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ORIGINAL ARTICLE

Clinical and Laboratory Characteristics of ABO-Incompatible Hemolytic Disease of the Fetus and Newborn

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SUMMARY

Background: **ABO incompatibility is the most common cause of hemolytic disease of the fetus and newborn (HDFN). We investigated HDFN at our institute and discuss clinical characteristics and considerations during transfusions and laboratory testing.**
Methods: **We reviewed the medical records of newborns with HDFN due to ABO incompatibility over a period of 5 years. Laboratory results such as the ABO blood type of mothers and newborns, direct antiglobulin test, hemoglobin, and total bilirubin were collected. History of transfusion and phototherapy were also taken.**
Results: **During the 5 years, 275 newborns were diagnosed with HDFN by ABO blood type testing and cross-matching. Group O mothers were predominant, with 259 newborns, followed by B and A types, with 11 and five newborns, respectively. For the newborns, group A was the most common, with 151, followed by group B with 108 and AB with 16 newborns. The most common type of incompatibility was O/A, accounting for 54.9%, followed by O/B, B/AB, and A/AB at 54.9%, 4.0%, and 1.8%, respectively. DAT was conducted on only half of the group O mothers, and among them, 38.5% had positive results. For the 16 newborns of non-O mothers, six underwent DAT, and all were negative. Further, 21.6% and 31.3% of newborns from group O and non-group O mothers received transfusions, and 49.4% and 43.8% received phototherapy, respectively.**
Conclusions: **Our findings highlight the importance of considering ABO HDFN even in DAT-negative neonates. Reverse typing may provide important diagnostic value, especially in transfusion settings with ABO compatibility with the maternal blood group.**
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INTRODUCTION

Hemolytic disease of the fetus and newborn (HDFN) occurs when maternal immunoglobulin G (IgG) antibodies cross the placenta and target fetal or neonatal red blood cells, leading to hemolysis. With the widespread use of Rh immunoglobulin prophylaxis, ABO incompatibility has become the most common cause of HDFN. The incidence of ABO HDFN varies by ethnic-

ity or region, with estimates ranging from 1% to 4%, as the distribution of maternal ABO groups differs among populations [1].

Diagnosis of ABO HDFN is usually based on evidence of maternal-fetal/neonatal ABO incompatibility, hyperbilirubinemia, and antibody-mediated hemolysis, often confirmed by a positive direct antiglobulin test (DAT) or serology [2]. However, due to the lack of a clear gold standard for diagnosis, overdiagnosis or underdiagnosis may occur [3,4].

Reverse typing, which detects anti-A or anti-B antibodies in neonatal serum, is typically omitted in neonatal blood group testing due to the immaturity of the neonatal immune system [1]. The American Association of Blood Banks (AABB) Standards and Korean Transfusion Guidelines both recommend using only front typing with anti-A/B reagents for ABO group determination in neonates under 4 months of age [5,6]. However, considering that maternal IgG antibodies are passively transferred across the placenta and are responsible for ABO HDFN, reverse typing may provide additional diagnostic value in identifying ABO antibody-mediated hemolysis in neonates.

No previous studies in Korea have specifically investigated ABO HDFN, except those that included ABO incompatibility as one of the causes of HDFN, such as Rh or other minor blood group incompatibilities [7,8].

In this study, we reviewed neonates who were diagnosed with ABO HDFN at a single tertiary medical center over the past 5 years. We aimed to investigate the clinical characteristics of ABO HDFN and evaluate the need for reverse typing for newborns.

MATERIALS AND METHODS

This was a retrospective study covering 5 years, from January 2019 through December 2023, at the Asan Medical Center, Seoul, Korea. Newborns who were diagnosed with ABO HDFN were included.

According to our laboratory's standard protocol, when a discrepancy is observed between front and back typing in a newborn's ABO blood group test, a retest is conducted, and the blood group is confirmed by a laboratory physician. If the newborn's ABO group is incompatible with the mother's, crossmatching is performed using the newborn's serum and the mother's test cells. ABO-HDFN is defined by any positive crossmatching result, including trace positivity. When ABO-HDFN is confirmed, an incompatible transfusion is registered in the electronic medical record. The blood group selection for transfusion in newborns with HDFN is summarized in Table 1.

The data we collected included the mother's ABO/Rh blood type, the newborn's ABO blood typing results, including front and back typing, crossmatching between the newborn and mother's blood groups, direct antiglobulin test (DAT), total bilirubin, and hemoglobin (Hb). Clinical information such as gender, age, transfusion history, and phototherapy treatment was also collected.

RESULTS

Within 5 years, from 2019 through 2023, a total of 273 newborns were registered with ABO HDFN, a total of 151 males (55.3%) and 122 females (44.7%). The age at registration ranged from 1 to 16 days, with a median age of 2 days. The gestational age averaged 36 + 2 weeks, ranging from 22 + 6 weeks to 40 + 3 weeks.

