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CASE REPORT

A Case of AB Para-Bombay Phenotype with Weak A Antigen Expression

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SUMMARY

Background: **The para-Bombay phenotype is characterized by H antigen partially or totally deficient on red blood cells and the presence of ABH substances in body fluids.**
Methods: **A patient with discrepant results in forward and reverse ABO phenotyping was further investigated by serological and molecular methods.**
Results: **Ortho gel and tube results showed weak A antigen expression and weak antibody reacting with A and B cells. Absorption-elution assay detected B antigen, and saliva test confirmed substances H were present. The patient was confirmed as A102B101 and Le(a+b+) phenotype.**
Conclusions: **These findings suggest that the case is AB Para-Bombay Phenotype (secretor).**
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KEY WORDS

para-Bombay phenotype, Lewis blood group, secretor, gene sequencing

INTRODUCTION

H antigen is the precursor of A and B antigens in the ABO blood group. The formation of H antigen on the red blood cell (RBC) membrane is dependent on FUT1 gene (the H gene), whereas the FUT2 (secretor gene) is responsible for the H antigen on epithelial cells and in body fluids.

The Bombay and para-Bombay phenotypes are characterized by the deficiency of H, A, and B blood group antigens on the RBCs, which are distinguished by the presence or absence of the FUT2/secretor gene [1,2]. Bombay phenotypes are H-deficient non-secretors, while para-Bombay phenotypes are H-deficient secretors. Para-Bombay individuals may occasionally have A and B antigens on red cells due to passive adsorption of A and B blood group substances from plasma [3].

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MATERIALS AND METHODS

Case Report

A 78-year-old male who presented with weakness in both lower limbs for more than 1 year and worsened for more than half a year was received at our hospital. The case was diagnosed with lumbar spondylolisthesis and referred for surgery. He had no previous history of surgery and transfusion.

A blood sample obtained from the patient was submitted to our division for blood typing. Blood grouping was done by column agglutination technology (BioRad, Cressier, Switzerland; Ortho-Clinical Diagnostics, Milan, Italy). The primary results of ABO blood typing were discrepant in forward and reverse grouping. In column agglutination technology of BioRad, cell grouping was O RhD positive and serum grouping was AB group. In column agglutination technology of Ortho, forward grouping showed 1+ agglutination with anti-A, no agglutination with anti-B and 4+ with anti-D, while reverse grouping was weak antibody reacting with A and B cells.

As there was no massive transfusion or blood stem cell transplantation in the patient's medical history, further serological diagnostic and molecular tests had to be followed.

Serologic analysis

ABO, Rh typing, and antibody screening was done by tube technique using monoclonal anti-A, anti-B, anti-AB, anti-H, and anti-D reagents and A1, A2, B, and O red cells (Shanghai Hemo pharmaceutical & Biological Co., Ltd.). Lewis blood group was tested using anti-Lea and anti-Leb (Sanquin, Netherlands) to deduce their secretory status. The absorption and elution test were conducted to detect trace amounts of ABH antigens on the surface of RBCs. The hemagglutination inhibition test was employed to analyze secreted ABH antigens in the saliva.

ABO and FUT1/2 gene analysis

DNA was isolated from EDTA-anticoagulated blood using a commercial system (QIAamp Blood DNA Mini Kit, Qiagen, Hilden, Germany). ABO blood group genotyping was detected using a sequence-specific priming (SSP) kit (Tianjin Super Biotechnology Development Co., Ltd.). For genomic DNA sequencing, ABO exons 1 - 7 and FUT1/2 genes, the samples were sent to Tianjin Super Biotechnology Development Co., Ltd. Exons 1 - 7 of the ABO and entire coding regions of the FUT1 and FUT2 genes were amplified and sequenced. The acquired nucleotides sequences of ABO exons 1 - 7 were compared with standard ABO polymorphisms from the dbRBC of NCBI and each SNP or mutation was analyzed and documented in the ABO gene. To distinguish mutations in the FUT1 and FUT2 genes, all obtained nucleotides sequences were compared with the reference sequences (FUT1 reference sequence: GenBank accession NG_007510.1; FUT2 reference sequence: GenBank accession no. NG_007511.1).

