Significance of prenatal joint detection of ABO antibody titers and irregular antibodies in pregnant women with type O blood

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Summary

Objective: To investigate the effects of blood transfusion and number of pregnancies on ABO antibody titers and irregular antibodies in pregnant women with type O blood. *Materials and Methods:* The study included 4,200 pregnant women with type O blood (their husbands were with non-O type blood) that were divided into transfusion group and non-transfusion group, according to whether they had a history of blood transfusion. The both groups were respectively divided into three subgroups (the number of pregnancies was one, two, and ≥ three). The ABO antibody titers and irregular antibodies were detected at the same time. The effects of ABO antibody titers and irregular antibodies on hemolytic disease of the newborn (HDN) were discussed. *Results:* There was no consistency of ABO antibody titers and existence of irregular antibody. The positive rates of irregular antibody of transfusion group and of the subgroup (number of pregnancies ≥ three) were far higher than that of non-transfusion group and of the subgroups (number of pregnancies < three), respectively. All pregnant women with positive irregular antibody in non-transfusion group were with HDN. *Conclusions:* For pregnant women with number of pregnancies ≥ three or with history of blood transfusion, the prenatal joint detection of ABO antibody titers and irregular antibodies is helpful for accurately reflecting the in vivo antibody type and level.

Key words: Antibody titers; Irregular antibodies; Hemolytic disease of the newborn; Blood transfusion; Indirect antiglobulin test.

Introduction

Hemolytic disease of the newborn (HDN) results from the blood group incompatibility between mother and fetus [1]. Maternal IgG antibodies are produced in response to the antigens derived from fetal red cells, cross the placenta, and are responsible for hemolysis and anemia. Severe degrees of fetal hemolysis result in fetal hydrops [2]. ABO-incompatibility occurs in 15-25% of all pregnancies, while the development of HDN in offsprings has a rate of one to four percent, depending on the ethnic constellation of the population [3]. The HDN due to ABO-incompatibility leads to early onset hyperbilirubinemia (within 72 hours of age), and is a high-risk condition because it may present with acute and rapid rise in total serum bilirubin (TSB) values [3, 4]. A strong association between the level of maternal IgG antibody titers and the need for invasive treatment for hyperbilirubinemia in ABO-incompatible neonates has been confirmed. The routine test for maternal IgG anti-A or -B titers in blood group O-mothers may therefore be considered as an additional step in risk assessment of neonates and be useful in the evaluation of the likely response to therapy [5].

Maternal alloimmunization to other red cell antigens remains as the cause of fetal disease since no prophylactic immunoglobulins are available to prevent the formation of these antibodies [6]. The mild to severe cases of fetal hemolytic disease have been reported, when anti-c, C, e, E, or Kell, Kidd, Duffy, MNS, Lutheran, Diego, Xg, P antibodies, as well as other private and public blood group systems are found in the sera of mothers [7].

So was it necessary that all pregnant women tested irregular antibodies? Most developed countries have guidelines for screening all pregnant women for irregular erythrocyte antibodies. According to the guidelines of the British Committee for Standards in Haematology, all pregnant women should be ABO and D antigen typed and screened for the presence of red cell antibodies early in pregnancy and at the 28th week of gestation [8]. According to guidelines in The Netherlands, it has been mandatory since 1998 to screen all pregnant women for the presence of irregular antibodies in the first trimester of pregnancy [9]. However, no such guidelines are followed in developing countries like China. Lee *et al*. [10] suggest that the routine antenatal antibody screening for Chinese women may not be worthwhile except in D antigen-negative subjects or those with a prior history of hemolytic disease of the newborn. Their views are supported by Wu *et al*. [11]. However, several cases of hemolytic disease of the newborn due to anti-c or other irregular antibodies have been published in a retrospective diagnosis, their mothers were D antigen-positive and without a prior history of hemolytic disease of the newborn [12-17]. Distinctly, only the routine antenatal antibody screening for Chinese women in D antigen-negative subjects or those with a prior history of hemolytic disease of the newborn was far insufficient.

