

THE BENEFIT OF THE DIRECT ANTIGLOBULIN TEST USING GEL TECHNIQUE IN ABO HEMOLYTIC DISEASE OF THE NEWBORN

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Abstract. The direct antiglobulin test (DAT) using the gel technique was included in the investigation of infants with hyperbilirubinemia in the first week of life. Twelve cases were preterm and 48 cases were full term infants. The patients were divided into 2 groups: the study group comprised 22 cases of blood group A or B infants born to blood group O mothers; the control group comprised 38 cases of ABO blood groups compatible with those of their mothers. The mean \pm SD (39 ± 26 hours) of the age at the onset of hyperbilirubinemia induced by ABO hemolytic disease of the newborn (HDN) was significantly earlier than that due to other causes. The positive rate of DAT in the ABO incompatible group was similar by both the conventional technique and the gel technique, 54.5% and 50% respectively. However, the scores by the gel technique were higher than those of the conventional technique. The gel technique is simple, reliable, involves less technical error and requires a small amount of blood sample. The grading system is clear-cut, especially grade 1+ or weak positive as compared to the conventional technique which requires examination under a microscope. Therefore, the DAT using the gel technique is beneficial to the diagnosis of ABO HDN. It should be included in the investigation of infants with hyperbilirubinemia especially in case of suspected ABO HDN.

INTRODUCTION

In the management of infants with hyperbilirubinemia, a difficult problem facing the physician is distinguishing between simple physiologic hyperbilirubinemia and hyperbilirubinemia that is a manifestation of an additional pathologic process. The diagnosis of ABO HDN is another difficult problem since it primarily depends on hematologic findings in the newborn. The direct determination of the type of antibody coated on the infant red cells is technically demanding. The agglutination result of the direct antiglobulin test (DAT) using the conventional spin-tube technique is found to be negative or weakly positive. In addition, inappropriate washing of red cells can cause a false negative result. Therefore, a good result requires a skillful technologist. Recently, the DAT using the gel technique was introduced; it was found to be sensitive and easy to perform. A minute amount of red cells can be added directly to the specific gel without any washing procedure, then the result can be read after centrifugation. (Lapierre *et al*, 1990; Bromilow, 1993; Malyska and Weiland, 1994). The availability of a panel of monospecific antibodies can identify the type of antibodies and/or complement coated on the red cells.

This study presents the cause and investigation of hyperbilirubinemia in newborns. The DAT by using the gel technique was included and the benefit to the diagnosis of ABO HDN was shown.

MATERIALS AND METHODS

Subjects

Sixty infants with hyperbilirubinemia in the first week of life from the Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand were included.

Methods

The laboratory investigation of hyperbilirubinemia included hematocrit, microbilirubin, reticulocyte count, peripheral blood smear, ABO and Rh blood group, G6PD screening test and the DAT. The DAT was simultaneously performed by unbiased blind test using both the conventional spin-tube technique and the gel technique at the Department of Pathology, Pramongkutklao Hospital, College of Medicine, Bangkok, Thailand. The grading of the agglutination reaction of both tech-

niques was scored as 12, 10, 8, 5, 3, 2 and 0 for 4⁺, 3⁺, 2⁺, 1⁺, weak positive (w⁺), weak microscopic positive (w⁺m) and negative, respectively (Marsh, 1972).

The conventional spin-tube technique: One drop of 2-5% suspension of red cells was washed three times manually with normal saline. Antihuman globulin reagent (DiaClon Coombs, DiaMed AG) was then added and the cell suspension was centrifuged at 1,000g for 15 seconds and was examined for the agglutination of red cells. The DAT reaction strength (American Association of Blood Banks, 1993) of each sample was recorded.

Gel technique: Fifty microliters of 1% red cell suspension in low ionic strength saline (LISS) solution was added into each microtube of ID-card (DC screening monospecific Coombs sera, DiaMed AG, Switzerland). The ID-card was centrifuged at 70g for 15 minutes. The DAT reactions were graded by the distribution of the agglutinates throughout the gel matrix and the negative reaction appeared as a discrete cell button at the base of the column according to the manufacturer (Dia Med-GB Ltd, Scotland).

