Case Report

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Hemolysis in reverse grouping: Evaluation and implication of high titer isoagglutinin of two blood donors

Suman Sudha Routray, Sukanta Tripathy, Gopal Krushna Ray

Abstract:

Hemolysis is a positive agglutination reaction and is primarily associated with high anti-A or anti-B antibody titers. This high titer may result in no agglutination due to the "prozone" phenomenon. Platelet concentrate of high titer has an adverse effect on the recipient of the non-identical ABO blood group. Similarly, the blood products with higher titers of isoagglutinin have recently increased the incidence of intravenous immunoglobulins-related hemolysis. In this Asian subcontinent, the impact of O blood donors with high antibody titers or ABO incompatible platelets is hardly addressed. Blood was collected from two healthy donors and subjected to blood grouping as done routinely. Hemolysis was observed in the reverse grouping with the "B-"cell. Blood grouping was repeated with the conventional tube technique (CTT) where there was no agglutination with the "B"-cell. Suspecting the "prozone" phenomenon, serial dilution of anti-B was done by CTT, and the titer was found to be 1:256 and 1:128 in both cases. Then, the reverse grouping was repeated with a diluted serum (1:8), and the blood group was confirmed to be A RhD-positive and O RhD-positive, respectively. The absence of agglutination in a reverse grouping is not only an indicator of weak antibody but also a presentation of the "prozone" phenomenon. This could be differentiated by doing the titer of isoagglutinin. Hemolysis due to high agglutinin levels should be documented and evaluated, and blood components should be properly labeled to ensure that the product is transfused to the same blood group patients.

Keywords:

ABO-incompatible, blood group discrepancy, immunohematology, prozone

Introduction

Blood group discrepancy refers to situations, in which the interpretation of a patient's or donor's ABO grouping results is ambiguous. These aberrant results occur when the forward or cell groupings are inconsistent with the reverse or serum groupings.[1] In general, confirmed blood groups are reported when both forward and reverse groups are concordant. Blood group discrepancies have been classified into four categories and described as Type I, II, III, and IV. Type I discrepancy refers to the unexpectedly weak or the absence of agglutination in the reverse grouping. This commonly observed discrepancy is due to weak/absent antibodies, but in some cases, the ""prozone"" phenomenon may be responsible for the lack of aggregation or hemolysis.[2] Hemolysis is a positive agglutination reaction and is primarily associated with high anti-A or anti-B antibody titers. The presence of high titers of ABO isoagglutinin has several implications. Due to the limited availability and very short shelf life of platelets, transfusion of ABO-mismatched platelets has been an accepted practice, especially in emergencies. However, non-identical ABO platelet transfusions have been shown to not only compromise the efficacy of such transfusions but also adversely affect the recipient. For similar reasons, during solid organ transplantation, ABO incompatibility remains a formidable barrier to cross. Few

Department of Immunohematology and Blood Transfusion, Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha, India

Address for correspondence:
Dr. Gopal Krushna Ray, All India Institute of Medical Sciences, Guwahati - 781 101, Assam, India.
E-mail: ray.gopal88@gmail.com

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blood centers screen platelet products for high titers of ABO isoagglutinin. Modern manufacturing methods of intravenous immunoglobulins (IVIG), caprylate fractionation, and/or chromatography have replaced the Cohn-like ethanol fractionation step, which has enhanced the recovery of immunoglobulin G (IgG) from pooled plasma to yield high-purity products with functional integrity but does not reduce the level of isoagglutinin.[3] As a result, these products with higher titers of isoagglutinin have recently increased the incidence of IVIG-related hemolysis.[4] In this Asian subcontinent, the impact of O blood donors with high antibody titers or ABO incompatible platelets is rarely discussed. Here, we describe two cases of blood group discrepancy characterized by hemolysis in the reverse grouping.

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Two healthy adult male donors were screened as per the drug and cosmetic act and selected for blood donation.[5]

Both forward and reverse blood grouping was performed by ABO and Rho (D) gel card (Matrix, Tulip Diagnostic [P] Ltd., India) [Figure 1]. The forward grouping was suggestive of A RhD-positive and O RhD-positive, respectively. However, in the reverse grouping, hemolysis was detected with the "B-"cell in both cases. The blood group discrepancy was resolved following different steps as depicted in Figure 2.

With the conventional tube technique (CTT), there was no agglutination reaction and the hemolysis could be hardly appreciated with the "B"-cell. Suspecting the "prozone" phenomenon, serial dilution of anti-B was done by CTT to measure the titer. The anti-B immunoglobulin M (IgM) titer of both donors at room temperature was 1:256 and 1:128, respectively [Figure 3]. An agglutination strength of 4+ was observed in the dilution of 1:8 in both donors with a negative reaction with the undiluted serum. Hence, the reverse grouping was repeated with a diluted serum (1:8), and the blood group was confirmed to be A RhD-positive and O RhD-positive, respectively. The serum was treated with 0.01M DTT (Dithiothreitol), then the anti-B titer (IgG) of O RhD-positive case was found to be 1:2 in the antihuman globulin phase [Supplement Figures 1 and 2], but no agglutination was detected in A RhD-positive case.[6] All three components prepared from both units were labeled as high-titer components to avoid ABO-nonidentical platelet transfusions and pooling of plasma for fractionation.

