Original Article

Association of Maternal ABO IgG Antibodies with Neonatal Jaundice due to ABO Incompatibility at Siriraj Hospital

Duangporn Sungnoon[1], Viroje Chongkolwattana[1], Sasijit Vejbaesya[1] and Kleebsbai Sanpakit[2]

[1]Department of Transfusion Medicine; [2]Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University

Abstract:

Objective: *To study the association between maternal ABO immunoglobulin G (ABO IgG) antibody titer and neonatal hyperbilirubinemia, neonatal direct antiglobulin test (DAT) results, the detection of ABO IgG antibodies in elution and neonate sera, and the implications for treatment of the neonate.* ***Materials and Methods:*** *Samples were collected from 240 couples with ABO blood group maternal-fetal incompatibility, with maternal indirect antiglobulin test (IAT) negative, and neonatal bilirubin level ≥ 3 mg/dL. Newborn samples were tested using DAT, IAT, and elution. Maternal samples were examined for ABO IgG antibodies using dithiothreitol (DTT) testing to distinguish IgM from IgG antibodies, and antibody titration.* ***Results:*** *There was a statistically significant association between maternal ABO IgG antibody titer and bilirubin level, neonatal DAT, ABO IgG antibody in newborn sera and ABO IgG antibody in eluate. The correlation coefficient r = 0.384, 0.336, 0.384 and 0.176 (p < 0.001), respectively. In negative DAT samples, 58.9% of Lui freeze-thaw elution test were positive.*

Conclusion: *The association between maternal ABO IgG antibody level and neonatal jaundice was statistically significant. In the routine care of these newborns, the blood bank should add testing for maternal ABO IgG antibody titer and elution testing of newborn serum for all ABO-incompatible groups to advise clinical decision-making. The early detection and treatment of neonatal hyperbilirubinemia is critical to prevent bilirubin-induced encephalopathy.*

Keywords : ● *ABO HDFN* ● *ABO IgG antibodies* ● *Neonatal jaundice* ● *Hyperbilirubinemia*

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Introduction

Hemolytic disease of the fetus and newborn (HDFN) occurs when maternal IgG antibody crosses the placenta to the fetus and coats fetal red cells. It may be harmful to the fetus with corresponding red cell antigen. Fetal red cells that are sensitized by maternal IgG antibody cannot continue to circulate and do their function; they are destroyed by the reticuloendothelial system. Bilirubin, the waste product of hemoglobin (as a result of red-cell destruction), is transported across the placenta for excretion by the mother. In the most severe cases of HDFN, hydrops fetalis may result in intrauterine death and stillbirth. Blood banks and transfusion services play critical roles in supporting the diagnosis and treatment of these conditions.[1-3] In addition to the Rh blood group system, several other blood group systems can cause

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Requests for reprints should be addressed to Viroje Chongkolwattana, Department of Transfusion Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkoknoi, Bangkok 10700, Tel. +66-2-419-8081, 081-636-0532 E-mail: sivjk@mahidol.ac.th

HDFN. The most common HDFN are caused by the ABO and Rh systems, followed by the Kell, Kidd, and Duffy blood group systems.[3]

Only antibodies of the immunoglobulin G (IgG) class transport across the placenta; most IgG antibodies are directed against paternal antigens on the fetal red blood cells. Before birth, the fetal bilirubin is processed by the maternal liver. In newborn, the liver is incapable of conjugating the amount of bilirubin that results from destruction of antibody-coated red blood cells. Unconjugated bilirubin is toxic to the developing central nervous system (CNS), causing brain damage referred to as "kernicterus".[4] In all but ABO HDFN, maternal antibodies reflect alloimmunization by pregnancy or transfusion. However, ABO antigens are not fully developed until after the first year of life. ABO feto-maternal incompatibility cannot be diagnosed during pregnancy and newborns are rarely symptomatic at birth. The severity of the disease is independent in the presence of a positive direct antiglobulin test (DAT) result or demonstrable anti-A, anti-B, or anti-A,B in the eluate of the infant's red blood cells. In ABO-HDFN, it is often possible to elute anti-A and/or anti-B from infant red cells, despite a negative DAT.[5]

