FOLLOW-UP STUDIES IN Rh(D) NEGATIVE WOMEN RECEIVING AND NOT RECEIVING ANTI-D IMMUNOGLOBULIN INJECTION

by

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Introduction

Entry of Rh(D) positive cells in the maternal circulation is the prerequisite for Rh(D) immunization. Besides full term delivery, spontaneous or induced abortion may also cause significant foeto-maternal haemorrhage and subsequent immunization. Since the introduction of anti-D immunoglobulin (anti-DIg) injection in the post partum period as a prophylaxis, the incidence of Rh(D) immunization has significantly diminished in the West (Freda *et al*, 1976). However, there are no reports from India about the impact of this therapy in reducing the risk of maternal isoimmunization. Majority of the published reports on the incidence of Rs(D) immunization are based on Rh(D) antibody testing performed during the antenatal period. However, it is essential to know the high risk factors. This is possible only by follow up of the individual woman.

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Present study gives the incidence of Rh(D) immunization after induced or spontaneous abortion, still birth and full term Rh(D) positive delivery. Attempt is also made to evaluate the role of anti-DIg and various obstetric factors.

Material and Methods

All the pregnant women attending antenatal clinic of Nowrosjee Wadia Maternity Hospital are routinely investigated by Institute of Immunohaematology (Blood Group Reference Centre) for ABO and Rh(D) group. Rh(D) negative women are periodically followed up for Rh(D) antibodies and cord blood samples of their infants are tested for ABO and Rh(D) group and direct antiglobulin test. Within 72 hours after birth of Rh(D) positive child, non-immunized women are advised appropriate dose of intramuscular anti-DIg injection. Generally 250 μg anti-DIg is administered after normal and 350 μg after complicated delivery.

Rh(D) negative women undergoing spontaneous or induced abortion are also routinely tested for Rh(D) antibodies and nonimmunised women are given 100 μg anti-DIg after abortion upto 10 weeks and 250 μg after abortion of more than 10 weeks.

During last 4 years, 2500 Rh(D) negative women were investigated. Follow Up after the obstetric event was possible in 1124 cases. Nine hundred and seven women were tested before their subsequent pregnancy, 68 during their subsequent pregnancy and 149 were investigated within 6 months after delivery as well as during subsequent pregnancy. Out of 1124 women, 512 were protected by anti-DIg while 612 patients could not afford this therapy (Non-protected). Three different brands of anti-Dig were commercially available and procured by the patients.

ABO and Rh(D) group, direct antiglobulin test and Rh(D) antibody titre studies were done using locally prepared reagents and methods described by Bhatia (1972).

Results

Table I gives the incidence of Rh(D) immunization among Rh(D) negative women, after spontaneous or induced abortion, still-birth and full term Rh(D) positive delivery. Data was divided on the basis of women receiving anti-DIg (protected) and those not receiving it (non-protected). Comparison between the protected and non-protected groups show the significantly high incidence (1:20) of Rh immunisation among non-protected as compared to 1:57 in the protected group. Among non-protected group the rate of Rh(D) immunization was equally high in patients undergoing induced abortion as in full term deliveries. Among the spontaneous abortion series, risk was very low when abortion was less than 20 weeks. Among the protected women the overall failure rate was 1:57 with 1:76 in full term series.

Table II gives the parity of the patients at the time of follow up. In the non-protected group highest risk of immunization was seen in second delivery and in nearly 70 per cent of all cases, immunization occurred after first or second delivery. Four out of 240 (1:60) protected primigravida developed antibodies after delivery.