Statistical methods

The statistical difference between the mean values was calculated with the paired student's *t*-test. A *p* value of less than 0.05 was considered significantly different.

RESULTS

Sixty infants with hyperbilirubinemia were included in this study. Twelve cases were preterm and 48 cases were full term infants. Their birth weight ranged from 1,180 to 5,170 g ($\bar{X} \pm SD$ 2,872 \pm 724), and the age at the onset of hyperbilirubinemia ranged from 17 to 120 hours after birth ($\bar{X} \pm SD$, 59 \pm 29). The female to male ratio was 1:1.1. Most of the patients were first-born children (35/60, 58%) and only two cases had a history of hyperbilirubinemia in their siblings. The evidence of hemolysis was mild to moderate with a range of few to grade 1⁺ positive of spherocytes on the peripheral blood smear. The level of microbilirubin at the onset ranged from 7.8 to 20.3 mg% ($\bar{X} \pm SD$, 14 \pm 3.6) and the maximum level was 24.7 mg%.

The patients can be divided into two groups: the study group composed 22 cases with blood group A or B infants born to blood group O mothers; the control group composed 38 cases with ABO groups compatible with their mothers. The age at the onset of hyperbilirubinemia, the level of microbilirubin and hematocrit of the two groups were not different (*p* > 0.05). However, the mean \pm SD (39 \pm 26 hours) of the age at the onset of hyperbilirubinemia induced by ABO HDN was significantly earlier than that due to other causes (*p* = 0.003). The hematocrit of the infants with ABO HDN was significantly lower than those of other causes (*p* = 0.01).

The positive rate of the DAT in the ABO incompatible group was similar by both the conventional technique and the gel technique, 54.5% (12/22) and 50% (11/22), respectively. However, the agglutination scores were higher in the gel technique than those of the conventional technique (*p* > 0.05). The positive rate of the DAT in the ABO compatible group either by the conventional technique (1/38, 2.6%) or the gel technique (4/38, 10.5%) was markedly lower than that of the ABO incompatible group. The discrepancy was found in six samples (Table 1). Two samples of newborns with blood group B born to blood group O mothers gave a weak microscopic positive result only by the conventional technique. The cause of hyperbilirubinemia was due to G6PD deficiency in one case. Four samples gave a weak or grade 1⁺ result only by the gel technique. One was an OA incompatible newborn and three were ABO compatible newborns who showed early signs of hyperbilirubinemia from 18 to 42 hours after birth. The antibody coated on the red cells which was demonstrated by the gel technique was only IgG. The other causes of hyperbilirubinemia were G6PD deficiency (10 cases), sepsis (6 cases), polycythemia (2 cases), cephalhematoma (2 cases), hereditary spherocytosis (1 case). The rest of the patients (29 cases) had inconclusive causes. Three cases had more than one cause, OB incompatibility with either G6PD deficiency (2 cases) or polycythemia (1 case).

Interestingly, one maternal serum showed grade 1+ in the indirect Coombs test. She was blood group O, Rh negative, and received 300 μ g of anti-D gamma globulin during the 32nd week of pregnancy. She gave birth to identical twins at the 38th week of pregnancy. Her babies were blood group A, Rh positive. Although anti-D was evidenced in

the cord blood by the elution technique, and the DAT showed weak positive using the conventional technique and the gel technique, hyperbilirubinemia was found in only one 5-day old twin. The peripheral blood smear revealed mild hemolysis. The level of microbilirubin was 15 mg% and decreased after one day of phototherapy. Therefore there was no evidence of Rh HDN.

The management included phototherapy in most of the patients for one to five days. The level of microbilirubin gradually decreased. Exchange transfusion was performed in two cases. One case was an ABO incompatible infant whose hemotocrit was 33% and microbilirubin was 10.4 mg%, in the first 24 hours of life. The other was a G6PD deficient infant whose maximum microbilirubin was 24.7 mg% at the age of four days. No serious neurological complication was found among these patients.

