Case Series

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The importance of testing for secretor status of ABH antigens: A case series

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Abstract:

While simple serological tests such as forward and reverse grouping can help identify rare phenotypes, their Confirmation is to be done with a battery of other serological tests including adsorption elution and nonserological tests such as saliva testing for secretor status. Two cases of H-deficient secretor state (para-Bombay) and one case of a weak B phenotype are presented here highlighting the importance of nonserological tests. Case 1: A 50-year-old male who is a repeat blood donor, with no significant history, had "O positive" by forward grouping, "B" by reverse, and no reaction with anti-AB antisera or anti-H lectin. Adsorption elution showed the presence of weak B antigen and saliva testing confirmed secretor status for B and H antigen. Case 2: A 25-year-old primigravida, with no significant history, had "O positive" by forward grouping, "B" by reverse, and no reaction with anti-AB antisera or anti-H lectin. However, adsorption elution showed the absence of any antigen on RBCs. The saliva testing confirmed secretor status for B and H antigen. Both of them were compatible (Coomb's major and minor) with "B" and "O" units and were grouped as para-Bombay B. Para-Bombay phenotypes can present with complete or partial suppression of ABH antigens and nonserological tests are valuable in their diagnosis. Case 3: A 27-year-old first-time blood donor, with no significant history, also had "O Positive" by forward grouping, "B" by reverse grouping, and no reaction with anti-AB antisera. Furthermore, 3+ reaction with anti-H lectin was noted. Saliva testing confirmed secretor status for B and H antigens. He was also compatible with "B" and "O" units and was grouped as "Weak B." Further classification was not possible as adsorption-elution tests could not be done.

Keywords:

H-deficient secretor, para-Bombay, secretor status, subgroups

Introduction

Performing both forward and reverse grouping for ABO grouping is an important step that helps immunohematologists suspect many rare phenotypes. These then have to be confirmed with further serological and nonserological workup. One of the most common and diagnostically valuable nonserological tests is saliva testing for secretor status of ABH antigens. Here, two cases of the para-Bombay B phenotype and one case of a weak B phenotype are detailed that show how the determination of secretor status can be a valuable tool in the evaluation of immunohematological discrepancies.

Case Reports

Case 1

A 50-year-old voluntary blood donor, who had donated five times before, donated for the 6th time at this South Indian Tertiary Care Hospital. He had reported his blood group as "O positive" during his predonation interview. A discrepancy was noted between his forward and reverse ABO grouping patterns and further evaluation was done.

Case 2

A 25-year-old primigravida came for her routine check-up. Her sample was sent for routine ABO grouping and RhD typing. The

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discrepancy in her ABO grouping and RhD typing was similar to that in case 1.

Case 3

A 27 year old man, a first time blood donor with no significant prior history showed a discrepancy in his ABO grouping and RhD typing, similar to that in cases 1 and 2.

The discrepancy seen in all the three cases is shown in Table 1.

The same battery of serological tests was conducted in an attempt to resolve the discrepancies in these three cases. The findings are summarized in Table 2.

While case 1 and case 2 showed identical reactions in the entire battery of tests, case 3 showed one major difference - a reaction of 3+ grade was noted with anti-H lectin, to be contrasted with no reaction in case 1 and 2.

After a thorough literature search and suspecting H-deficient B secretor state (para-Bombay B), adsorption with commercial anti-B antisera and heat elution was done as per procedures stated in AABB Technical Manual, 15th Edition.[1] The results are shown in Table 3. This would have been a logical step in the evaluation of case 3 as well, but could not be done due to the scarcity of the sample.

In case 1, a 2+ reaction was seen between the B-cells and the elute, signifying the presence of a weak B antigen on the red blood cells (RBCs), in case 2, this reaction was absent. No antigens were detected on repeating adsorption with two different commercial and donor derived anti B antisera and using Lui freeze thaw elution technique.

All three cases were recalled. It was elicited that none of them had any significant prior history of any major illness, drug intake, transfusion, or stem cell transplant. A sample of each of their saliva was collected to assess their secretor status by the technique mentioned in AABB Technical Manual 15th Edition.[1] The results in all the three cases were identical and are highlighted in Table 4.

The three cases were all compatible (Coomb's major and minor) with "B" and "O" blood. Fresh blood samples were obtained from cases 1 and 2 on their second callback and the entire battery of tests repeated with identical results. They were called back for a third time thereafter and counseled.

