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Original Article

Serological profile and hemolytic disease in term neonates with ABO incompatibility

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Abstract

Background Hemolytic disease of the newborn (HDN) due to ABO blood type incompatibility is one of the most common cause of neonatal hyperbilirubunemia that potentially leads to bilirubin encephalopathy. Data on ABO-hemolytic disease of the newborn (ABO-HDN), especially regarding umbilical cord blood serological profile, are limited.

Objective To identify the serological profile and hemolytic disease in term neonates with ABO incompatibility.

Methods This was a cross-sectional descriptive study, conducted at RSIA Budi Kemuliaan Jakarta.

Results We found 68 healthy term neonates with ABO incompatibility, nine of them had positive direct antiglobulin test (DAT) result, and 38 subjects had a positive result on DAT with elution method. The highest titer of IgG was 1:8. Hyperbilirubinemia was found in 30 (44%) subjects, and ABO-HDN was diagnosed in 28 (41%) subjects. Within the positive DAT group, eight out of nine subjects had suffered from hyperbilirubinemia and ABO-HDN. Meanwhile, within the positive DAT with elution method group; 24 subjects had suffered from hyperbilirubinemia with 23 of them having ABO-HDN. Based on the chi-square analysis; those with positive DAT with elution method had 3.2 times higher risk of suffering from ABO-HDN. Furthermore, there was a 3.6 times higher risk of sufferring from hyperbilirubinemia.

Conclusion In healthy term neonates with ABO incompatibility, the incidence of ABO-HDN is 41%. DAT serological examination with elution method is better than DAT in assessing risk for hyperbilirubinemia and ABO-HDN. **[Paediatr Indones. 2009;49:219-23]**.

Keywords: *ABO incompatibility, hyperbilirubinemia, DAT*

In the last decade, discharging healthy term neonates earlier from hospital is a common practice due to medical and socioeconomic reasons. Several studies show that neonates discharged in less than 72 hours of age, are significantly being re-hospitalized more often than those discharged at more than 72 hours of age.[1-4] Hyperbilirubinemia is reported as the most common cause of rehospitalization during the early neonatal period.[1-3] ABO incompatibility is one of the cause of hemolytic disease in newborns (HDN) that has been one of the most common risk factor of neonatal hyperbilirubinemia.[5-7] Term neonates with ABO incompatibility have a 2.9-4.2 times higher risk of suffering from hyperbilirubinemia on the first day. [8-9] This condition potentially leads to hyperbilirubinemia encephalopathy. [6,10-11]

Establishing diagnosis of ABO-HDN is not easy because there is no specific diagnosis. Diagnosis of ABO-HDN is usually presumptive when A or B blood-type neonate who is born from a mother with O

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blood-type suffers from jaundice in the first 24 hours of life.[6,10-14] History of hyperbilirubinemia or ABO-HDN in siblings can also support the diagnosis.[5,15] Data about ABO incompatibility, especially regarding to serological profile of ABO-HDN in Indonesia, is still limited. The objective of this study was to identify the profile of antiglobulin test (DAT), anti-A and anti-B immunoglobulin titer, and hemolytic disease in term neonates with ABO incompatibility.

Methods

This was a cross-sectional descriptive study, conducted at the delivery rooms of RSIA Budi Kemuliaan Jakarta, during October to November 2008. Subjects were recruited consecutively. We included healthy term neonates without any signs of infection or syndrome, A or B blood-type and born from a mother with O blood-type without rhesus incompatibility. We excluded small-for-age neonates, mothers with severe anemia and/or intra-labour infection, and mothers with a history of particular drug consumption (chloramphenicol, salicylic acetyl, and anti-malarial drugs).

Umbilical cord blood specimen from neonates was taken and collected in a EDTA tube, underwent DAT and DAT with elution method, and anti-A and anti-B immunoglobulin-G titer in Central Red Cross (CRC) laboratory. At the age of 24 hours, peripheral blood was taken from the heel and underwent hemoglobin, hematocrit, bilirubin, and peripheral blood examination to see the percentage of reticulocyte and microspherocyte.

