A Case Report of Occult Weak 'A' Subgroup: An Important Message for Renal Transplant Physicians

Abstract

Accurate ABO grouping is the cornerstone of a successful ABO-compatible organ transplant. While conventional methods identify blood groups accurately in most cases, rare and weak blood groups could occasionally be misread/missed. Weak A subgroups such as A3, Ax, Aend, Am, Ay, and Ael are often mistyped as group O. We present one interesting case of 'weak A' subgroup in a renal transplant donor, who was wrongly typed as 'O' Rh D positive by conventional grouping techniques. It was a near miss as the donor was almost selected for transplant for the patient with blood group B positive.

Keywords: *Anti A, B antisera, blood group discrepancy, weak subgroup*

Mitu Dogra, Rasika Setia, Vishal Saxena[1], Amit K. Yadav[1]

Departments of Transfusion Medicine and [1]Renal Sciences and Kidney Transplant, BLK-Max Super Specialty Hospital, New Delhi, India

Introduction

The ABO blood group system was discovered more than a century ago and still often raises uncertainty during sub-typing or detection of weaker variants. Accurate ABO grouping is the cornerstone for a successful ABO-compatible organ transplant. There are no fixed standards for how any laboratory should perform ABO blood typing. Majority of the laboratories adopt routine methods like conventional tube technique (CTT) or column agglutination techniques (CAT) using Anti-A, Anti-B and Anti-D antisera for forward typing; and A cells and B cells for reverse typing. Use of Anti-A, B antisera, Anti-A_1 lectin, Anti-H lectin and A_2 cells is purely optional[1] and is not used by most labs. While conventional methods identify blood groups accurately in most cases, rarer and weaker blood groups can occasionally be misread or inadvertently missed by routine testing methods. Weaker subgroups of A such as A_3, A_x, A_{end}, A_m, A_y, and A_{el}, due to weak reactions by conventional techniques, are often mistyped as group O and may potentially be dangerous when it comes to a solid organ transplantation.[2] Differentiation of weak A and B phenotypes require extended typing methods and specialized techniques. Anti-A, B antisera is more effective in detecting weakly expressed A and B antigens as compared to monoclonal reagents Anti-A or Anti-B.[3]

Case Report

A living donor kidney transplant was planned for a 69-year-old male with end-stage renal disease. The donor was a 50-year-old, healthy female. Both recipient and donor had undergone a preliminary pre-transplant workup at an NABL accredited pathology laboratory where blood grouping had been performed by CTT and the donor and recipient's groups reported as O RhD-positive and B RhD-positive, respectively. Additionally, the donor also had a previous blood group report of O RhD-positive from another well-established laboratory.

As per hospital transplant workup protocol, the donor's sample was sent to our blood bank for pre-transplant blood group confirmation. Grouping was performed using hemagglutination (HA) technique (twelve-well-typing), which included Anti-A, B antisera, Anti-A_1 lectin in forward typing and A_2 cells in reverse typing. Donor's red cells were nonreactive with monoclonal Anti-A and Anti-B antisera, and Anti-A_1 lectin, however, showed 2+ reaction with Anti-A, B antisera and strong agglutination reaction with Anti-H lectin (4+). On reverse grouping, the donor's serum showed reaction with A_1 cells (2+) and B cells (4+), but no reaction with A_2

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Address for correspondence:
Dr. Mitu Dogra, House No. E 50, Mansarover Garden, New Delhi - 110 015, India.
E-mail: drmithu25@yahoo.in

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and O cells, indicating presence of Anti-A_1 and Anti-B isoagglutinins [Table 1]. Blood grouping pattern observed on HA showed a type-II discrepancy suggestive of a weak A subgroup, which was missed by routine CTT and CAT. Blood grouping was repeated by extended CTT method, with additional Anti-A, B antisera and Anti-A_1 and Anti-H lectins and A_2 cells. Reaction pattern by extended CTT method showed findings similar to HA [Table 1]. To detect weak A antigen, Lui freeze–thaw elution procedure was performed on the donor's red cells. The Lui freeze–thaw elution technique is the most simple technique and very effective for eluting ABO antibodies. This procedure is based on the principle that weak ABO subgroups are too weak to be detected by direct agglutination techniques, even after application of cold temperature and antibody enhancement. Presence of such weak A antigens, B antigens, or both can be elicited by adsorbing monoclonal anti-A or anti-B antibodies to these red cells, followed by elution of bound antibody by rapidly freezing these red cells at a temperature less than -30°C. The extracellular ice crystals that form attract water from their surroundings, increasing the osmolarity of the remaining extracellular fluid, which then extracts water from the red cells. The red cells shrink, resulting in cell lysis. As the membranes are disrupted, the isoantibodies, if adsorbed, are dissociated from the red cell surface and come into the eluate. This eluate when reacted with group specific reagent A_1 or B red cells, show agglutination if any isoantibodies are present in the eluate, as the reagent red cells have adequate number of ABO antigens on their surface.[4]