RESULTS

Serological characteristic

Tube grouping results for the patient's RBCs showed no agglutination with monoclonal human anti-A, anti-B, and anti-AB at room temperature, and his serum did not react with A, B, and O cells. The results of the test tube method were consistent with those of the BioRad gel. However, Ortho gel test results showed weak positive A antigen on his RBC and weak antibody reacting with A and B cells in his serum. Further tests were performed to detect weak antigens and antibodies. When the tubes were incubated at 4℃, the results were similar to the Ortho gel method. Microscale A and B antigens on red cells were detected by the absorption-elution assay. The saliva test showed that H substances were present in the saliva (Table 1). The patient's RBCs agglutinated with monoclonal anti-Lea[a], but not with monoclonal anti-Lea[b], which showed his serological Lewis phenotype was Le(a+b-).

ABO genotype

The sequence of the ABO gene was 467C>T, 297A>G, 526C>G, 657C>T, 703G>A, 796C>A, 803G>C, and 930G>A by direct DNA sequencing and was assigned as A102B101.

Mutations in FUT1 and FUT2 gene

One heterozygous mutation (522C/A) and two nucleotide AG deletions (547_552delAG) of the FUT1 were identified by direct DNA sequencing. Sequencing results for the FUT2 gene indicated that the FUT2 genotypes were homozygous for se357 (357C>T), and heterozygous for the weak secretor allele (Se^w) se385-(385AT), responsible for the Le(a+b+) phenotype [4,5].

DISCUSSION

The patient in this case received further serological testing due to the discrepancy in forward and reverse grouping of ABO blood typing. Tube grouping (room temperature) and the BioRad gel method results did not detect A/B antigen, and the plasma was also not reactive with A, B, and O cells. However, Ortho gel test and the tubes incubated at 4℃ showed weak positive A antigen on his RBC and weak antibody reacting with A and B cells in his serum. Due to the addition of PEG, the sensitivity of Ortho gel test will be higher than the BioRad gel. The discrepancies in forward and reverse grouping of ABO blood typing required additional investigations. At the same time, we evaluated the result of the H antigen system and found that no H antigen was detected on his RBC, which represents the para-Bombay blood type. Next, we conducted the absorption-elution assay and saliva secretory test. The results of the absorption-elution

Table 1. Results of serological investigations performed and their interpretation.

Reaction condition	Cell (forward) grouping				Serum (reverse) grouping			
	Anti-A	Anti-B	Anti-AB	Anti-H	A cell	B cell	O cell	Autocontrol
Gel technique (BioRad)	-	-	NT	NT	-	-	NT	-
Gel technique (Ortho)	$1+^{w}$	-	NT	NT	+ -	+ -	NT	-
Tube technique (RT)	-	-	-	-	-	-	-	-
Tube technique (4°C)	$1+^{w}$	-	-	-	+ -	+ -	1+	-
Tube technique (37°C)	-	-	-	-	-	-	-	-
Absorption-elution test	NT	NT	NT	NT	1+	1+	-	NT
Saliva secretory test	NT	NT	NT	1+	-	-	NT	NT

RT - room temperature, NT - not tested.

assay showed the presence of B antigen on the RBC surface, and the saliva test results showed the presence of H substances.

Subsequent sequencing results proved that the patient was A1B type with FUT1 and FUT2 gene mutations. FUT2 gene polymorphism in various populations indicated ethnic specificity, and FUT2 mutations se357 and se385 are the most common in Chinese populations [6]. The FUT2 genotype result (Se^{w}, Le(a+b+)) was consistent with the subjects' secretor status, but not with his serological phenotype (Le(a+b-)). Because lower amounts of the enzyme are produced in mutant cases than in the wild-type cases, some anti-Leb reagents were too weak to detect the small amounts of Leb present in the RBCs of some Le(a+b+) individuals, and they may be misphenotyped as Le(a+b-) [7,8].

