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A *novel* observation on grouping anomaly: The phenomena mimicking the B_{el} genetic variant of the ABO blood groups

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Abstract:

BACKGROUND: Discrepancy in "forward/reverse" grouping leads to confusion in assigning ABO group to a person. It could be genetic in nature and classified according to the presence/absent of antigen on red blood cell (RBC) vis-a-vis corresponding alloantibody in plasma.

AIM: The aim of the study was to investigate the grouping anomaly found in a recently delivered woman who required transfusion.

MATERIALS AND METHODS: A standard protocol for investigation was followed.

RESULTS: A 27-year-old female, gravida 4, para 3, was grouped O on forward grouping, but her serum did not agglutinate Group B RBCs tested. Absorption-elution study gave an active eluate from her sensitized RBCs with anti-B or anti-A+B. Saliva showed H, but no B antigens indicating to her B_{el} phenotype. However, 2-week latter in the follow-up study, her serum revealed a presence of complement binding high titer anti-B. The problem of missing anti-B on the previous occasion was attributed to hemagglutination inhibition caused by accumulated complement macromolecules on RBCs that gave rise to physical hindrance in the formation hemagglutination clumps.

CONCLUSION: The unusual case of erroneous reversed grouping was attributed to complement-mediated hemagglutination inhibition. The positive eluate obtained from sensitized RBCs of the mother was considered to be due to a contamination of fetal RBCs in maternal circulation entered during her postpartum phase of pregnancy. It could also be due to a conversion of H to B antigen no matter in trace amount by the fetal B group-specific transferase percolated into maternal circulation.

Keywords:

Anomalous ABO group, complement-mediated hemaggltination inhibition, mimicking B_{el} phenotype

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Introduction

Reciprocal relation of the antigen on red blood cells (RBCs) and the corresponding antibody in plasma not only defines the ABO group of a person but also makes it mandatory to use homologous blood in transfusion. Forward grouping carried out on RBCs gets confirmed by reverse grouping performed on plasma. Discrepant results between the two may lead to confusion in assigning an appropriate group to a person. There are a number of causes for grouping anomaly including the genetic variants of the antigens. It is important to know whether the problem involved is *intrinsic* to the RBCs *per se* or due to the *extrinsic* factors present surround them. The genetic variants are usually intrinsic in nature, though they are often noticed as to extrinsic factor, for example, missing the expected antibodies in plasma. The genetic variants are classified as per the results obtained by testing RBCs with a battery of reagent antisera, the absorption/elution experiment,

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and the detection of secretary antigens in saliva.[1] With an advent in the development of molecular techniques, the weaker variants are better understood on the genetic mutations involved. The present study was aimed at finding the cause of grouping anomaly in a recently delivered woman who required transfusion in the face of the postpartum hemorrhage.

Materials and Methods

Patient's blood specimen was collected in ethylenediaminetetraacetic acid (EDTA) and plain vacutainer tubes. Forward grouping and absorption-elution experiments were carried out using RBCs from the specimen collected in EDTA, while reverse grouping was carried out using serum separated from the clotted blood specimen. The RBCs reagent used in reverse grouping was *in house* blood collection suspended in normal saline. Antisera used were of commercial origin (Tulip Diagnostics, India). Methods used throughout the study were the standard serological procedures.[2] To detect the weak B antigen on the patient's RBCs, a set protocol was followed. The battery of anti-B and anti-A+B reagents was used if any of the reagents gave direct hemagglutination. The absorption/elution experiments were performed to see if the antibody was absorbed by and eluted from her RBCs sensitized using appropriate antisera. Antibody elution was carried out by heat elution method on her thoroughly washed RBCs sensitized by appropriate antisera. The supernatant of the last wash, as washing procedure control, was run in parallel to the test on the eluate. The patient's saliva, boiled and diluted with equal volume of normal saline, was used in hemagglutination inhibition test to determine the secretor status. The family members of the index case and the additional cases studied were tested in an identical manner. Immunoglobulin nature of the antibody was determined by incubating the patient's serum with equal volume of 0.1M 2-ME (mercatoethanol) reagent at 37°C. Anti-M (IgM) and anti-D (IgG) were run as control in parallel to the test to validate the 2ME results. Ethical approval was taken verbally from the participants after explaining the purpose of our study to seek their cooperation to that they readily agreed upon.

