FACTORS INFLUENCING THE SEROPATHOLOGY AND SEVERITY OF ABO—HDN

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Abstract

Serological criteria for the diagnosis of ABO-HDN are presented. Incidence of ABO-HDN among liveborn infants was 1:171 and 1:81 respectively at Wadia and Mahim maternity hospitals. Analysis of 3548 cases of neonatal jaundice suggested that though first born, male and the infants with complicated deliveries were more susceptible to jaundice, incidence of these factors was not significantly increased in 477 cases of ABO-HDN. Incidence of ABO-HDN was comparable in LBW and FT infants. However the disease appeared more severe among LBW infants. ABO incompatibility could also increase the severity of disease in the jaundice infants with G6PD deficiency and associated maternal diseases. Risk of ABO-HDN was increased twice when mothers received tetanus toxoid injections during pregnancy.

Introduction

The need for better understanding of neonatal jaundice was realised when clinical and pathological observations suggested that almost any severe neonatal jaundice regardless of aetiology may lead to "Kernicterus". Since various obstetric, pediatric, environmental and genetic factors influence the risk of neonatal jaundice (Crosse 1966; Arias *et al* 1964; Gupte and Bhatia 1973), incidence of neonatal jaundice changes from country to country. Our earlier studies (Gupte and Bhatia 1973) suggested that the male, first born and low birth weight infants were more susceptible to jaundice. Complicated deliveries also played a significant role in increasing the risk (Gupte and Bhatia 1973).

While the cases of Rh(D)HDN are easily identified, diagnosis of ABO-HDN is difficult to make. Main problems are the occurrence of IgG anti-A and anti-B in normal individuals and negative direct Coomb's test on infants red cells, ABO-HDN may also be missed as physiological jaundice as it is often mild and slow to develop. Associated haematological alterations like increased osmotic fragility and spherocytosis, though a help in diagnosis, may not be associated with every case of ABO-HDN (Gupte and Bhatia 1973).

Present paper deals with the criteria for the diagnosis of ABO-HDN and the correlation of the levels of antibody titre with haematological alterations. Data is also presented for various factors which

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Accepted for publication 25-3-80.

influence the incidence and severity of the ABO-HDN disease.

Results and Discussion

Criteria for Diagnosis of ABO-HDN

Based on the findings in normal women (Gupte and Bhatia 1972) IgG anti-A or anti-B levels greater than 1:32 in O groups and greater than 1:16 in A or B group mothers were considered significant to identify cases of ABO--Haemolytic disease (ABO-HDN). Following criteria were used in assessing haematological changes in ABO-HDN.

1.	Moderate to severe anaemia	Hb less han 14 gm%.
2.	Severe Hyper-bilirubinemia	Serum bilirubin more than 16 mg%.
3.	Spherocytosis	Occasional spherocytes in blood smear.
4.	Incrased Osmotic fragility	More than 8% haemolysis in 0.55% buffered saline.
5.	Reticulocytosis	More than 5%.
6.	Normoblastosis	Occasional normoblasts in blood smear.

Incidence of ABO-HDN

Incidence of ABO-HDN among liveborn infants was calculated using random data collected at Wadia Maternity Hospital during 1969-1970. Based on serological criteria, Table I gives an overall incidence of ABO-HDN as 1:171 and in O-A and O-B combinations as 1:17 and 1:52 respectively (Gupte and Bhatia 1973).

Incidence of ABO-HDN may differ from hospital to hospital. Random series collected at Mahim Maternity Hospital showed overall incidence of ABO-HDN among liveborn infants as 1:81, while in O-A and O-B combinations it was 1:11 and 1:22 respectively (Bhatia and Gupte 1977). As seen in Table II the incidence of ABO-HDN was significantly increased at Mahim Hospital. Type of the patients attending Mahim Hospital (more muslims) and the routine use of tetanus toxiod during pregnancy may probably explain this increased incidence at Mahim Hospital.

