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***Retrospective Study***

**Rh-incompatible hemolytic disease of the newborn in Hefei**

Bi SH *et al*. Rh-HDN in Chinese

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**Abstract**

***Background***

Anti-D antibody is not the common cause of Rh-isoimmunization in Chinese neonatal jaundice. Recent change in national population policy has followed by an increase in Rh-isoimmunization related hemolytic disease of the newborn (HDN). Unfortunately, regional status of Rh-HDN is unavailable. We hypothesize that Rh-HDN in our region is most commonly due to anti-E antibody.

***AIm***

To investigate the prevalence of hemolytic disease of the newborn due to Rh-isoimmunization in Hefei City.

***Methods***

Retrospective review of data obtained from Children’s Hospital of Anhui and Hefei Blood Center between January 2017 and June 2019. Status of minor blood group antibody was studied in the corresponding mothers.

***Results***

Totally 4138 newborns with HDN admitted during the study period and 116 (2.8%) received blood exchange transfusion (BET). Eighteen newborns (0.43%) with proven Rh-incompatible HDN were identified. All were not the first-born baby. Thirteen mothers were RhD (+) (72%) and five were RhD (-). The distribution of Rh-related antibodies in mothers was ten anti-E (55%), five anti-D (27%), and for one anti-C, anti-c, and anti-E/c (6%) each. Thirteen (72.2%) were qualified for BET, relative risk for BET was 28.9 as compared to other types of HDN, but only 10 received due to parenteral refusal. All (100%) RhD related HDN received BET which is not significantly different from RhE related HDN (81.8%).

***Conclusion***

As expected, all Rh-incompatible HDN newborns were not the first-born. Contrary to the Caucasian population, anti-D induced HDN is not the most common etiology. In our region, anti-E (11/18, 61%) is the most common cause of Rh-HDN.

**Key words**: Rh-isoimmunization; Hemolytic disease of the newborn; Minor blood group

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**Core tip:** Rh-hemolytic disease of the newborn (HDN) is more commonly seen after the change of national population policy in China. Different from Caucasian, the most common antibody that causes Rh-HDN in Chinese neonates is the anti-E antibody. The severity of anti-E Rh-HDN seems no less than anti-D Rh-HDN since most of our indexed cases were qualified for blood exchange transfusion. This emerging medical problem requires a nationwide collaboration of research in order to establish evidence-based guidelines for Chinese population.

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**Introduction**

Hemolytic disease of the newborn (HDN), or erythroblastosis fetalis, is due to blood type incompatibility between the mother and the fetus. This incompatibility causes the mother’s immune system to generate IgG antibody against the blood type of the fetus. The IgG antibody binds to fetal red blood cells transplacentally to cause hemolysis. ABO isoimmunization is the most common etiology for HDN but minor blood group isoimmunization can also cause severe HDN. Different from ABO-HDN, minor blood group HDN usually will not occur in the first-born newborn unless the mother has prior abortion, inadequate transfusion, or miscarriage. During the era of one-child policy, we had extremely limited experience with minor blood group HDN. There were only a few reports about minor blood group HDN in China[1-3]. After discontinuation of the one-child policy, we believe that pediatricians will start to experience more minor blood group HDN.

Our clinical experience tells us that Rh-isoimmunization is the second most common cause of HDN in Chinese newborns[1,3]. Without early recognition, Rh-HDN can cause severe neonatal jaundice that can complicate with kernicterus or death. Severe Rh-HDN can also lead to fetal demise, miscarriage, or premature birth. Neutropenia and thrombocytopenia can be a clinical manifestation of newborns with Rh-HDN[4,5]. Occasionally, the fetal hydropic change can cause uterine atony, maternal preeclampsia, mirror (Ballantyne) syndrome[6], or difficulty in cross-matching. Early identification of the at-risk pregnancy and intrauterine intervention may offer a better outcome of the newborn and the mother. After the introduction of Rhogam in the 1960, problems from Rh-isoimmunization are almost eradicated[7]. However, we do not know whether Rhogam can offer similar benefit to our population or not. Up-to-now, our medical society still lacks adequate data to guide us to develop a rational management pathway. Our study is aimed to call for the attention to Rh-HDN in Chinese population.

