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Evaluation of anti-A and anti-B IgM and anti-AB IgG antibody titers in Group O blood Iraqi donors

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Abstract:

BACKGROUND: Blood group O is transfused as a universal blood group in emergencies. The red cells of blood Group O possess no major ABO antigens, while the blood Group O plasma contains naturally occurring IgM anti-A and anti-B and cross-reacting IgG anti-AB antibodies. Thus, the transfusion of blood Group O units may cause marked destruction of the recipients' A or B red cells manifested as severe acute hemolytic transfusion reactions owing to potent ABO hemolytic antibodies.

OBJECTIVES: This study was done to estimate the titers of anti-ABO antibodies in Group O blood donors and to suggest a possible scheme to introduce as a routine testing for high ABO antibodies' titers in blood Group O donors in Iraqi blood transfusion centers.

SUBJECTS AND METHODS: Two hundred blood Group O Iraqi healthy blood donors (192 males and 8 females, age range: 18–59) were chosen to evaluate the titer of ABO antibodies (IgM and IgG) in their serums using tube test.

RESULTS: The prevalence of high-titer anti-A and anti-B IgM antibody at 1:50 dilution in this study was 98.5% and 97.5%, respectively ($P < 0.05$). The prevalence of high-titer anti-AB IgG measured by dithiothreitol and 2-mercaptoethanol method of at least 50 was 63% and 74%, respectively ($P < 0.05$).

CONCLUSIONS: There is a high prevalence of high-titer anti-A and anti-B IgM and IgG antibodies among blood Group O Iraqi donors. The detection of IgG antibodies is more practical by using dithiothreitol method as it needs much shorter incubation time; however, 2-ME is more sensitive. Detection of high-titer anti-A IgM antibody in dangerous universal donors, by immediate spin at 1:50 dilution, can be used alone in practice, replacing the need to use all portfolio of tests of anti-ABO antibodies.

Keywords:

Anti-ABO antibodies' titer, Group O blood donors, hemolytic transfusion reactions, potent ABO hemolytic antibodies

Introduction

Whole blood (WB) transfusions have a long history in military medicine and were considered as an optimum resuscitation fluid for the hemorrhagic traumatized patients.[1] It was demonstrated that Type O WB was independently associated with improved outcomes when compared with traumatized patients who had been transfused with component therapy (red blood cells [RBCs] units and plasma units).[2]

Major advantage of transfusing Type O WB was to eliminate the risk of severe hemolytic transfusion reactions caused by the major ABO incompatibility; thus, it can be used in situations of limited resources for ABO testing; however, Group O donors have anti-A and anti-B antibodies and a cross-reacting anti-AB antibody in their

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serum/plasma and these passively acquired antibodies can destroy the recipient's own red cells.[3]

The widely use of fresh Group O WB in the life-threatening bleeding necessitates the assessment of the problem of hemolytic reactions secondary to the transfusion of ABO-incompatible plasma.[4] The antibodies of ABO system are mainly IgM and/or IgG types, which can agglutinate the RBC at or below the room temperature and causing complement activation at 37°C.[5] It is practically important to determine the ABO antibody titer in clinical situations like the transfusions of blood components containing a significant amount of incompatible plasma and in the transplantation of organs across the ABO barrier.[6]

The use of low-titer antibody blood Group O WB and other plasma-containing blood components are becoming a standard procedure in certain circumstances; however, there are no guidelines or consensus to follow regarding the context of transfusion or even the required techniques or titer threshold.[7]

The amount of antibody was appreciated by titration usually of IgM, antibody in a saline dilution (immediate spine), and of IgG isotype with an antihuman globulin technique. Titration of anti-A and anti-B antibodies had been used to select the safe universal Group O donors.[8]

The aim of this study was to evaluate titers of anti-A and anti-B IgM and anti-AB IgG antibodies in Group O blood donations and to suggest a possible scheme to introduce routine testing for high ABO antibody titer in blood Group O donors in Iraqi blood transfusion centers.

Subjects and Methods

This is a cross-sectional study enrolled 200 blood Group O Iraqi healthy blood donors from the National Blood Transfusion Center in Baghdad from July 2019 to October 2019. Donors' age ranged between 18 and 59 years with no history of previous blood transfusion. The study included donors of both sexes (192 males and 8 females), females had no history of pregnancy. All of the donors are negative for virology and bacteriology screen. The study was approved by the scientific committee and ethical committee of Al-Nahrain university college of medicine. A written informed consent was obtained from all participants in this study.