Results

The case a 27-year-old female, gravida 4, para 3, required transfusion to compensate the blood loss following postpartum. She was never grouped nor was transfused in the past. While grouping, her RBCs were typed Group O, but anti-B was missing in her serum. A repeat sampling was asked for confirmation of our preliminary observation and to carry out additional testing. The results obtained are summarized in Table 1. The discrepancy between forward and reverse grouping was obvious with the RBCs showing Group O but serum showing a strong agglutination of Group A cells but not with B cells suggesting to missing anti-B. Further investigations ensued to follow the protocol on testing for weaker form of B.

While her RBCs did not show a direct agglutination with the battery of anti-B and anti-A+B reagents, they gave the positive elution of anti-B upon exposure to anti-B or anti-A + B reagents, thereby suggesting a presence of weak B antigen on her RBCs [Table 2]. Her saliva showed a presence of H but not B antigen pointing toward a rare B_{el} phenotype. However, the follow-up investigations, carried out after a 2-week lapse, revealed her RBCs continued to be typed Group O, but her serum, this time, showed a presence of complement-mediated hemolytic anti-B with the hemagglutination titer of 1:512 [Table 1]. The anti-B titer was reduced to 1:128 after the treatment of her serum with 2-ME (results are not tabulated) indicating the antibody predominantly being IgG. Family study showed her parents, two sibs, and one daughter as Group O, while her other 2 children and her husband were typed as B.

In a separate study, seven Group O mothers with infants of Group B or A were tested in identical manner as that of the index case. Of these, five mothers had their infants with Group B, and the other two had infants group as A. Three of the five mothers with Group B infants and one of the two mothers with Group A infants showed the positive eluate for B and A, respectively [Table 2]. The remaining three mothers did not give the positive eluate in the absorption-elution experiment (results are not tabulated).

Discussion

Reciprocal relation of antigen on RBCs and antibody in plasma of an individual was a classic observation made by Landsteiner while discovering the ABO blood groups. The feature helps confirming blood group of an individual. An absence of an expected antibody in

Table 1: Results on the ABO blood grouping on patient during initial testing and follow-up investigation

Specimen	Forward grouping (on RBCs)			Reverse grouping (in serum)	
	Anti-A	Anti-B	Anti-(A+B)	A cells*	B cells*
Index case (initial)	0	0	0	+4	No agglutination or no trace of hemolysis
Index case (follow-up)	0	0	0	+4	Gross hemolysis; Agglutinin titer 1:512

*Suspended in normal saline. RBC=Red blood cell

Table 2: Eluate prepared from the red blood cell of the five mothers group O* sensitized by anti-B or anti-A tested with red blood cells of Group B or Group A, respectively

Mothers' Group O	Infants' group	Mothers' RBCs sensitized with Anti-B or anti-A as per the infant's group		
		Eluate#/reaction score		Reactivity in the last wash
1 (index)*	B	Anti-B	+1-2	0
2	B	Anti-B	+1-2	0
3	B	Anti-B	+1	0
4	B	Anti-B	+w	0
5	A	Anti-A	+1-2	0

*RBCs sensitized with Anti-A+B showed the same results as that of anti-B, #Antibody reactivity in eluate was inhibited by Group B or Group A. Secretor saliva in a ratio of 4:1. RBC=Red blood cell

plasma indicates to a presence of the corresponding antigen on RBCs, no matter it is in the weakest form. The weaker variants of A, B, and H antigens are of rare occurrence and classified as per reaction pattern obtained with grouping antisera, presence/absence of the expected antibodies in plasma, and the presence/absence of corresponding secretary antigens in saliva.[1] They are under the genetic control; hence, molecular typing could be a great help in understanding the mutation involved at the core level. The present case posed the unique kind of grouping anomaly, by presenting its serological features akin to the weaker variant B_{el}.

