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ABO Hemolytic Disease Leading to Hyperbilirubinemia in Term Newborns: Value of Immunohematological Tests

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Abstract

Introduction:
ABO hemolytic disease of the newborn is most common cause of neonatal jaundice; previous studies have shown a poor correlation between serologic tests and clinical course in affected infants. The aim of this study was to identify the value of direct and indirect Coombs' immunohematological tests, to identify the incidence of hemolytic disease in newborns (ABO-HDN).

Materials and Methods:
This two-year retrospective study of 1800 jaundiced term-neonates studied 355 cases due to ABO incompatibility divided into two groups: with and without hemolytic disease. Relation of laboratory parameters and immunohematological tests to severity of disease were studied. We did not analyze the maternal antibody titer or elusion test, and this was a limitation of the study.

Results:
In this study, 355 (19.7%) of all jaundiced newborn infants were ABO incompatibles; 98 (27.6%) of the newborns who had ABO incompatibility showed ABO-HDN (5.4% of total icteric patients). The positive direct antiglobulin (direct Coombs' test) and indirect antiglobulin (indirect Coombs' test) were diagnostic in 18.2% and 25.5% respectively in affected infants. The overall prevalence of immunohematological tests associated with ABO-HDN was 43.7%. There was significant correlation between positive antiglobulin tests and severity of jaundice (P=0.000); also there was a significant difference between indirect and direct Coombs' test and severity of jaundice (P=0.002).

Conclusion:
The antiglobulin tests, namely the indirect Coombs' (IC) test and direct Coombs' (DC) test are very useful to detect the newborns liable to serious jaundice.

Keywords:
Massage therapy, Newborn, Premature infant.

Introduction

This randomized clinical trial was conducted on 28-34 week infants hospitalized at the NICU of Beheshti Hospital during 2009-2010. The inclusion criteria were: Gestational age of 28-34 weeks and complete oral feeding or gavage of 150 ml/kg/day. Infants in all study groups were fed with milk and did not receive fluid infusion. Infants older than 7 days were selected if they had stable vital signs. The exclusion criteria were:

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Important congenital disorders, congenital heart disease, gastrointestinal disorders and need for mechanical ventilation during massage therapy. The number of infants receiving nutritional supplementation of mother's milk was similar in all 3 groups. The number of breastfed or formula-fed infants was also similar in the 3 groups. All the study phases and consent forms provided by parents were approved by the Ethics Committee of Isfahan University of Medical Sciences. The study was thoroughly explained to the parents and written informed consent was obtained. A code was allocated to each infant qualified for the study. These codes were written on small pieces of paper and placed in a box. A person not aware of the study randomly drew pieces of paper out of the box and this way all infants were randomly divided into 3 groups. Each group consisted of 20 infants. The first group received massage therapy by their mothers, and the second group by a nurse. The 3rd group did not receive massage therapy and only routine care was given to them. Massage therapy was started on the first day of the study and continued for 5 consecutive days, each time for 5 minutes. The first session was one hour after the morning feeding, the second session was 30 minutes after the mid-day feeding, and the 3rd session was 45 minutes after the completion of the 2nd session. Each session consisted of 5 minutes of tactile stimulation. The infant would be placed in prone position. The massage therapists would warm up their hands first and were quiet during therapy. Massaging was gentle using the ventral part of the fingers. The whole massage was divided into 5 sections of 1 minute each and each section to 6 fractions of 10 seconds each. In the first section, massage was started from the head to the posterior neck and back to the head. In the second and third sections, massage started from the posterior neck to the shoulders and back to the posterior neck. In the 4th section, massage started from the shoulders down to the buttocks and back to shoulders except for the vertebral area which was left untouched. The fifth section included simultaneous massage of both legs from the hip down to the soles and back. The sixth section included both hands from the shoulders to the wrists and back to shoulders simultaneously. The phases of each 5-minute session of massage included 5 times repeating of the 1 minute massage which contained head and neck massage for 10 seconds, 20 seconds shoulders massage, 10 seconds back massage, and 10 seconds massage of both legs. A special questionnaire was filled out daily for each infant containing daily weight, amount of fluid intake, number of defecations, medications, and amount of calories received (kcal/kg/d). Before initiation of study, the mothers and nurses who were going to massage the infants received necessary instructions and training. For accurate recording of information, a fulltime nurse was recruited to supervise the whole process.