Diagnosis of ABO-HDN is established if there was hemolytic condition, indicated by hyperbilirubinemia (total bilirubin >5 mg/dl), jaundice in the first 24 hours of life; in neonates with ABO incompatibility without rhesus incompatibility, presence of reticulocytosis (reticulocyte >4.6%) and microspherocyte in peripheral blood smear, and can be supported by positive DAT.

Data about sex, gestational age, birth weight, APGAR score, birth method, maternal age, primi/multigravida, existence of pre-ecclamptia and history of jaundice in siblings, were taken from each case. Statistical analysis was done using SPSS 15.0 and Epi Info 6.

Results

From 183 neonates born from mothers with O blood-type, 157 umbilical cord blood samples had been successfully collected, with 71 (45.2%) of them having A or B blood type. There were only 68 subjects that completed all examinations required and became subjects of this study.

Characteristics of subjects who fulfilled the criteria for this research were listed in **Table 1.** History of jaundice in siblings was found in 10 cases, with 8 of them suffering from ABO-HDN.

Table 1. Characteristics of subjects with ABO incompatibility pregnancy (n=68)

Characteristics	n(%)
Sex	
Male	31(46)
Female	37(54)
Birth weight	
<2500 grams	9(13)
≥2500 grams	59(87)
Growth in pregnancy	
Normal	65(96)
Big-for-dates	3(4)
APGAR score 1'/ 5'	
≤3	0(0)/0(0)
4-6	5(7)/0(0)
≥7	63(93)/68(100)
Delivery method	
Spontaneous	28(41)
Vacuum extraction	10(15)
Forcipal extraction	1(2)
Cesarian section	29(43)
Blood type of neonates	
A	38(56)
B	30(44)
History of jaundice in siblings	
Yes	10(15)
No	58(85)
Hemoglobin level (g/dl)	
<17.1	18(27)
17.1-21.5	44(65)
>21.5	6(9)
Reticulocyte (%)	
≤4.6	19(28)
>4.6	49(72)
Microspherocyte	
Yes	43(63)
No	25(37)
Bilirubin level (mg/dl)	
≤5	30(44)
>5	38(56)
Direct antiglobulin test (DAT)	
Direct (+)/(-)	9(13)/59(87)
Elution method (+)/(-)	38(56)/30(44)

Two mothers have history of blood transfusion, eight mothers have history of pre-eclampsia, and 41 mothers are multiparous.

Incidence of hemolytic disease in this study was 28 (41%) from 68 subjects. The highest titer of anti-A and anti-B IgG was 1:8. Distribution of DAT and DAT with elution method on the occurence of ABO-HDN and hyperbilirubinemia is listed in **Table 2** and **Table 3**.

Table 2. Distribution of DAT and DAT with elution method on ABO-HDN

DAT	ABO-HDN(%)	NonABO-HDN(%)
Direct		
Positive	8(12)	1(2)
Negative	20(29)	39(57)
Elution method		
Positive	23(33)	15(22)
Negative	5(7)	25(37)

Table 3. Distribution of DAT results on hyperbilirubinemia in term neonates with ABO incompatibility

DAT	Hyperbilirubinemia (%)	Non Hyperbilirubinemia (%)
Direct		
Positive	8 (12)	1 (2)
Negative	21 (31)	38 (56)
Elution method		
Positive	24 (35)	14 (21)
Negative	6 (9)	24 (35)

Statistical analysis showed that groups with positive DAT had a relative risk (RR) of 2.6 (95% CI 1.7 to 4.0; P <0.01) to suffer from ABO-HDN. This group also had RR of 2.5 (95% CI 1.5 to 6.7; P <0.001) to suffer from hyperbilirubinemia, while those with positive DAT using elution method had RR of 3.2 (95% CI 1.5 to 6.7; P <0.001).

Discussions

Pregnancy with ABO incompatibility was accounted for 15-40% from all pregnancies.[7,12] In this study, there was 71 (45%) neonates with ABO incompatibility from 157 mothers with O blood type. This result is smaller in comparison to the study by Kadri[16] at the same place in 1998. The actual prevalence of pregnancy with ABO incompatibility could not be calculated because there were 26 neonates from mothers with O blood type that had not undergone blood type examinations.

