

Blood group discrepancy in A_h para-Bombay phenotype: a rare blood group variant and its clinical significance

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Individuals with the rare para-Bombay phenotype have inherited defects in producing H associated with *FUT1* and/or *FUT2* genes. We report a case of blood group discrepancy in a para-Bombay patient from a tertiary care hospital of eastern India. A 31-year-old woman with rheumatic heart disease presented with fatigue and breathlessness and was then scheduled for valvuloplasty, for which a blood transfusion request was sent to the blood center. During pre-transfusion testing, red blood cell (RBC) testing showed group O, and serum testing showed strong reactivity with group B RBCs, weak reactivity with group O RBCs, and very weak reactivity with group A RBCs. Saliva inhibition testing and enzyme treatment of RBCs concluded the patient to be of “A_h para-Bombay” phenotype. The patient’s Lewis phenotype was Le(a–b+). This patient’s serum also had cold-reacting anti-IH along with anti-B. This case report highlights the importance of performing an advanced immunohematologic workup, including adsorption, elution, enzyme treatment, and saliva inhibition testing for identification of weak A or B subgroups as well as the rare para-Bombay blood group, when routine ABO typing, using forward and reverse grouping, is inconclusive. Accurate identification of blood group helps in preventing transfusion-related adverse events and encouraging safe transfusion practice. *Immunohematology* 2021;37:160–164. DOI: 10.21307/immunohematology-2021-026.

Key Words: para-Bombay phenotype, anti-IH, *FUT1* gene, *FUT2* gene, H-deficient phenotype, saliva inhibition test

Bombay and para-Bombay are rare, autosomal-recessive, H-deficient blood group phenotypes. Individuals with these phenotypes are unable to produce type 2H, which is the precursor for A and B.¹ The molecular basis of these phenotypes is due to mutations in *FUT1* (H gene) and/or *FUT2*, also known as the secretor gene. The important differentiating feature between these phenotypes is that para-Bombay individuals are homozygous for a non-functional *FUT1* and have at least one functional *FUT2*, having the genotype of either hh, Se/Se or hh, Se/se, making them secretors and responsible for the formation of H in the saliva or other tissues like gastrointestinal or genitourinary tissues. Occasionally, from the secretions, H, A, and/or B may sometimes get adsorbed onto the red blood cell (RBC) surface. Thus, para-Bombay individuals carry

negligible amounts of H, A, and/or B on their RBCs, which may or may not be identified by routine serologic testing.² Only a very few cases of individuals with the para-Bombay blood group presenting with a blood group discrepancy are reported. The para-Bombay phenotypes are even more rare than the Bombay phenotype, estimated at a ratio of 1:15.³ Moreover, the actual incidence of para-Bombay is still unknown in the Indian population. Blood grouping is challenging in such individuals, and proper understanding and performance of a detailed immunohematologic workup in such individuals play a vital role when determining appropriate transfusion therapy.

Case Report

A 31-year-old woman from a remote village in the state of Odisha, in eastern India, presented to the cardiology outpatient department with complaints of fatigue and breathlessness. She had been in treatment for rheumatic heart disease with mitral stenosis for the past few years. During her present visit, she was admitted to the intensive care unit for unstable blood pressure and tachycardia. After further evaluation, the cardiology team planned for balloon valvuloplasty, and a blood transfusion request was sent to the transfusion medicine department for any emergency requirement during the intervention. The patient was a mother of four children, all born via normal delivery, and was neither aware of her blood group or had any transfusions until this date. No blood group details of any of her family members were available.

Immunohematologic Workup

Patient blood grouping was done via a gel card (Matrix Octoplus Forward and Reverse Grouping Card with Sub Grouping; Tulip Diagnostics [P] Ltd., Goa, India). Her RBCs showed no reactivity with anti-A or anti-B, but showed strong reactivity with anti-D. But her serum testing showed 1+ reactivity with group O RBCs, no reactivity with group