**MATERIALS AND METHODS**

We prospectively initiated a collaboration between Anhui Provincial Children’s Hospital and Hefei Blood center for this cohort study. Blood types of parents and the newborns, hemolysis, and antibodies of minor blood groups were tested for all newborns admitted for neonatal jaundice. Both coagulated and anti-coagulated blood from mother and newborn was collected according to the Chinese National Standardized Protocols for Clinical Laboratory, 4th version, for saline cross matching, polybrene test, and Coombs test. Antibodies against Rh group including anti-D, anti-E, anti-e, anti-C, and anti-c were tested together with ant-A and anti-B. Blood was also tested for hemoglobin, non-specific antibody, reticulocyte count, direct Coombs test, and indirect Coombs test. The study started from January 2017 to June 2019. Consent for data collection was obtained from the parents. Due to limited case numbers, non-parametric test was used for comparisons between two groups. Fisher’s exact test was used to compare categorical data between two groups. Data were analyzed by Prism 8 for Windows v.8.1.2.

**Results**

Our hospital adopted the American Academy of Pediatrics guidelines published in 2004 to diagnose and manage neonatal jaundice[8]. During the study period there were totally 4138newborns admitted for neonatal hyperbilirubinemia and 116 (2.8%) of them received double-volume blood exchange transfusion (BET). There were totally 18 mother-newborn dyads in our study (0.4%) without ABO incompatibility. Among those 18 mothers, 3 were blood type A, 6 were blood type B, 5 were blood type O, and 4 were blood type AB. Thirteen mothers were RhD (+) and 5 were RhD (-) (Table 1). Direct Coombs test, free antibody test, and antibody release test were positive for all 18 index cases. Thirteen out of the 18 newborns (55.6%) were qualified for BET but 3 of them did not undergo the procedure due to refusal by their parents. This left only 10 newborns received BET which accounted for 8.6% of all newborns who received BET during the study period. Rh-HDN had much higher risk to be qualified for BET (relative risk = 28.9, odds ratio = 101.4; *p* < 0.0001 by Fisher exact test). All 3 newborns qualified for BET but did not receive the procedure were born to RhD (+) mothers with anti-E antibody. Among the 10 newborns received BET, all five newborns from RhD (-) mothers as compared to 5 from RhD (+) mothers. There was no difference in the percentage of requiring BET between newborns from RhD (-) mothers and RhD (+) mothers (5/5 versus 8/13, *p* = 0.25 by Fisher exact test). All 10 newborns tolerated the exchange transfusion and discharged home without complication. Fifteen newborns (83.3%) were the 2nd born while 3 (16.7%) were the 3rd born. The reticulocyte counts ranged between 1.12% and 25.3%. Though the median reticulocyte count was higher for newborns from RhD (-) mothers (18.53% versus 8.37%), the difference was not statistically significant (*p* = 0.40). There were 10 mothers with E antibody (55%), 5 with anti-D antibody (27%), one with both anti-E and anti-c antibodies (6%), and one each for anti-C (6%) and anti-c antibody (6%).

**Discussion**

Blood group isoimmunization has been known to be a major cause of hydrops fetalis since 1940s[9] and the most important cause of neonatal jaundice[8]. The most common etiology of blood group isoimmunization is ABO-isoimmunization while Rh-isoimmunization is the second most common etiology. However, before the introduction of Rhogam, Rh-isoimmunization used to be the most common cause of kernicterus[9]. After exposure to fetal blood with different blood type the maternal immune system can be sensitized to generate IgG antibody, an isotype that can cross placenta into fetal circulation, to hemolyze fetal red blood cells[10]. The introduction of postpartum Rhogam injection has successfully reduce the Rh (D) sensitization from 14% down to 1%-2% and the addition of antepartum Rhogam injection further reduces the sensitization down to 0.5%[11]. Unfortunately, the successful experience cannot apply to our population for since than 1% of pregnant women is RhD (-).

Although Rh-HDN only accounted for less than 1% (0.43%) of all HDN admission in hour hospital, the relative risk for requiring BET was 28.9-fold of other type of HDN. With roughly 7 cases per year (18 cases over 2.5 years) in our hospital, Rh-HDN is not an uncommon problem we are facing. Rh blood group system is one of the more than 40 known human blood group systems. There are two sets of nomenclatures for Rh blood group, one developed by Fisher *et al*[12] and the other by Wiener[13]. The Fisher system is more commonly used by clinicians which contains 3 classes of epitope (C, c, D, d, E, e) and are encoded by two adjacent gene loci on chromosome 1. Due to their proximity on the DNA, the 3 classes of epitope co-express in a complex pattern with at least 34 genotypes. The potency of antigenicity studied show D > E > C > c > e > d which explains the severity of neonatal jaundice caused by the corresponding antibody.