Most neonates were born through ceasarian section. This condition can be explained by the fact that RSIA Budi Kemuliaan is a referral hospital with high number of ceasarian section. According to Sarici *et al*[17], history of jaundice in siblings was a good predictor of significant hyperbilirubinemia and severe ABO-HDN. In this study, 10 subjects had history of jaundice in siblings, and 8 of them were also having ABO-HDN. Katz *et al*[15] reported that from 18 neonates with history of ABO-HDN in siblings, 16 of them had ABO-HDN with 10 neonates needing phototherapy.

Most of the subjects in this study had normal hemoglobin levels. This is in accordance with literature, that anemia in neonates with ABO incompatibility rarely occurs and was usually slight.[6,14] Reticulocytosis happen as an erythropoietin activity response to compensate a hemolytic condition. Normal levels of reticulocyte in term neonates ranged between 1.8-4.6%.[18] In this study, reticulocytosis was found in 72% cases, found to be much higher than the study by Iskandar[9] (37.5% cases). This difference was probably due to the higher cut-off of reticulocytosis used in the study by Iskandar (>7%).

Microspherocyte is the most commonly found feature in ABO incompatibility.[14] Pathogenesis of microspherocyte was predicted due to the decrease of erythrocyte surface caused by antibody phagocytosis on erythrocyte and erythrocyte membrane by macrophage.[6] Microspherocyte was found in 63% of cases in this study, in accordance with a previous study (60%).[9]

In this study, positive results in DAT was revealed in 13% cases. This number was smaller than other literature which show positive results in 18-40% cases;[14,19-21] however the method used in the previous studies was not explained. Positive results in DAT with elution method was revealed in 59% cases, in range of 35.1-79.2% cases in previous studies.[16,22-23] The large discrepancy between percentages in DAT group and DAT with elution method group maybe due to the field range of neonatal red blood cell antigen which is less than in adults. Besides that, the small amount of antibody molecule would also influence the result of DAT, which is relatively smaller than common literature. [24]

The highest titer of anti-A and anti-B IgG in this study was 1:8. This is smaller than in the study by Kadri; which stated highest titer of 1:32. High titer IgG in neonates had positive correlation with high maternal IgG titer, and numbers of mothers with high titer of IgG was higher in groups of mother with placenta inflammation.[16] In this study, the presence of intralabour infection signs was regarded as an exclusion criteria, therefore resulting in a lower titer of anti-A and anti-B IgG.

Term neonates with ABO incompatibility had 2.9-4.2 times higher risk to suffer from hyperbilirubinemia on the first day of life.[8,9] In this study, hyperbilirubinemia in the first 24 hours of life occurred in 44% of cases, and hemolytic disease in 41% cases. This incidence is higher than in Iskandar's study[9] which reported an incidence of 37.5% cases (15 out of 40 neonates), and Sarici[17] with 21.3% cases (29 out of 136 neonates). Cantayuda[22] reported 23 (53.5%) that 43 neonates had suffered from ABO-HDN. This difference was probably due to the different diagnosis criteria used.

In this study, neonates with positive result of DAT had a 2.6 times higher risk to suffer from ABO-HDN, meanwhile neonates with positive result of DAT using elution method had 3.6 times a higher risk. Maissels[25] stated that neonates from ABO incompatibility pregnancy with positive DAT had a 2 times higher risk to suffer from moderate hyperbilirubinemia (total bilirubin >13 mg/dL) compared to those without ABO incompatibility.

Neonates with positive DAT also had 2.5 times higher risk to suffer from hyperbilirubinemia, meanwhile those with positive DAT using elution method had 3.2 times higher risk to suffer from hyperbilirubinemia. Quinn *et al*[20] stated that positive DAT with or without elution method is useful in predicting the occurence of jaundice, but is not able to predict the severity of jaundice.

This study was designed as a cross-sectional descriptive study, but with some limitations in assessing correlation between variables. By adjusting the power of the study, analysis on some variables was done with this limited data. We suggest further analytical studies with higher numbers of samples and using control to assess corelation between several parameters related to incidence of ABO-HDN.

In conclusion, term neonates with ABO incompatibility in this study had highest titer of anti-A and anti-B IgG 1:8, positive result of DAT in 13.2% cases, and positive result of DAT with elution method in 58.8% cases. The incidence of ABO-HDN in this study was 41%. DAT with elution method is better than DAT in assessing the risk of hyperbilirubinemia and ABO-HDN.

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