Case Report

Detection of Weak "B" Phenotype While Resolving an ABO Discrepancy: A Rare Case of B Subgroup

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Abstract

Quantitative and/or qualitative differences in ABO phenotypes give rise to ABO discrepancies causing difficulties in establishing the accurate phenotype. These discrepant serological reactions result in significant delays in blood bank. The correct typing of the blood group is imperative to prevent ABO incompatibility issues. However, the discrepant results encountered in routine settings are a challenge to immunohematologists. The variants of the ABO occur very rarely particularly true for B subgroups. Here, we present a case study of a healthy blood donor, discrepant results in forward and reverse blood grouping led us to perform adsorption-elution test. The presence of anti-B in the eluate confirmed the presence of weaker variant of B antigen on the surface of red blood cells. Correct identification of blood group is mutually important for donor and recipient to prevent the occurrence of any transfusion reaction. The serologically determined weak B phenotypes, however, require further confirmation through genomic analysis.

Keywords: *ABO discrepancy, adsorption elution, B group variant, subgroups*

Introduction

The ABO blood group system is the most important and widely studied blood group system of transfusion medicine. It is considered the backbone of transfusion medicine due to the consistent natural occurrence of antibodies (anti-A, anti-B, and anti-A, B) which are capable of destroying red blood cells (RBCs) in individuals whose red cells lack the corresponding antigens. The ABO locus for blood group-specific enzymes is encoded on the long arm of chromosome 9; the A and B blood group antigens are produced by enzymes that modify glycoproteins found on the surface of RBCs. The precursor glycoprotein to both the A and B antigens is known as the H antigen. As with other blood group systems, the ABO system has variant phenotypes or subgroups that primarily result from the expression of an alternate weak allele at the ABO loci. The associated immunogenic reactions such as hemolytic reactions due to a mismatch necessitate the correct ABO typing of both blood donors and recipients.[1,2]

The definite blood group is reported based on the concordance between forward and reverse grouping as well as the strength of the agglutination reaction. However, not always straightforward, particularly in cases where a weak or negative reaction is observed.[3] Many factors other than ABO subgroups for instance altered expression of ABO antigens, reduced production of naturally occurring ABO antibodies, medications, presence of allo/autoantibodies, and/or titer of antibodies may interfere with testing. Thus, all factors are needed to be considered to ensure accurate ABO typing and safe blood transfusion.[4] According to published reports, subgroups of A are comparatively frequent, whereas subgroups of B are exceptionally rare. It is also evident from a recent study, where A group variants were identified with the highest frequency of 44.6%, whereas only 2.3% were detected as the B subgroup.[5] However, there is no large study published from our region regarding subgroups and secretor status in our population. Studies have shown that group B is the second-most common group in our population preceded by the O group. Due to the high frequency of the B group, although not proven to date, subgroups of B may be frequent in our population as compared to other parts of the world. Subgroups can also occur in AB phenotypes. Here, we present a case of a voluntary nonremunerated blood donor, whose ABO typing revealed a discrepancy between forward and reverse blood grouping. After performing laborious testing, he was identified to have a variant of B, i.e., B3 (AB3) which is a rare subgroup of B with a very low overall incidence.

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Case Report

A 24-year-old healthy male donor donated blood voluntarily to the blood center of Indus Hospital, Karachi. He fulfilled the predefined donor selection criteria with unremarkable history of any acute or chronic disease. His general physical examination and predonation vitals were found to be appropriate for blood donation. The blood sample was sent for initial screening against transfusion transmissible infections and ABO and Rh typing. The screening results revealed the absence of HIV, HCV, HBV, syphilis, and malarial parasite in the blood. The standard tube testing technique was used to perform blood grouping. Monoclonal antibodies anti-A (IgM), anti-B (IgM), and anti-D (IgG + IgM) antisera from Diagast were used for forward grouping while 3%–5% red cells suspension from the donors' sample was used to perform reverse blood grouping. His Rh status was clearly negative which was repeated and rechecked to exclude the possibility of weak D. However when assessed for ABO blood group determination, discrepancy was observed in forward and reverse grouping. In particular, the RBCs showed negative agglutination with anti-B indicating blood group A. Further, the serological test with serum was suggestive of the AB blood group. To resolve this discrepancy, the procedure was repeated along with two additional tubes for auto control and O cells. After that reverse grouping was performed by increasing the incubation time, i.e., 60 min at 4°C; however, no change in reactions was observed. Missing reactions in reverse grouping led us to repeat testing with increased serum-to-cell ratio to detect the presence of low titer antibodies. Still, the ABO group could not be identified. The results are summarized in Tables 1 and 2.