Regarding the mother's blood type, type O was the most common, with 258 (94.5%) mothers, and their newborns having type A 150 and type B 108, respectively; 10 mothers had type B and five mothers had type A, respectively, and all of their newborns were in group AB (Table 2).

The DAT was performed in 241 out of 273 newborns. Among the tested newborns, 175 (72.6%) had negative results, representing 64.1% of all newborns. All 66 DAT-positive newborns were born to type O mothers, showing a trace of weak positive reactions. Newborns of mothers with non-O blood types were all DAT-negative (Table 3).

The clinical characteristics of newborns showed that those of blood group O mothers had a mean hemoglobin level of 16.2 g/dL (range: 8.8 - 23.1) and a mean total bilirubin level of 6.3 mg/dL (range: 0.2 - 23.4). Newborns of non-O blood group mothers had a mean hemoglobin level of 16.8 g/dL (range: 14.4 - 20.6) and a mean total bilirubin level of 5.2 mg/dL (range: 3.4 - 9.2). During hospitalization, 57 newborns (20.9%) received red blood cell (RBC) transfusions, including 53 from type O mothers and four from non-O mothers. For jaundice treatment, 134 newborns (49.1%) underwent phototherapy, including 128 newborns of type O mothers and six newborns of non-O mothers (Table 4).

Approximately half of the newborns showed trace levels in crossmatching results. Among the grades ranging from 1+ to 4+, only three newborns showed 3+ positivity, while all others were 2+ or lower, and none showed 4+. Stronger crossmatching positivity was observed in newborns involving group O mothers compared to non-O group mothers (Table 5).

DISCUSSION

This study analyzed the clinical characteristics of neonates diagnosed with ABO HDFN and assessed the necessity of reverse typing in neonatal ABO blood group testing. Over the past 5 years, 273 neonates were diagnosed with ABO HDFN, and the majority (94.5%) were born to group O mothers. This finding aligns with previous studies, which report that group O mothers are more likely to have IgG subclass anti-A and anti-B antibodies compared to non-group O mothers [9].

Among the 241 neonates who underwent DAT testing,

Table 1. Blood group selection for transfusion in hemolytic disease of the fetus and newborn.

Blood group		Blood group selection	
Mother	Newborn	RBC	Platelet/FFP
O	A	O	A
	B	O	B
A	AB	A	AB
B	AB	B	AB

RBC red blood cell, FFP fresh frozen plasma.

Table 2. Blood group of mothers and newborns with hemolytic disease of the fetus and newborn.

		Newborn			
		A	B	AB	Subtotal
Mother	O	150 (54.9%)	108 (39.6%)	0	258
	A	NA	0	5 (1.8%)	5
	B	0	NA	10 (3.7%)	10
	Subtotal	150	108	15	273

NA not available.

Table 3. Results of direct antiglobulin test.

Blood group		DAT results		Not tested	Subtotal
Mother	Newborn	Positive [a]	Negative		
O	A	42 (28.0%)	90 (60.0%)	18 (12.0%)	150
	B	24 (22.2%)	70 (64.8%)	14 (13.0%)	108
Non-O	AB	0	15 (100.0%)	0	15
Total		66 (24.2%)	175 (64.1%)	32 (11.7%)	273

[a] Positive results include trace.
DAT direct antiglobulin test.

Table 4. Clinical characteristics of newborns of each blood group of their mothers.

Mother's blood group	Hemoglobin (g/dL) [a]	Total bilirubin (mg/dL) [a]	RBC transfusion [b]	Phototherapy [b]
O	16.2 (8.8 - 23.1)	6.3 (0.2 - 23.4)	53 (20.5%)	128 (49.6%)
Non-O	16.8 (14.4 - 20.6)	5.2 (3.4 - 9.2)	4 (26.7%)	6 (40.0%)
Total	16.2 (8.8 - 23.1)	6.3 (0.2 - 23.4)	57 (20.9%)	134 (49.1%)

[a] Mean (range).
[b] Number of newborns who underwent RBC transfusion or phototherapy (percentage among same blood group mothers).

Table 5. Crossmatch results[a] of newborns' serum and newborns' blood group red blood cells.

Mother's blood group	Trace	1+	2+	3+	4+
O	123	103	19	3	0
Non-O	11	4	0	0	0
Subtotal	134 (51.0%)	107 (40.7%)	19 (7.2%)	3 (1.1%)	0 (0.0%)

[a] **All crossmatching results were positive. However, 10 newborns recorded as "positive" without a specific grade notation were excluded from this table.**

175 (72.6%) newborns were DAT-negative, consistent with previous reports showing high negative rates exceeding 70% [10-12]. Given this high proportion of negative results, our findings further support the argument that DAT alone should not be used as a screening tool for ABO HDFN.