A RBCs, and strong reactivity with group B RBCs. Thus, forward typing showed her RBCs as group O and reverse typing supported her being an A of some type. Blood grouping was repeated with washed RBCs and the same blood grouping card, which gave the same results, along with no reactivity with anti-H (Fig. 1A). The standard test tube method (forward typing performed using commercially available antisera from Tulip Diagnostic [P] Ltd. and reverse typing performed using in-house prepared 3–5% pooled group A, group B, and group O RBCs) at both room temperature (RT) and 4°C revealed similar results in forward typing but weak reactivity with the group O and group A RBCs along with strong reactivity with group B RBCs in the reverse typing at RT incubation. The same in-house prepared pooled RBCs were used in both phases of reverse typing. However, the strength of reactivity with group O and group A RBCs was enhanced when incubated at 4°C for 1 hour (Fig. 1B and C). The autologous control at 4°C and at RT was negative (not shown in Fig. 1). Hence, based on the results summarized in Table 1, forward typing showed a picture of Bombay/para-Bombay phenotype, and reverse typing suggested the presence of anti-B, along with the possibility of anti-H, anti-IH, or other cold-reacting antibodies in the patient's serum. Serum typing was performed at 37°C and antihuman globulin (AHG) phase by using AHG (Tulip Diagnostic [P] Ltd.) and showed the same strength with group B RBCs, weak reactivity with group O RBCs, and no

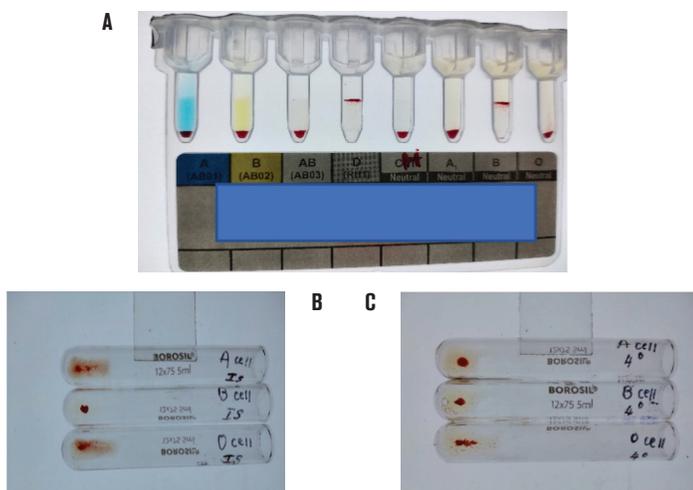


Fig. 1 (A) Blood group discrepancy using gel cards (Matrix Octoplus Forward and Reverse Grouping Card with Sub Grouping; Tulip Diagnostics [P] Ltd.). (B) Serum grouping by tube method (by in-house prepared pooled red blood cells) at room temperature. (C) Serum grouping by tube method (by in-house prepared pooled red blood cells) at 4°C.

Table 1. Results of forward and reverse typing in gel card and standard test tube method

Method	Forward typing					Reverse typing		
	Anti-A	Anti-B	Anti-D	Anti-AB	Anti-H	O RBCs	A RBCs	B RBCs
Matrix gel system*	0	0	4+	0	0	1+	0	4+
Test tube method (RT)	0	0	4+	0	0	1+	1+	4+
Test tube method (4°C)	0	0	4+	0	0	3+	4+	4+

RBCs = red blood cells; RT = room temperature.

*Testing performed using Matrix Octoplus Forward and Reverse Grouping Card with Sub Grouping; Tulip Diagnostics (P) Ltd.

reactivity with group A RBCs, suggesting the possibility of an immunoglobulin (Ig)G and/or (Ig)M component of anti-B with high thermal amplitude along with anti-H or anti-IH with wide thermal range. There was no pattern of reactivity identifying an antibody specificity observed with commercially available reagent RBCs for antibody screening (ID-DiaCell I-II-III; Bio-Rad, Cressier, Switzerland), since the testing showed panreactivity at 4°C, RT, and the AHG phase. The serum was then tested with RBCs of different phenotypes for differentiation and identification of cold-reacting antibodies, using the method described in the AABB technical manual.⁴ The reactivity pattern fit with the anti-IH pattern (Table 2). Furthermore, the patient's serum was tested with saliva containing H substance, but the patient's anti-IH did not show any inhibition by the H substance. This finding is a typical picture of anti-IH, which, unlike anti-H, is not inhibited by salivary H substance.⁵ Therefore, it was concluded that the patient's serum had anti-B, along with anti-IH.

