STUDY OF FOETAL ERYTHROCYTES IN MATERNAL CIRCULATION IN NORMAL AND ABNORMAL PREGNANCY DURING ANTENATAL PERIOD AND DELIVERY

by

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Introduction

With the recognition of the formation of antibodies by the mother against antigens of the baby in cases of ABO or Rh incompatibility and the ever increasing clinical importance of erythroblastosis foetalis, investigators have been searching for a mechanism by which the interaction between the mother and the baby could be explained. Foeto-maternal haemorrhage during advanced pregnancy or during labour may result in antigen antibody reaction causing maternal sensitization. Presence of foetal erythrocytes in maternal circulation is a conclusive proof of this mechanism and this is the basis of undertaking the present work.

Material and Methods

One hundred and fifty form the present study and a total of 300 slides were studied, one at term and the other within six hours of delivery. In all cases a detailed history was taken. As a routine, haemoglobin and complete urine examinations were carried out. ABO and Rh grouping of the mother and the baby was also done.

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Principal: The staining method is based on the fact that foetal haemoglobin is more resistant not only to alkali but also to acid as compared to adult haemoglobin which will be eluted but foetal haemoglobin will remain in the erythrocyte which is stained and counted.

The calculation of the amount of transplacental haemorrhage was done. Smear showing 1-4 cells per 1000 maternal ghost cells were grouped as mild and haemorrhage amounting to 0.4 ml. 5-7 foetal cells per 1000 ghost cells were grouped under intermediate and haemorrhage amounting to 0.4-2.5 ml. 8-15 foetal cells per 1000 maternal cells were grouped as severe haemorrhage amounting to 2.5-10 ml. 15-23 of more foetal cells per 1000 adult cells the haemorrhage was said to be massive and calculated blood loss was more than 10 ml.

Observations

The 150 cases were divided into two main groups.

A. Normal:

Normal pregnancy—81 cases

Subgroups:

a. Primigravida —31
b. Multigravida —50

Normal labour—45 cases

B. Abnormal:
Abnormal pregnancy—69 cases
a. Primigravida —24
b. Multigravida —45
Abnormal labour—105.

An attempt was made to study the effect of parity on transplacental haemorrhage during the antenatal period only as occurrence of foetal cells in these cases was affected by labour to some extent.

TABLE I

Showing Incidence of Foeto-maternal Haemorrhage in Primiparae and multiparae

Parity	Foetal cell positive cases	Foetal cell negative cases	Total
Primipara	12	43	55
Multipara	39	56	95
Total:	51	99	150

TABLE II

Shows the Distribution of Cases According to ABO and Rh Grouping in 100 Mothers and 100 Babies

	A	B	O	AB	Total
Mother	23	35	30	12	100
Baby	22	25	40	13	100
Total	45	60	70	25	200

Hence the difference between the sample mean in the blood group of the mother and the baby is not significant.

Perusal of the above Table shows that most frequently encountered blood group was 'O' in both the mother and the baby, next common blood groups in order of frequency were B and A and least commonly observed group was 'AB'. Incidence of Rh negative mothers was 3.3%, whereas none of the babies were Rh negative.

Levine (1940) for the first time described the compatible and incompatible mating according to blood group.

TABLE III

Shows Compatible and Incompatible Mating

Compatible				Incompatible			
Mother	Baby	No. of cases	Percentage	Mother	Baby	No. of cases	Percentage
A	A	1	2.2	O	A	10	18.8
A	O	9	19.1	O	B	16	30.2
B	B	3	6.3	O	AB	4	7.6
B	O	21	44.6	A	B	4	7.6
O	O	1	2.2	A	AB	8	15.0
AB	AB	—	—	B	A	8	15.0
AB	A	1	2.2	B	AB	3	5.7
AB	B	1	2.2				

TABLE IV

Showing Incidence of Foetal Cell Positive Cases in Normal Pregnancy and Labour

Total No. of cases	Positive at term		Positive after delivery	
	No.	%	No.	%
30	11	36.6	16	53.3

TABLE V

Showing Foetal Cell Positive Cases in Abnormal Pregnancy

Type of cases	Total No.	Positive at term		Positive after delivery	
		No.	%	No.	%
Antepartum haemorrhage	25	10	40	14	56
Toxaemia	16	7	43.7	9	56.3
Twins	8	3	37.5	5	62.5
Hydraminor	5	2	40	3	60.0
Anaemia	15	6	40	8	53.3

TABLE VI

Showing Evidence of Foetal Cell Positive Cases in Abnormal Labour

Type of cases	No.	Positive at term		Positive after delivery	
		No.	%	No.	%
Caesarean section	50	16	32	31	62
Forceps	40	13	33	23	57.5
Manual removal of placenta	10	2	20	7	70.0
Medical induction	5	2	40	3	60.0

TABLE VII

Showing Incidence of Foetal Cell Positive Cases in Compatible and Incompatible Cases

Type of cases	No.	Positive at term		Positive after delivery	
		No.	%	No.	%
Compatible	47	20	42.5	27	57.4
Incompatible	53	17	32.07	26	49.05

The above Table shows a higher incidence of foetal cell positive cases in the compatible mating, both at term and after delivery, 42.5% and 57.4% respectively as compared to incompatible mating where the incidence at term and after delivery is found to be 32.07% and 49.05% respectively.