Incidence of Rh(D) immunization on the basis of type of liveborn delivery is given in Table III. In the protected group, majority of the failures have occurred in the women having instrumental deliveries like forceps, caesarean sections or other complication such as breech presentation or manual removal of placenta (4/96). Non-protected series also shows

TABLE I

Incidence of Rh(D) Immunization on the Basis of Outcome of the Pregnancy

Series	Induced Abortion	Spontaneous Abortion upto 20 wks.	Spontaneous Abortion >20k Wks.	Still-birth	Liveborn delivery	Total
Protected	11(2)	23(—)	7(1)	16(-)	455(6) 1:76	512(9) 1:57
Non-Protected	114(6) 1:19	64(2) 1:32	7(1)	10(1)	417(21) 1:20	612(31) 1:20
Total	125(8) 1:16	87(2) 1:44	14(2)	26(1)	872(27) 1:32	1124(40) 1:28

Figures in the ( ) give number of patients developing Rh(D) antibodies.

TABLE II

Parity-wise Incidence of Rh(D) Immunization among the Follow-up Patients

Series	Parity				Total
	I	II	III	>III	
Protected	240(4) 1:60	160(2) 1:80	73(2) 1:37	39(1) 1:39	512(9) 1:57
Non-Protected	169(7) 1:24	179(15) 1:12	145(5) 1:29	119(4) 1:30	612(31) 1:20

Figures in the ( ) give no. of patients developing antibodies.

TABLE III

Incidence of Immunization on the Basis of Type of Liveborn Delivery

Series	Full term normal	Premature normal	Forceps or Vacuum	Caesarean Section	Other Compli-cations	Not known	Total
Protected	353(2) 1:177	24(1)	30(1)	(30(1)	12(1)	6(—)	455(6) 1:76
Non-Protected	259(11) 1:24	31(1)	14(1)	13	32(4)	68(4)	417(21) 1:20

Figures in the ( ) give nos. of patients developing Rh(D) antibodies.

the high risk of immunization after complicated deliveries (6/90) compared to full term normal delivery (11/259).

Data was also analysed on the basis of sex of the child. No correlation was observed between the sex of the child and the maternal immunization.

Table IV gives the period after which Rh(D) negative women were followed up. In the non-protected group nearly 50 per cent women produced antibodies within 8 months after the obstetric event. Immunization was more frequent in the women when tested during their subsequent pregnancy. Three women who had no evidence of immunization up to

TABLE IV

Correlation Between Period of Follow-up and Rh(D) Immunization

Series	Period of Follow up		During Subseqeunt Pregnancy
	within 2 months	2 to 12 months	
Protected	30(—)	425(6) 1:71	54(3) 1:18
Non-Protected	109(5) 1:22	492(10) 1:49	163(16) 1:10

Figures in the ( ) indicate no. of women developing antibodies.

NOTE: 149 women were tested twice, within 6 months after delivery as well as during their subsequent pregnancy.

6 months post delivery, did develop Rh(D) antibodies during the second trimester of the subsequent pregnancy. Similar pattern with greater risk of immunization during subsequent pregnancy was observed in the protected group.

Role of ABO incompatible pregnancy protecting Rh(D) immunization is evaluated in Table V. Out of 28 women producing Rh(D) antibodies, only 3 (7.5 per cent) were having ABO incompatible pregnancy. In the non-protected group incidence of immunization with ABO incompatible pregnancy was 1:107 and with compatible pregnancy 1:16.

TABLE V

Influence of ABO Compatibility on Rh(D) Immunization

Series	Mother—Child Combinations								Total
	ABO Incompatible				ABO Compatible				
	O-A	O-B	Other	Total	0-0	$A<\frac{A}{O}$	$B<\frac{B}{O}$	$AB<\frac{AB}{AB}A$	
Protected	33(—)	38(1)	66(1)	137(2) 1:69	79(2)	95(—)	96(2)	48(—)	318(4) 1:80
Non-Protected	32(—)	36(1)	39(—)	107(1) 1:107	81(6)	89(5)	103(7)	37(3)	310(21) 1:15

Figures in the ( ) indicate no. of women developing Rh(D) antibodies.