ABO-HDFN is complex because anti-A and anti-B antibodies are mainly IgM. Since only IgG antibodies cross the placenta, pregnant women with high levels of IgG anti-A, anti-B, or anti-A,B, with ABO-incompatible fetuses will give birth to infants with ABO-HDFN.[6] However, neonatal jaundice generally develops a few days after birth and the degree of jaundice is depended on many factors, including the strength of maternal IgG antibody. The disease usually requires treatment, such as phototherapy and/or the administration of immunoglobulin, but in severe cases, exchange transfusion may be needed to reduce hyperbilirubinemia.

ABO incompatibility is now the most common cause of HDFN among different populations.[7,8] It occurrs in 20–25% of pregnancies and infants at risk are those with blood group A or B, born to mothers with O blood type. In Thai populations, ABO HDFN was found in 63.9%.[9] Maternal IgG anti-A or anti-B titer measurements are not routinely performed for the assessment the risk of HDFN. However, the presence of these maternal IgG antibodies is helpful in a diagnosis of ABO-HDFN[10] and may identify newborns at risk. In addition, IgG anti-A or anti-B titer levels ≥ 512 have also been suggested as a risk factor for hyperbilirubinemia.[5]

Materials and Methods

A total of 240 paired mothers and newborns with ABO-HDFN defined as negative maternal indirect antiglobulin, neonatal bilirubin levels ≥ 3 mg/dL and neonatal age ≤ 7 days, were collected and transferred to the Department of Transfusion Medicine, Siriraj Hospital. The newborn samples were tested for DAT, IAT, and elution tests. The maternal serum samples were tested for ABO IgG antibodies using dithiothreitol (DTT) to distinguish IgM from IgG antibodies, and antibody titration.

Descriptive statistics were used to describe the variables; mean and standard deviation (SD) for continuous variables; percentage for categorical variables and the association between maternal ABO IgG antibodies and neonatal bilirubin levels were analyzed by Pearson correlation coefficient. All tests were two-sided and p-values of less than 0.05 were considered statistically significant. The analysis was performed using SPSS for windows (version 18.0 SPSS Inc., Chicago, IL, USA). The study was approved by the Ethics Committee of the Faculty of Medicine Siriraj Hospital, Mahidol University (No 244/255 EC1).

Results

Of the 240 paired mother and newborn samples, the mean maternal age was 32 years with age ranging from 14 to 48 years and the gestational mean was 37.71 ± 1.53 weeks. For the ABO group of mother, the most common was group O 71.7% followed by group A 15.0% and group B 13.3%, respectively.

For the newborn, there were group B 55.0%, group A 30.4%, and 14.6% of newborns were in blood groups AB; 44.6% of newborns were female and 54.4% male. Most newborns were 2 days old (range: 1-7 days). The mean bilirubin level was 13.33 mg/dL (SD 2.66). One hundred and sixty four (68.33%) neonates were treated with phototherapy during day 1 to day 6 and most of them required 2 days for phototherapy. Hospital stay was varied from 3 to 8 days. None of them needed an exchange transfusion (Table 1).

When the ABO titer was more than 1:2,048, about 73% of newborns had bilirubin levels > 15 mg/dL. There was a statistically significant relationship between maternal ABO IgG antibody titer and bilirubin level ($r = 0.384$, $p < 0.001$). The correlation was higher in maternal group O ($r = 0.472$, $p < 0.001$), but no significant association was found in maternal non-group O ($r = 0.217$, $p = 0.125$). Of 240 neonates, only one had a bilirubin level > 20 mg/dL; this neonate was born to a mother with a maternal ABO antibody titer of 1:4,096. In the two groups with maternal ABO antibody titers < 1:2,048 and > 1:2,048 ; 48 (21.4%) and 18 (69.2%) neonates had bilirubin levels of 15-20 mg/dL (Table 2).

