Case Report

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Detection of a rare subgroup of A phenotype while resolving ABO discrepancy

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Abstract:

Weaker subgroups of ABO blood group system give rise to discrepancies between forward and reverse grouping and cause diagnostic difficulties in routine blood banking. Weaker subgroups of A blood group that have been reported so far include A3, Aend, Ax, Am, Ay, and Ael. We report a case of a 54-year-old patient whose red cells showed a discrepancy between cell and serum grouping on initial testing. Serological investigation included absorption elution tests and saliva testing after performing initial blood grouping. Molecular genotyping of the ABO gene was performed by DNA sequencing of exons 6 and 7 of the ABO gene. The serological characteristics of the patient's red cells were similar to Ax subtype. The patient was a secretor and only H substance was present in the saliva. Serum did not show the presence of anti-A1. Molecular genotyping confirmed the ABO status as Aw06/O13. The weak A phenotype identified in the propositus had serological characteristics similar to Ax and showed the ABO genotype Aw06/O13. Although Aw06 allele has been previously reported in the Indian population, this is the first study to report O13 allele in the Indian population.

Keywords:

Aw06, Ax, O13, subgroup, weaker variants

Introduction

Due to their immunogenicity, the ABO antigens are of prime importance in blood transfusions, hemolytic disease of the fetus and newborn due to ABO incompatibility, and organ transplantation. Correct typing of ABO blood groups of blood donors and recipients is therefore essential. The antigens are encoded by the ABO gene which is located on chromosome 9. It consists of seven exons and six introns. The three main alleles encoded by this gene are A, B, and O.[1]

ABO antigens are routinely detected using hemagglutination-based methods. The presence of weaker subgroups of A and B gives rise to discrepancy in cell and serum grouping. Weaker subgroups of A are defined as those Group A participants whose erythrocytes give weaker reactions or are nonreactive serologically with anti-A antisera than A_2 RBCs.[2] These weak phenotypes, in majority of cases, result from expression of a variant A allele present at the ABO loci. These can be divided into two categories depending on whether the cells are agglutinated with anti A-A_3, A_{end}, and A_x are agglutinated, while A_m, A_y, and A_{el} cells are not. The above weaker phenotypes can be serologically differentiated from each other using the following techniques:[3]

a. Cell grouping using anti-A, anti-B, anti-A, B, and anti-H and serum grouping to detect ABO antibodies
b. Testing with different batches of anti-A reagent
c. Adsorption-elution experiments with polyclonal anti-A and anti-A + B from Group B and Group O individuals, respectively

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d. Secretor status for the presence of H and/or A antigen in saliva
e. Molecular genotyping of ABO gene, especially exons 6 and 7 as they encode for 77% of glycosyltransferase activity.

The weak A phenotype identified in the present case had serological characteristics similar to A_x blood type and showed the ABO genotype Aw06/O13. Although Aw06 allele has been previously reported in the Indian population, this is the first study to report O13 allele in the Indian population.

Case Report

A 54-year-old gentleman was admitted in the cardiology intensive care unit for angioplasty. Blood sample was sent to the blood center for ABO grouping and Rh typing. Discrepancy was observed in cell and serum grouping. The patient's red cells were nonreactive with anti-A, anti-B, and anti-A_1. However, it showed weak reaction with anti-A, B and strong agglutination reaction with anti-H (++++). The patient's serum showed the presence of anti-B antibody [Table 1]. Blood grouping pattern observed was suggestive of the presence of weaker subgroup of A, indicating type II discrepancy.[1]

Blood grouping was repeated and technical errors were ruled out. To detect the presence of weak A antigen, heat elution was performed on the patient's red cells. The eluate showed microscopic agglutination with three different A group cells. To detect the presence of soluble substances, secretor status was determined using patient's saliva. The patient was found to be a secretor, having only H substance detectable in the saliva. These serological reactions obtained were consistent with reactivity pattern of A_x subtype. Molecular genotyping of the ABO gene was carried out by DNA sequencing of fragments specific for exons 6 (479 bp) and 7 (975 bp) as described by Cai *et al*.[4] Briefly, exons 6 and 7 were amplified using the following polymerase chain reaction (PCR) program: an initial denaturation of 3 min at 95°C; 30 amplification cycles consisting of 30 s at 95°C, 30 s at 56°C, and – 30°C to 60 s at 72°C; and then, a final extension step of 8 min at 72°C. The primer sequences are: For exon 6: Forward primer 5′ tggaagggtggtcagagga3′ and reverse primer 5′ctggagaaggagctgggtt 3′ and For Exon 7: 5′ tgggaagaggatgaagtgaat 3′ and reverse primer 5′ caacaggacggacaaagga 3′. The PCR products were then purified from agarose gel using a gel extraction kit (Biobasic) and subsequently sequenced on the ABI Prism 310 Genetic Analyzer (Applied Biosystem, CA, USA). The data were analyzed using Sequencing Analysis Software, Version 5.2 (Applied Biosystem, CA, USA) (ABI 377, Applied Biosystems, Foster City, CA, USA). The ABO genotype of the patient was found to be Aw06/O13.

