Journal of Thrombosis and Circulation Open Access

Sood et al., J Thrombo Cir 2016, 2:1
DOI: 10.4172/2572-9462.1000104

Research Article Open Access

Antibody Titers Study in Group O Blood Donors: Tube and Column Agglutination Techniques

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Abstract

Background: O blood group transfusions to patients of all blood groups has continued since long. Clinical significance of ABO antibody titre in ABO-I kidney transplantation is well known. Few studies done on group O blood/apheresis donations, for detecting ABO antibody titers in collected plasma components. The passively acquired antibodies may destroy recipient's own red cells and tissue grafts, cause acute hemolysis, hemoglobinemia, jaundice, progressive anemia, spontaneous agglutination, positive direct antiglobulin test and increased osmotic fragility of the patient's red cells.

Objective: To evaluate agglutinin levels in group O blood donations. Group O donor population, randomly selected and titrated using tube technique and gel card technique to identify titer levels for Anti A, Anti B, Anti AB antibodies. Both IgM and IgG titer levels evaluated.

Methods: Plasma samples from 200 randomly selected blood group O donors were tested by ABO antibody titration using conventional tube technique and AHG gel card column agglutination technique (CAT). ABO antibody levels categorized as those higher than 16 and those lower than 16. After treatment with Dithiothretiol (DTT) for characterization of only IgG class, titres levels were again tested in same O group blood/apheresis donors. Statistical analyses performed using various tests.

Results: Males constituted 88% of O group donors studied and 12% were females. ABO antibody titer categorized as 0 to ≤16 and titer >16 for both IgM and IgG antibody for Anti A, Anti B and Anti AB. Both test tube and CAT used for testing.

Estimates of prevalence of titers by CAT: Anti A IgM ≤ 16 in 62%; >16 in 38% (p-value < 0. 001); Anti A IgG ≤ 16 in 32%; >16 in 68% (p-value < 0. 001); Anti B IgM ≤ 16 in 69%; >16 in 31% (p-value < 0.001); Anti B IgG ≤ 16 in 35%; >16 in 65% (p-value < 0.001); Anti AB IgM ≤ 16 in 30%; >16 in 70% (p-value < 0.001); Anti AB IgG ≤ 16 in 27%; >16 in 73% (p-value < 0.001); And estimations of prevalence for titers using tube technology: Anti A IgM ≤ 16 in 44%; >16 in 56% (p-value < 0.045); Anti A IgG ≤ 16 in 44%; >16 in 56% (p-value < 0.045); Anti B IgM ≤ 16 in 51%; >16 in 49% (p-value < 0. 0.389); Anti B IgG ≤ 16 in 36%; >16 in 64% (p-value < 0.001); Anti AB IgM ≤ 16 in 34%; >16 in 66% (p-value < 0.001); Anti AB IgG ≤ 16 in 4%; >16 in 96% (p-value < 0.001). No significant co-relation found between age and titer or gender and titer, by both technologies. Mean anti-A and anti-B and anti-AB titers in group O plasma were, respectively,163.28,113.42 and 166.77 for IgM antibody and 174.50,152.98 and 311.63 for IgG antibody by tube method and 34.01,33.30 and 63.77 for IgM Antibody,108.41,103.10 and 272.46 for IgG antibody by CAT (p < 0.0001).

Conclusion: Study confirms that titration of ABO antibodies in blood banks will increase safety in non-identical ABO transfusions and transplants. No significant correlation established between titer and age or titer and gender.

Keywords: ABO blood-group system; Blood donors; Blood transfusion; Agglutinin levels; Antibody titre; Agglutination tests/methods; Antigen-antibody reactions

Introduction

Blood/Blood Component transfusion is comparable to mini-transplant. There are cells containing antigens and serum containing antibodies which interact and react in the form of Immunological or Serological reaction. Immunohematology in blood banks studies the cell antigens and naturally occurring as well as immune antibodies in serum. Most currently used techniques in blood banks are still based on the principle of interactions between antigen and antibody and subsequent agglutination of red blood cells [1].