The serum of para-Bombay individuals may contain anti-H, anti-HI, and anti-A/anti-B [9]. Transfusion of the same type of blood is the most ideal choice, but this blood type is rare and very difficult to obtain. In the absence of the para-Bombay blood group, the ABO blood groups with the weakest reaction to the patient's serum at 37°C should be selected for transfusion [10-12]. For our patient, only weak anti-H antibodies are present, and there is no reactivity at 37°C. If appropriate, autologous blood transfusion can be considered; if not, ABO red blood cell transfusion of the same type can be selected.

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Declaration of Interest:

The authors declare that they have no conflict of interest.

References:

1. Bhende YM, Deshpande CK, Bhatia HM, et al. A "new" blood group character related to the ABO system. Lancet 1952 May; 1(6714):903-4 (PMID: 14918471).

2. Kelly RJ, Ernst LK, Larsen RD, Bryant JG, Robinson JS, Lowe JB. Molecular basis for H blood group deficiency in Bombay (Oh) and para-Bombay individuals. Proc Natl Acad Sci USA 1994 Jun; 91(13):5843-7 (PMID: 7912436).

3. Roback JD, Grossman BJ, Harris T, Hillyer CD, editors. Technical Manual-American Association of Blood Banks. 17th ed. Bethesda: AABBPress; 2011, 375 pp.

4. Yu LC, Yang YH, Broadberry RE, Chen YH, Chan YS, Lin M. Correlation of a missense mutation in the human Secretor alpha 1,2-fucosyltransferase gene with the Lewis(a+b+) phenotype: a potential molecular basis for the weak Secretor allele (Sew). Biochem J 1995 Dec;312 (Pt 2):329-32 (PMID: 8526839).

5. Henry S, Mollicone R, Fernandez P, Samuelsson B, Oriol R, Larson G. Homozygous expression of a missense mutation at nucleotide 385 in the FUT2 gene associates with the Le(a+b+) partial-secretor phenotype in an Indonesian family. Biochem Biophys Res Commun 1996 Feb;219(3):675-8 (PMID: 8645240).

6. Yip SP, Lai SK, Wong ML. Systematic sequence analysis of the human fucosyltransferase 2 (FUT2) gene identifies novel sequence variations and alleles. Transfusion 2007 Aug;47(8):1369-80 (PMID: 17655580).

7. Chang JG, Yang TY, Liu TC, et al. Molecular analysis of secretor type alpha(1,2)-fucosyltransferase gene mutations in the Chinese and Thai populations. Transfusion 1999 Sep; 39(9):1013-7 (PMID: 10533829).

8. Henry SM, Woodfield DG, Samuelsson BE, Oriol R. Plasma and red-cell glycolipid patterns of Le(a+b+) and Le(a+b-) Polynesians as further evidence of the weak secretor gene Se(w). Vox Sang 1993;65(1):62-9 (PMID: 8362517).

9. Scharberg EA, Olsen C, Bugert P. The H blood group system. Immunohematology 2016 Sep;32(3):112-8 (PMID: 27834485).

10. Matzhold EM, Wagner T, Drexler C, Schonbacher M, Kormoczi GF. Aberrant ABO B Phenotype with Irregular Anti-B Caused by a Para-Bombay FUT1 Mutation. Transfus Med Hemother 2020 Feb; 47(1):94-7 (PMID: 32110200).

11. Kim MS, Kim JS, Park H, et al. The First Case of Para-Bombay Blood Type Encountered in a Korean Tertiary Hospital. J Korean Med Sci 2019 Oct 14;34(39):e258 (PMID: 31602828).

12. Lin-Chu M, Broadberry RE. Blood transfusion in the para-Bombay phenotype. Br J Haematol 1990 Aug; 75(4):568-72 (PMID: 2207009).