Discussion

Weaker variants of A and B arise due to inheritance and expression of variant alleles at the ABO locus. They are mostly identified using hemagglutination-based methods. However, due to variation in reagents and techniques used, these weaker phenotypes are often mistyped as O group. If a donor of weaker subgroup is mistyped and labeled as O group and transfused to O group recipients, it may lead to decreased survival of the red cells due to the presence of ABO antibodies in the recipient's serum. Hence, it is important to characterize these weaker subgroups as accurate determination of the ABO type would help in better management of "O" group RBCs and "AB" plasma for transfusion in discrepant cases.[5]

In the present case study, the patient's group was serologically identified as A_x type. The red cells of A_x individuals distinguish themselves by giving negative or weak agglutination by anti-A sera, negative reactions with anti-B sera, but remarkably strong reactions with anti-A, B sera. The A_x phenotype has been associated with many different A variant alleles.[6] The patient's red cells in the present study showed negative reaction with anti-A sera but remarkably strong reaction with anti-A, B. Serum, however, did not show the presence of anti-A_1. The molecular genotype was found to be Aw06/O13. This finding is similar to that reported by Seltsam *et al*., 2002, who found that serological characteristics of RBCs of three patients were similar to subgroup A_x and the serum contained weakly reactive anti-A_1. The ABO genotype of all the three patients showed the presence of variant A allele, Aw06.[7]

Table 1: Serological and molecular findings observed on testing the patient

Blood grouping										Group	Absorption-elution			Secretor status	Molecular genotyping of exons 6 and 7 of the ABO gene
Forward						Reverse									
Anti-A	Anti-B	Anti-A, B	Anti-A_1	Anti-H	Anti-D	A cells	B cells	O cells	A_1 cells		Anti-A	Anti-B	Anti-A + B		
0	0	Weak	0	++++	++++	0	+++	0	0	Ax type	++	0	+++	A and H substances present	Aw06/O13

++,+++,++++ the grades of agglutination reactions 2+, 3+ and 4+

Aw06 is identical to the reference allele, ABO(*) A101, except for a single-base substitution in exon 7 at position 502, where C was replaced by G causing an amino acid exchange from arginine to glycine at position 168 [Figure 1]. Aw06 has been reported previously in the Indian population and known to cause weak expression of A antigen.[8]

O13 is deletional O allele which is characterized by single-point mutations 261delG; 297A>G; 646T>A; 681G>A; 771C>T; and 829G>A in exon 6 and 7 of the ABO gene. This allele has been reported at low frequencies in several populations, and interestingly, its frequency increases from north to south for Basques (from France and Spain, 0% ±5% of all O alleles), Berbers (from Morocco, 9%) and Akans (from Ivory Coast, 19%).[9,10]

Conclusion

Discrepancies in blood typing can be avoided through detailed serological tests and analysis. In the present case, the weak A phenotype having serological characteristics similar to A_x blood type showed the ABO genotype Aw06/O13. Although Aw06 allele has been previously reported in the Indian population, this is the first study to report O13 allele in the Indian population. Molecular genetic approaches help in the correct determination and characterization of weaker ABO subgroups and helps decision making in routine serology.

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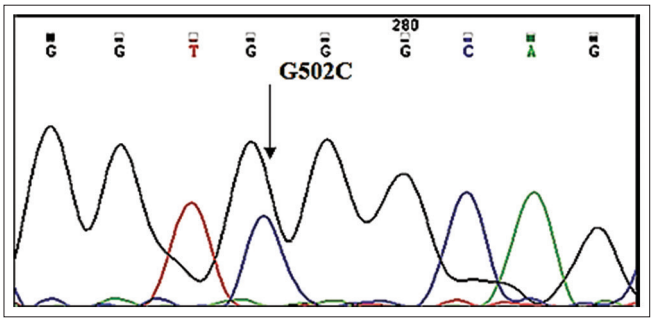

Figure 1: Electropherogram showing 502G>C mutation (heterozygous) identified in the patient A = Adenine, C = Cytosine, G = Guanine, T = Thymine

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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Nil.

Conflicts of interest

There are no conflicts of interest.

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