The additional investigation including adsorption elution agglutination assay was performed to identify the correct ABO blood group [Figure 1]. Figure 1 shows a general scheme representing the workflow to resolve ABO discrepancy by adsorption elution test.

The obtained pattern of elution with appropriate valid controls is depicted in Table 3 indicating anti-B reactivity, hence is suggestive of the presence of weaker B subgroup. The donor was labeled as AB variant "AB_3" Rh Negative on the basis of eluate results.

Discussion

According to published reports, subgroups of A are comparatively frequent in the Western population, whereas subgroups of B are exceptionally rare. Due to reaction patterns, the subgroup of B may be typed as group O since B antigen is very weakly present on RBCs. Weak expression of A, B, and H antigens can be inherited or acquired. These antigenic changes are observed in leukemias, myelodysplastic syndromes, and some cases of Hodgkin's lymphomas. The transfusion of ABO-incompatible blood may lead to serious consequences for instance hemolytic transfusion reactions in the recipient. Notwithstanding the devised criteria of blood typing, ABO discrepancies thus pose a challenge for safe blood transfusion.[4]

Although possessed by only a small fraction, (0.02%) the importance of accurate subgroup typing cannot be disregarded particularly when considering the massive donations per year.[6] Here, we describe a case of ABO discrepancy which on extensive workup was identified as the B group variant. Initial typing results indicated strong agglutination with anti-A on forward grouping, whereas reverse grouping noted the blood group AB. It was not until the adsorption elution was performed, the donor was identified with the blood group B variant, B3. The adsorption elution is helpful in identifying the presence of weaker antigens on the surface of RBCs and has been in extensive use since it is highly sensitive and specific.

The subgroup of "B" occurs rarely compared to their counterparts in the A group. Whereas, the B3 phenotype is the most frequent subgroup observed for B allele. Since the B blood group (33.37%) is frequent in our region, the chances for identifying the B variant subgroups may be high compared to the A group variant.[7] B3 alleles arise due to amino acid substitutions in the glycosyltransferase B enzyme. B3 shows a mixed field agglutination, Bx shows a weak agglutination pattern, whereas Bel cells do not agglutinate with anti B. It requires a depth of knowledge and expertise to identify these subgroups. While resolving ABO discrepancy, meticulous attention should be paid to the strength of agglutination.

A higher frequency of weak B subgroups (1:8885) has been reported in the Indian population, whereas it is reported to be 1:116,667 for French donors.[8] In the past 5 years, B group variants have been reported by Kaur *et al*. and Jain *et al*. from India.[4,8] Further Chaurasia *et al*. and Khan *et al*. reported three and one cases of the B subgroup, respectively.[2,9] Infrequent reporting of these cases could be due to the dearth of knowledge and the complex classification of subgroups. Moreover, most of the laboratories in this region are performing only forward grouping; hence, there are chances that they can miss the ABO discrepancy secondary to subgroup.

In general, subgroups occur very rarely and their detection in routine setting is not possible. It is of paramount importance to characterize such variants to avoid the associated ill effects. The advanced techniques, i.e., adsorption and elution although helpful in this regard, definite identification and exact typing generally require molecular testing. Here, we also suggest that

Table 1: Initial ABO typing

Forward grouping						Reverse grouping			
Anti-A	Anti-B	Anti-D 1st choice*		Anti-D 2nd choice*		A1 cells	B cells	O cells	Auto control
++++	O	O		O		O	O	NT	NT
		Weak D 1st choice	CC	Weak D 2nd choice	CC				
		O	MFw	O	MFw				