ABO blood group antigens are expressed throughout the body [2]. These antigens are also present on embryonic kidney cells [3], vascular endothelium, convoluted distal tubules, and collecting tubules [4] (Figure 1).

Two situations need blood bank strategies to minimize the biological effects of ABO antibodies:

1) Acute hemolytic transfusion reactions (AHTRs) have been reported, though not commonly, after transfusion of group O single-donor apheresis platelets (SDPs) to group A, B, and AB recipients due to the presence of unusually high titers of antibodies which can be found in the plasma of some group O donors and the large plasma volume of SDPs. Random donor platelets from group O donors have also been implicated in these reactions [5,6] (Figure 2).

2) Increasing need for ABO-Incompatible renal transplant for

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Received January 06, 2016; **Accepted** February 09, 2016; **Published** February 16, 2016

Citation: Sood R, Neelima, Kumar D, Kumar T, Kumar V, et al. (2016) Antibody Titers Study in Group O Blood Donors: Tube and Column Agglutination Techniques. J Thrombo Cir 2: 104. doi: 10.4172/2572-9462.1000104

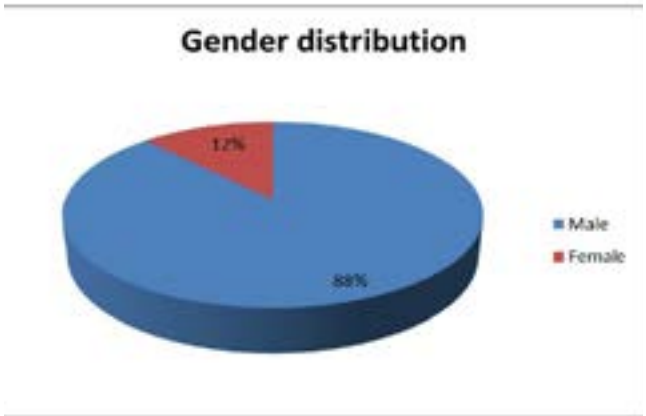

Figure 1: Gender distribution of blood donors studied.

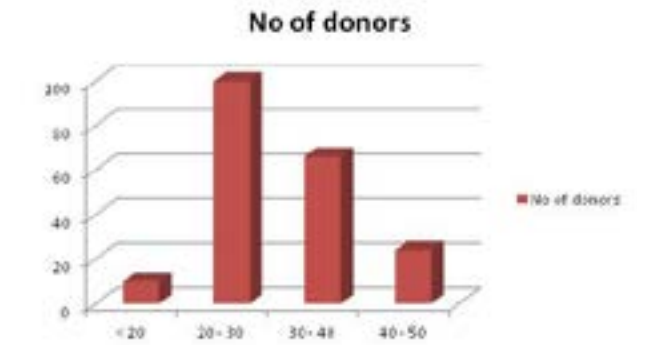

Figure 2: Number of donors in different age groups.

end stage renal failure patients because of shortage of identical organ and blood donors. Removing ABO barrier expands the donor pool, increases availability of organs for transplantation, decreases time on organ waiting list, and ultimately facilitates transplantation before patients develop comorbid conditions. ABO-I renal transplants are occurring through a preparative regimen including the use of therapeutic plasma exchange (TPE), double-filteration plasmapheresis, or immunoadsorption and immunosuppressive therapy to reduce circulating ABO antibody titers, antibody titers to ≤16. This permits better engraftment of ABO-I kidney transplants [7-10]. All individuals undergoing ABO-I renal transplantation at Johns Hopkins Hospital receive posttransplant TPE to prevent rebound of anti-A and/or anti-B titers. This results that majority (>75%) of individuals have posttransplant ABO antibody titers that remain at low levels (titer ≤ 16). ABO-I is currently an American Society for Apheresis (ASFA) category II indication for TPE [11,12]. Other institutions have documented high post transplant ABO antibody titers which correlate with increased graft loss [13,14].

In addition to acute hemolysis, other effects of incompatible plasma include hemoglobinemia, jaundice, progressive anemia, spontaneous agglutination, positive direct ant globulin test and increased osmotic fragility of the patient's red cells [15].

Hence the transfusion of group O plasma to other blood group recipients has been a matter of debate and discussion.

