ISO-IMMUNIZATION IN PREGNANCY

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The problem of iso-immunization in pregnancy was studied at Nowrosjee Wadia Maternity Hospital from 1963 to 1965. This problem can be handled successfully by systematic investigations, proper follow-up of the baby and timely treatment as described below.

Functioning of the Iso-Immunization Clinic

At the antenatal outdoor, blood samples were collected routinely from all antenatal cases, registered at the hospital, for blood group and Rh testing. In cases found to be Rh negative, the importance of regular check-up at the clinic was stressed. The patient's blood was collected for antibody titre and genotype. The blood was screened for atypical antibodies.

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Received for publication on 19-10-66.

Husband's blood group and genotype were done.

V.D.R.L. tests of both patient and husband were done.

Haemoglobin of the patient was recorded.

Previous children's blood group and genotype were done (this was subsequently discontinued as it proved impracticable).

The patient's past obstetric history was recorded and the history of past blood transfusions, if any, was obtained.

Antenatal clinical examinations were done and the complaints treated at this and subsequent visits.

Investigations for Rh Negative Women

Routine blood group and Rh were investigated antenatally for 24,289 women. Of these, 1,128 (4.6%) were Rh negative. Other workers have quoted as follows:

Mollison and Cutbush	17% in English population
Sacks et al	13.5% in American population
Sheth	3.7% in Bombay population
Hazel Cameons et al	5.2% in Bombay population

The ABO group distribution in Rh positive and negative cases had no significant difference.

The incidence of immunization among the Rh negative was 5.7%.

The genotypes of all these women were done and found to be as follows:

rr	.. ..	94.4%
R'r	.. ..	4.2%
R''r	.. ..	1.4%

The findings of Stratton and Renton have shown a higher incidence of homozygous fathers when the babies suffered from haemolytic disease. As Rh negative women with homozygous husbands have no chance of escaping Rh positive foetuses, one may expect that their chances of getting immunized are very much greater, but our findings (perhaps due to the small series studied) indicate that the incidence of homozygous or heterozygous husbands was the same for both immunized and non-immunized women. From non-immunized women, 50% of husbands were homozygous and 50% heterozygous. From immunized women, 45% of husbands were homozygous and 55% heterozygous.

Of the couples with Rh negative wives, 39% were ABO incompatible and 61% were ABO compatible. Of the wives who were imunised to D antigen, 16% had ABO incompatibility with their husbands, whereas amongst non-immunised women 33% had ABO incompatibility with their husbands. This may be explained by the reason that the incompatible cells from the foetus entering the maternal circulation are broken down by anti-A and anti-B antibodies and thus their survival is shortened in the maternal blood stream, resulting in decreased formation of the anti-D antibodies.

Factors causing Iso-Immunization

(1) The most important and yet totally avoidable factor causing immunization was found to be Rh positive blood transfusions to Rh negative women. All 13 women who reported previous blood transfusions, were found to be immunized. All these transfusions were administered in well-known hospitals in Bombay, during the last 5 years. The obstetric outcome following these transfusions has been tragic. Of 29 pregnancies in these 13 women, 4 ended in stillbirths, 11 in neonatal jaundice ending in death, 8 affected babies were saved by exchange transfusion and 5 babies were jaundiced but survived. The only baby that escaped jaundice was Rh negative. It is very necessary that blood banks store Rh negative blood and meticulously ensure that Rh negative females always receive Rh matched blood.

(2) When foetal cells pass out of the placental blood vessels and reach the maternal circulation, these antigens cause antibodies to be produced in the mother. Such foeto-maternal haemorrhages and subsequent immunization may be caused by abnormal obstetrics, such as intrauterine manipulations, curettage for abortion, attempts at expression prior to manual removal of placenta and prolonged labour. Of 49 patients who had undergone abnormal obstetrics in previous pregnancies 24 (50%) developed immunization while of 309 cases studied with previous normal

deliveries only 28 (9%) developed immunization.

Zipursky et al and Wimhofer et al have done foetal cell counts in maternal blood following normal and abnormal labour of various kinds. Their data confirm that operative procedures that disturb and damage the placental site cause transplacental haemorrhage.

