SIGNIFICANCE OF MATERNAL ANTI-D LEVEL IN THE MANAGEMENT OF RH HEMOLYTIC DISEASE OF THE NEWBORN

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SUMMARY

To determine the cut-off level of anti-D concentration for invasive interventions, 90 Rh immunized mothers were investigated. The results were correlated with severity of Rh HDN, judged by the outcome of pregnancy, cord haemoglobin (Hb), postnatal indirect serum bilirubin (ISB) and requirement of exchange transfusion (ET). Repeated estimations in 20 women suggested that by 28th week of gestation the peak level is attained. Good correlation was observed between Rh titre and anti-D concentration ($r = 0.79$ $P<0.01$). Both the parameters showed a direct correlation with severity of Rh HDN but there was a wide scatter of values. Stillbirth was associated with >4 ug/ml anti-D level. When maternal anti-D concentration was <4 ug/ml, none of the infants had cord Hb below 10g/dl and peak or pre ET ISB was <18 mg/dl. Hence invasive interventions like amniocentesis and cordocentesis are necessary when anti-D concentration is >4.0 ug/ml or 20 IU/ml.

INTRODUCTION

The antenatal assessment of severity of Rhesus haemolytic disease of the newborn (Rh HDN) is generally based on past obstetric history, Rh titre, amniotic fluid analysis and ultrasonography (USG) examination. Amniocentesis involves certain risks therefore it is important to ensure its necessacity. Morely et al (1977) have analysed the levels of anti-D concentration recommended by several workers, to carry out amniocentesis. Vast variation from 0.5 to 5.3 ug/ml, is observed in these levels. Therefore we decided to establish our own value of

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anti-D concentration.

With the advent of USG it is possible to collect fetal blood sample by cordocentesis to diagnose fetal anaemia. According to Nicolaides and Rodeck (1992), the decision of cordocentesis should be based on the maternal anti-D level.

In the present communication, attempt is made to correlate anti-D concentration with severity of Rh HDN to decide the cut-off value for amniocentesis and cordocentesis. Simultaneously we have correlated parity and Rh titre with anti-D concentration.

MATERIAL & METHODS

The study includes 90 Rh immunized mothers referred during 1988 to 1992. There parity, past obstetric history, particularly of still birth/hydrops fetalis, neonatal jaundice and anaemia was recorded by personal interview.

The Rh antibody titre was carried out periodically. Maternal anti-D concentration was measured once at 32 to 34 weeks gestation for all cases while 20 women were tested at two weeks interval from 20 weeks till delivery.

Standard methods were employed for ABO and Rh(D) grouping, direct antiglobulin test, Rh titre (indirect antiglobulin technique), Hb and serum bilirubin estimations. The immuno radiometric assay reported by Gupte et al (1994) was used for anti-D quantitation.

Mean, standard deviation (SD), coefficient of variation (CV) correlation coefficient (r), student's 't' test ANOVA test (F) and chisquare tests were applied to evaluate scientific data, using standard formulae.

RESULTS

Anti-D concentration of each serum was measured twice and if the difference between the duplicate results exceeded six times the SD of the mean, the test was repeated again. Generally the CV was between 5 to 10%.

The repeated estimations of anti-D cocentration in 20 women suggested that the concentration remained almost the same in 11 cases. Nine cases showed a gradual increase of about one to three micrograms,

TABLE I
CORRELATION OF MEAN ANTI-D CONCENTRATION WITH ORDER OF PREGNANCY

Gravida	I	II	III	IV	>V	Total
n	5	14	15	24	25	83
Mean anti-D concentration ug/ml	4.7	5.3	6.2	6.8	7.7	6.5
+ SD	3.4	3.0	2.4	2.6	2.3	2.7

TABLE II
CORRELATION BETWEEN Rh ANTIBODY TITRE AND ANTI-D LEVEL, WITH RESPECT TO SEVERITY OF Rh-HDN

Anti-D concentration ug/ml	IU/ml	No Exchange Transfusion Rh titre			Exchange Transfusion Rh titre			Still birth Rh titre		
		<32	64-128	>256	<32	64-128	>256	<32	64-128	>256
<4	<20 16	13	1	-	2	-	-	-	-	-
4-8	21-40 39	1	4	-	8(1)	19	2	1	2	2
>8	>40 28	-	-	2	1(1)	8	6(1)	-	2	9
Mean + SD Anti-D level		4.2 ± 2.1			6.7 ± 2.2			8.8 ± 2.3		
Range		1.9 to 9.5			2.6 - 11.5			4.2 - 11.5		
Mean + SD Log 2 Rh titre		4.6 ± 2.0			6.2 ± 1.4			7.9 ± 1.4		
Range		1:2 to 1:256			1:16 to 1:512			1:32 to 1:1024		

Figures in the parenthesis indicate number of deaths within a week after ET.