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In this study, the ABO antibody titers and irregular antibodies were detected in 4,200 pregnant women with type O blood. The effects of blood transfusion and number of pregnancies on ABO antibody titers and irregular antibodies were investigated, and the significance of prenatal joint detection of ABO antibody titers and irregular antibodies was analyzed for those who had transfused and were pregnant more than three times.

Materials and Methods

Subjects

The study included 4,200 pregnant women with type O blood (RhD positive) who were given perinatal care in the present hospital from January 2008 to December 2011. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of People's Hospital of Henan Province. Written informed consent was obtained from all participants. Their husbands were with non-O type blood. They were divided into transfusion group and non-transfusion group, according to whether they had a history of blood transfusion. The non-transfusion group was divided into three subgroups (the number of pregnancies was one, two, and ≥ three, respectively).

Methods

The detection of ABO antibody titers and irregular antibodies was conducted on the screened red blood cells (RBCs) and husband's RBCs using incomplete antibody test card (indirect antiglobulin test, IAT), according to the standard protocol. The maternal serum was treated with 0.2 M 2-mercaptoethanol. The antibody titers were presented with the reciprocal of dilution factor, using titers ≥ 64 as positive result. The result of irregular antibody test was presented with negative or positive antibody. For positive antibody, its type was initially determined according to the reactivity pattern of screened cells, and then verified with panel cells. The detection and determination were continued until the birth of fetus, and then the ABO-HDN was identified and diagnosed.

The diagnosis of ABO-HDN hemolytic disease in this neonate was based on the parameters as follows: onset of hyperbilirubinemia within 24 hours of birth, fetomaternal ABO incompatibility, laboratory evidence of erythrocyte sensitization, i.e., positive direct coomb's test (DCT) and exclusion of other causes of early onset of jaundice namely G-6-P-D deficiency and Rh-isoimmunization [18].

Results

General data

In 4,200 pregnant women, 356 cases (8.49%) of which the newborns were diagnosed with HDN, two cases that had positive irregular antibody in non-transfusion group were with HDN, four cases with positive irregular antibody in transfusion group, and three cases were with HDN.

Antibody titers

Results of ABO antibody titers and irregular antibodies detection in 4200 pregnant women are shown in Table 1. The total positive rate of irregular antibodies was 0.14% (6/4200). The positive rate of irregular antibodies in transfusion group was 0.49%, which was far higher than that in non-transfusion group (0.059%). The detection results in 3,382 pregnant women without blood transfusion are shown in Table 2. Two cases with positive irregular antibody were the pregnant women of whom the number of pregnancies was ≥ three.

Table 1. — *Detection results of ABO antibody titers and irregular antibodies in 4,200 pregnant women.*

Groups	Total cases	Cases of ABO antibody Titers < 64	Cases of ABO antibody Titers ≥ 64	Irregular antibodies Titers < 64	Irregular antibodies Titers ≥ 64	Positive rate
Non-transfusion	3,382	2,004 (50.25%)	376 (40.69%)	1	1	0.059%
Transfusion	818	522 (63.81%)	294 (35.94%)	1	3	0.49%

Table 2. — *Detection results of ABO antibody titers and irregular antibodies in non-transfusion group.*

Number of pregnancies	Total cases	Cases of ABO antibody Titers < 64	Cases of ABO antibody Titers ≥ 64	Irregular antibodies Titers < 64	Irregular antibodies Titers ≥ 64	Positive rate
1	642	424 (66.04%)	218 (33.96%)	0	0	0
2	926	611 (65.98%)	313 (33.80%)	0	0	0
≥ 3	1,814	1,069 (58.93%)	745 (41.07%)	1	1	0.11%

As seen in Tables 1 and 2, the positive rate of ABO antibody titer ≥ 64 was 30%-40%. There were cases with positive irregular antibody (titers < 64 and ≥ 64) in pregnant women both in transfusion group and in non-transfusion group. This suggested that there was no consistency of ABO antibody titers and existence of irregular antibodies. In six cases with positive irregular antibody, five cases had anti-E and (or) anti-C antibody and one case had anti-C antibody.