With this logic in mind, obstetricians should handle Rh negative cases with delicateness during their labour. Modifications in technique to consciously avoid placental trauma are surely indicated in light of these findings.

(3) During the antenatal period a poor standard of nourishment or an anaemic condition may increase the possibility of retroplacental haemorrhages. Toxaemia and consequent increased blood pressure may also lead to retroplacental haemorrhage and leak of foetal cells into maternal circulation. Out of 52 immunized cases, 7 (13%) showed moderate oedema, 14 (27%) patients had blood pressure above 135/90, 3 patients had marked hydramnios, 4 patients were severely anaemic (haemoglobin below 50%). In contrast, of 306 non-immunized women, only 6 (2%) had oedema, 9 (3%) had hypertension, 2 had hydramnios and 4 were severely anaemic.

Pathological changes leading to damage of foeto-maternal cell barrier in placenta are important factors facilitating the passage of foetal cells into maternal circulation, specially with the onset of uterine muscular activity. Any injury on the abdominal wall leading to a slight separation of the placenta can also cause entry of foetal cells into the maternal circulation. One of the patients in our series (Mrs. K) had no antibodies at 32 weeks. She reported an injury on the abdominal wall by a large ball which caused mild vaginal bleeding. One week later her antibody titre had risen to 1 : 64.

History of Previous Babies and Present Foetal Outcome

Our findings may be summarised as follows: (Ref. Table 1).

It is now well-known that the factors which led to poor foetal outcome in past pregnancies would most often lead to similar or poorer outcome in subsequent pregnancies. Exceptions would be cases where the outcome of past pregnancies was marred by additional complications such as toxaemia, prolonged labour or other obstetric complications. Also exceptions would be cases when the baby happens to be Rh negative.

Work is being done in other countries to improve the outcome of pregnancies by preventing formation or decreasing the antibodies in the mother, by retarding the passage of antibodies to the foetus and by reducing the destructive effect of antibodies on foetal cells. Intrauterine transfusions to prolong the life of the foetus to about 36 weeks are also being done.

Maternal Antibody Titre and Outcome of Pregnancy

Our findings may be summarised as follows: (Ref. Table 2).

The significant relationship between the maternal antibody titre and the foetal outcome has been well established. Our data here confirm

TABLE 1

PAST FOETAL OUTCOME		PRESENT FOETAL OUTCOME					
Past outcome	No. of women	Unaffected babies	Babies affected, no exchange required	Babies affected, exchange done	Babies affected and died	Full term stillbirths	Premature stillbirths
Normal healthy babies	16	7	8	—	1	—	—
One or more babies jaundiced but recovered	12	—	7	3	1	1	—
One or more babies jaundiced and permanently affected or died	21	1*	1	14	3	—	2
One or more babies full-term still-born	4	—	—	3	—	—	1
One or more babies prematurely still-born	1	—	—	1	—	—	—
Total	54	8	16	21	5	1	3

* Rh negative.

TABLE 2

MATERNAL ANTI-BODY TITRE		PRESENT FOETAL OUTCOME					
Titre	No. of women	Unaffected babies	Babies affected, no exchange required	Babies affected, exchange done	Babies affected and died	Full-term stillbirths	Premature stillbirths
1 : 16 or less	8	7	1	—	—	—	—
1 : 32	10	4	5	1	—	—	—
1 : 64	8	—	4	3	1	—	—
1 : 128	16	—	1	12	3	—	—
1 : 256	5	—	1	3	—	—	1
1 : 512	3	—	—	2	—	—	1
1 : 1024 or more	—	—	—	—	—	—	—
Total	50	11	12	21	4	—	2

that the higher the antibody titre, the poorer the prognosis for the foetus. Antibody titre done repeatedly reveals cases of increasing titre and such increasing titre indicates worsening prognosis for the foetus. Although the titre is a significant investigation it is not a sufficiently reliable guide for judging cases for premature induction of labour. As can be seen from chart above, there are instances when the titre and the foetal outcome are not consistently related.

