

Haemolysin test for characterization of immune ABO antibodies

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Background & objectives: Antibodies with haemolytic properties are common within the ABO system. These lytic antibodies are immunoglobulin G (IgG) and in high titres cause haemolysis during blood transfusion. Information on Immunoglobulin types and concentration of ABO haemolysins in Indian population is lacking. The present study was undertaken to know the usefulness of haemolysin test for characterization of immunoglobulin class of ABO antibodies.

Methods: Serum samples from 187 O group blood donors were screened for A and B haemolysins. Thirty five samples were treated with dithiothreitol (DTT) for characterization of Ig class. Antibody titre was compared with grade of haemolysis.

Results: Of the 51 strongly haemolytic serum samples, 32 (62.8%) had IgG titres of ≥ 64 after treatment with DTT. There was significant association ($P < 0.05$) between grade of haemolysin and anti B IgG titre.

Interpretation & conclusion: Haemolysin test was found to be a useful screening test to identify group O donors with high levels of IgG anti A and/or anti B for blood transfusion purposes.

Key words ABO incompatible blood - DTT assay - haemolysins - immune antibody titre - immunoglobulin class

Though a large fraction of A and B antibodies belongs to IgM class of immunoglobulins (Ig), anti A and anti B haemolysins are IgG. These lytic antibodies cause haemolysis of red cells of recipients when O group blood/plasma is transfused to non O group patients, and are known to cross placenta and cause haemolytic disease of the new born (HDN)¹. Variable proportions of dangerous group O donors are reported in literature and in most studies it is found that approximately 10-20 per cent of group O donors has high titre (critical titre) of anti A or anti B²⁻⁵. There are reports^{2,6-10} of acute intravascular haemolysis after transfusion of incompatible O group blood/plasma components. Such data pertaining to immune haemolysis during blood transfusion from our population are scarce. In our country as adequate supply

of blood is not available, it is not uncommon to transfuse group O blood to non O group patients. With limited pre-transfusion work up, passively transferred antibodies in group O blood/plasma could result in intravascular haemolysis in such patients. The present study was therefore undertaken to know the usefulness of a simple test like haemolysin for identification of immune anti A and anti B.

Material & Methods

This pilot study with a duration of 3 months was undertaken on blood donors who came to donate voluntarily at the Blood Center of the Sree Chitra Thirunal Institute for Medical Sciences and Technology,

Thiruvananthapuram, during 2001. The study protocol was approved by the Institutional Ethics Committee and informed consent was obtained from all individuals. Of the 525 O group donors without any history of transfusion or pregnancy, 187 blood donors (185 males and 2 females age group 18-48 yr) were randomly selected for the study. Blood group was determined by cell and serum grouping and O group serum samples were tested for haemolysins. Test for haemolysins and scoring was done as per standard procedure¹¹. Haemolysin was graded as follows: 3+ (complete haemolysis) and, 2+ (partial >50% but not complete), 1+ (trace haemolysis), and negative (no visible haemolysis). Serum samples having a score of 3+ and 2+ were considered strongly haemolytic. Of the 187 samples tested for haemolysins, 35 were treated with dithiothreitol (DTT) for characterization of Ig class. DTT assay and interpretation were done as per accepted procedure¹². DTT is preferred as it is more resistant to oxidation in air and is more efficient as a reducing agent than 2-mercaptoethanol (2-ME). Serum dilutions beyond the end of agglutination were further tested by indirect antiglobulin test (IAT)¹³. DTT cleaves disulphide bonds of pentameric IgM, abolishing their agglutinating and complement binding activities and permits detection of IgG antibodies in the serum. Anti A and anti B antiserum for cell grouping were obtained from Tulip Diagnostics, India and pooled cells for serum grouping were prepared in-house. IAT was performed using anti-human globulin serum obtained from Dominion Biologicals, Canada.

Geometric mean of antibody titre was determined for anti A and anti B haemolysins. Chi-square test was used to determine the association between grade of haemolysis and antibody titre in the 35 DTT treated samples.

