

# A Case of Mild Hemolytic Disease of the Fetus and Newborn - The First Case of Anti-Di<sup>b</sup> Identified on Prescreening Test during Pregnancy -

Ji Won Choi<sup>1</sup>, Moon Jung Kim<sup>2</sup>, Myung Han Kim<sup>3</sup>, Jung Ran Park<sup>1</sup>, Hyun Ok Kim<sup>1</sup>

*Department of Laboratory Medicine, Yonsei University College of Medicine<sup>1</sup>, Blood Service Headquarters<sup>2</sup>,  
Central Blood Laboratory Center<sup>3</sup>, Korean Red Cross, Seoul, Korea*

The Di(a+b-) phenotype is extremely rare among Caucasians and mostly confined to mongoloids. The clinical significance of anti-Di<sup>b</sup> is incompletely recognized. The authors report here a mild case of hemolytic disease of the fetus and newborn (HDFN) due to anti-Di<sup>b</sup>. The mother was Di(a+b-) with anti-Di<sup>b</sup>, which was detected by antenatal screening. She delivered a Di(a+b+) infant with a positive direct antiglobulin test and mild hemolytic disease. The infant was treated by phototherapy and subsequently recovered without the need for exchange transfusion. We suggest that the screening program as routine prenatal care is necessary. (**Korean J Blood Transfus 2010;21:165-170**)

**Key words:** Anti-Di<sup>b</sup>, Hemolytic disease of a fetus and newborn, Antenatal screening

## Introduction

The Diego blood group mainly consists of 2 independent pairs of antigens, called Di<sup>a</sup>/Di<sup>b</sup> and Wr<sup>a</sup>/Wr<sup>b</sup>.<sup>1)</sup> The Di<sup>a</sup> antigen is very rare in Caucasians, but is relatively common in mongoloid populations, including South American Indians (7~54%), Chinese, Korean, and Japanese (5~8%) populations.<sup>2)</sup> In contrast, the Di<sup>b</sup> antigen is common in all populations and the Di(a+b-) phenotype is almost exclusively found in mongoloid persons with a few other ethnic cases on record. Anti-Di<sup>b</sup> was first discovered in 1967 by Thompson and coworkers while investigating the cause of a delayed hemolytic transfusion reaction. Its clinical significance

is still not entirely known.

There have been about 30 reported cases of hemolytic disease of the fetus and newborn (HDFN) due to anti-Di<sup>b</sup>, mostly in the Japanese population<sup>3)</sup> and three additional cases among Koreans have also been reported.<sup>4-6)</sup> We report a baby born to a Di(a+b-) Korean mother who suffered mild HDFN due to anti-Di<sup>b</sup>, while in the previous 3 cases of similar disease in Korea, anti-Di<sup>b</sup> had been found after HDFN occurred to an infant without antenatal screening for irregular antibodies. In the present study, anti-Di<sup>b</sup> on antenatal screening was observed, enabling the perinatal community occasion to prepare for the potential problem of HDFN and to secure compatible Di(b-) blood. Two units of autologous

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책임저자 : 김 현 옥 120-752 서울시 서대문구 성산로 250 연세대학교 의과대학 진단검사의학교실

TEL: 02) 2228-2444, FAX: 02) 364-1583, E-mail: hyunok1019@yuhs.ac

blood were prepared by mother.

### Case Report

A 33-year-old Korean woman (gravida2, para1) presented in her second pregnancy. Anti-Di<sup>b</sup> was found antenatal through irregular antibody screening on IUP 15 weeks. The mother's serum had a positive indirect antiglobulin test (IAT) (3+) and her red blood cells (RBCs) tested negative with direct antiglobulin test (DAT). Her serum also reacted with the entire panel of RBCs. Because of the suspicion of HDFN due to an antibody against a high-frequency red cell antigen, the mother's red cells antigens were tested for Lu<sup>b</sup>, k and Di<sup>a</sup> which can be the cause of HDFN. Her phenotype was found to be: Lu(b+), k(+), Di(a+). The Di<sup>b</sup> phenotyping was performed using a monoclonal anti-Di<sup>b</sup> antibody from the Japanese Red Cross Blood Center (Osaka, Japan), and Di<sup>b</sup> phenotype was negative. In the 36th week of gestation, her antibody titer came in at 256 with Di(a+b+) RBCs.