DISCUSSION

The diagnosis of ABO HDN is difficult because there is no specific test. The peripheral blood smear shows evidence of mild to moderate hemolysis. Although the spherocytes are often found, they can also be found in other hemolytic diseases and in hereditary spherocytosis. The demonstration of sensitized red cells in the infant is technically difficult. The blood sample obtained from the infant, especially preterm, is rather small.

The introduction of the gel technique is helpful. The gel technique is simple, reliable, involves less technical error and requires a very small blood sample. The grading system is clear-cut, especially for grade 1⁺ or weak positive result as compared to that of the conventional technique which requires examination under the microscope. The positive agglutination result of the gel technique can be visualized and remains unchanged for a week at 4°C. A photocopy of the positive or negative result can be taken and kept as laboratory data.

Six samples had discrepant results. Two samples (#1, 2 in Table 1) gave a weak positive result only by the conventional technique. The possibility of a false positive result was considered since G6PD deficiency was proved to be the cause of hyperbilirubinemia in one case (# 2). The weak

Table 1

The discrepancy in the results between the conventional spin-tube technique and the gel technique.

No.	Birth weight		Onset of hyperbilirubinemia			Maternal blood gr	Infant blood gr	G6PD screening	DAT	
	Sex	(g)	Delivery	Age (hr)	Hct (%) / MB (mg%)				conventional	Gel
1	F	2,740	NL	64	49/17	O	B	N	w ⁺ (m)	-
2	M	2,890	NL	17	55/7.8	O	B	def	w ⁺ (m)	-
3	F	2,810	NL	66	57.5/16.4	O	A	N	-	1 ⁺
4	F	3,660	C/S	18	47/7.3	O	O	N	-	w ⁺
5	M	3,900	NL	42	65/12.3	O	O	N	-	w ⁺
6	F	2,630	C/S	38	76/15.7	A	A	N	-	w ⁺

NL = normal labor, C/S = cesarean section, N = normal, def = deficiency, MB = microbilirubin

positive result by the conventional technique was detected under the microscope. The chance of a false positive or a false negative result was rather high (Mollison *et al*, 1993). Another four cases (# 3-6) gave a weak positive or grade 1⁺ result only by the gel technique. Although the pathogenesis of hyperbilirubinemia was inconclusive in three cases (# 4-6), # 3 was OA incompatible with her mother. However, because the chance of a false positive result by the gel technique is also possible, the further elution test for the demonstration of antibody coated on red cells should be included.

In this study, the positive DAT rates in the ABO HDN by the gel technique and the conventional technique were similar. However, the scores by the gel technique were higher than by the conventional technique. The gel technique can demonstrate IgG coating on red cells and is reproducible even by an inexperienced technologist. The disadvantage of the gel technique was also demonstrated in this study. The severity of hyperbilirubinemia was not predicted by the positive rate of the DAT either by the conventional technique or the gel technique. In some cases, the IgG coating on the infant red cells might not be the cause of hyperbilirubinemia, for example, anti-D was coated on the cord blood of the twin infants whose mother received anti-D gamma globulin during the pregnancy. The administration of anti-D gamma globulin during pregnancy has been shown to cause no harm to either the mother or fetus (Rh Conference, 1977).

Even though the infant has ABO incompatible with the mother, ABO HDN does not occur exclusively. Other causes should be searched for. An inconclusive cause is not uncommon. Since the hepatic mechanism for the disposal of bilirubin is

immature, hyperbilirubinemia is commonly found in the newborn infant. However, the clinical course of hyperbilirubinemia is not severe. Most of the patients show a good response to the phototherapy. Few patients who have a high level of microbilirubinemia and/or severe anemia require exchange transfusion. The definite diagnosis of the etiology of hyperbilirubinemia is helpful for proper management.

In conclusion, the DAT using the gel technique is beneficial to the diagnosis of ABO HDN. It should be included in the investigation of infants with hyperbilirubinemia.

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