Puzzle on missing anti-B

Usually, the *typical* prozone phenomenon occurs with high-titer antibody molecules overwhelmingly blocking all the antigenic sites on the RBCs so as to prevent cross-linking among the antibody-coated RBCs.[3] On the other hand, an *atypical* prozone effect, as was found with anti-B in the present case, could occur due to the complement activation[4] that forms macromolecule complex of C1qrs on the RBC surface that keeps the intracellular distance that is greater than required for cross-linking among the antibody-coated RBCs.[5] Reports on the serological hindrance caused by complement-fixing antibodies such as anti-Jka[6] and anti-Lea[7,8] have been documented. In the present case, the high-titer complement fixing hemolytic anti-B was observed in the follow-up study provided an evidence as to complement mediated serological hindrance might have been responsible for the failure to detect anti-B in reverse grouping on earlier occasion. The use of reagent RBCs suspension fortified with citrate or EDTA would have obviated the anomaly in the first place. The family study further confirmed the patient's blood group as O as both her parents being typed Group O.

Observation on the RBCs

The present case was initially classified as a rare variant B_{el} by virtue of an absence of expected anti-B in an apparently Group O individual, a positive eluate from her RBCs sensitized with anti-B or anti-A+B, and the presence of H but not the B antigen in her saliva.[1] It was the follow-up study that revealed the presence of high-titer complement binding anti-B, thus confirming her RBCs lacked B antigen. In view of these conflicting observations, it was indeed baffling to find the positive eluate from her RBCs sensitized with anti-B and anti-A+B in the initial testing. It was probable that the baby's RBCs grouped B might have entered the maternal circulation through fetomaternal bleed[9] and so was responsible for the positive eluate. However, the mother's RBCs did not show the mixed field agglutination pattern with anti-B or anti-A+B reagents in the forward grouping ruling out this hypothesis. Although speculative in nature, we thought of an alternative hypothesis based on literature review. Conversion of H antigen to B on Group O RBCs by α-D-galactosyl transferase has been demonstrated under the experimental condition *in vitro*.[10,11] as H being the precursor substrate for group-specific transferases responsible to add specific oligosaccharides to form respective antigens.[12,13] Conversely, individuals with Bombay (Oh) phenotype, that lack H, do not produce A or B antigen, in spite of having normal complement of respective group-specific transferases for want of H.[14] The level of the group-specific transferases in cord blood was found as much that is being present in adults.[15] Based on this knowledge, it is conceivable that, in the present case, that the B group-specific transferase of the baby might have percolated into maternal circulation, converting a few of the H receptors to B antigen on maternal Group O RBCs. While under experimental conditions, one requires the UDP-sugar and Mn-ions to be added for the group-specific transferases to convert H on Group O RBCs to A or B antigens,[10,11] such conversion *in vivo* has not been reported in literature with the best of our knowledge. However, this requirement may be met with their natural presence in the body of the UDP-sugar[16] and the Mn ions.[17] The amount of glycosyltransferase entering into maternal circulation might get diluted to yield a fewer antigenic sites to be appreciated as an extremely weak B_{el}-like phenotype.

While in the follow-up study, her RBCs did not show the positive elution with anti-B or anti-A+B that might be due to her RBCs with passively acquired B being eliminated through immune mechanism. Similarly, the three mothers with no positive elution in our study might have lost the RBCs with acquired antigens through immune clearance.

Conclusion

The unusual case of erroneous reverse grouping with missing anti-B may be attributed to the complement-mediated hemagglutination inhibition.

The positive eluate for B obtained from maternal RBCs sensitized with anti-B or anti-A + B could be due to the contaminant fetal RBCs entered the maternal circulation through the fetomaternal leak. It could also be due to the development of B antigen on maternal RBCs through the conversion of some amount of H by the passively transferred B group-specific transferase of the fetal origin. If the later hypothesis holds true, the present report deserves its due merits of being first ever reported in literature. In any circumstance, observations made through investigating this case, one should keep in mind while evaluating the results on investigating the rare weaker variants of the ABH antigens among the pregnant or recently delivered women.

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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