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Case Report

Immunohematological study of the first pediatric patient with the Bombay phenotype in Medellín, Colombia

Catalina Gómez Piedrahita[a,*], Leidy Alejandra Toro Espinosa[b], Daniel Téllez Paz[b], Ana Claudia Perón[c], María Angélica Patiño Perez[d], Carlos Arturo Vallejo Rios[a]

[a] Bacteriologist, Blood Bank, Hospital Universitario San Vicente Fundación, Medellín, Colombia
[b] Scientific and Technical Advisor at Biocientífica, Professor at Universidad de Antioquia, Colombia
[c] IHD Scientific Affairs Manager for Latin America, Immunohematology Division, Bio-Rad, Brazil
[d] Medical Director, Blood Bank, Hospital Universitario San Vicente Fundación, Medellín, Colombia

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ABSTRACT

The Bombay (Oh) phenotype is a rare phenotype in which red blood cells lack the H antigen as a result of a point mutation in the *H* gene. Oh patients are a challenge in transfusion medicine. We present a case of a pediatric patient with the Bombay phenotype who was carried to the emergency department of the Hospital Universitario San Vicente Fundación in Medellín, Colombia. The patient presented gastrointestinal hemorrhage and required transfusion therapy. Pretransfusion and molecular immunohematological analyses identified the Bombay phenotype. The patient was transfused with Oh red blood cells imported to Colombia from the Hematology and Hemotherapy Center of Ceará (Hemoce) in Fortaleza, Brazil. This first case of an Oh individual in Colombia highlights the need to look for donors with rare phenotypes to fulfill the transfusion requirements of the population.

1. Introduction

The H antigen (ISBT 018) [1,2] is the acceptor carbohydrate for the monosaccharides that define the A and B antigens of the ABO blood group system (ISBT 001). The H antigen is not the primary product of the *H (FUT1)* gene. *FUT1* codes for an α-2-L-fucosyltransferase, which links an α-L-fucose to the terminal galactose in carbohydrate chains bound to proteins or lipids present on the red blood cell (RBC) surface. The immunodominant fucose constitutes the sugar that defines the H antigen [4]. Another gene, the *FUT2* codes for a fucosyltransferase that binds an α-L-fucose to the terminal galactose in the type 1 carbohydrate precursor chains that are attached to lipids and proteins in body secretions [4].

The Bombay (Oh) phenotype is a rare phenotype with a prevalence of 1 in 10,000 in India and 1 in 1,000,000 outside that country [1]. This phenotype results from the homozygous inheritance of autosomal recessive mutant *FUT1* alleles (*hh*). The *h* alleles have point mutations that give rise to absent or non-functional fucosyltransferases, and the lack of the H-antigen on the RBCs' surface [3,6]. In addition, these patients have a deletion of the coding region of the *FUT2* gene. The classical Bombay phenotype results from a Tyr316- > ter mutation in the coding region of *FUT1* [7,8]. The mutation introduces a stop codon in the mRNA, which translates into a truncated enzyme that lacks 50 amino acids at the C-terminal end, and has no enzymatic activity. In Caucasians, the Bombay phenotype can be caused by different mutations. (9)

In Oh individuals, the lack of H antigen on red blood cells (RBCs) results in the absence of A and B antigens of the ABO blood group system. In routine tests for blood classification, Oh individuals are typed as O group persons; however, their sera strongly agglutinate RBCs from O group donors in the crossmatch tests [1]. The plasma of Oh individuals contains anti-A and anti-B antibodies, and an unusual high titer of anti-H antibodies; these can be of the IgM isotype, hemolytic, and very reactive with all types of RBCs, except Bombay-phenotype RBCs. Therefore, Oh individuals can only be transfused with auto- or (Oh) isologous blood units [2].

Here, we present a case of a pediatric patient, admitted to the emergency department of the Hospital Universitario San Vicente Fundación (HUSVF) in Medellín, who was found to be a Bombay-phenotype individual during medical assistance.

1.1. Case report

A 15-month-old female patient was admitted to the emergency

* Corresponding author.
E-mail address: catalina.gomez@sanvicentefundacion.com (C. Gómez Piedrahita).