A study of the maternal family showed the presenting woman to be Di(a+b-), while her parents, a sister, a brother, and the baby were all Di(a+b+). Di<sup>a</sup> antigen was determined on maternal and familial RBCs by the transfusion research institute, Korean Red Cross (KRC); Di<sup>b</sup> antigen was determined on familial RBCs by cross-matching to the antiglobulin phase of mother's serum. The antibody present in maternal serum did not react with Di(b-), O red cells, thus specifically confirming the maternal serum antibody to be anti-Di<sup>b</sup>.

The presenting woman had a history of Cesarean sections, and, ten years prior, reported having re-

ceived RBC transfusion during such a surgical operation. Her previous newborn was healthy, although suffered some fetal respiratory difficulty at birth. In general, when antenatal patients with irregular antibodies present, their husband should be promptly tested for the presence of relevant antigen. If found, physician should immediately determine the severity of such disease as can be caused by the presence of this antibody. Because frequencies of the Diego blood group phenotype in Koreans are, respectively, 0.25% for Di(a+b-), 9.75% for Di(a+b+), and 90% for Di(a-b+),<sup>7)</sup> the authors presumed the paternal phenotype to be Di(b+).

We also concluded that the fetus in this instance was at risk for HDFN, and that the perinatal needs of the mother might be severe, so we prepares Di(b-) blood for either exchange- or red cell-transfusion. Since all family members' RBCs (*in toto*) were positive for Di<sup>b</sup> antigen, baby's mother donated her RBCs twice (at IUP 36 and IUP 37 weeks), so as to be prepared in the event that HDFN surfaced. Under the assumption that her baby might be group B, this would mean that the maternal blood with which we were dealing would, due to ABO incompatibility, automatically become in appropriate for exchange transfusion of the coming baby. So we requested KRC to procure compatible blood at least two days before delivery. Interestingly, among 600 O+ blood units tested, we found only one (1) donor with group O red cells that proved antigen-negative for the mother's anti-Di<sup>b</sup> antibody, yet which was at the same time was compatible by cross-matching with maternal plasma.

At 38 weeks of gestation, a male baby was born by Cesarean section. The neonate's and the mother's

red cells typed B, RhD positive, and A, RhD positive, respectively. Neonatal red cells reacted positively (3+) in the DAT while the serum reacted positively (2+) in the IAT. Eluate from these red cells were reactive with the entire panel of red cells we'd tested, so we deduced anti-Di<sup>b</sup> in the neonate's serum from the results mentioned above. Laboratory examination at birth showed a hemoglobin level of 19.6 g/dL, reticulocyte count at 2.87%, and a bilirubin of 4.1 mg/dL. Bilirubin, in fact, rose up to 9.7 mg/dL on the neonatal day two (ND2) (reference range of total bilitubin: 2~6 mg/dL for 24 hr after birth, 6~7 mg/dL for 48 hr, 4~12 mg/dL for 3~5 day). There was no specific finding on peripheral blood smear. There was no hepatosplenomegaly nor neurologic abnormalities to be recorded or evaluated. Phototherapy was started on ND2, immediately after admission, and the patient remarkably recovered without the subsequent need for exchange transfusion. Her newborn was discharged on the 6th day of life in good health without anemia.