Table 3 shows the results of the neonate DAT tests and the ABO IgG antibodies in newborn sera and eluate; 33 cases (13.7%) had positive DAT results and 207 (86.3%) were negative. In the negative DAT samples, 122 cases (58.9%) were positive and 85 cases (41.1%) were negative by Lui freeze-thaw elution test. Moreover, IgG anti-A and anti-B inneonate serum were detected in this group, 86 cases (41.5%) were positive by IAT and 121 cases (58.5%) were negative. Both Lui-freeze-thaw elution test and IAT for anti-A, anti-B, were negative in 66 cases (31.9%); most of these patients 52 cases (78%) were non-group O mothers of whom 141 (68.1%) were positive. In the positive DAT samples, 25 cases (75.8%) of Lui freeze-thaw elution tests were positive and 8 (24.2%) negative. The IgG anti-A, anti-B titer were detected in this group; 31 cases (93.0%) were positive by IAT and 2 cases (6.1%) were negative. Both Lui freeze-thaw

Table 1 Demographic data of 240 paired mothers and newborns with ABO-HDFN

	Mother	**Newborn**
N	240	240
Mean age (range)	32 (14-48) years	2 (1-7) days
ABO blood group ; n (%)		
A	36 (15.0)	73 (30.4)
B	32 (13.3)	132 (55.2)
O	172 (71.1)	-
AB	-	35 (14.6)
Gestation age (weeks)	37.71 ± 1.5	
Gender ; n (%)		
Male ; n (%)		133 (54.4)
Female ; n (%)		107 (44.6)
Birth weight (g) mean ± SD		2908.80 ± 498.3
Bilirubin mean (min-max) mg/dL		13.33 (4.8-23.1)
Treatment		
No treatment; n (%)		76 (31.7)
Phototherapy; n (%)		164 (68.3)
Period of phototherapy days ; (range)		2 (1-6)
Length of stay days ; (range)		5 (3-8)

Table 2 Association between maternal ABO IgG antibody titer and neonatal bilirubin level (n = 240)

Maternal ABO IgG antibody titer*	Neonatal bilirubin level mg/dL n (%)				
	< 10	10.1-15	15.1-20	> 20	Total
1:4	2 (33.3)	1 (16.7)	3 (50.0)	0 (0)	6
1:8	4 (30.8)	4 (30.8)	5 (38.5)	0 (0)	13
1:16	4 (23.5)	10 (58.8)	3 (17.6)	0 (0)	17
1:32	2 (14.3)	10 (21.4)	2 (14.3)	0 (0)	14
1:64	0 (0)	22 (84.6)	4 (15.3)	0 (0)	26
1:128	2 (4.6)	33 (76.7)	8 (18.5)	0 (0)	43
1:256	3 (9.4)	24 (75.0)	5 (15.6)	0 (0)	32
1:512	5 (13.9)	27 (75.0)	4 (11.1)	0 (0)	36
1:1,024	1 (3.7)	15 (58.6)	11 (40.7)	0 (0)	27
1:2,048	0 (0)	6 (33.3)	12 (66.7)	0 (0)	18
1:4,096	0 (0)	1 (12.5)	6 (75.0)	1 (12.5)	8

r = 0.384 p-value < 0. 001 * IgG anti-A or IgG anti-B

Table 3 Summary of DAT, ABO IgG antibody in newborn serum and newborn eluate

DAT	Serum n (%)		Eluate n (%)		Eluate and Serum n (%)	
	Negative	Positive	Negative	Positive	Negative	Positive
Negative	121 (58.5)	86 (41.5)	85 (41.1)	122 (58.9)	66 (31.9)	141 (68.1)
Positive	2 (6.1)	31 (93.9)	8 (24.2)	25 (75.8)	1 (3.0)	32 (97.0)

Eluate = IgG antibody in newborn eluated by Lui freeze-thaw elution test

Serum = IgG antibody in newborn serum by IAT for anti-A, anti-B

elution test and IAT for anti-A, anti-B were negative for 1 case (3.0%) and positive for 32 cases (97.0%).