During last ten years, 3548 infants suffering from neonatal jaundice were investigated at B.G.R.C. units at Wadia Maternity Hospital. One thousand, three hundred and eighty-nine (39.1%) infants were ABO incompatible with their mothers (see Table III). This incidence is significantly higher compared to the 28% ABO incompatibility in normal

TABLE I

Incidence of ABO-HDN at Wadia Hospital

(Data recalculated from Gupte and Bhatia 1973)

	Mother-Child Combinations			Total No.
	O-A	O-B	A $\langle$ B, AB or B $\langle$ A, AB	
No. of deliveries	1044*	1044*	1934*	15235**
No. of Jaundiced infants	147 (1:7)	86 (1:12)	122 (1:16)	1000*** (6.5%)
ABO-HDN	63 (1:17)	20 (1:52)	6 (1:322)	89 (1: 171)

* Calculated from ABO frequencies of the normal deliveries in the same hospital.

** Total No. of livsborn infants during the period of studies.

*** Total No. of jaundiced infants during the study.

TABLE II

Incidence of ABO-HDN Among ABO Incompatible Mother-Child Combinations at Wadia and Mahim Hospitals

Mother-Child Combinations	Wadia [15235]		Mahim [7444]	
	No. of Jaundice Infants	Incidence of ABO-HDN	No. of Jaundice Infants	Incidence of ABO-HDN
O-A	147 (14.1)	63 (6.0)	83 (7.8)	42 (9.0)
O-B	86 (8.2)	20 (1.9)	75 (13)	26 (4.5)
Other ABO Incompatible Categories	20 (6.25)	6 (0.3)	70 (7.5)	24 (2.6)

Figures in the [ ] represent total No. of liveborn infants.
Figures in the ( ) give incidence among ABO-incompatible liveborn infants.

TABLE III

Incidence of ABO-HDN in the Series of 3548 Jaundice Infants

Mother-Child Combinations	Jaundice Infants.		Cases of ABO-HDN	
	Ob. No.	Exp. No.**	No.	%
O-A	618*	238	270	56.6
O-B	387*	238	123	25.8
$A < \frac{B}{AB}$ or $B < \frac{AB}{B}$	384	518	84	17.6
Total ABO Incompatible	1389*	994	477	100.0

* Statistically significant $P<0.01$.
** Based on total jaundice infants (3548).

population. It is apparent that significant increase is mainly in ABO incompatible jaundice infants of O group mothers. Since frequency of A and B groups among patients at Wadia Maternity Hospital is similar, the incidence of ABO-HDN among A and B group infants was expected to be the same. However, ABO-HDN was more frequently observed in A group infants of O mothers. Out of 477 cases of ABO-HDN, 84 (17.6%) infants belonged to A or B group mothers.

Seropathology of ABO-HDN

ABO-HDN is often marked by stricking haematological changes such as spherocytosis and increased osmotic fragility. With the increasing severity of anaemia one may find reticulocytosis and normoblastosis (Gupte and Bhatia 1973). All these haematologic changes are considered significant while analysing the data. Table IV gives the analysis on the basis of the titre of IgG anti-A or anti-B in mothers. Direct correlation was observed with the titre of IgG anti-A or B and incidence of haemolytic anaemia. Haematological changes were equally frequent in A or B group infants of O group mothers, though severity of neonatal jaundice was more often in O-A combination than in O-B and other ABO incom-

TABLE IV

Correlation Between IgG-A/-B Titre Levels and Haematological Abnormalities

Series	Ig G Titre level	Number Tested	Percentage of cases having					
			Hb <14gm%	Bili-rubin >16mg%	Sphe-rocy-tosis	Increased Osmatic fragility	Reti-cuto-cyto-sis	Normo-blas-tosis
O-A	<1:32	154	11.7	10.3	5.1	6.7	14.7	5.8
	1.32	116	16.4	17.3	16.1	19.3	24.7	15.0
	>1.32	270	23.0	30.7	34.0	45.7	45.7	22.0
O-B	<1.32	124	11.3	8.1	7.1	10.1	12.1	6.1
	1.32	83	8.7	8.5	18.5	20.4	29.6	7.4
	>1.32	123	21.3	20.4	35.2	36.1	45.4	15.7
A < B, AB or B < A, AB	<1:32	220	5.4	6.0	1.8	8.4	2.4	1.2
	1.32	40	7.5	12.5	5.4	10.8	2.7	2.7
	>1.32	44	11.4	20.4	12.8	7.7	7.7	5.7
ABO Compatible Jaundice Full Term	—	900	7.23	7.2	4.7	3.1	3.0	2.4

patible combinations. While IgG titre levels > 1:32 were considered significant for diagnosis of ABO-HDN, altered haematological picture in some of the low titre cases may also suggest mild haemolytic disease in these cases.