Premature induction

By premature induction, the incidence of stillbirths (due to haemolytic diseases) can be decreased. At the same time prematurity carries with it other risks for the baby due to greater susceptibility to infections etc. For deciding on premature induction of labour, besides the criteria of past history and maternal antibody titre, spectrophotometric data of amniotic fluid are useful. Thirty-five amniocenteses were done, of which 31 were successful. To help us interpret the spectrophotometric data, the graphs developed by Liley were used. Although this experience with amniotic fluid investigations is too limited to draw any conclusions, this study undoubtedly helped us to judge the foetal prognosis more accurately.

Cord blood investigations

The maternal antibody titre and cord blood findings have significant relationship (Ref. Table 3).

Cord blood findings are indicative of the babies' condition at birth, as seen in the following chart (Ref. Table 4).

TABLE 3

TITRE		CORD BLOOD FINDINGS					
		Bilirubin greater than 3.5 mg. per cent		Bilirubin less than 3.5 mg. per cent		Bilirubin greater than 3.5 mg. per cent	Haemoglobin less than 12 gm.%
Titre	No. of women	Haemoglobin less than 12 gm.%	Haemoglobin more than 12 gm.%	Haemoglobin less than 12 gm.%	Haemoglobin more than 12 gm.%		
Col. No. 1	2	3	4	5	6	Total of 3 and 4	Total of 3 and 5
1 : 16 or less	7	—	1	1	5	1	1
1 : 32 and 1 : 64	16	7	3	1	5	10	8
1 : 128 or more	22	17	2	—	3	19	17

TABLE 4

CORD BLOOD FINDINGS		CONDITION OF BABIES					
Bilirubin	Haemoglobin	No. of babies	Unaffected babies	Babies affected, no exchange required	Babies affected, exchange done	Babies affected and died	*Stillbirths
Greater than 3.5 mg. per cent	Less than 12 gm.%	24	1	1	17	4	1
	Greater than 12 gm.%	4	—	2	2	—	—
Less than 3.5 mg. per cent	Less than 12 gm.%	1	—	—	1	—	—
	Greater than 12 gm.%	14	6	7	1	—	—

* For all the stillbirths, the cord blood haemoglobin was less than 8 gm.% and bilirubin more than 4 mg. per cent.

Cord blood haemoglobin readings are sometimes lower than baby's true haemoglobin because of squeezing the cord too late for collection or because of clots in the oxalate bulb. Haemoglobin readings by heel prick (since it is capillary blood) are higher than baby's true haemoglobin. Of babies delivered at N. W. Maternity Hospital the average cord blood haemoglobin was 13 gms.% and the average heel prick haemoglobin 15.2 gms.%. A re-check of haemoglobin by heel prick is useful to detect errors in cord blood collection.

In the above charts, 12 gms.% has been arbitrarily set as the critical point to differentiate the good haemoglobin from the poor. In other countries, 14 gms.% is usually considered the dividing point.

Direct Coomb's test

A positive result of a direct Coomb's test definitely indicates the sensitisation of foetal cells due to maternal antibodies. Babies whose blood show DCT positive need close observation.

All the 25 cases that showed DCT

Result of Coomb's test	Babies' condition		
	Baby not affected	Baby affected moderate jaundice	Baby with severe jaundice needing exchange transfusion
DCT positive ..	—	13	25
DCT negative ..	7	3	—

strongly positive needed exchange transfusion. All the babies of non-immunized mothers showed DCT negative.

Bilirubin estimation

The most important follow-up investigation after birth, to judge the necessity and timing for treatment, is the indirect bilirubin level. At the present time micromethods of bilirubin estimation are well established and as only 0.3 ml. of blood is required, from a heel-prick, this estimation can be done more frequently, even 6-hourly, if need be.

The method used by us follows the method of "Micro-estimation of Indirect Bilirubin in Plasma" by C. S. Shaw and J. C. Thompson of Group Laboratory, Lewisham Hospital, London (Nov. 1957).

Bilirubin levels were noted against a time base on a graph which then gave a ready picture of the rate of rise of bilirubin from which the need for exchange transfusion was assessed. An indirect bilirubin level of 20 mg. per cent was considered the critical level beyond which the infant may develop kernicterus. (See figure)

Serial haemoglobin estimations should be done to detect anaemia in good time. Anaemic babies suffer the risk of death from cardiac strain.