As compare to ABO-HDN, the Rh-HDN has more aggressive hemolysis as reflected by more of the patients require BET. It is believed that fetal red blood cell (RBC) express less A/B antigen on the cell membrane and A/B antigen also express on other cell types which can decrease the binding of antibody to the RBC.

The D gene (D or d) is located on short arm (p) of chromosome 1 with 94% population as RhD (+) and 6% RhD (-) globally. Caucasian has the highest (15%) RhD (-) than Black (8%) and Asian population (< 1%)[14] which makes the Rh (D) isoimmunization extremely rare in Chinese population. C (C or c) and E (E or e) are co-express due to their proximity. The genotype distribution of C (68%Caucasian, 27%Black, and 93%Asian), c (80%Caucasian, 96%Black, and 47%Asian), E (29%Caucasian, 22%Black, and 39%Asian), and e (98%Caucasian, 98%Black, and 96%Asian)[15] is in agreement with the previous reports suggest isoimmunization against E and c is more common in the Chinese population[1,3]. In our results, only 5 HDN were associated with anti-D antibody. However, all 5 HDN with anti-D antibody received BET as compared to 8 out of 13 (61.5%) in anti-D negative newborns who qualified for BET although 3 did not receive it due to parenteral refusal.

Since Rh-HDN is very rare in Chinese population and rarely occurs in the first pregnancy, unless there was a prior abortion or miscarriage, so our medical community really lacks the knowledge of this morbidity especially during the one-child policy era. Rh (D) mediated HDN is just one kind of the Rh-HDN which can be prevented or managed by Rhogam. Unfortunately, there is no role for Rhogam in C, c, E, e antibody mediated HDN. With the recent reversion of one-child policy we can expect the number of CcEe-mediated HDN may increase and we need to prepare for this change. We pediatricians need to be aware that Rh (+), as we commonly call for those RhD (+) pregnant women, do not guarantee that there is no risk for their newborns to develop Rh-HDN. We also need to know that first-born newborn is not completely protected from Rh-HDN if the mother had prior abortion, transfusion, or miscarriage. Our obstetric colleagues are recommended to provide at least 500 I.U. Rhogam injection to pregnant women at 28 wk’ and 34 wk’ gestation, or one 1500 I.U. injection at 28 wks’ gestation, to RhD (-) pregnant women, followed by a 500 I.U. injection within 72 h after delivery to prevent Rh (D) sensitization if they are Rh (D)-negative with a Rh (D)-positive sex partner.

In the presence of ABO incompatibility, the chance to develop Rh sensitization decreases dramatically by at least 2.4-fold[16]. This protective effect is believed to come from the higher antigenicity of the ABO blood group. It is interesting that none of our newborns complicated with ABO incompatibility, but clinical significance deserves more extensive multi-institutional studies in the future. During clinical work-up, caution needs to be paid for that direct Coombs test can be negative in severe Rh-HDN due to extremely high titer of the antibody[17]. Contrary to Rhogam, Anti-E antibody is presently not available for preventing and treating anti-E mediated HDN. However, non-specific intravenous immunoglobulin can be used to ameliorate the severity of hemolysis and hence the jaundice[18].

In conclusion, Rh-HDN is an infrequent cause of HDN but can elicit severe hemolysis. In the present era, we need to be more familiar with the spectrum of this disease since most of our Rh-HDN is not due to anti-D antibody and cannot be prevented by the Rhogam injection. Our results only represent our regional experience. An extensive collaboration between pediatrician, obstetricians, and transfusion experts is required for a better understanding of Rh-HDN that can help us to establish a proper guideline in management.

**Article Highlights**

***Research background***

Before the discontinuation of the national population policy one-child policy–Rh-hemolytic disease of the newborn (HDN) was a rare cause of severe neonatal jaundice in China. Different from Caucasian population, RhD (-) is extremely rare in Chinese. We experienced a dramatical increase in Rh-HDN since the discontinuation of the national population policy which we believe will impact our management of neonatal jaundice nationwide. The lack of our own epidemiologic data will hinder our generation of public health policy judging from the severe consequence of bilirubin induced neurologic deficit.

***Research motivation***

To share our experience with our colleagues to encourage a statewide or nationwide collaboration to study Rh-HDN in Chinese.

***Research objectives***

The investigate the distribution of Rh antibodies in Chinese HDN and the clinical manifestation.

***Research methods***

Retrospective chart review of prospectively collected cohort over 18 mo in one free standing Children’s Hospital.