Discussion

The "prozone" phenomenon is a state of excess antibodies that prevent cross-linking of red cells by

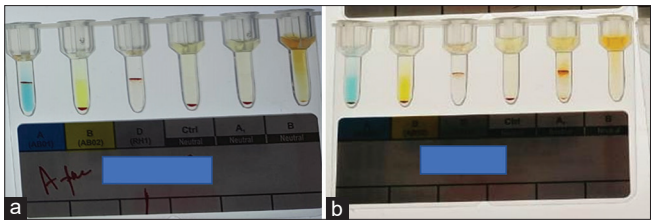


Figure 1: Forward and reverse grouping by Gel card showing hemolysis in reverse grouping with "B"cell in A group (a) and O group (b)

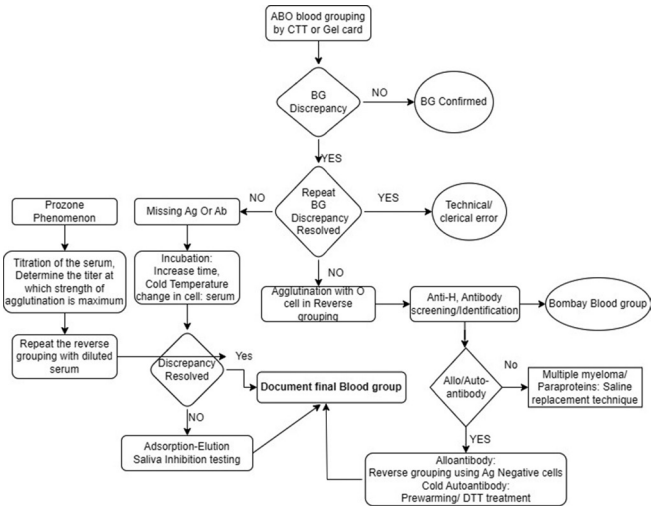
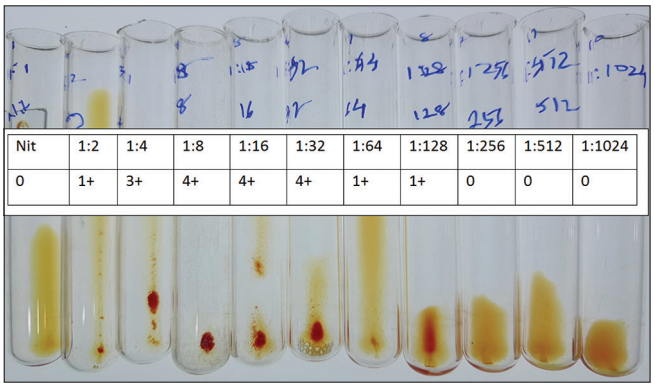


Figure 2: Flow diagram showing steps to resolve blood group discrepancy



Nit	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
0	1+	3+	4+	4+	4+	1+	1+	0	0	0

Figure 3: Serial dilution of anti-B isoagglutinin showing a titer of 1:128 and "prozone" phenomenon

blocking most antigenic sites through steric hindrance.[7] Thus, the first tubes of serial dilutions from these two donors either showed no agglutination or showed an agglutination strength of 2+ or less. The reddish hue of the reverse grouping in the gel card [Figure 1] is due to the hemolysis of red blood cells. High agglutinin titers above a critical value of 64 (IgM) result in *in vitro* hemolysis.[8] However, the absence of hemolysis in the tube method (nit) could not be explained. In the Indian population, more than 50% of the donors have a titer of ≥1:128 of anti-A and anti-B in their sera,[9] whereas in the Brazilian population, it is found in only

9% and 5% of donors, respectively.[10] High levels of isoagglutinin in Asian and African populations have been attributed to increased incidence of mosquito bites and parasitic infections.[11] The level of antibodies in a donor population depends on the ethnic background, environmental factors, vaccination status, and foreign antigenic exposures in the form of pregnancy and blood transfusions. Improved environmental hygiene, reduced parasites, intestinal infections, and consumption of processed foods might be attributed to low titers of these antibodies in the Japanese population.[12] The type I blood group mismatch is resolved by increasing the serum-to-cell ratio, increasing the incubation time, and decreasing the incubation temperature.[1] If the discrepancy is due to the "prozone" phenomenon, resulting from a high antibody titer, then diluton of the serum or plasma is done to reduce steric hindrance and bring the antibody concentration within the equivalent range to elicit a positive reaction. While resolving blood group discrepancies, it is recommended to document the patient's age, transfusion history, and disease diagnosis before a detailed immune-hematological work-up. In male donors (50 years and older), titers of these agglutinins decrease with age, in contrast to female donors, which show an inverse relationship with age.[9] Pregnancy may be responsible for elevated antibody levels in women, although the exact mechanism is unknown. Unlike the United States, India has no regulatory policies to prevent hemolytic transfusion reactions (HTR) of plasma incompatible components and no consensus on critical titers.[13] To bridge the huge gap between platelet demand and supply, nonidentical ABO platelet transfusions have been accepted in most cases. Similarly, residual plasma is routinely donated for plasma fractionation, and the presence of high-titer antibodies in plasma always has the inherent potential to cause HTR after infusion of such products.

Conclusion

Hemolysis due to high agglutinin levels should be documented and evaluated, and blood components should be properly labeled to ensure that the product is transfused to the same blood group patients. Agglutinin titers in this region need to be evaluated in larger populations to define critical titers that will help develop guidelines for increased safety in transfusion and transplantation practices.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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Supplementary Figure 1: Titer of anti-B (IgG) after DTT treatment of the O group serum. IgG = Immunoglobulin G

Supplementary Figure 2: Titer of anti-B in AHG phase after DTT treatment(O group serum)