Case 3 refused to provide another blood sample or to return for a third callback.

Final ABO and RhD types:

Table 1: Discrepancy noted in forward and reverse grouping in the three cases (page 2)

Anti-A	Anti-B	Anti-D	Result	A-Cell	B-cell	O-cell	Result
0	0	4+	O positive	4+	0	0	B

*Forward grouping shows O Positive, reverse grouping, however, is consistent with B, +Anti-A, anti-B, and anti-D antisera (Monoclonal, Eryclone, Tulip Diagnostics, Goa)

Table 2: Serological tests employed to resolve ABO discrepancy (page 2)

Test	Case 1	Case 2	Case 3
Ruling out type I ABO grouping discrepancy (weak/missing antibody)			
Reverse grouping with increased serum: Cell ratio	0	0	0
Reverse grouping with three different sets of pooled "B cells"	0	0	0
Reverse grouping at 37°C	0	0	0
Reverse grouping at 4°C	0	0	0
Ruling out type II ABO grouping discrepancy (weak/missing antigen)			
Forward grouping with increased cell: antisera ratio (5:1)	0	0	0
Forward grouping with two different commercial anti-B antisera (combined ABO monoclonal antibodies, J. Mitra, New Delhi and Span Clone ABD agglutinating antisera, Span Akrey Healthcare, Surat)	0	0	0
Forward grouping with three different donor-derived anti-B antisera	0	0	0
Anti-AB antisera	0	0	0
Anti-H lectin	0	0	3+

Table 3: Results of adsorption and elution findings in cases 1 and 2 (page 2)

	B-cells		O-cells		Interpretation
	Final wash	Eluate	Final wash	Eluate	
Case 1	0	2+	0	0	Weak B antigen present on RBC
Case 2	0	0	0	0	B antigen absent on RBC

RBC=Red blood cell

Case 1: Para-Bombay B positive, Case 2: Para-Bombay B positive, Case 3: Weak B positive.

Discussion

Reverse (serum) grouping plays an important role in identifying subgroups and H-deficient states such as Bombay phenotype. Two of the cases discussed here are referred to as para-Bombay phenotype. H (and subsequently A/B antigens) are either absent or greatly reduced on the RBC surface and cannot be detected by routine serology.

In such cases, adsorption elution must be performed. Here, the concerned antisera (anti-A or anti-B) are

Table 4: Saliva testing for assessing secretor status-findings in the three cases (page 2)

	Anti-B (1:512)	Anti-H (1:32)
Saline control	2+	2+
Known secretor saliva	0 (B)	0 (O)
Known nonsecretor saliva	2+ (O)	Saliva from Bombay individual was not available
Test	0	0
Interpretation	Secretor of B-antigen	Secretor of H-antigen

adsorbed onto the RBCs by prolonged incubation at 4°C. The cells are then washed with ice-cold normal saline, the last wash preserved, and cells incubated with bovine albumin for 10 min at 56°C, causing elution of the adsorbed antibodies. The eluate so obtained is tested in parallel with the final wash forward grouping is performed with three populations each, of cells expressing(in this case, concerned antigen being B, "B cells" express the antigen) and not expressing the concerned antigen (in this case, O). Reaction with final wash must be negative with both categories of cells for the test to be valid. If eluate tests are negative, it indicates nonadsorption of antibodies (from antisera, during incubation) due to the absence of suspected antigen.

ABH antigens are also secreted in most body fluids such as saliva. Assessing this secretor status is a valuable tool in diagnosing rarer phenotypes as demonstrated in the cases, particularly the 3rd case where adsorption elution could not be done. When present in saliva they neutralize the respective antibodies in appropriately diluted antisera, such that when indicator cells having that antigen is added to these tubes, they do not agglutinate. The presence of agglutination indicates the absence of the concerned antigen in the saliva.

H, *Se*, and *AB* are the three genes at three separate loci that control the occurrence and the expression of the A and B antigens. The active alleles at these loci, *H and Se*, produce transferases that act at the cellular level to form the H antigen on red cells and secretions, respectively. H antigen is the substrate on which A and B antigens are formed.