Obstetric Factors Influencing ABO-HDN

Several workers have observed that male, first born, lowbirth weight and the infants undergoing complicated deliveries are more susceptible to neonatal jaundice (Gupte and Bhatia 1973; Lucey 1960, Barton 1962 and Lee *et al* 1977). Data on ABO-HDN was therefore analysed to see the influence of these factors on jaundice of ABO incompatibility (Table V).

Though first born, male and lowbirth weight ABO-HDN infants had greater risk, the incidence of these parameters was higher in the series of ABO compatible jaundice infants thus suggesting no higher risk to ABO-HDN cases. Further analysis suggested that increase in the male and lowbirth weight infants was marginal in O-A combination. First born infants, however, were increased in all the ABO incompatible combinations Since ABO antibodies are naturally occuring and even low titre IgG antibodies are present normally, first born child is often effected by ABO-HDN.

Though complicated deliveries increased the risk of neonatal jaundice among ABO compatible infants, present data did not show greater risk of the ABO-HDN to the infants of women with complicated delivery.

TABLE V

Correlation of Sex, Para, Birth Weight and Complicated Deliveries with the Incidence of ABO-HDN

Series	Total No. of Infants	Male	First Born	Low Birth Weight	Complicated** Deliveries
ABO-HDN	477	266	193	71	42
		% 55.8	40.5	14.5	8.8
ABO* Compatible Jaundice	1828	1074	941	394	291
		% 58.8	51.5	21.6	15.9
Normal	15235	7762	3611	1872	995
		% 50.9	23.7	12.3	6.5

* Excluding cases of Rh(D)HDN, G6PD deficiency, enclosed haemorrage, septicemia etc.
** Complicated deliveries include forceps, vacuum extractions caesarean sections and abnormal presentations.

Assoiated Factors influencing Severity of ABO-HDN

Low Birth Weight

Because of the immaturity of liver and various other complications associated with low birth weight (LBW), infants are more susceptible to neonatal jaundice. As seen in Table VI, severe anaemia was more frequently associated with LBW infants. Analysis on the basis of serum bilirubin levels suggested that values less than 12.5 mg% were more often observed in full term (FT) infants. However, since LBW infants are generally treated even when serum bilirubin levels do not reach upto 16 mg%, incidence of hyperbilirubinemia was higher in FT infants.

Other Aetiological Factors

Cases of neonatal jaundice due to Rh(D) incompatibility, G6PD deficiency, enclosed haemorrhage and maternal diseased conditions like syphilis, diabetes and hepatitis were analysed to assess the severity of the disease among ABO incompatible infants (Table VI). Out of 300 jaundiced neonates with above aetiological factors, 70 (23.3%) were associated with ABO incompatible pregnancy.

TABLE VI

Haemoglobin and Serum Bilirubin Levels in full Term and LBW Infants Suffering from ABO-HDN

	Full Term (406)		Low Birth Weight (71)	
	No.	%	No.	%
Hb <14 gm %	75	18.5	21	29.6
Serum Bilirubin mg%				
upto 12.5	213	52.5	32	45.0
12.6-16.0	86	21.2	24	33.8
>16.0	107	26.3	15	21.2

Incidence of ABO incompatible pregnancy was considerably reduced in Rh (D) HDN cases. It is known that ABO-incompatibility gives protection to Rh (D) immunisation. High incidence of ABO incompatibility among G6PD deficient, jaundiced infants probably suggests that ABO incompatibility could trigger the haemolytic stress in G6PD deficient infants. Increased incidence of ABO incompatibility was also observed in the cases with enclosed haemorrhage and maternal diseased conditions like syphilis, diabetis and hepatitis. In all these conditions, ABO incompatibility did not increase the severity of anaemia (Hb < 14 gm%) but increased the severe hyperbilirubinemia.