TABLE III
CORRELATION OF CORD BLOOD Hb AND POSTNATAL SERUM BILIRUBIN WITH MATERNAL ANTI-D CONCENTRATION.

Anti-D ug/ml	Cord blood Hb g/dl <10	10-14	>14	No Exchange transfusion Peak ISB mg/dl ≤18	>18	Exchange Transfusion PreET ISB mg/dl ≤18	>18
<4	-	5	11	14	-	2	-
4-8	11	15	3	5	1	13	19
>8	6	6	2	-	1	5	7
Total	17	26	16	19	2	20	26

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until 28 weeks of gestation, later on the level remained constant.

The comparison of Rh antibody titre and anti-D concentration results showed the correlation coefficient (r) to be 0.79, standard error 0.08 and P<0.01.

Table I shows the gradual increase in anti-D concentration with increase in gravida. The ANOVA test showed significant variation in mean values (F = 89.2, P<0.005).

Out of 90 mothers investigated 7 delivered Rh(D) negative infants while 83 had Rh(D) positive infants having positive antiglobulin test. Hence only 83 cases were analysed. Sixteen (19.3%) mothers had Still Birth (SB). Out of 67 liveborn infants 46 were treated with one to four ET.

Table II shows a gradual rise in anti-D titre and concentration with increase in severity. The comparison by "t" test, between the mean anti-D cocentration and Log 2 Rh titre did not show significant difference (P>0.5). Majority of untreated cases had Rh titre upto 1:32 and anti-D concentration below 4 ug or 20 IU/ml. All SBs were associated with >4 ug/ml anti-D concentration. In SB category 11 (68.7%) out of 16 mothers had Rh titre >1:256 and anti-D cocentration >8 ug. One SB occured when Rh titre was 1:32. ANOVA test applied to compare mean anti-D concentration in different groups of severity revealed significant difference (F=60.7, P=<0.005).

Cord blood Hb was measured for 59 liveborn infants only. TABLE III shows that the severe anaemia (Hb <10 g/dl) and hyperbilirubinemia (ISB>18 mg/dl) were associated with >4 ug/ml anti-D concentration. The chisquare test showed the statistically significant (P<0.001) inverse relationship between cord blood Hb and anti-D concentration.

DISCUSSION

Mollison (1993) has suggested several parameters for prenatal assessment of severity of Rh HDN. The amniotic fluid analysis has been considered important as it can predict severity before accumulation of ascitic fluid. Nicolaides and Rodeck (1992) have recommended fetal blood sampling for diagnosis of anaemia if maternal anti-D concentrations is >15 IU or 3 ug/ml. As amniocentesis and cordocentesis involve certain risks, it is essential to ensure that these procedures are really necessary. Morley et al (1977) carried out discriminant analysis of levels reported by different workers and suggested that the amniocentesis should be performed when anti-D level is >0.8 ug or 4.0 IU/ml. Since Rh immunized pregnant women generally have levels beyond this value (minimum level observed in this study is 1.9 ug/ml), one will have to carry out amniocentesis in every case.

Generally Rh immunised mothers reach their highest potential of anti-D production very early during pregnancy and there is not much difference in the levels at 28th and 32nd weeks gestations. Our follow up study on 20 women confirmed this fact.

As expected, parity showed a significant association with anti-D concentration thus emphasizing the importance of past obstetric history. Gupte and Bhatia (1985) observed excellent correlation of Rh titre with severity in the first affected pregnancy, but in the subsequently affected pregnancies the correlation was poor.

Inspite being a semiquantitative visual technique, Rh titre results significantly

correlated with anti-D quantitation. (r=0.79, P<0.01). The mean values of these two tests in different categories of severity were also comparable by 't'test. However the predictive value of Rh titre appears to be slightly poorer than anti-D quantitation, as low titre was frequently associated with severe disease. In ET group 11 (23.9%) cases had titre <1.32 but low anti-D cocentration (<4 ug/ml) was observed only in two cases. Similarly one still-birth case was associated with 1:32 Rh antibody titre. The broad range of Rh titre and anti-D concentration was observed in different categories of severity. Nicolaides and Rodeck (1992) have also reported a wide scatter of values. The high degree of correlation is not expected from these parameters as they are not functional tests. Garner et al (1992) have emphasized the limitations of serological tests, particularly in selected cases of severe Rh-HDN. Several factors like Rh genotype of fetus, rate of antibody transfer across placenta, IgG subtupe, lytic ability of anti-D, number and function of effector cells, erythropoietic activity in response to red cell destruction etc influence the severity.

On the basis of outcome of pregnancy, cord Hb postnatal serum bilirubin and requirement of exchange transfusion it is concluded that the severe disease occurs when anti-D concentration is >4 ug or 20 IU/ml. Hence we recommend amniocentesis and cordocentesis beyond this level.

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