The patient's RBC phenotype was found to be Le(a-b+) using anti-Le^a and anti-Le^b murine monoclonal antisera (Immucor, Norcross, GA), although the strength of reactivity

Table 2. Results of reactivity pattern of cold antibodies with different RBCs

Serum reactivity observed with:	At RT	At 4°C
Group O adult RBCs (O I)	1+	3+
Group O cord RBCs (O i)	0	0
Group A ₁ adult RBCs (A ₁ I)	1+	4+
Enzyme-treated group O RBCs	1+	3+
Bombay (O _h) RBCs	0	0
Autologous RBCs	0	0 to weak

RBCs = red blood cells; RT = room temperature.

was very weak (Fig. 2A). Secretor status was also performed by saliva inhibition study according to the AABB technical manual.⁶ The patient was a secretor of A and H substance, but the presence of A on RBCs could not be demonstrated by cold adsorption and heat elution methods, even after best efforts were tried, like increasing the cell-to-serum ratio and overnight incubation. Papain (LIQUIPAP; Tulip Diagnostics [P] Ltd.) treatment of the patient's RBCs, per AABB recommendations,⁷ showed augmented reactivity against both polyclonal sera derived from in-house healthy blood donors and monoclonal anti-A (Tulip Diagnostics [P] Ltd.), (Fig. 2B and C). Thus, the patient's blood group was identified and confirmed to be A_h para-Bombay.

Discussion

The H on RBCs is synthesized by α -(1,2)-fucosyltransferase enzyme regulated by two different but closely linked genes, *FUT1* and *FUT2*. *FUT1* (H gene) regulates expression on RBCs, and *FUT2* (Se gene) is accountable for H in secretions in different tissues. Among ABO carbohydrate blood group systems, Bombay and para-Bombay are rare, autosomal-recessive, H-deficient phenotypes due to homozygous amorphous alleles of the *FUT1* gene (hh). In these individuals, the type 2 precursor chain remains unchanged, prohibiting them from synthesizing the "H structure," which is the precursor for A and B.⁸ RBCs of these individuals are typically serologically negative for H, A, and B along with the presence of naturally occurring anti-H, anti-A, and anti-B in their plasma. The distinguishing feature of para-Bombay is the presence of soluble type 1 ABH substance in mucosa and

secretions. Serologically, the para-Bombay phenotypes can be distinguished from Bombay phenotype as outlined in Table 3. The *FUT2* gene present in para-Bombay converts type 1 precursor chain to type 1H, which on further action by A or B enzymes is converted to type 1A or type 1B, respectively. Trace amounts of ABH on RBCs may be detectable because of adsorption of these antigens from plasma; but in some instances, even adsorption and elution studies cannot detect them.⁸ We also encountered no or very negligible expression of A on the RBCs, resulting in a blood group discrepancy in this para-Bombay case. Although the patient was a secretor for A and H substances, adsorption and elution studies could not demonstrate the presence of A on RBCs. Only by the enzyme enhancement method was the presence of A established.

We noted a very weak strength of reactivity while phenotyping for Le^b with Le^b antisera. (Immucor), Lewis antigens are not intrinsic to RBCs and are adsorbed into the membrane of RBCs from the plasma. This fact explains why Lewis typing of RBCs is difficult and sometimes confusing because of weak hemagglutination caused by low titer or low specificity of the reagents. The distribution and expression of Lewis phenotypes in a population also vary with ethnicity, gender, and demographic region.^{9,10}

The ability of para-Bombay individuals to express ABH in tissues like genitourinary epithelium or gastrointestinal epithelium draws special attention when ABO mismatched solid organ transplantation is being considered. Townamchai et al.¹¹ reported that a group AB para-Bombay kidney donor was unintentionally misclassified as blood group O. If that misclassification had not been corrected, transplanting the donor tissue of this group AB para-Bombay into a group O blood

