Case Report

A_2B with Clinically Significant Anti-A_1 : A Case Report on the Incidental Finding

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ABSTRACT

The purpose of this article is to report an incidental finding of a clinically significant anti-A1 antibody while detecting blood group of an elderly patient. As this antibody is reactive at 37□, this may cause destruction of transfused A1 red cells and this is the cause of clinical significance. Usually anti-A1 antibodies in plasma are naturally occurring antibodies, not clinically significant because they react best below room temperature, not at body temperature sometimes causing discrepancy during routine blood grouping and crossmatching. For this particular case, some precautions should be taken before blood transfusion to avoid hemolysis.

Keywords: *ABO blood group, Rh typing, Agglutination reaction*

INTRODUCTION

As of October 2024, the International Society of Blood Transfusion (ISBT) has recognized 47 blood group systems containing 366 red cell antigens[1]. Four main blood groups; A, B, AB and O are enlisted in ABO system, which was discovered in 1900 by Karl Landsteiner, an Austrian-American biologist, physician and immunologist. The ABO blood group is determined by the presence or absence of an antigen on red cell membrane and the absence or presence of a corresponding antibody in plasma[2]. The expression of ABO antigen is controlled by three separate genetic loci[3]. Numerous mutations are found in A, B and O genes, but the most common mutation is A_2. The A_2 gene has two nucleotide different from the A_1 gene which results in diminished enzymatic activity and consequently, weakened antigen expression[4].

Distinction between A_1 & A_2 made by testing red cells with the lectin from Dolichos biflorus[5]. Typically blood group AB individuals express both A and B enzymes and carry both antigens on their RBCs. Because the A and B glycosyltransferases are not 100% efficient, blood group A, B, and AB individuals also express some H antigen[6]. The relative amounts of H antigen are found in the following sequence of phenotypes: O > A_2 > B > A_2B > A_1 > A_1B > Para-Bombay > Bombay[7]. This H antigen can be detected by testing red cell with anti-H lectin which is commercially available.

The incidence of ABO groups varies very markedly in different parts of the world and among different races[8]. Anti-A and anti-B are usually detectable within 3 to 6 months after birth[9]. At the age of 5 years, the titer of anti-A and anti-B antibodies reaches a maximum and persists throughout adulthood. The titer of IgM anti-A and anti-B antibodies may gradually decline with advanced age[10]. The frequency of the common A subgroups varies greatly among different populations. In A and AB blood groups among Caucasian population, approximately 80% are A_1 or A_1B and 20% are A_2 or A_2B[11,12].

According to an Indian study report, the frequency of A_1 and A_2 subgroups, among A blood group was 98.14% and 1.07%, respectively whereas, in AB blood group, the frequency of A_1B was 89.28% and that of A_2B was 8.99%. This report describes the proportion of A_2B among AB blood group as significantly higher than that of A_2, in group A blood group[13] and approximately the same distribution is obtained by Bangera[14].

CASE REPORT

A 78 year old male, diagnosed case of β thalassaemia trait, who has never received any blood transfusion came to check his blood group as part of routine investigations. For ABO grouping and RhD typing, 3ml blood sample in an EDTA tube was received in the Transfusion Medicine Department. Red cell and plasma were separated by centrifugation at 4000 rpm using a tabletop centrifuge machine. A 3% cell suspension was prepared from the washed red cells of the patient. Reagent A cell, B cell and O cell were prepared from in-house pooled A cell, B cell and O cell. Blood grouping was done by both the column agglutination method using Ortho BioVue ABD forward and reverse cassettes and conventional slide tests. Forward grouping revealed AB blood group whereas reverse grouping showed B blood group (due to agglutination with A cell which was an unexpected reaction). Reaction pattern is shown in the Table 1