Regarding crossmatching, positive results were classified into five grades (trace and +1 to +4). All neonates underwent crossmatching, and except for 10 newborns recorded as "positive" without notation of any grade, 263 newborns had documented positivity grades. Most results were weakly positive, with 51.0% of newborns classified as trace and no newborns observed at 4+ grade. The likely reason for the weak crossmatching results could be that the antibodies were already diluted in the neonatal circulation or already bound to RBCs. However, additional tests were not conducted to investigate this further.

ABO HDFN is generally self-limiting and rarely leads to severe clinical manifestations due to the underdevelopment of fetal ABO antigens and the neutralization of isohemagglutinins by tissue and soluble antigens [1,9]. However, in our study, 20.0% required RBC transfusions, and 49.1% required phototherapy. Since group O individuals have a higher proportion of IgG among the ABO antibody subclass compared to non-group O individuals, they were expected to have less favorable laboratory findings. Additionally, a previous study suggested that DAT-negative neonates with ABO HDFN are at a lower risk of developing hyperbilirubinemia requiring phototherapy [11]. In our study, all neonates born to non-group O mothers were DAT-negative, whereas only approximately 70% of neonates born to group O mothers were DAT-negative. This difference in DAT positivity rates may also be relevant when considering the risk of hyperbilirubinemia and the need for phototherapy. However, due to the small number of non-group O mothers and the lack of data on maternal and neonatal antibody titers, further investigation and statistical analysis were not feasible.

While HDFN caused by various unexpected antibodies has been reported in South Korea, studies specifically focused on ABO HDFN remain limited. In 1998, Song et al. conducted a retrospective analysis on 79 newborns with HDFN, out of which 20 newborns (25.3%) were due to ABO incompatibility [8]. In 2019, Shin et al. investigated HDFN, specifically in patients who underwent DAT, and observed that 28.4% (86 newborns) of the 303 neonates had ABO HDFN [7]. Although these previous studies were not exclusively focused on ABO HDFN, their findings align with our results in two key aspects. First, most neonates diagnosed with ABO HDFN were born to group O mothers, consistent with our study's finding that 94.5% of neonates involved group O mothers. Second, the high rate of negative DAT results in newborns with ABO HDFN was reported in these studies, with Shin et al. documenting an 80.2% DAT-negative rate among neonates with ABO HDFN. This aligns with our finding that 72.6% of neonates who underwent DAT testing were DAT-negative, reinforcing the notion that DAT alone is insufficient as a screening tool for ABO HDFN.

Despite its potential diagnostic value, reverse typing is often omitted in neonatal blood group testing due to the immaturity of the neonatal immune system [1]. The AABB standards specify that front typing using only anti-A/B reagents is sufficient for determining the ABO blood group in neonates. However, for a pre-transfusion test, assessing anti-A/B antibodies in the newborns' serum or plasma is required, but only when a non-group O newborn is to receive non-group O red blood cells that are incompatible with the maternal ABO group [5]. A survey by Crowe et al. reported that 82.1% of healthcare institutions in North America exclusively transfuse group O RBCs to neonates, regardless of their blood type, and thus do not perform reverse typing [13]. Similarly, Korean transfusion guidelines permit ABO front typing alone in neonates under 4 months, with no requirement for serological testing [6]. This policy is reflected in the national health insurance system. However, unlike the AABB standards, there are no provisions regarding testing anti-A/B antibodies in newborns' serum or plasma as part of pre-transfusion testing. In other words, Korean guidelines do not include any recommendations for reverse typing, even in specific transfusion scenarios. This could pose a problem in a newborn whose blood group is incompatible with the mother's ABO group and requires an RBC transfusion. Therefore, we recommend performing reverse typing as a part of routine ABO blood group testing or at least pre-transfusion testing for neonates. If the neonates have incompatible ABO antibodies for their front typing

results, crossmatching should be proactively conducted using RBC reagents corresponding to the maternal ABO group. This approach will help determine whether HDFN is present and guide the selection of compatible RBCs for transfusion (as shown in Table 1). Without reverse typing, there is a risk of missing the cause of incompatibility, even when crossmatching continues with RBC products based solely on the ABO blood group determined by front typing. This can cause confusion for the laboratory staff and may lead to delays in transfusion or hemolysis.

In conclusion, reverse typing for neonates should be performed more actively, especially when transfusion is necessary. Reverse typing should be included in transfusion guidelines and insurance policies when revising them to ensure the diagnosis of ABO HDFN and safe transfusions. Furthermore, medical staff and caregivers should be well informed to avoid confusion regarding incompatible RBC transfusions.

Declaration of Interest:

The authors have no conflicts of interest to declare.

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