Discussion

Fetal blood type genes are generated 50% from father and 50% from the mother and regulate the production of antigen with different blood types. All blood type antigens in mother cannot stimulate the body to produce antibodies. Only the blood type antigens from father which the mother lacks can stimulate antibody production. Bakkeheim *et al*. [5] have demonstrated the association between the level of maternal IgG antibody titers and the incidence of hyperbilirubinemia in ABO-incompatible neonates. The presence of these IgG antibodies supports the diagnosis of ABO-HDN [18], and antibody titre levels below 512 identify a reduced risk for severe hyperbilirubinemia and subsequent kernicterus, whereas for higher titre levels, the risk is markedly increased. [5].

In this study, the positive rate of ABO antibody titers ≥ 64 is 30%-40%, but the incidence of HDN is only 8.49%,

which is slightly different with 20.41% in reported data [5]. It can be found that, for pregnant women with ABO-incompatibility, most of their newborns have no HDN, even the ABO antibody titer is ≥ 64 in late pregnancy. This may be related to the existence of ABO antibody subgroups. In addition, for pregnant women with ABO antibody titers < 64, the HDN also occurs. The cause may be associated with the presence of irregular antibodies or IgG subclasses. High matenal antibody concentrations with prevailing IgG2 subclass (which does not induce monocyte-driven cell destruction) have been reported to cause slight hemolytic conditions. On the other hand, when the antibody levels are lower, but the IgG1 and IgG3 are predominant, moderate or severe hemoltic disease is also developed [19].

The detection of ABO antibody titers in pregnant women is mainly applicable for determining the IgG antibody for ABO blood types, but not for other antibodies. It is suggested to use husband's RBCs to detect the ABO antibody titers. The reason is that, the RBCs can furthest reflect all the antibodies responding to husband's antigens in pregnant women, but not the simple ABO antibodies. The irregular antibody screening is mainly used to confirm the presence of IgG antibodies outside the ABO system. In this study, the positive rate of irregular antibodies in 4,200 pregnant women is 0.14%. This is in accordance with the findings of Lurie *et al*. [20] and Adenijii *et al*. [21], who have reported the alloimmunization rates among Rh-positive women of 0.2% and 0.15%, respectively. The positive rate of irregular antibodies in transfusion group is 0.49%, far higher than that in non-transfusion group (0.059%). Two cases with positive irregular antibody were the pregnant women of whom the number of pregnancies is ≥ three. Though these results may be affected by specimen volume, or associated with the pregnant women status (there are more than half of pregnant women have over three times of pregnancy), the change trend of irregular antibody production can at least be seen.

As the other antigens outside ABO system are weak, the produced antibodies cannot change the trend of ABO antibody titers in pregnant women. This results in the inconsistency of ABO antibody titers and existence of irregular antibody. The positive irregular antibody exists in pregnant women with negative ABO antibody. However, the majority of irregular antibodies are generated from the immune system, which makes the screening of positive irregular antibodies more clinically significant. Shilpa *et al.* [12] report that, the dilution titer of 1:4 is associated with fetal hydrops, and no other irregular antibody or any other cause of non-immune hydrops can be attributed to. As reported by Hackney *et al*. [14], the critical titer of 1:32 without supplementation with ultrasound and the critical titre of 1:16 supplemented with ultrasonographic features of hydrops are considered significant.

For pregnant women without history of blood transfusion, the irregular antibodies are produced by the stimulation of antigens from husband's RBCs. If the effects of previous pregnancy are excluded (they are firstly pregnant or the corresponding antigens are negative in previous pregnancy), the incidence of HDN is 100%. However the severity of HDN is closely related with the antibody titers and affinity. For pregnant women with history of blood transfusion, the type of positive irregular antibody should be firstly confirmed, and then the antigens in husband's RBCs are detected using the standard serum. If the corresponding RBC antigen is negative, the effect of antibody on fetus can be ignored. More attention should be highly paid to the case in which the corresponding RBC antigen is positive. In this study, five cases of six pregnant women with positive irregular antibody have anti-E and (or) anti-C antibody, and one case had anti-C antibody. These are consistent with the report of Wu *et al*. [22].

For pregnant women with number of pregnancies ≥ three or with history of blood transfusion, the prenatal joint detection of ABO antibody titers and irregular antibodies is helpful for accurately reflecting the in vivo antibody type and level. It has an important significance in diagnosing and treating HDN, and should be used as a routine test in perinatal period.

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