Naturally occurring antibodies directed against ABO antigens are key mediators of antibody- mediated rejection (AMR) in cases of renal transplantation across ABO barriers [16,17]. AMR resulting from the generation of newly formed anti-donor antibodies can occur weeks to years after transplantation and can lead to the development of transplant glomerulopathy and chronic rejection [8,18].

AMR differs from hyperacute rejection which occurs within minutes to hours after transplant. Hyperacute rejection is due to already existing circulating antibodies directed against ABO, HLA, or other alloantibody-to-donor endothelial surface antigens [16].

Data pretainning to titer levels of anti-A, anti-B and cross-reacting Anti AB antibody in group O population is scarce. Minimal research has been performed to determine the optimal pretransplant titer. The post-transplant ABO antibody titer should also be monitored as the antibody mediated rejection (AMR) due to ABO antibodies can be reversed with therapeutic plasma exchange and CMVIg treatments [17,18].

Major challenges in respect to standardized, prospective screening are absence of a recognized reference method and critical end titer that will reasonably differentiate safe from high-titer donors for both clinical settings.

Some centres use the goal of a titer of 16 or less at the anti-human globulin (AHG) phase before transplant surgery [12,7,18]. Others uses a lower-titer goals [19,20] or higher titer goals of 64 or more [21].

Aim

Aim of this study was to evaluate agglutinin levels in group O Blood donations, both IgM and IgG. Thus, group O donations were randomly titrated to identify the best source of products for apheresis and exsanguinous transfusion.

Material and Methods

Materials used included A, B and AB pooled red cells (3% and 0.8% cell suspension-prepared in house), DTT Dithiothretiol (make-Sigma Diagnostics), AHG test serum (make-Tulip Diagnostics) and donors EDTA plasma samples, Neutral cards (ID Cards BIORAD) and AHG cards (ID Cards BIORAD). Anti A, Anti B and Anti AB antiserum (Make-Tulip Diagnostics).

Titer calculation is a semi-quantitative test for representing the approximate concentration of antibodies in the serum being tested. Anti A, Anti B and Anti AB antibody titers were determined by testing two-fold serial dilutions of the test serum with commercially available A and B indicator red cells. The highest serum dilution ratio that showed 1+ reactivity indicated the anti A and anti B antibody titers. IgG titers were measured using serum samples treated with Dithiothretiol while IgM titers were determined from untreated samples.

Plasma Samples

Samples from 200 group O blood donors were tested for ABO antibody titer using the conventional tube technique at 37°C incubation followed by DTT treatment and AHG incubation. Same samples were also tested by the gel card column agglutination Technique using Neutral cards(ID Cards Bio-Rad) and after DTT treatment using AHG cards(ID Cards Bio-Rad).

Samples were selected from donors with no past history of any Transfusion or pregnancy.

Use of A, B and AB pooled Indicator cells:

For test tube titration, 3% concentrated cells were used. For AHG card titration, 0.8% concentrated cells were used. We tried to keep the same donor units for pooled cell preparation.

Preparation of serial dilutions of Antiseras - Titration Procedure:

Serial dilutions were prepared in normal saline 0.9% using fresh plasma samples of healthy donors who have no history of any previous transfusion or pregnancy and were labelled as master titer tubes.

DTT treatment

Plasma samples of the same donors, as above, were treated with Dithiothretiol (DTT) for characterization of only IgG class antibodies after destruction of IgM antibodies by the DTT. Using test tube method and Gel card technology again the titer values were tested.

DTT assay and interpretation were done as per the accepted procedure [22].

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). DTT cleaves disulphide bonds of pentameric IgM, abolishing their agglutinating and complement binding activities and permits detection of IgG antibodies in the serum.

Reading

Immediately after half hour incubation at 37c for test tube samples, samples were centrifuged at 3400 rpm for 15 seconds. Agglutination was considered positive if the red cells remain agglutinated after gentle shaking. The highest dilution causing agglutination was assumed to represent antibody titer.