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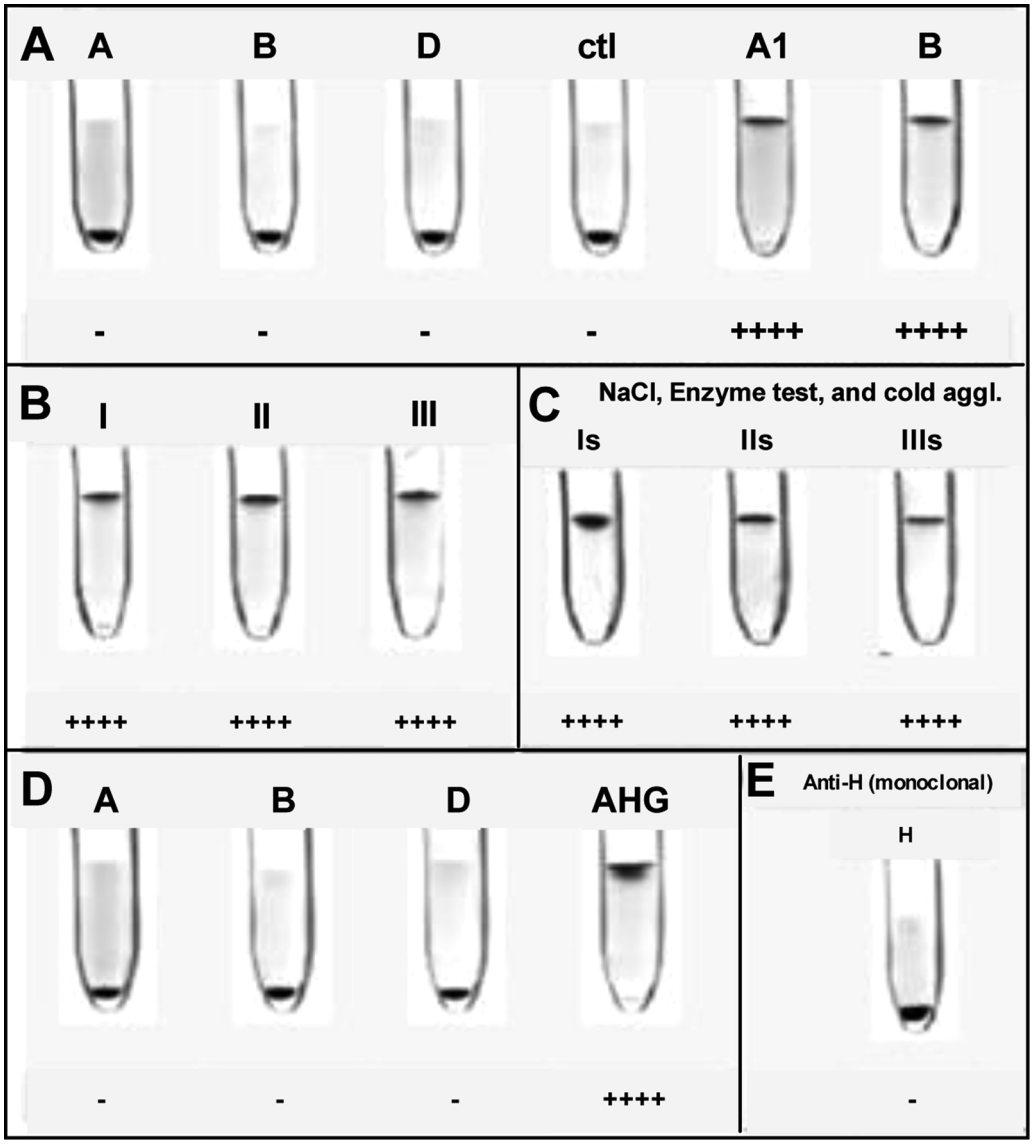

Fig. 1. A. Patient's ABO and Rh (D) blood typing: O, Rh (D) negative. **B.** Antibody screening in patient's serum in the LISS/Coombs cards at 37°C: positive. **C.** Antibody screening in patient's serum in the NaCl cards at 22°C: positive. **D**. ABO and Rh(D) typing of RBCs from a donor and the corresponding incompatible crosstmatch test with the patient's serum. **E.** Patient's H-antigen typing with mAbs: negative.

department of the HUSVF for active gastrointestinal hemorrhage from an ulcer at the duodenal bulb. The patient required transfusion therapy, but pretransfusion analyses showed unexpected serological reactions and crossmatch incompatibility. Additional blood samples were drawn from the patient, her parents, and relatives. All the adult relatives signed an informed consent and the study was approved by the Research Ethics Committee of the HSVF.