Physiologic responses were controlled by measuring the heart rate and respiration rate per minute. In case of emergence of signs or symptoms of physiologic distress such as the heart rate over 200 beats/min, the massaging would be stopped for 15 seconds or until reaching the baseline values. A warning alert sound would warn the therapist about the presence of unfavorable physiologic responses. Mothers received necessary information and training in this respect.

The head nurse supervised the massaging by the mothers and nurses. Data were entered via SPSS version 15 software and one way analysis of variance (ANOVA) and Kruskal Wallis test were used for data analysis.

Material and Methods

This study was performed in 1800 jaundiced, term, neonates at the Ghaem and

Imam Reza Medical Centers from March 2007-March 2009. All healthy full-term, jaundiced newborns with blood groups A or B born to mothers with blood group O without a Rhesus factor incompatibility at two medical centers through this period, were studied. Neonates with a hemolytic condition other than ABO incompatibility, positive maternal history of autoimmune diseases, drug usage, condition such as severe bruising, sepsis, Down's syndrome, Glucose-6-phosphate dehydrogenase deficiency or a positive DAT from any cause other than ABO isoimmunization in the newborn, were excluded. The study was approved by our ethics committee. Total cell blood count, peripheral blood smear, hemoglobin, hematocrit, reticulocyte count, blood group including Rhesus factor, DC, IC tests, Glucose-6-phosphate dehydrogenase activity, serum total and direct bilirubin levels were analyzed. Demographic data were collected from records. Gestational age, chronologic age and weight at admission, birth weight, and sex, feeding pattern, maternal age, parity, delivery route and history of jaundice were recorded. The ABO-HDN was diagnosed if there was significant early onset jaundice in newborns that were A-B incompatible with their mother's and they met one or more of the hemolytic criteria (without other cause of hemolytic diseases and anemia), such as: decrease of hemoglobin and hematocrit, increase of indirect bilirubin>0.5-1 mg/h, reticulocytosis >7%, spherocytosis in peripheral blood smear, and positive Coombs' tests. Severity of jaundice was defined according to total serum bilirubin levels (mg/dl); severe jaundice was considered when it was≥25 mg/dl, significant jaundice considered when the neonate needed hospitalization for treatment of jaundice. Statistical data were analyzed with independent t-test and descriptive analysis and chi-square and Fisher exact test by SPSS 13, ($P \leq 0.05$ was considered significant).

Results

During a two year period, 1800 newborns jaundiced infants were admitted to two medical centers; 355 (19.7%) comprised blood group A or B newborns who were born to group O mothers. Based on the inclusion criteria and definition of ABO-HDN, they were divided into two groups: Newborn infants with ABO-HDN and those without ABO-HDN. Demographic characteristic of newborns is shown in (Table 1).

Table 1: Demographic characteristic of newborns with and without ABO-hemolytic disease.

	Sex (%)			**Blood group (%)**			**Parity (%)**				
	Female	Male	Total	B	A	Total	1	2	3	>3	Total
ABO incompatibility without ABO hemolytic disease	105 (67.7)	152 76.5	257 72.4	136 75.6	117 66.9%	253 71.5	122	71	29	26	248 69.9
ABO-incompatibility with ABO hemolytic disease	50 32.3	48 24	98 27.6	44 24.4	58 33.1%	102 28.5%	50	32	17	8	107 30.1
Total	155 56.3	200 43.7	355 100	180 50.7	175 49.3	355 100	172 48.5	103 29.5	46 13.0	34 9.5	355 100

All patients had term gestation and were fed breast milk. The mean bilirubin levels was:22.33±7.52 mg/dl (range 5-48 mg/dl), mean reticulocyte count was 2.52±3.05% (range 0.2-18%), mean hemoglobin was 13.38±2.72 g/dl (range 10-21 g/dl), mean hematocrit was 39.3±7.66% (range 27-61%), mean age of newborn at admission was 4.03±3.84 days, mean maternal age was 26.10±4.9 years (P: 0.63). 98 (27.6%) of the

newborns who had ABO incompatibility showed ABO-HDN.