Exchange transfusion

Babies developing unsafe levels of bilirubin or severe anaemia were treated by exchange transfusion. Exchange transfusions serve to remove the anti-D antibodies, remove the Rh positive cells, remove the bilirubin from the plasma and improve the haemoglobin level. Twenty-one exchange transfusions were done in this series, of which 19 were successful as the babies survived, while 2 died during the process of transfusion or soon after the transfusion. The babies whose exchange was timed early fared definitely better than babies whose exchange was done rather late. Early exchange transfusion removes the principal source of pigment—the incompatible circulating red cells—and helps to prevent high concentrations of bilirubin accumulating in the body. With late exchange transfusion lowering of a high serum bilirubin level is difficult because the interstitial fluid is saturated with pigment while only that in the circulation is readily removed by exchange transfusion.

The best results of exchange transfusion are obtained if the donor blood is compatible with the mother's

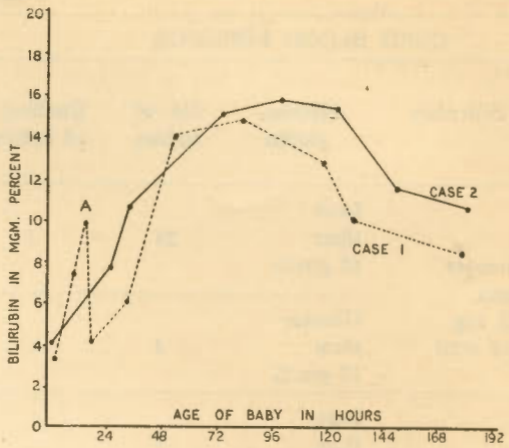

Fig. 1

In case 1, the rate of rise of bilirubin was fast enough to require an exchange transfusion at point 'A'; while case II had a gradual rise of bilirubin, and was followed up until the bilirubin level fell and did not need exchange transfusion

serum, if it is fresh so that the red cells transfused to the baby have maximum life and if the blood has a good level of haemoglobin.

Transfused babies were watched regularly until their serum bilirubin was found to be steadily decreasing. Haemoglobin levels were followed up every 2 weeks for 2 months to detect cases of late anaemia; 66 per cent of the babies remained anaemic for about 3 months with haemoglobin less than 70%. At a later stage, they were normal in health.

Five of the babies to whom exchange transfusions were given developed umbilical hernia.

Pathology of placenta

The pathology of 80 placentae was studied. In the placentae of immunized women, we observed the changes studied by Robert Burstein, Taylor, Crawford and others, namely hypertrophy and hyperplasia with partial persistence of cytotrophoblast. We also observed hydropic degeneration of villi with proportionate increase in syncitial knots. The average placental weight of immunized patients was 14% higher than the average of non-immunized patients.

Other causes of neonatal jaundice referred to us

During the course of 2 years, 9 babies with ABO incompatibility were found to have developed jaundice of a marked nature. Of these 4 needed exchange transfusion.

We had one typical case of glucose 6 phosphate dehydrogenase deficiency which was treated with exchange transfusion.

Eleven cases of neonatal jaundice due to prematurity were referred to us. Of these 4 survived and the rest died. Avoidance of death due to kernicterus is possible in such cases by careful exchange transfusion.

Summary

4.6 per cent of all antenatal patients were Rh negative. Of these 5.7% were immunized.

The principle factors causing isoimmunization are incompatible blood transfusion and damage to foetomaternal cell barrier in the placenta.

The outcome of pregnancy has definite relationship to the outcome of past pregnancies and maternal antibody titre.

The study of amniotic fluid would help to judge cases which deserve premature induction to avoid stillbirth.

Cord haemoglobin of 12 gms.% or less and cord bilirubin of 3.5 mg.% or more indicate the need for close observation of the baby.

Serial estimation of bilirubin after birth is most important to judge the necessity and timing of exchange transfusion.

Acknowledgements

This study was sponsored by Sir Mangaldas Mehta Research Grant of Bombay University.

We are grateful to Dr. B. N. Purandare, Hon. Principal Medical Officer, Nowrosjee Wadia Maternity Hospital and Dr. Jer Master for their guidance and help.

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