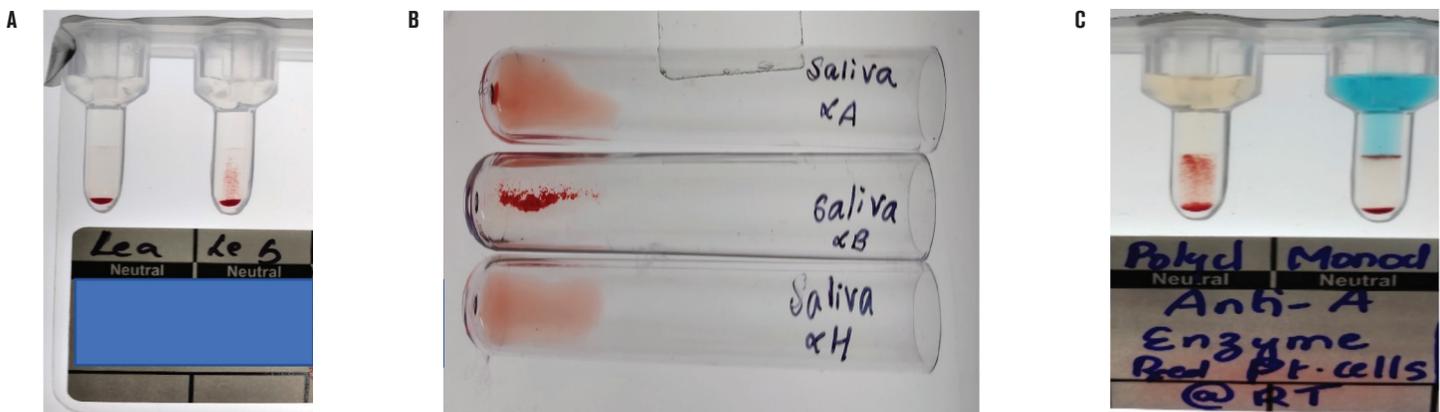


Fig. 2 (A) Le(a-b+) phenotype (secretor). (B) Saliva secretor status showed secretion of A and H substances. (C) Enzyme-treated patient red blood cells (Papain, LIQUIPAP; Tulip Diagnostics [P] Ltd.) showed reactivity with anti-A.

Table 3. Immunohematologic (serology) workup to distinguish Bombay and para-Bombay phenotypes

Characteristic	Bombay	para-Bombay
Forward typing	No reactivity with anti-A or anti-B	No or weak reactivity with anti-A and/or anti-B
Reverse typing	Strong reactivity with group O, group A, and group B RBCs	Weak reactivity with group O, group A, and/or group B RBCs
Reactivity with anti-H lectin	No reactivity	Weak reactivity
Reactivity with group O i _{cord} RBCs	Strong reactivity	No reactivity
Saliva secretor status	Nonsecretor of H, A, and B substances	Usually secretor of H, A, and/or B substances
Le ^b phenotype (indication for secretor status)	Le(b-)	Le(b-) or Le(b+)
Antibody testing with panel RBCs	Panreactivity: equal strength	Panreactivity: differential strength
Adsorption elution studies for H, A, and/or B antigens	Negative	May be negative or weakly positive

RBCs = red blood cells.

group patient would have resulted in detrimental hyperacute rejection ensuing immediate intravascular thrombosis and sudden allograft loss due to the reaction between anti-A or anti-B of the recipient's serum and A or B on the para-Bombay donor's kidney epithelial cells. Hence, successful ABO-incompatible renal transplantation in such a group O recipient required pre-transplantation desensitization because of the donor's ABO group being group AB para-Bombay.¹¹

The molecular basis of occurrence of Bombay and para-Bombay phenotypes are proposed to possess mutations in *FUT1* and *FUT2*, more frequently nonsense and missense mutations.¹² There are more than 30 *FUT1* and 5 *FUT2* alleles associated with weak H expression and 25 *FUT1* and 22 *FUT2* alleles associated with loss of H expression.¹³ Some well-known mutations for *FUT2* are c.385A>T (p.Ile129Phe) in *FUT2*01W.02* alleles in the Asian population and nonsense mutation c.428 G>A (p.Trp142Ter) found in the *FUT2*01N.02* allele and reported in African and European populations.¹⁴ In addition to naturally occurring anti-A and/or anti-B, para-Bombay individuals can also produce anti-H, anti-IH, or both. These antibodies are predominantly of IgM type and exhibit a broad thermal range (4–37°C) as we encountered in this case.¹⁵ Determining the presence of these antibodies is important because they are sometimes potent enough to cause rapid intravascular RBC destruction.¹⁶ Anti-IH, a weak H-like antibody, is almost always present in the serum of these individuals. This antibody is nonreactive with cord RBCs and is not inhibited by secretor saliva.⁸ Unlike anti-H and anti-I, it reacts only in the presence of both H and I together. Agglutination with group O I_{adult} RBCs and no agglutination with group O i_{cord} RBCs and O_h I_{adult} Bombay RBCs suggests the specificity of anti-IH in our patient.⁴ The titer of the cold antibody in this patient was 8 at 37°C and RT and 32 at 4°C.

Since the cold antibody is reacting at 37°C, it is considered clinically significant. In view of transfusion therapy, this patient could be transfused with only Bombay or para-Bombay blood group RBCs due to preformed antibodies in the serum. In this case, the patient underwent the intervention without any need for transfusion.

Consequently, systematic investigation of any blood group discrepancy with detailed immunohematologic workup has established a vital role in planning transfusion therapy in such rare phenotype individuals.

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