After 37°C incubation, the fresh plasma samples from same donor were treated with DTT solution, in ratio of 1:1. One drop of AHG was added to each test tube, the test tubes centrifuged at 3400 rpm for 15 seconds. Again antibody titer reading was taken.

Plasma samples from master titer tubes were used for testing using the neutral gel column agglutination cards and AHG Column agglutination cards after DTT treatment of the master titer tube plasma.

Statistical analysis

Descriptive statistics were used to describe the age, gender and anti-A and/or anti-B titers in serum. Statistical analyses were performed using the Mann-Whitney test, Wilcoxon test, Chi-square test and Pearson's Correlation coefficient

Results

This study with duration of 12 months was undertaken on blood donors who came to donate voluntarily at the blood centre of Artemis Health Institute, Gurgaon, between Oct 2012 to March 2013 and Saket City Hospital starting October 2013 till March 2014.

Of the 200, O group donors without any history of transfusion or pregnancy, 70 group O blood donors from Artemis Hospital and 130 group O blood donors from Saket City Hospital were randomly selected for the study. This allowed us to study a wider range of population. Blood group was determined by cell and serum grouping and O group plasma samples were tested for antibody titers, both IgM and IgG titers. In the study population, 88% males and 12% females, between age group 18-50 yrs were studied. ABO antibody titer was categorized as 0 to ≤16 and titer >16 for both IgM and IgG antibody for Anti A, Anti B and Anti AB. Both test tube and column agglutination technology were used for testing.

Estimates of the prevalence of titers using column agglutination technology were as follows:

Anti A IgM ≤ 16 in 62% cases, >16 in 38%cases (p-value < 0. 001); Anti A IgG ≤ 16 in 32% cases, >16 in 68% cases (p-value < 0. 001); Anti B IgM ≤ 16 in 69% cases, >16 in 31% cases (p-value < 0.001); Anti B igG ≤ 16 in 35% cases, >16 in 65% cases (p-value < 0.001); Anti AB IgM ≤ 16 in 30% cases, >16 in 70% cases (p-value < 0.001); Anti AB igG ≤ 16 in 27% cases, >16 in 73% cases (p-value < 0.001); And the estimations of prevalence for the titers using tube technology were as follows:

Anti A IgM ≤ 16 in 44% cases, >16 in 56% cases (p-value < 0.045); Anti A IgG ≤ 16 in 44% cases, >16 in 56% cases (p-value < 0.045); Anti B IgM ≤ 16 in 51% cases, >16 in 49% cases (p-value < 0. 0.389); Anti B igG ≤ 16 in 36% cases, >16 in 64% cases (p-value < 0.001); Anti AB IgM ≤ 16 in 34% cases, >16 in 66% cases (p-value < 0.001); Anti AB igG ≤ 16 in 4% cases, >16 in 96% cases (p-value < 0.001).

No significant co-relation was found between age and titer (p-value > 0.103) or gender and titer (p-value> 0.215), by both the technologies.

Mean anti-A and anti-B and anti-AB titers in group O plasma were, respectively,163.28,113.42 and 166.77 for IgM antibody and 174.50,152.98 and 311.63 for IgG antibody by tube method and 34.01, 33.30 and 63.77 for IgM Antibody,108.41,103.10 and 272.46 for IgG antibody by gel (p < 0.0001).

Discussion

Significance of a blood group system in clinical blood transfusion practice depends upon the frequency of its antibodies and on the possibility that such antibodies will destroy incompatible cells in vivo [23]. Blood group O red cells can be given to A, B, or AB recipients, however studies have shown high frequency of potentially lytic anti-A and anti-B and cross-reacting anti-AB antibody, (mostly IgG) in their serum [24-26]. Some sera may contain all the three classes and non-stimulated individuals contain predominantly IgM (Table 1).

Pregnancy and incompatible transfusions

Changes in the characteristics of anti-A or anti-B occur as a result of further immunization with pregnancy or by incompatible transfusions. They are serologically detectable through increases in titers, agglutinin avidity and hemolytic activity. In the study, samples from donors having history of previous blood transfusion or pregnancy were avoided.

In our study we avoided samples from females with pregnancy history.

Number of donors and age interval

Donors were randomly selected in the mentioned time period.