Rh(D) antibody titre levels of the patients developing Rh(D) antibodies are given below:

Series	Rh(D) titre		
	upto 1:8	1:16-1:64	>1:64
Protected	5	2	2
Non-protected	22	7	2

The distribution of patients with low and high titre was similar in both the groups.

Discussion

Since the realization of the importance of Rh factor as a cause for haemolytic disease of the newborn the incidence of immunization has been reported by several workers (Woodrow, 1970). Most of these studies are based on the detection of antibodies during antenatal check up. In our earlier studies we have reported the frequency of Rh(D) immunization as 1:29 among Rh(D) negative women attending antenatal clinic of Nowrosjee Wadia Maternity Hospital (Bhatia and Gupte, 1977). The use of anti-D immunoglobulin injection to prevent Rh(D) immunization is the major landmark in the eradication of Rh(D) HDN. Because of the extensive use of this therapy, incidence of Rh(D) immunization has been considerably reduced in west (Jennings, 1976). However, no reports are available from India about the impact of this prophylactic therapy on reducing the incidence of Rh immunization. Present study gives the comparative incidence of Rh(D) immunization after obstetric events like spontaneous or induced abortion, still birth and full term Rh(D) positive delivery. Overall risk of immunization in the present series is 1.8 per cent in the women protected by anti-DIg and 6.5 per cent in those not receiving it. Thus nearly 75 per cent women who otherwise would get immunized were protected by anti-DIg. Among the patients not receiv-

ing anti-DIg the incidence of immunization as 1:20 in full term deliveries is comparable to the earlier reports from U.K. (Walker, 1958). The incidence of immunization is also similar (1:19) in women undergoing medical termination. The similar incidence was also reported by Goldman and Eckerling (1972). The risk of immunization was reported low (1:32) in the patients with spontaneous abortion (Queenan *et al,* 1971). The similar lower risk was observed in our studies for those having early abortions. The risk is comparatively higher for those women having abortion after 20 weeks of gestation.

In our earlier study we observed (Mehta *et al,* 1976) that majority of the women who get immunized develop the antibodies after the first or second delivery. This observation, which has the support from other reports, (Woodrow, 1970) is also noticed in the present series of the nonprotected women. However, among the protected women the immunization is more often among multiparous women, suggesting thereby that they probably had the primary response in their previous pregnancy when they had not received anti-DIg.

Since the higher incidence as well as larger quantum of transplacental haemorrhage (TPH) is more likely to be associated with complicated deliveries, the incidence of Rh immunization is also increased after such deliveries (see Table III). Even in the protected group, 4 out of 6 failures were associated with complicated deliveries. It is therefore advisable that Rh(D) negative women with complicated deliveries should be identified and protected with higher dose of anti-D Ig, if necessary, after being investigated for TPH.

Even though ABO incompatible pregnancy protects Rh immunization (Levine, 1943) the present report supports this concept among nonprotected women. However, 2 of the protected women were immunized inspite of their ABO incompatible pregnancy. Both of them had complicated deliveries and possibly had massive TPH, volume which could not be eliminated with their low titre anti-A and anti-B.

In the large series reported by Clarke and McConnell (1972) the failure rate in the women with ABO compatible pregnancy and protected with anti-DIg was reported as 0.4 per cent within 6 months after the delivery and 1.0 per cent by the end of the second Rh(D) positive pregnancy. In our series of 512 women receiving anti-DIg the failure rate was 1.5 per cent up to 12 months and 1.7 per cent by the end of subsequent pregnancy. This higher rate of failure in our series could be due to several brands and batches of anti-DIg during the period of 4 years and some of which may be of questionable quality. It is also likely that some of them, specially multiparous women, probably had primary response in their earlier pregnancy or some of them had massive TPH and needed larger dose of anti-DIg. It is also possible that prophylactic anti-DIg was given too late in some of the cases. Four of our failure cases were primi gravida. The possible TPH during third trimester is probably the cause of primary response.

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