The data showed a relationship between maternal ABO antibody titer and newborn DAT. Even when maternal titer was high (1:2,048), DAT was undetectable in some newborns. Statistical analysis found a significant relationship between maternal ABO IgG antibody titer and neonatal DAT (r = 0.336, p-value < 0.001). Additionally, a relationship between maternal ABO antibody titer and ABO IgG antibody titer in newborn sera was demonstrated. There were significantly higher positive IgG antibody titer results in newborn sera when maternal ABO antibody titer were high (r = 0.384, p-value < 0.001). A weak association was found between maternal ABO IgG antibody titer and ABO IgG antibody titer in eluate (r = 0.176, p-value = 0.006). Of the neonates with maternal antibody titer levels > 2,048, 21 cases (80%) needed phototherapy, but there was no significant association between maternal ABO IgG antibody titer and treatment (r = 0.183, p-value = 0.198) (Table 4).

Discussion

ABO incompatibility is a commonly occurred in 20-25% of pregnancies. Infants with blood groups A or anti-B, born to mothers with type O blood, are at risk.[4] ABO-HDFN occurs almost exclusively among neonates of group O mothers and high antenatal IgG anti-A or anti-B titer. Hemolysis due to anti-A is found more often among Western populations than anti-B.[5] Several studies have established that ABO HDFN is more common among Africans and African Americans, and neonate of mixed racial origin, than Caucasian infants.[4] In contrast to anti-D antibodies, anti-A and anti-B antibodies cause hyperbilirubinemia in most cases without severe neonatal anemia, explained by

Table 4 Association between maternal ABO IgG antibody titer and newborn DAT, ABO IgG antibody in newborn serum, eluate, and phototherapy

Maternal ABO IgG antibody titer*	ABO IgG antibody in							
	Newborn DAT(n)		Newborn serum (n)		Newborn eluate (n)		Phototherapy (n)	
	Negative	Positive	Negative	Positive	Negative	Positive	Yes	No
1:4	6	0	5	1	5	1	4	2
1:8	12	1	12	1	4	9	8	5
1:16	17	0	12	5	10	7	12	5
1:32	13	1	10	4	4	10	9	5
1:64	21	5	13	13	12	14	17	9
1:128	36	7	23	20	20	23	27	16
1:256	27	5	7	25	8	24	23	9
1:512	31	5	19	17	14	22	25	11
1:1,024	25	2	14	13	10	17	18	9
1:2,048	14	4	6	12	4	14	13	5
1:4,096	5	3	2	6	2	6	8	0
Total	**207**	**33**	**123**	**117**	**93**	**147**	**164**	**76**

* IgG anti-A or IgG anti-B

the relatively few group-A or -B antigens on neonatal red blood cells, and the presence of A and B antigens on cells of other tissues and in body fluids.[11] Besides anti-D antibody, ABO incompatibility and other alloantibodies have reportedly caused severe HDFN among Asian countries. Reports from Sri Lanka, Bangladesh, and India have described unusually severe ABO-HDFN, requiring multiple exchange transfusions.[12] In a study from Saudi Arabia, nearly one third of infants with ABO incompatibility required exchange transfusions and the use of intravenous immunoglobulin (IVIG) in such cases reduced the need for exchange transfusion,[13] similar to a case of RhD-HDFN in Iran.[14] An unusual report also described severe ABO-HDFN incompatibility in a Bangladesh group B Rh positive mother with an A_1B infant.[15]

In this study, we found the prevalence of ABO-HDFN in maternal blood group O was 71.7%. Samung et al. showed ABO-HDFN rates in maternal blood group O and non-group O, of 63.9% and 36.1%, respectively.[9] Both groups showed mild hemolytic disease; however, most newborns were not anemic and showed minimal hemolysis. Apart from early phototherapy, these cases require no transfusion; however, these newborns are at risk of developing severe, late anemia by 3-6 weeks of life. In this study, 68.0% of newborns needed phototherapy. Neonates of group O mothers (67.6%) needed similar treatment to non-group O mothers (68.2%). The length of stay for neonates of group O mothers did not differ from non-group O mothers. The length of stay for most newborns (31.5%) was 5 days (group O mothers = 32.7%; non-group O mothers = 24.9%).