Prevalance of various titers

Titer of an antiserum is the reciprocal of the highest dilution in which agglutination occurs (Tables 2-5) Number of blood donors showing prevalence of various titer values was as follows:

In this study, mean anti-A and anti-B and anti-AB titers in group O plasma were, respectively,163.28,113.42 and 166.77 for IgM antibody and 174.50,152.98 and 311.63 for IgG antibody by tube method and 34.01,33.30 and 63.77 for IgM Antibody,108.41,103.10 and 272.46 for IgG antibody by gel (p < 0.0001).

Prevalence of less than 55% haemolysins was reported by Kulkarni et al. in Zaria (Northwestern Nigeria) [24] in 1985 and Olawumi and

Age Interval(in years)	No of donors	% of donors
< 20	10	5.00%
20 - 30	100	50.00%
30 - 40	66	33.00%
40 - 50	24	12.00%

Table 1: Donors were randomly selected in the mentioned time period.

TITER VALUE	Anti-A IgM	Anti-B IgM	Anti-AB IgM M
0	2	5	6
1	3	2	0
2	15	10	0
4	10	13	0
8	21	11	12
16	11	26	15
32	15	13	22
64	13	11	23
128	9	7	11
256	0	1	8
512	1	1	3
1024	0	0	0
2048	0	0	0
4096	0	0	0
8192	0	0	0

Table 2: Titer of IgM antibodies in group O plasma (gel card).

TITER VALUE	Anti-A IgG	Anti-B IgG	Anti-AB IgG
0	2	5	5
1	3	2	0
2	11	8	0
4	4	7	0
8	10	9	0
16	14	6	6
32	7	13	13
64	16	17	13
128	16	15	24
256	8	14	19
512	8	2	13
1024	1	2	5
2048	0	0	2
4096	0	0	0
8192	0	0	0

Table 3: Titer of IgG antibodies in group O plasma (gel card).

Olatunji in Ilorin (Southwestern Nigeria) [25] in 2001. The higher prevalence rate observed in this study could be due to the methods and the fact that large population of group "O" donors were screened.

Scenario in various countries

The true biological reason for evolution and phenotypic variations of the ABO antigens and related carbohydrate epitopes remains obscure. ABO antibody levels depend on the ethnic background and environmental factors. In Japan anti-A and anti-B titers decreased over 15 years (1986-2001) and titers of more than 100, as measured using the saline method, are rare. Similar to North Americans, the Japanese population eats more processed food than other Asiatic populations (Laotian and Thai populations) [26].

TITER VALUE	Anti-A IgG	Anti-B IgG	Anti-AB IgG
0	0	0	0
1	0	0	0
2	1	1	5
4	2	2	5
8	10	13	9
16	19	20	17
32	24	25	16
64	15	15	18
128	11	16	18
256	6	3	5
512	8	0	2
1024	2	2	2
2048	1	2	1
4096	1	1	1
8192	0	0	1

Table 4: Titer of IgM antibodies in group O plasma (using test tube).

TITER VALUE	Anti-A IgG	Anti-B IgG	Anti-AB IgG
0	0	0	0
1	0	0	0
2	1	1	5
4	2	2	5
8	10	13	9
16	19	20	17
32	24	25	16
64	15	15	18
128	11	16	18
256	6	3	5
512	8	0	2
1024	2	2	2
2048	1	2	1
4096	1	1	1
8192	0	0	1

Table 5: Titer of IgG antibodies in group O plasma (using test tube).

The concern of ABO incompatibility in organ transplantation in countries such as Sweden [27] and Japan [28] encouraged the development of more sensitive titration techniques (gel hemagglutination and flow cytometry, respectively). Japanese groups have pre-transplant preparative regimens that include titration, immunosuppression and splenectomy associated to a therapeutic plasma exchange (TPE) program. However, the research in this area is limited and basic and also a standardized clinical protocol for TPE is lacking [12].

In the United States, in transfusion medicine, from 10 to 40 percent of all PLTs transfusions are plasma-incompatible. Reported donor anti-A titers implicated in HTRs range from 32-16,384 in saline (median 512), to 32-32,000 (median 4096) in the anti-globulin phase [6]. Screening experience in the United States is currently limited to approximately 2% of institutions [6].

