A LABORATORY SURVEY OF A-B-O BLOOD-GROUP INCOMPATIBILITY AND NEONATAL JAUNDICE*

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It is well known that A-B-O blood-group incompatibility is responsible for hyperbilirubinaemia[1-4] in a large number of infants. If the haemolytic disease due to Rh sensitization is compared with that caused by the A-B-O system, it is suggested that the underlying immunological mechanism must be similar. With Rh sensitization, methods to determine the degree of sensitization of the mother and the severity of the icterus of the newborn are both straightforward and reliable. The Rh antigen only occurs on the red blood cells, whereas the A substance is present in many tissues and also in various molecular forms.[5]

It is also well known that unaffected A-B-O incompatible infants can be born to highly immunized mothers and, conversely, that severely jaundiced infants may be born to apparently poorly immunized mothers.[6-8] With the study of a series of cases at this laboratory from 1 July 1966 to 30 June 1967 an attempt was made to correlate the serological analysis of the maternal blood and the infant's blood with the severity of the observed haemolytic disease of the newborn.

In addition, in order to demonstrate the incidence of G-6-PD deficiency as the cause of neonatal jaundice in Cape Town, an initial routine screening test for G-6-PD-deficient mothers and for babies was included, and on all the positive cases a further variety of tests to establish the diagnosis were performed.

The criteria for inclusion in the series were as follows:

1. A total serum bilirubin value of $\geqslant$ 12 mg./100 ml. during the first 7 days postnatally.
2. Cord blood examintion had to show:
   (*a*) Anti-human-globulin test: Positive
   (*b*) Total serum bilirubin $\geqslant$ 3 mg./100 ml.
   (*c*) Haemoglobin $\leqslant$ 12 mg./100 ml.
3. The mothers and/or babies had to be G-6-PD deficient.

MATERIAL AND METHOD

The series took as the sources of its information 8,208 routine maternal and cord bloods, and also specimens where a special request for investigations of the neonatal jaundice was made. In this series 602 cases were included (of which 500 were non-White), and only those 500 will be considered for the rest of the paper.

Routine Management of Blood Tests on Mothers and Infants

The mother's blood group and haemoglobin value are determined antenatally as a routine. The maternal serum is screened for the presence of atypical antibodies in all cases. The baby's blood group and direct anti-human-globulin (AHG) test are determined on umbilical cord blood. Whenever the materno-foetal blood groups are incompatible the total serum bilirubin and haemoglobin values are also determined as a routine.

Jaundice

If the baby becomes jaundiced, its serum bilirubin is determined colorimetrically by Powell's method,[9] as often as considered necessary.

If any of the criteria listed are present, the case is investigated more fully as follows:

(*i*) Elution tests are done according to Landsteiner and Miller,[10] on the infant's red blood cells; with determination of A-B-O or Rh antibody specificity against group A Rh-negative, group B Rh-negative and pooled group O Rh-positive cells.[11]

(*ii*) A direct AHG test is done, by the Rosenfield's method, using suitable wide-spectrum serum.[12]

(*iii*) The infant's direct reacting and total serum bilirubin are determined.[9]

(*iv*) The haemoglobin value and reticulocyte count of the infant's blood are determined, and microscopic examination of the baby's peripheral blood smear is done.

(*v*) Glucose-6-phosphate-dehydrogenase (G-6-PD) activity of the infant's blood is determined by the brilliant-cresyl-blue dye test of Motulsky and Campbell-Krant.[13]

(*vi*) More extensive investigations of the maternal serum for atypical antibodies are carried out, and whenever the foeto-maternal blood groups are A-B-O incompatible, a 2-mercapto-ethanol test as described by Adinolfi and as modified at the Natal Blood Transfusion Service is done.[14-17]

Serum bilirubin is determined regularly until the jaundice subsides.

The range of tests undertaken in individual instances is determined by the suitability of the specimens.

RESULTS

The 500 non-White cases in this series were divided into 5 main groups according to the materno-foetal blood-group combinations: O-A, O-B, other A-B-O incompatible combinations, A-B-O compatible combinations and the incomplete group, i.e. where the maternal blood was not submitted for investigations. The division is shown in Table I. The figures in brackets indicate the number of cases where the red blood cells of the baby were serologically affected as a result of Rh sensitization (that is, either a positive AHG test or a positive eluate test).

Table II details the findings in the 3 incompatible groups.