*Weak D testing performed by two different anti-sera to confirm Rh status. MFw: Mixed field weak reaction, NT: Not tested, CC: Check cells, ++++: One solid agglutination

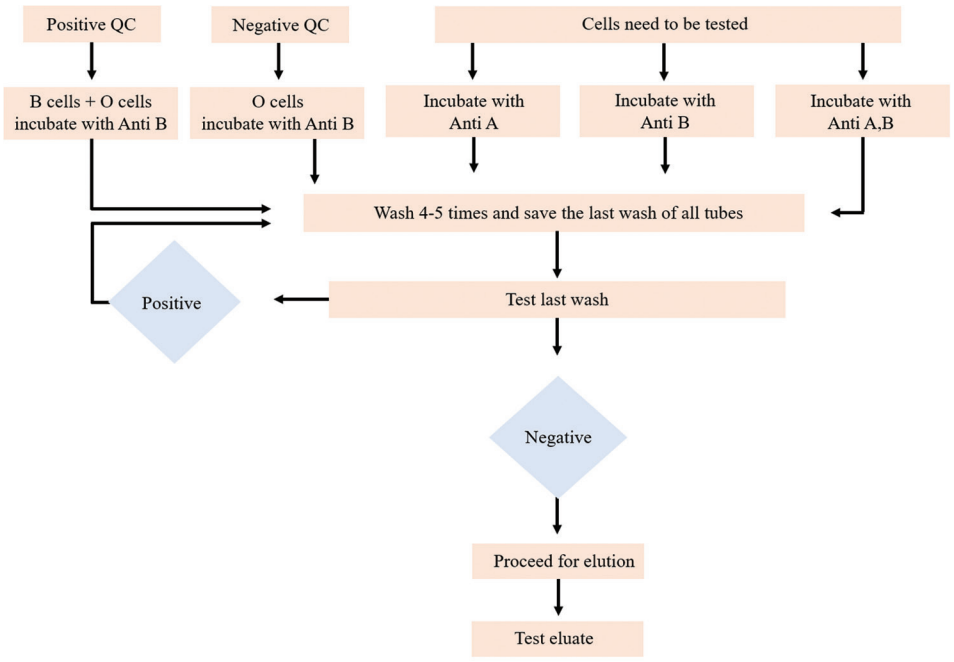

Figure 1: A general scheme representing the workflow to resolve ABO discrepancy by adsorption elution test

Table 2: Additional workup for ABO typing

Forward grouping			Reverse grouping			
Anti-A	Anti-B	Anti-D	A1 cells	B cells	O cells	Auto control
++++	O	O	O	O	O	O

++++: One solid agglutination, O: Negative, no agglutination/ hemolysis

Table 3: Testing on eluate performed after adsorption and heat elution

Testing phase	Anti A	Anti B	Anti A, B
Immediate spin	++++	MF++	++++

MF: Mixed field, ++: Medium agglutinates, clear background, ++++: One solid agglutination

the confirmation of the B3 variant in the present case should be done through genomic analysis which in turn might result in the identification of novel mutations.

There may be qualitative as well as quantitative differences in the B antigen of B3 red cells in contrast to normal B cells. Definitive identification of subgroups usually performed by reference laboratories using adsorption-elution methods and/or molecular testing which often have longer turnaround times. From a transfusion perspective, attention needs to be paid on transfusion indications in such cases. When these individuals present as recipient, they should be transfused with group O red cells, group compatible plasma, and platelet components. In case of a blood donor with weaker/unexpected reaction, the unit should not be issued until the discrepancy is resolved.

In conclusion, the presence of a weak B phenotype is an uncommon phenomenon. The availability of advance testing such as secretor studies and molecular analysis is required to identify these cases. Reference laboratories should provide these services and facilitate other basic laboratories/blood banks to resolve and diagnose such cases. As there is no rare blood group donor registry in Pakistan, therefore, every time such cases pose a diagnostic challenge to blood banks and sometimes remain unidentified due to the unavailability of advance testing and expertise. There is a need to develop a rare donor registry and implementation of confirmatory testing to prevent incidents that may arise as a consequence of unresolved discrepancies.

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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Conflicts of interest

There are no conflicts of interest.

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