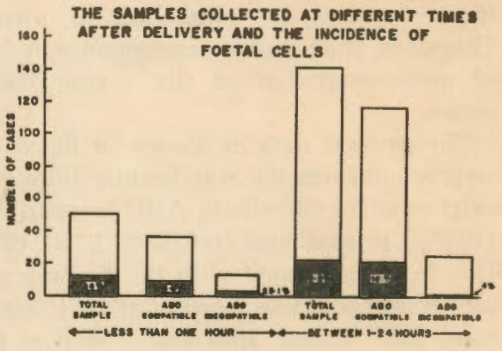

Fig. 3

Discussion

If Rh positive red cells of incompatible ABO group are injected into Rh negative subjects the stimulation of antibody against Rh antigen is less likely to occur, whereas if they are injected into compatible ABO group the stimulation of antibody against them is more likely (Stern

et al 1956). Similar observations are also reported in cases of iso-immunisation due to Rh compatibility between mother and foetus. The mechanism whereby ABO incompatibility partially protects against Rh immunisation is not clearly understood. The two possible explanations are (1) clonal competition for antigen and (2) antibodies of the ABO system bringing about isolation of red cells in some part of reticulo-endothelial system where antibody formation is interfered (Mollison, 1967).

The assessment of foetal blood loss with the help of the ratio between foetal and maternal erythrocytes depends on blood volumes, haematocrit and MCV values of both mother and foetus. Moreover, in vivo the time of entry of foetal erythrocytes into maternal circulation and their survival is also an important factor to be considered. It is quite possible that the foetal cells found in the smear may be due to the total effect of repeated or intermittent transplacental spills. All these factors cannot be controlled in vitro. Therefore, the possible assessment may be of underestimation of the foetal blood losses.

The present data as shown in figure 2 clearly indicates the significantly high incidence of foetal cells in ABO compatible (19.5%) pregnancies ($p\ 0.05 > 1 > 0.02$). This is in agreement with the findings of earlier workers like Lewi *et al* 1961, Finn *et al* 1963, Fraser 1964 and Cohen *et al* 1964, 1967. Taneja *et al* 1969 reported 56% of incidence in which the number of cases showing less than 1:1000 foetal cells is not stated. The unsually high incidence is probably explained due to inclusion of many cases which showed less than 1:1000 foetal cells.

Figure 3 shows the percentage incidence observed according to varying periods lapsing between delivery and collection of maternal samples. When this period was less than one hour, the incidence of 24.32 per cent is almost similar in ABO-compatible and 23.08 per cent in ABO-incompatible cases. There is a significant difference ($p\ 0.01 > 1 > 0.001$) in the percentage of these two different categories when the samples were examined in the period between one hour and 24 hours. This clearly indicates the importance of time of sampling in evaluating the data for finding the incidence of foetal cells. In other words, period between the delivery and collection of maternal samples plays a significant role in determining the incidence of foetal haemorrhage.

On the basis of quantitation of foetal blood in maternal circulation, 34 cases were classified into mild, intermediate, severe and massive types. In ABO-compatible group it shows that out of 30 positive cases, 26 (86.6 per cent) belong to mild type in which calculated foetal loss is upto 0.4 ml. Cohen *et al* 1964 reported 251 out of 303 positive cases i.e. 82.5 per cent of minimal and slight category in which foetal blood loss was also upto 0.4 ml. In the ABO incompatible group, 3 out of 4 cases are of mild type and one is of intermediate type. Cohen *et al* 1967 reported 37 cases of minimal and slight category out of 38 positive cases. Our observations are in agreement with these findings.

Table II shows that there is no difference in the incidence of foetal cells between those having normal labour and those with obstetric interference. Workers like Cohen *et al* 1964 and Brown 1963 did not find any differences in the incidence between pregnancies conducted naturally and by caesarean section. On the other hand earlier workers like Finn *et al* 1963 (23.5% showing foetal cells in cases of caesarean sections against 5.2% in normal), Wimhofer *et al* 1962 (four times higher foetal cells than that of nor-

TABLE II

Effect of obstetric interference in ABO-Compatible cases

Nature of labour	Total No. of cases	Foetal cells detected	Foetal cells not detected	Percentage of foetal cells detected
Natural	106	23	83	21.70
Episiotomy	23	2	21	8.69
Complicated cases—manual removal of placenta or caesarean	25	5	20	20.00
Total	154	30	124	19.48

mal) and Zipursky *et al* 1963a (11 out of 102 foetal cells in cases with obstetric interference against only 1 out of 121 with normal) claimed that manual removal of the placenta and caesarean section increases the incidence of foetal blood loss in maternal circulation.

The data when classified on parity basis did not reveal any significant pattern. In ABO-incompatible cases, data did not show any relation between the titre and positive findings.

Summary

Two hundred cases were investigated to assess the quantity of foetal blood which goes into maternal circulation by the acid-elution technique. Thirty-four cases showed the presence of foetal cells in different numbers. The positive cases were classified into mild, intermediate, severe and massive categories. Out of these 192 cases, 154 were of homospecific i.e. ABO compatible and 38 heterospecific —ABO incompatible. In homospecific, (30 out of 154) 17.7% cases were positive while in heterospecific (4 out of 38) 10.53% cases were positive. The interval between delivery and sampling was an important factor. Obstetric interference did not contribute to the incidence of foetal cells when compared with the normal cases.

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