The severity of HDFN is associated with maternal antibody titer and number of antigen sites on the surface of newborn red blood cells. ABO-HDFN can be diagnosed by testing ABO grouping, Rh typing (both maternal and newborn) and antibody screening and identification in the mother, especially for IgG antibody, which can cross the placenta to the fetus. DAT and IAT tests should be used, since both are sensitive. Most ABO-HDFN cases give negative or weakly positive DAT results. In 207 DAT negative cases, ABO IgG antibodies were found in 122 eluates and in 86 sera. Because there are relatively few group A or

B antigens on neonatal red blood cells and the level of IgG anti-A and anti-B are low in titer.

The association between maternal ABO IgG antibody titer and level of neonatal hyperbilirubinemia was statistically significant ($r = 0.384$, $p < 0.001$), similar to previous study by Bakkeheim, et al. ($r = 0.47$, p-value < 0.0001).[16]

In this study, DAT yielded 13.75% positive and 86.25% negative results while Chanachaisuwan, et al. study found that 42 (9.2%) infants were born to group O mothers, 8 infants had positive DAT and 34 infants had negative DAT. Anti-A and/or anti-B could be eluted from 31 infants (73.8%). They concluded that the elution test is often more useful than DAT in assessing ABO HDFN.[17] Cheepsattayakorn, et al. reported that a positive DAT was 36.67% and an IAT for ABO IgG antibodies were 66.67%.[18] Asian and African infants have higher prevalence of positive DAT tests than Caucasians.[19] Ratanasirivanich et al. reported that positive DAT tests were higher prevalence among Thai neonates and Caucasian neonates. Antibodies in the ABO system were the most common cause of DAT positivity in both ethnic groups. In this study, statistical analysis revealed a significant relationship between maternal ABO IgG antibody titer and neonatal DAT grading ($r = 0.308$, p-value < 0.001). Concerning these two data, high maternal ABO titer was strongly associated with newborn positive DAT. When DAT was 2+, maternal ABO IgG antibody titer was likely to be > 1:128. In the routine laboratory, we recommend performance of the elution test for newborn samples for ABO incompatibility.

Regarding the relationship between maternal ABO antibody titer and ABO IgG antibody in newborn serum, a significantly higher positive result for IgG antibody was found in newborn serum (correlation coefficient = 0.384, p-value < 0.001). We found 48.7% positive by IAT for ABO IgG antibody in newborn serum, which was lower than the 66.67% found in the study by Cheepsattayakorn et al.[18] However, the presence of these IgG antibodies supports a diagnosis of ABO-HDFN. An ABO IgG antibody titer in newborn sera of > 512 by IAT should indicate ABO-HDFN.[20] Incompatible newborn infants with maternal IgG anti-A or anti-B titer ≥ 512, bilirubin level ≥ 4 mg/dL or positive direct Coombs' cord blood test indicate a "high risk" category, who should be hospitalized for frequent evaluation and appropriate therapy.[5]

Conclusion

A correlation was found between maternal ABO IgG antibody titer and level of neonatal hyperbilirubinemia, neonatal DAT, detection of ABO IgG antibody in elution and neonate sera. In the routine case of these newborns, blood banks and transfusion services should include testing for maternal ABO IgG antibody titer and neonatal red blood cell elution, to inform clinical decision-making, together with other laboratory data, i.e. neonatal DAT, and indirect antiglobulin testing for free anti-A, anti-B in neonate serum. Early detection and treatment of neonatal hyperbilirubinemia is critical to prevent bilirubin-induced encephalopathy. The results of this study will be helpful for pediatricians to provide better care and treatment for newborns with ABO-HDFN.