***Research results***

Rh-HDN accounted for 0.43% (18 out of 4138) of all HDN and 72.2% (13/18) were qualified for BET. No mother received antenatal Rhogam injection. The most common antibody involved was anti-E (55%, 10/18). The risk for BET was similar between anti-D (100%) and anti-E (81.8%) Rh-HDN.

***Research conclusions***

Anti-E antibody is the most common cause of Rh-HDN in Chinese. Our limited experience showed the severity of RhE neonatal jaundice is no less severe than the RhD neonatal jaundice.

***Research perspectives***

More extensive study in Rh-HDN is warranted after the change of our national population policy. The severity of Rh-HDN to both pregnant women and fetus deserve our attention. Collaboration among perinatology, neonatology, hematology, and immunology is needed to provide the best care for our next generation.

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Grade B (Very good): 0

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Grade D (Fair): 0

Grade E (Poor): 0

**Table 1 Blood group information of the parents and the newborn, maternal pregnancy status, and laboratory data of the newborns**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Case** | **Blood type (F)** | **Blood type (M)** | **Blood type (N)** | **Direct Coombs** | **Free antibodytest** | **Antibody releasetest** | **Minor blood group** | **BET** | **Rh(D)** | **Gestation** | **Reticulocyte count (%)** | **Gestational age (weeks)** | | **Age** | | | **T-bil (µmol/L)** | **Direct-bil (µmol/L)** | **Hb** |
| **test** | **(g/L)** |
| 1 | O | O | O | + | + | + | E | Y | - | G3P3 | 4.10 | | 41+0 | | 5d | 406.1 | | 100.8 | 133 |
| 2 | O | B | B | + | + | + | E | N1 | + | G4P2 | 4.50 | | 39+5 | | 33h | 370.7 | | 20.1 | 145 |
| 3 | O | O | O | + | + | + | D | Y | - | G5P2 | 19.17 | | 39+3 | | 25h | 326.6 | | 66.3 | 74 |
| 4 | A | B | AB | + | + | + | E | N | + | G5P2 | 11.99 | | 38+6 | | 3d | 361.0 | | 30.0 | 105 |
| 5 | A | A | A | + | + | + | E、c | Y | + | G3P2 | 18.25 | | 37+1 | | 24h | 469.3 | | 30.5 | 83 |
| 6 | B | A | AB | + | + | + | E | Y | + | G3P2 | 25.30 | | 40+3 | | 32h | 471.1 | | 75.6 | 76 |
| 7 | A | B | A | + | + | + | E | N1 | + | G3P2 | 3.95 | | 37+6 | | 3d | 407.0 | | 18.1 | 208 |
| 8 | O | O | O | + | + | + | D | Y | - | G3P3 | 3.63 | | 40+6 | | 5d | 365.7 | | 28.7 | 120 |
| 9 | A | AB | AB | + | + | + | E | N | + | G2P2 | 1.12 | | 40+2 | | 8d | 228.9 | | 21.4 | 122 |
| 10 | O | AB | B | + | + | + | D | Y | + | G4P3 | 8.07 | | 39+0 | | 20h | 282.1 | | 33.1 | 137 |
| 11 | A | B | B | + | + | + | E | Y | + | G4P2 | 22.97 | | 38+4 | | 11h | 257.4 | | 28.1 | 97 |
| 12 | O | O | O | + | + | + | E | N1 | + | G2P2 | 15.62 | | 39+4 | | 21h | 347.9 | | 16.3 | 121 |
| 13 | O | O | O | + | + | + | C | N | + | G2P2 | 12.20 | | 39+2 | | 3d | 275.4 | | 10.0 | 106 |
| 14 | A | B | AB | + | + | + | D | Y | - | G2P2 | 18.53 | | 40+0 | | 10h | 249.9 | | 14.6 | 113 |
| 15 | O | AB | B | + | + | + | E | Y | + | G2P2 | 8.67 | | 39+2 | | 10h | 447.4 | | 21.3 | 93 |
| 16 | A | B | B | + | + | + | c | N | + | G2P2 | 2.88 | | 37+6 | | 6d | 312.5 | | 32.4 | 160 |
| 17 | A | A | A | + | + | + | D | Y | - | G2P2 | 18.53 | | 39+4 | | 14h | 292.4 | | 26.2 | 105 |
| 18 | B | AB | B | + | + | + | E | N | + | G3P2 | 4.50 | | 39+0 | | 3d | 288.6 | | 21.5 | 138 |

1Newborn received intensive phototherapy after meeting threshold of BET due to refusal by parents. F: Father; M: Mother; N: Newborn; BET: Blood exchange transfusion; T-bil: Total bilirubin.