Rarely, individuals may lack both *H* and *Se* alleles (genotype *hh* and *sese*). They have no H and therefore, no A or B antigens on their red cells or in their secretions (O_h phenotype). However, some *hh* individuals may demonstrate H, A, and B antigens in their secretions due to at least one *Se* allele which may get adsorbed onto the RBC surface and be detectable by adsorption elution only.

In these individuals:

- The H antigen on RBCs may be present in small amounts (detectable only by adsorption-elution techniques and not routine serological techniques) and may get converted to the A and/or B antigens depending on the genes present or may be completely absent.

These are called the para-Bombay phenotypes. Cases have been described of Para-Bombay A, B, and even AB phenotypes.[2]

Weak/subgroups of B, though rarer than those of A, are recognized by their varying strengths of reaction with anti-B and anti-AB. These result from alternate alleles and polymorphisms at the B locus. A combination of serological and nonserological tests proves valuable in such cases as well, and depending on their results, weak B is categorized as - B_3, B_x, B_m, or B_{el}.[2] Even B_y phenotype has been described.[3]

This report compiles three cases of decreased H (and B), absent H (and B), and normal H but reduced B antigens, respectively, on RBC surfaces with anti-A in the serum and normal amounts of B and H substances in the saliva. Case reports have described each of these phenotypes separately, one at a time.[4-9] This compilation and comparison of the three possible phenotypes of H (and B) antigenic expression and of their individual findings on different tests will hopefully provide an insight to others facing such cases regularly, more conveniently.

It is also interesting that neither of the first two cases had any anti-H or anti-IH activity, even at 4°C.

It has been established previously in the literature[10] that units of the compatible ABO group are safe for transfusion in these people. In the present cases, ABO groups B and O were found to be compatible (Coomb's major and minor) with all three patient samples. Molecular and family studies are warranted, but could not be done in these cases due to financial limitations.

Conclusion

Confirming ABO grouping with both forward and reverse groupings is a simple and effective first step to suspecting many rare phenotypes. Further confirmation may need a battery of serological tests including adsorption-elution and even nonserological tests such as saliva testing for ABH secretor status. The cases described highlight that H-deficient secretor states (para-Bombay) or ABO subgroups may be present with complete or partial suppression of ABH antigen expression on RBCs, but the presence of these antigens

in secretions. Assessing secretor status is of immense value in such cases.

Declaration of patient consent

The authors certify that besides the routine consent, an additional written consent from the participants was obtained permitting the use of their details in this report under the condition of anonymity.

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Conflicts of interest

There are no conflicts of interest.

References

1. Methods. In: Brecher EM, Leger MR, Linden JV, Roseff SD, editors. Technical Manual. 15th ed. Maryland: AABB Publications; 2005. p. 735-8, 773-5.
2. Harmening D, Forneris S, Tubby BJ. The ABO blood group system. In: Harmening D, editor. Modern Blood Banking and Transfusion Practises. 6th ed. Philadelphia: FA Davis Company; 2012. p. 119-35.
3. Lenkiewicz B, Seyfried H. First example of by phenotype. Mater Med Pol 1990;22:26-8.
4. Mathai J, Sulochana PV, Sathyabhama S. Para Bombay phenotype – A case report. Indian J Pathol Microbiol 1997;40:553-4.
5. Yashovardhan A, Chaitanya Kumar IS, Sreedhar Babu KV, Suresh Babu B, Verma A, Siddhartha Kumar B, *et al*. Para-Bombay phenotype report of a rare bloodgroup. J Clin Sci Res 2012;3:141-3.
6. Subramaniyan R. AB Para-Bombay phenotype: A rare blood group variant and its clinical significance. Hematol Transfus Cell Ther 2018;40:96-7.
7. Patel JN, Donta AB, Patel AC, Pandya AN, Kulkarni SS. Para-Bombay phenotype: A case report from a tertiary care hospital from South Gujarat. Asian J Transfus Sci 2018;12:180-2.
8. Chacko MP, Mathan A, Daniel D. Para-Bombay: A blind spot in blood grouping? Asian J Transfus Sci 2011;5:182-3.
9. Yoshida A, Yamato K, Davé V, Yamaguchi H, Okubo Y. A case of weak blood group B expression (Bm) associated with abnormal blood group galactosyltransferase. Blood 1982;59:323-7.
10. Lin-Chu M, Broadberry RE. Blood transfusion in the Para-Bombay phenotype. Br J Haematol 1990;75:568-72.