Positive DC and IDC were detected in 18.2% and 25.5% respectively in affected infants. The overall prevalence of immunohematological tests associated with ABO-HDN was 43.7%. Maternal age, gravity, parity (P=0.65) and infant birth weight (P=0.06), blood group type (P=0.09), sex (P=0.06), gestational age, admission age, history of neonatal jaundice, hemoglobin, hematocrit, and reticulocyte count did not have significant relationships to severity of disease between the two groups of ABO-incompatibility.

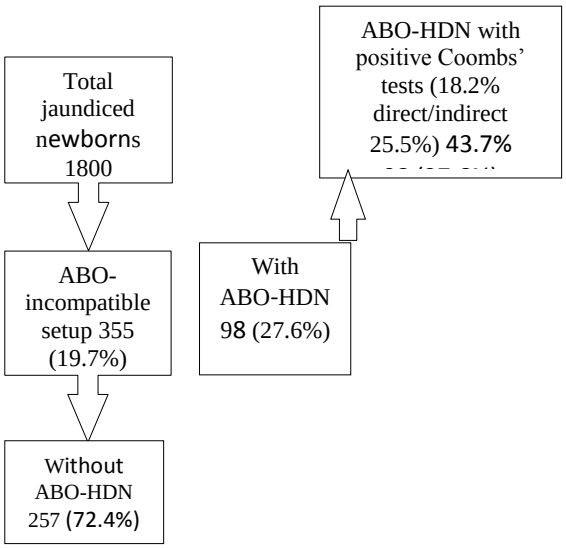

Fig 1: Frequency of immunohematologic tests in ABO-incompatibilityTable 1

Discussion

Our data based on the measurement of immunohematological tests in selected jaundiced term neonates showed that 19.7% of newborns with hyperbilirubinemia were ABO incompatible. The authors found 25-34% ABO incompatibility in clinically jaundiced neonates (7,8); in another series ABO incompatibility was the etiologic reason for 20% of exchange transfusion in newborns (7,9). In our study 5.4% of newborns with significant hyperbilirubinemia manifested ABO-HDN. Sarici and colleagues reported, the incidences of ABO incompatibility (14.8%), significant hyperbilirubinemia in ABO incompatibility (21.3%) and severe hemolytic disease of the newborn as a result of ABO incompatibility (4.4%) in infants with type O-A and type O-B incompatibility (4,10). The ABO-HDN is more common and often more severe in infants of African descent (10,11), this difference must be attributable to the race. It is commonly held that Coombs' test may be negative in ABO hemolytic disease (12). There is a wide spectrum in the manifestation of ABO -HDN with the possibility of there being some correlation of the severity of disease with the strength of the DC test (12). We found that with clinically significant ABO -HDN the DC test was positive in 18.2%. The overall prevalence of immunohematological tests associated with ABO-HDN was 43.7% and there was significant correlation between positive direct/indirect antiglobulin tests and severity of jaundice in newborns that did and did not develop ABO-HDN. As Herschel reported , it seems that DC as determined by the gel test, will be positive in neonates who have ABO incompatibility and clinically significant hemolysis as a result of isoimmunization (12), There was significant difference between indirect and direct Coombs' test (P=0.002) in our study. Also as mentioned, the authors observed, positive Coombs' test is not correlated with severity of disease (1,13).

The incidence of a positive cord blood DC was found to be 5.5%. It is considered to be an important test in identifying babies who are at risk of hemolytic disease of newborns; recommendation for testing are discussed but remain controversial in practice (14). Although the reticulocyte count, a positive DC and the presence of a sibling with neonatal jaundice were determined to be the good predictors for the development of severe HDN in a selective high risk population of full-term healthy newborns with ABO incompatibility (4), there were no significant differences in other demographic findings in this study.

Conclusion

The antiglobulin tests, including indirect Coombs' (IC) test and direct Coombs' (DC) test are very useful to detect the newborns prone to serious jaundice.

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