The O-A and O-B Groups

More than half of each group became jaundiced. If a positive 2-mercapto-ethanol (2-ME) test was found in the maternal blood, a large percentage of the infants had indications that their red blood cells were serologically affected.[18] This can be observed from a study of Table II. In 127 of the 157 in the O-A group and in 39 of the 66 in the O-B group a positive 2-ME test was demonstrated, and in 132 of the O-A group and 27 of the O-B group the infant had a positive direct AHG test or a positive eluate.

On the other hand, the 2-ME test (which demonstrates the presence and titre of immune alpha- and/or beta-agglutinins of γ G type) was of no aid as a predictor of jaundice.[19]

*Date received: 22 November 1968.

TABLE I. 5 MATERNO-FOETAL BLOOD GROUPS

	O-A	*O-B*	*Other A-B-O incomp.*	*A-B-O comp.*	*Incomplete group*	*Total*
Jaundiced	80 (3)	36 (1)	15 (2)	126 (16)	70	327 (22)
No jaundice	77	30 (1)	13 (1)	39 (16)	14	173 (18)
Total of series	157 (3)	66 (2)	28 (2)	165 (32)	84	500 (40)
Observed % occurrence of materno-foetal combinations	31·4%	13·2%	5·6%	33%	16·8%	100%
Expected random distribution of materno-foetal combinations	8·75%	6·05%	7·58%	77·64%		100%

Figures in brackets = number of cases with Rh sensitization.

TABLE II. DETAILED ANALYSES OF INCOMPATIBLE GROUPS

Group	*Total*	*2-ME positive*	*2-ME negative*	*2-ME not done*	*Rh ab.*	*AHG positive*	*Eluate pos.*	*Eluate neg. AHG pos.*	*Exchange transfusion*
O-A group									
Jaundiced	80	65	5	7	3	36	58	3	24
No jaundice	77	62	3	12		60	71		
Total	157	127	8	19	3	96	129	3	
O-B group									
Jaundiced	36	19	3	10	1	7	16	1	12
No jaundice	30	20	4	9	1	20			
Total	66	39	7	19	2	27	16	1	
A-B-O incompatible group (not O-A or O-B)									
Jaundiced	15	2	7	3	2	1	1	2	0
No jaundice	13	1	12	2	0	5	3	5	
Total	28	3	19	5	2	6	4	7	

As shown in Table II, a positive AHG test[6] or a positive eluate[11] was not very helpful in the prediction of jaundice. The same was observed with a low cord-blood haemoglobin,[4] a high total serum bilirubin and the reticulocyte count,[20] although the figures are not detailed. The onset of early jaundice clinically remained the most important single indication that exchange transfusion would probably become necessary.[3] Four babies in the O-A group and 3 babies in the O-B group were G-6-PD deficient and all 7 became jaundiced.

The birthweights were not considered upon inclusion into the series, and only 9 prematures were found in the O-A group and 4 in the O-B group (taking the definition of 'premature' as less than 5½ lb. birthweight).

When jaundice occurred in the O-B group it tended to be slightly more severe than in the O-A group. This was indicated by the observation that an exchange blood transfusion was required in 12 out of the 36 jaundiced cases in the O-B group, whereas it was needed in only 24 of the 80 O-A jaundiced cases.

The Other A-B-O Incompatible Combinations

In this group of 28 cases there were 3 cases with positive 2-ME tests. Two of these developed jaundice but the direct AHG test and eluate were negative; in the other case the direct AHG test and eluate were positive but the infant did not develop jaundice. The AHG test was positive in 7 cases, but in the 2 cases that became jaundiced the maternal serum contained anti-Rho antibodies and anti-Rho was identified in the eluate. In none of these cases, therefore, were there indications that the A-B-O incompatibility could be incriminated as the cause of the jaundice. No exchange blood transfusions were required in this group.

Incomplete Group (i.e. maternal blood not submitted)

This group of 84 included a wide variety of different aetiological factors. A positive eluate was found in 19 cases and these could have been due to an A-B-O incompatibility. Thirteen premature infants were included and 6 cases of G-6-PD deficiency were demonstrated.

A-B-O Compatible Group

Results in the A-B-O compatible group are shown in Table III. Of these 165 cases, 32 demonstrated Rh sensitization with evidence that the infant's red blood cells

TABLE III. A-B-O COMPATIBLE GROUP

Description	*No.*	
Total cases	165	
Rh antibodies	32	
Jaundiced: Rh antibodies	16	21 prematures
G-6-PD deficiency	9	
AHG test positive	1	
No blood group incompatibility demonstrated	100	
Total	126	
No jaundice: Miscellaneous group	23	4 prematures
Rh antibodies	16	
Total	49	

were affected, compared with 8 cases out of the 251 in the A-B-O incompatible group. The 9 infants with G-6-PD deficiency in this group all developed jaundice.