Table 1 : Blood Group in Column Agglutination Technology

Anti-A	Anti-B	Anti-D	O cell	A cell	B cell
+	+	+	-	+	-

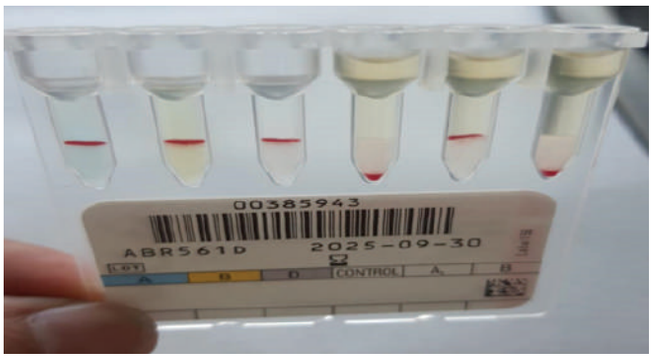

Figure : Blood Group in Column Agglutination Technology

Without solving this ABO discrepancy, blood group could not be confirmed. Patient's sample and identification, possible contamination in pooled cells used for reverse grouping as well as lot number and expiration date of gel card; all were rechecked. Repeat blood grouping both forward (testing patient's cell with reagent) and reverse (testing patient's plasma with in house prepared A cell, B cell and o cell) by traditional test tube method at 4ºC, room temperature (22-25ºC) and 37ºC showed similar results.

Table 2 : Blood group at different temperatures

	Anti-A	Anti-B	Anti-AB	Auto control	Anti-D	A cell	B cell	O cell
At 4℃	+	+	+	neg	+	+	neg	neg
At 22-25℃	+	+	+	neg	+	+	neg	neg
At 37℃	+	+	+	neg	+	+	neg	neg

On further testing with anti-A1 lectin, no agglutination reaction was observed. As there was no agglutination reaction with anti-A1 lectin, his ABO blood group was confirmed as A_2B which is a subgroup of AB blood group. Reaction with anti-H lectin showed positive reaction.

However, to exclude any chance of possible hemolytic transfusion reaction upon blood transfusion if required at any time, thermal amplitude of anti-A1 antibody was checked at 4°C, 22-25°C and 37°C temperature. To see the strength of the agglutination reaction, titration was done by double dilution method after a serial dilution of serum (1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128….). Antibody titre obtained was 1:32 at 37°C and 1:64 at both 4°C and 22-25°C. The presence of this anti-A_1 antibody was marked as clinically significant as it was reactive at 37°C. Family history of this patient could not be obtained.

DISCUSSION

A_1 and A_2 are the most common sub-types of A blood group. Other less prevalent sub-types are A_3, A_x, A_{el}, Aend. Individuals with A_1 sub-type express A_a, A_b, A_c and Ad antigenic determinants, whereas A_2 sub type have only A_a and A_b antigenic determinants. Absence of A_c and A_d determinants is assumed to be the cause of anti-A_1 antibody development in A_2 sub-type[15]. Approximately 0.4% of A_2 and 25% of A_2B individuals possess anti-A_1 antibody which is naturally occurring IgM cold antibody, reacts best below room temperature, causing discrepancy in blood grouping but does not cause any transfusion reaction[16]. However, in some cases this anti-A^1 antibody is reactive at 37ºC and

can cause hemolytic transfusion reaction if A_1 cell is transfused[17,18].

In this particular case, thermal amplitude of this anti-A_1 antibody is very wide (4- 37ºC). Reactivity at 37ºC temperature is the cause of clinical significance as it may cause hemolysis if A_1B cells are transfused to this individual. This patient was advised to accordingly that if blood transfusion is required at any time, A_2B blood group should be selected as well as crossmatching by IAT(Indirect Antiglobulin Test) method is a must. As it is very difficult to find out a donor of A_2B blood group, PRBC of O blood group and plasma or plasma components from AB blood group can be transfused.

CONCLUSION

In addition to standard ABO and Rh typing, extended antigen typing may be needed. It is also advisable to perform an antibody screen along with their thermal amplitude on the recipient's sample prior to blood transfusion, allowing for the selection of corresponding antigen-negative blood products when necessary. Furthermore, maintaining accurate records of patient's antibody history and transfusion reactions can help improve safety protocols and guide healthcare providers in making informed transfusion decisions. Continued education and training for staff involved in blood transfusion practices are imperative to ensure adherence to guidelines and to minimize the potential for adverse events.

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