## Discussion

It has been shown that Di<sup>b</sup> antigen is well developed on RBCs at birth and anti-Di<sup>b</sup> can contribute to severe jaundice in the presence of a Di<sup>b</sup> mismatch. Anti-Di<sup>b</sup> is usually formed during a previous pregnancy characterized by mismatch, or typically after problematic blood transfusion. In the case under study here, this 33-year-old mother with a transfusion of 10 years prior, and in her second pregnancy, proved to be "at risk." Hemolytic disease of the fetus and newborn is characterized by the destruction of fetal and newborn red cells by maternal allo-

antibodies specific for inherited paternal red cell alloantigen. The rate of hemolysis and severity of disease are determined by maternal IgG subclass, the amount of placental and circulating antibody, and the number of antigenic sites that exist on the affected red cells.<sup>8)</sup> Generally, HDFN caused by anti-Di<sup>b</sup> has been considered to be associated with mild to non-existent hemolysis<sup>9-11)</sup> although previous reports of Di<sup>b</sup>-related HDFN strongly suggest that the severity of this disease varies considerably, ranging from no symptoms to severe jaundice requiring exchange transfusion (ET).

Mochizuki, et al., only a few years ago reported a significant correlation between maternal anti-Di<sup>b</sup> titer and the severity of disease in the fetus and newborn. In this study,<sup>3)</sup> it was discovered that, among reported cases, approximately 60% of affected babies born to mothers having an anti-Di<sup>b</sup> titer of 64 or greater needed ET and/or high dose intravenous immunoglobulin (IVIG). In our case, a highly elevated maternal titer of anti-Di<sup>b</sup> (256) was associated with elevated risk of severe hyperbilirubinemia in the mismatched newborn. Thus, having prior awareness in mothers of excess paternal antigen should compel researchers and clinicians to determine the likely severity of peri- and postnatal disease, particularly HDFN and its ramifications.

Concerning treatment for severe cases, phototherapy, ET, or both, have routinely been performed. In most cases it has proven difficult to obtain the necessary Di(b-) RBCs needed for effective transfusion. Because the Di(b-) blood group phenotype is very rare, the authors were not surprised to find that of 600 crossmatch tests run on cross-matched blood obtained from our regional center, compatible Di(b-)

RBCs were found but once! So, in search of greater supplies of compatible rare blood, KRC is currently testing numerous units randomly selected from the complete array of our holdings in storage – a procedure that is usually time intensive and occasionally even fruitless. In our case, it was also very difficult to find compatible Di(b-) blood: among 600 O+ blood units randomly selected, once again only one turned out to be compatible. There was no need for transfusion because the HDFN due to anti-Di<sup>b</sup> was mild, But based on our experience that to find compatible Di(b-) blood is very difficult, We concluded that a donor-registration system which notates rare blood types should be established at the national level if such disease-defeating blood is to be effectively and safely employed. This study provides further support to previous research in which we advocated for the registration and collection of rare blood types via emergency donation, particularly and especially when such donors can be identified and/or pre-screened.<sup>12)</sup>

Table 1 summarizes three previous reports relative

to anti-Di<sup>b</sup> related HDFN in Korea. As noted, while HDFN due to the anti-Di<sup>b</sup> is often associated with very mild hemolysis, three previous cases in Korea showed up the potential for severe hemolytic consequences and hyperbilirubinemia serious enough to require IVIG and/or ET. Unlike these previous cases, our patient displayed no obvious hemolysis and presented with a mildly increased total bilirubin. Indeed, our case resembled those of Habash et al<sup>10)</sup> and Lenkiewicz and Zupanska<sup>11)</sup> in that a strongly positive infant DAT was present, yet without observable signs of hemolysis. Another important difference between historical Korean cases and the present one is that anti-Di<sup>b</sup> detection through antenatal screening was uniquely here performed. Generally, after newborns have shown signs of anemia and/or hyperbilirubinemia, anti-Di<sup>b</sup> is detected – and typically as a function of hemolysis evaluation. A critical insight then into this disease cluster, and what can be done about it preventatively, organizes itself around proper periodic antenatal screening. For patients with irregular anti-