Results

Of the 187 group O blood donors tested, anti A haemolysin was present in 31 (16.6%) and anti B in 20 (10.7%) donor samples; 104 individuals (55.6%) had haemolysis of grade 3+ and 2+ against both A and B cells. Trace and negative hemolysis results were obtained for 32 samples (17.1%). Geometric mean titre was high for IgG than IgM (85.4 and 53.5 for IgG anti B and A as against 16.00 and 31.2 for IgM anti B and A). Mixture of IgM and IgG antibodies were predominant in untreated serum samples. After treatment with DTT, IgG titres remained high in a number of strongly haemolytic serum samples. Of the 51 (27 anti A and 24 anti B) strongly haemolytic serum samples, 32 (62.8%) had anti A or anti B in titres ≥ 64 . Comparison of strongly haemolytic serum and antibody titre revealed significant ($P < 0.05$) association between grade of haemolysin and anti B IgG titre (Table). Weak activity of haemolysins was not considered as it was not found to be hazardous in clinical practice. Though comparison of pre and post DTT titres of anti A and anti B haemolysins showed no association between IgM (pre DTT) and IgG (post DTT) levels, high IgG titres was associated with haemolytic serum samples.

Discussion

High frequency of strongly haemolytic anti A and anti B haemolysins is reported from Asian and Black populations compared to Caucasians¹⁴⁻¹⁷. The higher grade of haemolysins in these populations has been attributed to mosquito bites and intestinal parasitic infections¹⁸. High titres of ABO haemagglutinins in O group individuals can also be the consequence of vaccination or other antigen exposures¹⁹.

Table. Comparison of haemolysin grades with immunoglobulin titres

	Anti-A				Anti-B			
	IgM		IgG		IgM		IgG	
Titre/Grade	<64	≥ 64	<64	≥ 64	<64	≥ 64	<64	≥ 64
0 & 1	3	2	4	1	9	0	7	2
	(9.4)	(6.3)	(12.5)	(3.1)	(27.3)	(0)	(21.2)	(6.1)
2 & 3	18	9	12	15	24	0	7	17
	(56.3)	(28.1)	(37.5)	(46.9)	(72.7)	(0)	(21.1)	(51.5)*
Total	32		32		33		33	

Figures in parentheses are percentages. * $P < 0.05$

There is scarcity of similar data on the Ig types and concentration of ABO antibodies in Indian population. High frequency of strongly haemolytic group O sera was observed in the present study. This may be due to environmental factors as 81 per cent of donors reported having taken tetanus toxoid injection with 38.3 per cent of them donating within 1 yr of immunization. Incidence of ABO HDN in the population is 1.9 per cent (Sulekha. C, personal communication). Contreras *et al*²⁰ found that all O group donors had low titres of IgG anti A and anti B before immunization which increased three-fold after immunization. Increase in antibody response with increasing number of toxoid injections has been reported in normal adults and pregnant women²¹. Correlation between IgM (pre DTT) and IgG (post DTT) levels has been reported earlier in Zimbabweans¹⁴. We did not find such a correlation.

Given the limited supply and short shelf-life of platelets and for optimal blood inventory management, it is a common practice to transfuse ABO incompatible platelets in emergency. Haemolysis following such transfusions due to passively transferred antibodies causes significant morbidity even when plasma volume is reduced^{2,6-10}. Haemolysis has also been reported following transfusion of other products containing ABO incompatible plasma as in packed red blood cells or plasma products⁸. It has been hypothesized that ABO immune complexes formed after transfusion of mismatched platelets and/or plasma, cause changes in cellular immunity and lead to adverse reactions. Elevated ABO isoagglutinin titres were demonstrated in patients with consistently poorer response following mismatched transfusions^{22,23}. Hence, it is important to avoid transfusion of blood containing high titres of immune anti A and anti B antibodies to non O group recipients. As per the requirements of the American Association of Blood Banks (AABB) Standards²⁴ transfusion service should have a policy concerning transfusion of components containing significant amounts of ABO incompatible antibodies. Taking into consideration the finding of the present study that strongly haemolytic samples have high titres of IgG, a simple screen for donor haemolysin is suggested which can decrease the risk of transfusion if platelets/plasma from donors with minor incompatibility are used. Besides detecting strong haemolytic serum, the test also identifies donors who are likely to have high levels of IgG anti A and anti B.

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