Case Report

In-house preparation of lectin panel and detection of Tn polyagglutination

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Abstract:

Polyagglutination is a condition in which red cells are agglutinated by ABO-compatible adult human sera, but not by cord blood sera and may be acquired or inherited. Lectins are invaluable reagents in the investigation of red cells polyagglutination. We prepared in-house lectin panel and confirmed Tn polyagglutination in a pregnant lady. The lady was anemic and refused blood transfusion elsewhere due to serological discrepancy. We found ABO discrepancy and an incompatible minor cross-match in the initial investigation and suspected polyagglutination. Confirmation of polyagglutination was done using adult and cord sera. We then used the in-house lectin panels to detect the type of polyagglutination. The agglutination pattern with the various lectins was suggestive of Tn polyagglutination, which was further supported by the enzyme study. Most blood banks in India lack commercial lectin panels because of cost and procurement difficulty. Lectins play an important role in the diagnosis and differentiation of polyagglutination and immunohematological management of patient. The important and basic lectins can be prepared in-house using specific raw seeds following standardized protocol.

Key words:

Dolichos biflorus, lectin, mixed field agglutination, polyagglutination, Tn antigen

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Introduction

Lectins are carbohydrate binding proteins obtained primarily from seeds. Many lectins agglutinate red blood cells (RBCs) and have been used as alternates to human sera for blood typing. They are invaluable reagents in the investigation of RBC polyagglutination.[1] Polyagglutinable RBCs are red cells agglutinated by a large proportion of human adult serum, regardless of blood group and are usually nonreactive with their autologous serum or serum from cord blood samples.[2] Polyagglutination may be acquired by the exposure of cryptic carbohydrate structures in RBC membranes due to the action of microbial enzymes (TF, Tk, Th, and others) or may be persistently acquired, e.g., Tn syndrome, or may be related to inherited genetic alterations.[3] These carbohydrate antigens responsible for polyagglutination are characterized by specific patterns of interaction with lectins.[4] Here, we shared our experience of preparation of in-house lectin panel and confirmation of polyagglutination (Tn) in a pregnant lady.

In-House Preparation of Lectin

We prepared in-house lectins from seed extracts as discussed elsewhere.[1] Raw seeds of *Dolichos biflorus, Arachis Hypogaea*, *Glycine max* and *Salvia sclarea* were purchased from the metropolitan seed and flower market, Kolkata, India.

Briefly each variety of seeds was covered with saline and soaked for 12 h and then grinded in a blender until the particles look liked coarse sand. In a small beaker, ground seeds were mixed with 3-4 times their volume of saline. The mixture was incubated at room temperature for 12 h, stirred occasionally and then transferred to the centrifuge tube. The tube was centrifuged for 5 min to obtain a clear supernatant.

In addition for *D. biflorus*, one drop of 5% cell suspension of known A_1, A_2, A_1B, A_2B, B, and O red cells were added to appropriately labeled tubes. One drop of the extract supernatant was added to each tube, centrifuged and inspected for agglutination. The lectin agglutinated A_1 and A_1B red cells strongly and A_2, A_2B, B, or O red cells moderately. To use this as a reagent, the lectin was further diluted to 4% using saline which agglutinated the A_1 and A_1B red cells (3+ to 4+) only, but not the A_2, A_2B, B, or O red cells. As recommended,saline extracts of each seed were then stored in the refrigerator for further use.[1]

Case Report

A 25-year-old pregnant female, primigravida at term was admitted in a rural hospital with hemoglobin (Hb) of 6.8 g/dl. The doctor on duty advised two units of blood transfusion. In rural India, most blood banks have no blood component facility and rely on whole blood. The blood group of the patient was detected as "O" positive. While performing minor cross-match all blood units

Table 1: ABO discrepancy in the patient sample

Red cells	Forward typing					Reverse typing			Interpretation
	Anti-A	Anti-B	Anti-A, B	Anti-A_1	Anti-H	A_1 cell	A_2 cell	B cell	Group
Normal sample	0	0	0	0	++++	++++	++++	++++	Group "O"
Patient sample	+ (mf)	0	++ (mf)	++	+++	++++	++++	++++	Discrepancy

mf: Mixed field

available then showed incompatibility. Denied blood transfusion the patient was referred to our hospital, which is a tertiary care multispeciality organization with blood component facility. On investigation, we found the patient's blood group as "O" positive, but with her red cells strongly reacting (4+) with anti-A_1 lectin and showing a mixed field agglutination with anti-A [Table 1]. Antiglobulin test both direct and indirect were negative. Two units of compatible washed "O" positive packed RBCs (PRBCs) were issued to the patient for transfusion. Owing to minor cross-match incompatibility and patient's cells agglutinating with anti-A and anti-A_1, we suspected polyagglutination and performed further investigations for confirmation as discussed before.[4] Six samples of fresh normal "AB" group adult sera and three samples of cord blood sera were tested with the patient red cells at both 22°C and 4°C. Three samples of group "O" red cells were used as controls. The sera study confirmed the presence of polyagglutination as described in Table 2. We used our in-house lectin panel to detect the type of polyagglutination. The agglutination pattern with the various lectins was suggestive of Tn polyagglutination [Table 3].[1,4] Papain treated polyagglutinable cells of the patient showed reduced agglutination strength (weak+ to +) with the adult sera used. This finding also supported the presence of Tn polyagglutination.[4]

Table 2: Confirmation of polyagglutination

Red cells	Adult sera (6 samples)						Cord sera (3 samples)		
	1	2	3	4	5	6	1	2	3
Normal "O" sample 1 (22°C and 4°C)	0	0	0	0	0	0	0	0	0
Normal "O" sample 2 (22°C and 4°C)	0	0	0	0	0	0	0	0	0
Normal "O" sample 3 (22°C and 4°C)	0	0	0	0	0	0	0	0	0
Patient (22°C)	++	+	+	++	+	+	0	0	0
Patient (4°C)	++	++	+	+++	++	++	0	0	0

Table 3: Detection of polyagglutination type using in-house lectin panel

Red cells	*Dolichos biflorus*	*Arachis, Hypogaea*	*Glycine max*	*Salvia sclarea*
Normal "O" sample 1	0	0	0	0
Normal "O" sample 2	0	0	0	0
Normal "O" sample 3	0	0	0	0
Patient*	+++	0	++	++

*Agglutination pattern with lectin panel suggestive of Tn polyagglutination

Discussion

Polyagglutination is a rare condition and can be detected using cord blood sera, normal adult sera, and lectins. Most blood banks in India lack commercial lectin panels because of cost and procurement difficulty. Owing to suspicion of polyagglutination in the pregnant lady, we purchased raw seeds and prepared lectins following standard protocol. The young lady under study was anemic with no significant complains except the pregnancy related symptoms. Except for the Hb and hematocrit, the other hematological and biochemical parameters of the lady were normal. She was instantly managed with compatible washed PRBCs. Lectin study was suggestive of Tn polyagglutination, which was further supported by the enzyme test. Since Tn polyagglutination may be a preleukemic condition, we advised the patient's physician for her regular hematological monitoring.[5,6] Tn polyagglutination is caused by a mutation in the hematopoietic tissue and is considered permanent and irreversible. The mixed field appearance with anti-A was the result of the mutant clone with normal hematopoietic tissue and the biochemical similarity of Tn antigen with Group A determinant.[7,8] We conclude that lectin panels can be easily developed in blood bank. The various raw seeds available in the market can be converted to lectins following the correct standard procedure and validation process. Lectins play an important role in the diagnosis and differentiation of polyagglutination and immunohematological management of the patient.

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