2. Methods and results

Immunohematological studies were performed with the semi-automated ID System platform (DiaMed/BioRad). Forward and reverse ABO blood group typing showed the patient's ABO phenotype was O, and the results for Rh phenotyping were Rh(D) negative (dd) (Figure 1A) and Ccee. The direct antiglobulin test (DAT) had negative results.

Irregular antibody screening (IAS) with a three-red blood cell panel (ID-Diacell I-II-III; Diamed/BioRad) in LISS/Coombs cards at 37 °C and NaCl cards at 22 °C showed strong agglutination (4+) with all cells (Fig. 1B and C and Table 1). The IAS with an 11-red blood cell panel (ID-Diapanel; Diamed/BioRad) was negative for autoantibodies and

Table 1
Antibody investigation in the patient's serum at different temperatures.

CAT	ID-DiaCell I-II-III (all)	ID-DiaPanel 11 cell (all)	Autocontrol
LISS/Coombs at 37 °C	4+	4+	0
NaCl at RT	4+	4+	0
NaCl at 4 °C	4+	4+	0
NaCl/enzyme at 37 °C	4+	4+	0

CAT, column agglutination technique. RT, room temperature.

strongly positive (4+) with all cells in LISS/Coombs cards at 37 °C and NaCl cards at 22 °C, 4 °C, and papain treatment (Fig. 2 and Table 1); the alloantibody detected has a titer of 512. Initially, 16 crossmatch tests were performed with O Rh(D) negative RBCs, but all of them were incompatible (4+) (Fig. 1D). Additionally, patient's serum reacted strongly (4+) with umbilical cord RBCs (Oi) in LISS/Coombs card at 37 °C.

The Red Blood Cell extended phenotype with the ID-cards ID-

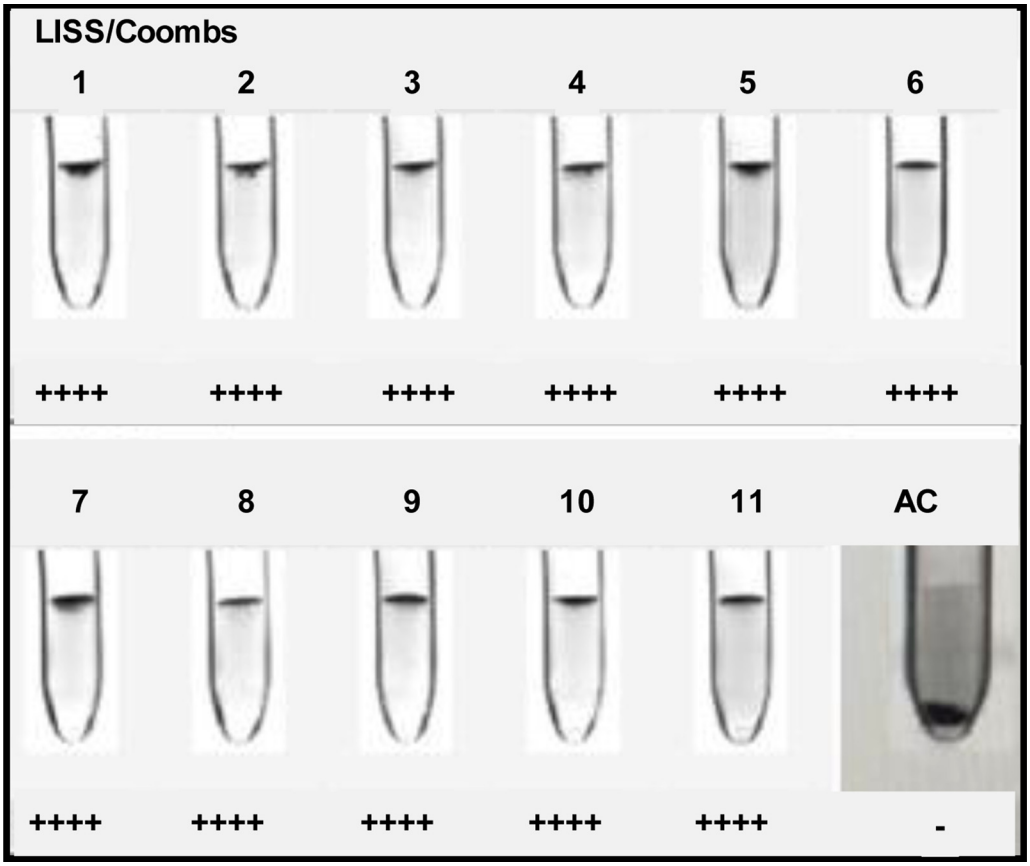

Fig. 2. Antibody screening in patients' serum. LISS/Coombs cards at 37 °C: positive; autocontrol: negative.