TABLE VII

Severity of Neonatal Jaundice in the ABO Incompatible Infants having Associated Aetiological Factors

	Rh (D) HDN [159])		Enclosed Haemorrhages [57]		G6PD Deficiency [33]		Maternal Disease [51]	
	ABO Comp.	ABO Incomp.	ABO Comp.	ABO Incomp.	ABO Comp.	ABO Incomp.	ABO Comp.	ABO Incomp.
No. of cases	147	12 (7.5)	35	22 (38.6)	18	15 (45)	30	21 (41)
Hb < 14 gm%	86	3	2	1	4	3	10	5
Serum Bil. mg%								
upto 12.5	84	3	20	14	10	8	21	7
12.6-16.0	26	4	10	2	2	1	6	8
>16.0	37	5	5	6	6	6	3	6

Figure in [ ] indicate total number of cases in the particular condition.
Figures in ( ) indicate percentage.

toxoid injections, studies were done using Haffkine, Glaxo and Biological Evans toxoid preparations. Anti-A and anti-B levels were gradually increased as the number of toxoid injections increased (Gupte and Bhatia, 1979). Risk of ABO-HDN was significantly increased among the O group women receiving tetanus toxoid compared to the non-toxoid group (Table VIII). As indicated earlier overall incidence of ABO-HDN was two times more at Mahim Maternity Hospital (1:81) where tetanus toxoid was used routinely, compared to the incidence (1:171) at Wadia Maternity Hospital where none of the pregnant ladies received tetanus toxoid injections. It may, therefore, be suggested that O group women with ABO incompatible husbands, particularly those with history of neonatal jaundice in previous children should be excluded from tetanus immunisation if they give history of tetanus toxoid injections.

Role of Tetanus toxoid influencing The risk of Neonatal Jaundice

Injections of vaccines and toxins are known to increase the levels of IgG anti-A and anti-B (Moullec 1938; Springer *et al* 1961 and Gupta and Bhatia 1979). Thus, to evaluate the risk of ABO-HDN among antenatal patients taking tetanus

Treatment and Mortality

ABO-HDN showed spectrum of the

TABLE VIII

Incidence of Neonatal Jaundice and ABO-HDN in O-A and O-B Mother-child Combination in Toxoid and Non-Toxoid Series

Mother-Child Combinations	Toxoid Series [559]			Non-Toxoid Series [378]		
	Number of patients	Number of Neonatal Jaundice	Number of ABO-HDN	Number of patients	Number of Neonatal Jaundice	Number of ABO-HDN
O-A	115	34 (29.6)	23 (20)	83	9 (10.8)	2 (2.4)
O-B	119	24 (20.6)	13 (10.6)	72	7 (9.8)	2 (2.8)

Figures in the parenthesis ( ) indicated percentage value.
Fgures in the bracket [ ] indicate total no. of O group women.

severity. Among low birth weight infants, severity was more compared to full term infants. In the present series, out of 1389 ABO incompatible jaundiced infants, 72 required exchange transfusion. In 65 cases, mothers were group O and 48 of them had A and 17 and B group children. There were total of 23 deaths, however in 13 cases ABO-HDN may be the cause of death. Eight children had kernicterus, 5 of them were B group infants of O mothers.

Conclusions

Based on the Indian normal data, ABO-HDN is identified serologically if the titre of IgG anti-A and anti-B is greater than 1:32 in O group and 1:16 in A and B group mothers of ABO incompatible infants, developing jaundice within 48 hours of life. Marked haematologic changes like anaemia associated with spherocytosis and increased osmotic fragility are significant of the ABO-HDN and more often associated with the severe disease and increased titre of IgG anti-A and anti-B. ABO-HDN is more often in A-B group infants of O group mothers. The overall incidence of ABO-HDN is 1:170 in total liveborn infants and 1:17 and 1:22 among A and B group infants of O mothers.

Though various obstetric factors as a rule increase the risk of neonatal jaundice, the incidence is not significantly increased if associated with ABO incompatibility. However, there is evidence that ABO incompatbility could increase the risk of neonatal jaundice and severity of the disease in low birth weight infants, G6PD deficiency and associated maternal diseases. Risk of neonatal jaundice and ABO-HDN is twice among the O group women receiving tetanus toxoid during third trimester. Data reported gives 72 cases of ABO-HDN receiving treatment, 13 deaths due to ABO-HDN and 8 children developing kernicterus. These figures should point out that the concept that ABO-HDN, generally mild should not create a sense a complecancy from the prompt attention and treatment.

Acknowledgements

Authors are thankful to the Dean Dr. (Miss) D. N. Patel, Ex.-Dean Dr. B. N. Purandare and Ex. Pathologist Dr. N. M. Purandare for the facilities to undertake present studies at Nowrosjee Wadia Maternity Hospital. Thanks are also due to the Municipal Corporation, Health department and Dr. (Mrs.) Lalita Chopra for the permission to carry out

the similar studies at Mahim Maternity Hospital.

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