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ความสัมพันธ์ของ ABO IgG Antibodies ของแม่กับภาวะตัวเหลืองในทารกแรกคลอดอันเนื่องมาจากหมู่เลือด ABO ไม่ตรงกันในโรงพยาบาลศิริราช

ดวงพร สังข์นุ่น[1] วิโรจน์ จงกลวัฒนา[1] ศศิจิต เวชแพศย์[1] และ กลีบสไบ สรรพกิจ[2]

[1]*ภาควิชาเวชศาสตร์การธนาคารเลือด* [2]*ภาควิชากุมารเวชศาสตร์ คณะแพทยศาสตร์ ศิริราชพยาบาล มหาวิทยาลัยมหิดล*

บทคัดย่อ

วัตถุประสงค์ *เพื่อศึกษาความสัมพันธ์ระหว่างความแรงของ ABO IgG แอนติบอดีในซีรั่มของแม่ที่มีหมู่โลหิตเข้ากันไม่ได้กับทารกกับอาการเหลืองของทารกโดยวัดจากระดับบิลลิรูบิน DAT ของทารก การตรวจพบ ABO IgG บนผิวเม็ดเลือดแดงและในซีรั่มของทารก รวมถึงการให้การรักษาแก่ทารก* ***วิธีการศึกษา*** *เก็บตัวอย่างเลือดมารดาและทารกจำนวน 240 ราย ที่มีหมู่เลือด ABO ไม่ตรงกัน โดยมารดาไม่มีแอนติบอดีอื่นนอกเหนือจากระบบ ABO ทารกมีระดับบิลลิรูบินเท่ากับหรือสูงกว่า 3 mg/dL ตัวอย่างเลือดทารกตรวจ direct antiglobulin test (DAT), indirect antiglobulin test (IAT) และ elution ตัวอย่างเลือดมารดาตรวจ ABO IgG แอนติบอดีโดยใช้ dithiothreitol (DTT) แยก IgM ออกจาก IgG จากนั้นทดสอบความแรงของแอนติบอดีโดยวิธี titration* ***ผลการศึกษา*** *มีความสัมพันธ์อย่างมีนัยสำคัญทางสถิติระหว่างความแรงของ ABO IgG แอนติบอดีของมารดากับระดับบิลลิรูบินของทารก DAT ของทารก การตรวจพบ ABO IgGแอนติบอดีใน ซีรั่มและอีลูชั่นของทารก โดยได้ค่า r เท่ากับ 0.384, 0.336, 0.384 และ 0.176 ($p < 0.001$) ตามลำดับ นอกจากนี้ยังพบว่าทารกที่ DAT ให้ผลลบแต่ให้ผลบวกใน eluate พบ ABO IgG แอนติบอดีถึงร้อยละ 58.9* ***สรุป*** *การตรวจพบและให้การรักษาอาการ hyperbilirubinemia ในทารกแรกเกิดเป็นสิ่งสำคัญในการป้องกันการเกิด bilirubin encephalopathy จากข้อมูลที่ได้นี้ จะสามารถใช้เป็นความรู้พื้นฐานเพื่อทำความเข้าใจความสัมพันธ์ระหว่างอาการและแนวทางการวินิจฉัยผู้ป่วย ABO hemolytic disease of the fetus and newborn (HDFN) ได้อย่างถูกต้อง โดยผลจากการศึกษานี้จะช่วยกุมารแพทย์ในการดูแล และรักษาทารกที่เป็นABO HDFN ได้ดียิ่งขึ้น*

Keywords : ● *ABO HDFN* ● *ABO IgG antibodies* ● *Neonatal jaundice* ● *Hyperbilirubinemia*

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