Antigen Profiles I and III (DiaMed/BioRad) showed the patient's RBCs were: $Fy^a(+)$, $Fy^b(-)$, $Le^a(+)$, $Le^b(-)$, S(-), s(+), M(-), N(+), P1(+), Lu^a (-), $Lu^b(+)$. Therefore, the relevance of antibodies against other public antigens was dismissed.

Finally, the patient's RBCs typing with anti-H mAbs (DiaClon; cell lines: H-86-50 + H89/8; DiaMed/BioRad) evidenced the lack of this antigen (Figure 1E) [10].

2.1. Transfusional approach

In the search of compatible blood, patient's relatives within the first degree of consanguinity were tested for the presence of H-antigen in their RBCs; all of them were positive. Additionally, in the Colombian database, there were no donors with RBCs compatible with the Bombay phenotype of the patient. Consequently, the Brazilian Ministry of Health and the Colombian Ministry of Social Protection cooperated to import to Colombia the compatible Oh blood component from the Hematology and Hemotherapy Center of Ceará (Hemoce) in Fortaleza, Brazil.

2.2. Molecular analysis

Blood samples drawn from the patient and her parents were sent to the Office of Medical Services, Division of Laboratory Medicine Department, Clinical Immunology and Transfusion Medicine in Sweden, for additional molecular and flow cytometry analyses.

H typing by polymerase chain reaction with allele-specific primers (ASP-PCR) revealed a c.826C > T mutation in the *FUT1* gene resulting in a p.Gln276Stop substitution and the absence of α2-fucosyltranferase. This mutation was consistent with the Oh phenotype and the presence of the anti-H antibody. Genotyping of the *FUT2* locus showed the patient was homozygous for the nonsense mutation c.428 G > A, resulting in a non-secretory phenotype. Therefore, the patient was classified as a true Bombay-phenotype individual [6]. The patient's parents were heterozygous for both mutations.

Flow cytometry analyses showed that the anti-CD173 (anti-H mAb; clone BRIC231) did not react with the patient's RBCs and just moderately with the RBCs of the parents.

3. Discussion

The case here described refers to a patient whose forward and reverse typing for the ABO blood group system did not show any discrepancy; however, the patient's serum exhibited an unexpected reaction (4+) with O RBCs. H-antigen typing with anti-H lectin showed the patient's RBCs were H-negative, suggesting the Bombay phenotype, which was confirmed by the Column Agglutination Technique with mAbs [10]. The patient was positive for k, Kp^b, and Lu^b antigens, which are considered public antigens in the population. The IAS with the 11-cell panel of RBCs (all from O donors with high expression of the H antigen), in the NaCl and Coombs Cards, suggested an alloantibody of wide thermal range. The negative results in the DAT, the absence of autoantibodies, the positive reaction of the patient's serum with cord blood Oi cells, and the compatible crossmatch with Oh RBCs allowed us to confirm the presence of an anti-H alloantibody.

Molecular analysis evidenced the presence of mutations in the *FUT1* and *FUT2* genes, commonly found in Bombay-phenotype individuals [3]. The mutation in the *FUT1* gene reported in this case (c.826C > T) has been previously described in studies defining the molecular basis of the Bombay and para-Bombay phenotypes. This mutation predicts a premature stop codon in the mRNA, resulting in an altered protein (Gln-276*ter) that is 90 amino acids shorter than the wild-type enzyme [6].

The patient was successfully transfused with Oh packed RBCs and had a satisfactory clinical recovery. This case report highlights the need

to implement in Colombia an active search for donors with rare blood group phenotypes to fulfill particular transfusion requirements of the population.

Contribution of the authors

Gómez, C., Toro, L.A., Téllez, D., and Patiño, M.A carried out the immunohematological research, samples remission, analysis of the medical history, and wrote the manuscript. Perón, C. and Vallejo, C.A. managed the international cooperation and reviewed the manuscript.

Declaration of Competing Interest

The authors have disclosed no conflict of interest relevant to the manuscript submitted to Transfusion and Apheresis Science.

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