Discussion

The present work is aimed to assess the effect of normal and abnormal pregnancy and labour on transplacental escape of foetal erythrocytes. The incidence of foetal cell positive cases was found to be 53.3% after delivery, whereas various workers have reported the incidence of positive postpartum cases varying from 17-56% as shown in Table XII.

The wide variation in the incidence of foetal cell positive cases noted by various authors apart from individual observation was dependent upon the concentration of alcohol used as fixative and also the type of buffer used. It was shown by Cohen *et al*, (1964) that the incidence of foetal cell positive cases was found to be 59.5% when 80% alcohol was used at the Ph of 3.3. On the other hand, the incidence of positive cases of the same group

TABLE VIII

Shows the Amount of Foetomaternal Haemorrhage in Normal Pregnancy, Labour and Abnormal Labour During Antepartum Period at Term

	Normal pregnancy and labour				Abnormal labour during antepartum at term							
	Ante-partum		Post partum		Caesa-rean section		Forceps		MRP		Medical induc-tion	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Mild 0-4 ml.	8	72.6	1	6.2	10	62.5	8	61.5	1	50	2	100
Moderate (0.4-2.5) ml.	2	18.2	10	62.5	5	31.2	5	38.4	1	50	—	—
Severe (2.5-10 ml.)	1	9.1	5	31.2	1	6.2	—	--	—	—	—	—

TABLE IX

Shows Amount of Foetomaternal Haemorrhage in Abnormal Labour in Postpartum Period

Amount of haemorrhage	Caesarean section		Forceps		M.R.P.		Medical induction	
	No.	%	No.	%	No.	%	No.	%
Mild	2	6.4	—	—	2	28.5	2	66.6
Moderate	16	51.6	13	56.8	4	57.1	1	33.3
Severe	13	42.0	10	43.1	1	14.3	—	—

TABLE X

Shows Transplacental Haemorrhage in Abnormal Pregnancy at Term

Amount of haemorrhage	A P H		Pre-eclampsia		Twins		Hydraminos		Anaemia	
	No.	%	No.	%	No.	%	No.	%	No.	%
Mild	7	70	7	100	2	66.6	2	66.6	5	83.3
Moderate	3	30	—	—	1	33.3	1	33.3	1	16.6
Severe	—	—	—	—	—	—	—	—	—	—

TABLE XI

Shows Amount of Transplacental Haemorrhage in Abnormal Pregnancy in Postpartum Period

Amount of haemorrhage	A.P.H.		Pre-eclampsia		Twins		Hydraminos		Anaemia	
	No.	%	No.	%	No.	%	No.	%	No.	%
Mild	—	—	—	—	1	20	—	—	1	16.6
Moderate	11	78.5	6	66.6	3	60	3	60	5	83.3
Severe	3	21.4	3	33.31	1	20	—	—	—	—

TABLE XII

Incidence of Positive Ante Partum and Post Partum Cases by Various Author

Author	Year	Antepartum foetal cell positive cases in percentage	Post partum foetal cell positive cases in percentage
Taylor and Kullman	1961	18	17
Keeman and Pearse	1963	7	19
Pilkington et al	1966	20	39
Ghosh & Agarwal	1970	44	56
Present series	1972	36.6	53.3

of cases dropped to 29.8% when 100% alcohol was used as fixative and the same buffer was taken at the Ph of 3.5.

Different scanning time was taken by different authors for studying the slides. Zipursky *et al,* (1959) considered those slides positive which showed one or more foetal cells in 2 minutes scanning whereas Finn *et al,* (1961) considered the slides to be negative which showed only one foetal cell in five minutes scanning. The same criterion was adopted in the present series and the incidence of positive cases was found to be 36.6% at term and 53.3% after delivery (vide Table IV). Same criterion was considered by Freese *et al,* (1963) who found the incidence of positive cases to be 43.8% and 15.6% in the antepartum and 56.9% and 31% in the postpartum in the same group of their cases.

For control, 25 peripheral films of infertile healthy females were studied and none showed foetal cells.

Zipursky *et al,* (1959), Pilkington *et al,* (1966) and Ghosh and Agarwal (1970) reported similar findings. On the other hand, Taylor and Kullman (1961) Zipursky *et al* (1963) and Cohen *et al,* (1964) found 2%, 4.3 and 3.03% foetal cells, respectively in their study of males and non-pregnant females. Clayton *et al* (1962) found small percentage of foetal cells in male and non-pregnant females.