In one report, in the United States [29], the median IgM titer was 32 in gel out of 100 group O apheresis donors, and 28% of samples were labelled as high-titer using 64 or greater as the cut-off. However, the plasma: RBC ratio used for buffered gel testing in this report was 25 uL plasma to 50 uL RBC (Red Blood Cells) s, which was lower than recommended by the manufacturer.

Cooling et al. [30] tested 124 pools of group O platelets (each pool containing 5 platelet concentrates) using buffered gel technique and found the median anti-A titer to be 64 and the median anti-B titer to be 32. Our screening in buffered gel with the initial dilution of 1:150 classified 50% of our group O units as high-titer. This does not necessarily imply that the median titer is 150, since a single dilution does not always yield equivalent results to those obtained by serial two-fold titration testing [31].

Many countries outside North America have adopted universal screening. Although plateletpheresis components account for a minority of total platelet use in much of Europe, still European strategies have defined a safe level of iso hemagglutinins for their donors and a cut-off determination to label products when the titer is high and thereby restrict its use [32].

A critical IgM titer is 64-100 in tube or gel, and a critical IgG titer is 200-512 [33,34]. This correlates with the mean titer value in our study.

Screening for high-titer anti-A and/or anti-B in the United Kingdom using a single dilution cut-off of 1 in 100 by automated direct agglutination has not eliminated platelet-mediated HTR, and Scotland now uses a dilution cut-off of 1 in 50 [31].

Data from the hemovigilance system in the United Kingdom suggest that platelets account for 20% (9/44) of acute HTRs overall, [36] and 33% (3/9) of acute HTRs in children [37].

Other factors and titer levels

Unfortunately, we do not have data of the location and ethnic background of our donor population; therefore, the reasons that young women have high Anti-B titer levels could not be investigated. We believe that the socioeconomic status and genetic background may explain the results [38].

Age and titer levels: Although the relationship between ABO antibody levels and age of donors based solely on agglutination was first observed in 1929, [38] this is the first study that stratifies Brazilian blood donors by age [39]. Our study showed no significant co-relation was found between age and titer (p-value > 0.103) or gender and titer (p-value > 0.215), by both the technologies.

A further complexity is that the tests of centers differ in almost every detail, including the use of donor and/or patient plasma or serum, the medium used or dilution, incubation

time, centrifugation time and speed, use of polyclonal vs. monoclonal secondary step antibody for indirect agglutination [38]. All these details may affect titer results for transfusions and organ transplants.

Rieben et al. [38] showed that donor to donor variation did not correlated exactly to age-related changes for all measured parameters. The authors determined isotypes and IgG subclasses of ABO antibodies from sera of 235 Swiss blood donors by enzyme-linked immunosorbent assay (ELISA). Indeed, they studied titers of up to 40 and found considerable variations between antibody levels by ELISA in different sera with the same agglutination titer.

Issues to consider for a universal platelet screening program include the serologic method (manual tube versus gel versus an automated platform), the choice of IgM or IgG titers, determining the threshold for a critical titer, performance of serial titrations versus testing a single pre-determined dilution, and interlaboratory variation in antibody titrations [32,39].

Conclusions

This study concludes that titration of ABO antibodies in blood banks will increase safety in non-identical ABO transfusions. The Transfusion Medicine speciality should consider measures to increase ABO-identical PLT transfusions and physicians should be aware of potential adverse outcomes when transfusing non-identical ABO PLTs. Although most cases recover with appropriate supportive care, reactions can be fatal. Platelet-mediated HTRs may be under-recognized [40], because patients receiving platelet transfusions are frequently anemic at baseline, and symptoms and signs associated with hemolysis may be mild or atypical and not appreciated as such, particularly in outpatients. Distinction should be made between IgM and IgG type of antibody titer levels [9,41,42].

It is important to implement a practical strategy to reduce the risk of passive hemolysis caused by plateletpheresis products. Studies on the data showing hemolysis in such incompatible transfusions can further highlight the significance of titer levels.

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