The eluate showed: a) agglutination with reagent A_1 cells, b) failed to agglutinate reagent O and A_2cells, indicating a weak A subgroup with Anti-A_1 isoantibodies, possibly indicating A_{end} or A_x subgroup. Serologically, A_{end} is almost similar to A_x and even adsorption–elution tests fail to differentiate between the two and need saliva testing for discrimination. To detect presence of soluble substances, secretory status was determined using the donor's saliva. She was found to be a secretor, having only H substance detectable in the saliva, excluding the possibility of A_{end} phenotype [Figure 1a and b]. The serological reactions and saliva findings obtained were consistent with reactivity

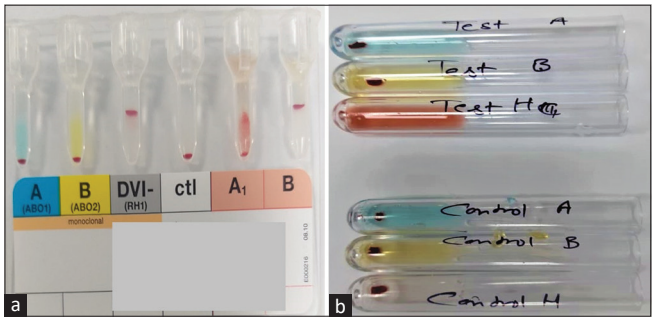

Figure 1: (a and b) Part A suggests blood group of donor falsely as O-positive on column agglutination technique. Part B suggests that the donor is secreting H substance in her saliva

Table 1: Serological, adsorption-elution and saliva test findings observed on testing the donor via Hemagglutination (HA) method and extended conventional tube technique (Extended CTT)

Method	Blood grouping									Adsorption-Elution				Secretory status	Group
	Forward				Reverse			Anti A		Anti B	Anti A, B				
	Anti-A	Anti-B	Anti A, B	Anti- A1	Anti- H	A1 cells	A2 cells	B cells	O cells	A1 cells	A2 cells	B cells	A1 cells	A2 cells		
HA	0	0	3+	0	4+	3+	0	4+	0	2+	0	0	2+	0	H substance present	Ax Type
Extended CTT	0	0	2+	0	4+	2+	0	4+	0							
CAT	0	0	N/A	N/A	N/A	2+	N/A	4+	N/A							

Key: 0=No red blood cell agglutination; 1+to 4+=Grades of red blood cell agglutination; N/A=Not applicable

Table 2: Serological reactions of weak subgroups of a phenotype

Weak subgroups	Forward grouping			Lectin		Reverse grouping		Substances present in saliva of secretors	Presence of A transferase in Serum	Number of Antigen sites RBC *10^3
	Anti A	Anti B	Anti-A, B	Anti-A_1	Anti-H	A Cells	B Cells	O Cells		
A_3	++mf	0	++mf	0	3+	No	Yes	Sometimes	A, H	Sometimes	35
A_x	wk/0	0	2+	0	4+	0/wk	Yes	Almost always	H	Rarely	5
A_{end}	wk mf	0	wk mf	0	4+	No	Yes	Sometimes	H	no	3.5
A_m	0/wk	0	0/+	0	4+	No	Yes	No	A, H	Yes	1
A_y	0	0	0	0	4+	No	Yes	No	A, H	trace	1
A_{el}	0	0	0	0	4+	Some	Yes	Yes	H	no	0.7

*Number of Antigen sites are important as more antigenic sites are responsible for rejection of graft

pattern of A_x phenotype [Table 2]. One of the donor's children, her son, who was available on site was also tested and found to be A_2B RhD-positive with anti-A_1 isoagglutinins. The donor's husband's blood group was confirmed to be B RhD-positive. Finally, the donor was reported as Weak A subgroup RhD positive-A_x phenotype.

Discussion

Principles of ABO compatibility are fundamental to any solid organ transplantation. Naturally occurring IgM anti-A and/or anti-B isoantibodies present in the serum of recipient constitute a major barrier against ABO-incompatible solid organ transplantation and are overcome by adopting various desensitization protocols;[5] however, the risks of infection and rejection are higher in such transplants. Although group A_2 or weaker subgroup donors have been shown to be equivalent to group O solid organ donors for non-O solid organ transplant recipients,[6] a donor of weaker subgroup, if mistyped as O group and selected as a solid organ donor for across group recipients, may lead to graft rejection due to presence of high anti-A or anti-B isoagglutinins in the recipient's serum. Differentiating subgroup of A enables transfusion and transplant facilities to plan solid organ transplantations appropriately. In the present case study, the donor's group was serologically identified as A_x type. The red cells of an A_x individual distinguishes themselves by giving negative or weak agglutination by monoclonal anti-A antisera, negative reactions with monoclonal anti-B antisera, but remarkably strong reactions with anti-A, B antisera. Weaker variants of A and B arise due to inheritance and expression of variant alleles at the ABO locus and are mostly identified using hemagglutination-based methods.[7] Hence, accurate ABO typing is vital for a successful organ transplantation and should be confirmed with additional studies when initial blood grouping shows a discrepancy. Detailed workup on blood groups can help identify weaker subgroups of A and B or rare Bombay and para-Bombay phenotypes so that appropriate desensitization protocols can be followed.[8]

Conclusion

To conclude, a nephrologist's awareness of the methodology used for blood grouping of a potential solid organ donor and correct serologic interpretation of the donor's weak A, B antigens is very important for clinical decision-making, the desensitization protocol to be used, and the patient's well-being.

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Conflicts of interest

There are no conflicts of interest.

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