DISCUSSION

The O-A and O-B Groups

In Table I the observed incidence of the materno-foetal O-A combination is almost 4 times and that of the O-B group more than double the expected random distribution of these combinations in the non-White population. Statistically this, therefore, demonstrates a correlation between O-A and O-B groups and haemolytic disease of the newborn.

To explain the fairly common occurrence of an unaffected A-B-O incompatible baby from a highly sensitized mother, a protective mechanism[21,22] of the foetus against the harmful maternal antibodies has been postulated. Two ways of efficient protection have been suggested:

1. *Weakness of A antigen at birth.* Experimental evidence has recently confirmed the existence of two red blood cell populations in newborn infants.[23,24] By use of the immunofluorescent technique it was shown that the maternal antibodies will lyse and therefore depress the expansion of red blood cells possessing a well-developed antigen. To compensate, another A antigen develops against which the maternal anti-A will be unable to react.

2. *Specific blood group substances.* The presence of specific blood group substances (SSBGS) in the infant's serum competing with the cellular A antigen could be another means of protection.[25] The sensitization of the foetal erythrocytes by the maternal anti-A is weakened in the presence of the homologous SSBGS. The effect is most pronounced with cells having a weak antigenic strength.[22] Furthermore, Kochwa *et al.*[17] observed in the 7S fraction of certain immune sera, antibodies which proved highly resistant to inhibition in the sera of mothers with a history of having had children with A-B-O erythroblastosis. However, the antibodies from women with A-B-O incompatible pregnancies but delivering healthy children were readily inhibitable with SSBGS.[6]

Schellong[26] pointed out that premature infants are protected from A-B-O haemolytic disease, presumably due to the fact that A and B antigens are even weaker than in mature infants.

Other A-B-O Incompatible Combinations

The expected random distribution of these materno-foetal blood group combinations (A-B-O incompatible, but not O-A or O-B) in the non-White population in Cape Town is 7·58%, as compared with 5·6% in this series. Statistically, therefore, there is no correlation between this group and haemolytic disease of the newborn.

In addition γ G anti-A and anti-B occur more commonly in group O mothers than in B or A mothers[27] (hence the small number of positive 2-ME tests in this group). It has been shown[27] in volunteers that injection of SSBGS produced predominantly γ G class antibody in group O individuals, whereas subjects of group A or B tended to produce γ M class antibody. It is the immune alpha- or beta-agglutinins of γ G class which serologically affect the infant's red blood cells in haemolytic disease of the newborn.

Bowden and Williams[28] reported an unusual case of A-B-O haemolytic disease in a group B infant from a group A_2 mother. Zuelzer and Cohen[29] reported 2 cases of group A_2 mothers with affected group AB infants.

The Incomplete Group

In most of these cases the baby was delivered at home, the infant was brought to the children's hospital with neonatal jaundice by a relative, the mother's blood was not submitted, and if there was no antenatal record of the mother at the laboratory the investigations as to the cause of the jaundice were incomplete. This group particularly demonstrates the value of routine antenatal blood group tests.[2]

The A-B-O Compatible Group

This group confirms the observations of Nevanlina and Vainio[30] and other observers[31] that an A-B-O compatible infant was much more likely to immunize its mother to the Rh system than one which was incompatible. They also found that Rh-affected children were equally commonly A-B-O compatible and incompatible, that is to say, once the mother had been immunized to Rh, A-B-O incompatibility had no protective effect.[32] This protective effect of A-B-O incompatibility to Rh sensitization is stronger in group O women.[33] Murray *et al.*[34] estimated that O-A incompatibility gave 90% and O-B incompatibility 55% protection.[35]

G-6-PD Deficiency and Neonatal Jaundice

In this series of 500 non-Whites there were 7 cases with G-6-PD deficiency and jaundice in the O-A and O-B groups, and in these the A-B-O incompatibility could have been the major cause of the jaundice. No other cause was demonstrated for the jaundice in the 9 cases of the A-B-O compatible group and the 6 cases in the incomplete group, who were G-6-PD deficient. G-6-PD deficiency appears to be a fairly common cause of neonatal jaundice in Malaya,[36] in Greece[37] and in Nigeria.[38]

SUMMARY

A study was made of A-B-O blood group incompatibility in a series of non-White infants with haemolytic disease of the newborn over a 1-year period. Examination of the infant's blood was correlated with the severity of the observed haemolytic disease, and an attempt was made to establish whether antibody specificities could be found in the maternal sera of mothers of jaundiced infants, which would otherwise be absent. It was concluded that there was no safe prediction method.

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