**Table 1.** Reported cases of HDFN due to anti-Di<sup>b</sup> in Korea

Year reported	Pregnancy Hx	Trans-fusion Hx	Ab screening test in antenatal care	Anti-Di <sup>b</sup> titer	Diego blood type of newborn	Treatment for newborn	Jaundice at birth	Hb level (g/dL)	T. bil level (mg/dL)	Reference No.
1994	G3P1	+	NT	NT	Di(a+b+)	Transfusion	+	5.2 (ND7)	13.6 (ND7)	5
1999	G3P2	+	NT	NT	Di(a+b+)	ET+transfuion	+	7.8 (ND3)	20.0 (ND3)	6
2008	G2P0	+	NT	1,024	Di(a+b+)	Phototherapy +IVIG	+	8 (ND3)	16.1 (ND3)	4
This case	G2P1	+	Anti-Di <sup>b</sup> was detected	256	Di(a+b+)	Phototherapy	-	17.2 (ND4)	9.7 (ND4)	

Abbreviations: ND, neonatal day; Hb, hemoglobin; T. bil, total bilirubin; ET, exchange transfusion; NT, not tested; Hx, history; Ab, antibody.

bodies, as we show here, can readily be detected, and their attending physicians can be alert as to potential hemolytic problems in the immediate future, thus providing crucial laboratories with the valuable time they need to find, and best utilize, acceptable donors. Koelewijn et al<sup>13)</sup> evaluated the effect of a first trimester antibody screening program on the timely detection of HDFN caused by antibodies other than anti-D. They conclude that a single first-trimester RBC alloantibody screen detects the important health problem of severe HDFN and screening program seems justified as part of routine prenatal care.<sup>13)</sup> On the other hand, There was another suggestion about the clinical significance of prenatal antibody screening test. Chung et al suggest that routine prenatal antibody screening may not be necessary for all pregnant women except Rh(D) negative women or those who have a history of HDN. Because among the positive results of antibody screening test, no one had evidence of HDN in their study.<sup>14)</sup>

### Summary

Di(a+b-) 혈액형을 가진 사람만이 혈청에 항-Di<sup>b</sup>를 생산할 수 있지만 이런 혈액형은 백인에서는 매우 드물며, 대부분 몽고인종에 국한되어 나타난다. 세계적으로 항-Di<sup>b</sup>가 신생아용혈성질환을 유발한다는 보고는 있지만 이에 대한 임상적 의의는 확실히 확립되어 있지 않다. 저자들은 임신 15주째 산전관리를 받던 산모에서 항-Di<sup>b</sup>를 검출하였고 고빈도 항원에 대한 항체인 경우 적합한 혈액을 구하는 것이 쉽지 않을 뿐 아니라 그 항체에 의한 신생아용혈성질환의 발생 여부도 예측할 수 없는 상태에서 교환수혈을 위한 O형

Di(a+b-)를 준비하려고 하였다. 대한적십자사 중앙 혈액검사센터에서 산모의 혈청과 600단위의 O형 농축적혈구와 교차시험을 시행하였고 한단위의 적합 혈액을 찾을 수 있었다. 또한 B형인 산모로부터 2단위의 혈액을 자가혈액으로 예치하여 준비하였다. 태어난 환아는 Di(a+b+) 혈액형이었지만 경미한 신생아용혈성질환을 보였을 뿐 광선요법만으로 호전되어 교환수혈 없이 퇴원하였다. 국내에서 이미 보고된 항-Di<sup>b</sup> 검출 3예는 신생아용혈성질환이 의심되는 환아에서 분만 후 산모의 혈청에서 검출된 증례였다. 본 증례는 산전관리에서 발견되어 6개월 동안 Di(b-) 혈액을 혈액은행에서 수혈용 혈액과 교환수혈용 혈액을 준비하는 과정의 경험을 국내의 첫 증례로 보고하면서 국내에서도 희귀혈액에 대한 헌혈자 등록제의 필요성을 제안하고자 한다.

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