In the present study the foetal cell score upto 4/1000 was detected at 36 weeks' of gestation, like Zipursky *et al,* (1963), Cohen *et al,* (1967), Ghosh and Agarwal (1970) and Parikh *et al,* (1971) who all noted a score of 4/1000 upto 36 weeks.

Correlation with Parity

Freese *et al,* 1963, Cohen *et al,* (1964) Parikh *et al,* (1971) did not find any correlation with parity, whereas in the present series we found a slightly higher incidence of foetal cell positive cases in multiparae (vide Table 1) Taylor and Kulman (1961) Zipursky *et al* (1962) noted a higher incidence in primiparae.

Table III shows that there is higher incidence of foetal cell positive cases in ABO compatible group as compared to ABO incompatible, but other workers did not find any correlation between the foetal cell appearance and the blood groups of the mothers and the babies (Freese *et al,* 1963; and Pilkington *et al,* 1966) (Knox and Walker; 1957; Finn *et al,* 1961; Reepmaker *et al,* 1962; Fraser and Raper 1962; Zipursky *et al,* 1963; Parikh *et al,* 1971) have, on the other hand, reported a lower incidence of foetal cell positive cases with ABO incompatible group. In the present study, there was no statistical significant difference found in both the groups of cases.

In the present study, 62.5% cases show-

ed a moderate amount of transplacental haemorrhage in postdelivery period (vide Table VIII). The same has also been reported by Klienhauser *et al,* (1957), and (1960) Queeman *et al,* (1962), Freese *et al,* (1963) and Cohen *et al,* (1964).

The incidence of foetal cells in abnormal pregnancy (vide Tables IV-V). Comparison of the incidence of foetal cell positive cases in abnormal pregnancy and labour with normal pregnancy and labour there is no significant difference. Similar observations were made by Zipursky *et al* (1963), Zilliacus (1964) and Ghosh and Agarwal (1970) but Knox *et al* (1961) found that toxaemia increases the risk of transplacental haemorrhage by 50%.

Foetal Cell Appearance in Abnormal Labour

Vide Table VI, we observed that in abnormal labour the incidence of foetal cell positive cases after labour is definitely higher 64.2%, than 53.3% seen in normal labour. Various workers like Taylor and Kullman (1961) Finn *et al,* (1961) Zipursky *et al,* (1963), Zilliacus (1964) Montague and Krevan (1966) also reported a high range between 12.2% to 70%.

In caesarean section the incidence of foetal cell positive cases is 62% (vide Table VI) as compared to 53.3% of normal labour. Montague and Kreven (1966), Ghosh and Agarwal (1970) reported 70% and 65% cases, respectively. Wimhofer *et al,* (1962), Finn *et al,* (1963), Pilkington *et al,* (1966) have also observed a higher incidence. On the other hand, Taylor and Kullman (1961) Zipursky *et al,* (1963), Cohen *et al,* (1964) and Parikh *et al,* (1971) did not find any increase.

There was no increase in the incidence of foetal cell positive cases with forceps application in the present series. The incidence was noted to be 57.5% after forceps as against 53.3% after normal labour. Taylor and Kullman (1961), Zipursky *et al,* (1963), Pilkington *et al,* (1966) and Ghosh and Agarwal (1970), did not observe any significant difference between the incidence of foetal cell positive cases after forceps delivery or after normal labour.

In the present study, in cases of medical induction, there was a higher incidence of 60%) foetal cell positive cases in the postpartum period and 40% in the antepartum, but as the number of cases was small, no definite conclusion can be drawn.

Summary

1. At term the incidence of foetal cell positive cases was 51 out of 150 cases (36.3%).

2. A sudden increase in the incidence of foetal cells was found in the postpartum period. Out of 150 cases 88 had foetal cells in the peripheral blood smear (53.3%).

3. In abnormal pregnancy, foetal cell positive cases at term were 40.5%.

4. In caesarean section, out of 50 cases 31 were positive (62%).

5. In forceps delivery, out of 40 cases 13 were foetal cell positive.

6. In cases where manual removal of the placenta was done, out of 10 cases 7 were foetal cell positive, i.e. 70% and after medical induction out of 5 cases 3 had foetal cells in the peripheral blood smears i.e. 60%.

7. Out of 100 cases where ABO grouping of the mother and baby was studied, 47% belonged to compatible group and 53% were incompatible.

8. The commonest incompatible combination was between the mother's group O and baby's group B in all the cases of the present study, i.e. 30.2%; the next

most frequent combination was between mother's group O and baby's group A.

9. The commonest compatible combination was between B group of the mother and O group of the baby, 44.6%.

10. The incidence of foetal cell appearance was higher in ABO compatible group.

11. In majority of the cases there was mild type of transplacental haemorrhage in the antepartum period at term, both in normal and abnormal groups.

12. Moderate type of transplacental haemorrhage was seen in the postpartum period of normal as well as the abnormal group.

13. In the control group of cases, 25 infertile females were taken as negative control and fresh cord smears from the newborn babies as positive controls. Negative controls showed no foetal cells, whereas positive control showed no adult cells.

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