Titration of antibodies was done by direct dilutions of donors' serums with normal saline at 1:25, 1:50, 1:75, and 1:100 ratios. The titer of IgM antibody was estimated by using the immediate spin tube method and IgG antibody titer was performed using indirect antiglobulin test, after treating the serum once with dithiothreitol and another with 2-mercaptoethanol.[9] Cutoff points for a positive test were 1:50 and 1:100 dilutions, as described in Oh and Goodman BBGuy Essentials 073.[10]

Titration of IgG antibody needs elimination of IgM antibody to permit quantification of IgG antibody by destruction of the J chain of the IgM molecule with IgG molecule remain intact, and this can be attained by treatment of the serum with sulfhydryl reagents such as DTT and 2-ME (DTT (Dithiothreitol) and 2-mercaptoethanol (2-ME))[11] the serum incubated once at 37°C with DTT for 30 min[11] and another sample of the same serum incubated with 2-ME for 2 h[12] to achieve complete IgM destruction).

The IS tube method was performed by adding about one drop of the red cells of known A and B blood group red cells, diluted to 5% suspension, to each labeled tube containing a diluted serum for detection of IgM anti-A and anti-B antibodies, then the tubes were immediately centrifuged at 1000 rpm for 1 min. The reactions read macroscopically and the titer was defined as the inverse of the last dilution that produced at least 1+ agglutination.[13]

The evaluation of IgG antibody titer included the addition of 5% of A-red cells suspension to the sulfhydryl-treated serum after direct dilution in saline. The tubes were incubated at 37°C for 30 min and then washed three times with saline solution. Antihuman globulin reagent, polyspecific, was added and the tubes were centrifuged at 1000 rpm for 1 min. The reactions were read macroscopically.[13] The negative reactions of antihuman globulin phase were assessed by check cells that prepared locally (using sensitized cells to IgG that had been incubated at 37°C for 30 min, as these cells coated by IgG only after destruction of IgM).

Statistical analysis

Statistical analysis was done using Statistical Package for the Social Sciences (version 23.0 for Linux®) (IBM Corp., Armonk, Ny, USA) for this study. Qualitative data represented as numbers and percentages, while continuous numerical data represented as mean ± standard deviation. $P < 0.05$ considered statistically significant.

Results

The mean age of participants in this study was 35 ± 9.72, around 40% of blood Group O donors are between 28 and 37 years old. Donors with high-titer antibody identified when their serums show 1+ agglutination at cutoff points (50 or 100 titers).

The prevalence of high-titer anti-A and anti-B IgM antibody of at least 50 titers in this study was 98.5% and 97.5%, respectively, and of at least 100 titer was 83.5% and 84%, respectively, with $P < 0.05$, as shown

in Tables 1 and 2. The prevalence of high-titer anti-AB IgG measured by DTT and 2-ME method of at least 50 titers was 63% and 74%, respectively, and of at least 100 titer was 21.5% and 35.5%, respectively, with $P < 0.05$, as shown in Tables 3 and 4.

At 1:50 dilution, IgM anti-A antibody represented most donors with a high titer, as 62.5% of donors had a high titer of IgM anti-A and anti-B and IgG (DTT and 2-ME). About 11% of donors showed no reaction for IgG antibody in the DTT method but had positive reaction strengths for IgG antibody by 2-ME method. The difference among variables presented in Table 5 was significant.

Table 1: Titers of IgM anti-A antibody

Reaction Strength	IgM anti-A antibody No. (%)			
	(1:25)	(1:50)	(1:75)	(1:100)
Negative	2 (1%)	3 (1.5%)	10 (5%)	33 (16.5%)
Positive	198 (99%)	197 (98.5%)	19(95%)	167 (83.5%)
P- value*	-	< 0.001	< 0.001	< 0.001

* Calculated using paired-samples *t*-test

Table 2: Titers of IgM anti-B antibody

Reaction Strength	IgM anti-B anti-body No. (%)			
	(1:25)	(1:50)	(1:75)	(1:100)
Negative	3 (1.5%)	5 (2.5%)	8 (4%)	32 (16%)
Positive	197 (98.5%)	195 (97.5%))	192 (96%)	168 (84%)
*P-value**	-	195 (97.5%)	< 0.001	< 0.001

* Calculated using paired-samples *t*-test

Table 3: Titers of IgG (DTT) antibody

Reaction Strength	IgG (DTT) antibody No. (%)			
	(1:25)	(1:50)	(1:75)	(1:100)
Negative	48 (24%)	74 (37%)	110 (55%)	157 (78.5%)
Positive	152 (76%)	126 (63%)	90 (45%)	43 (21.5%)
*P-value**	-	< 0.001	< 0.001	< 0.001

* Calculated using paired-samples *t*-test

Table 4: Titers of IgG (2-ME) antibody

Reaction Strength	IgG (2-ME) antibody No. (%)			
	(1:25)	(1:50)	(1:75)	(1:100)
Negative	23 (11.5%)	52 (26%)	83 (41.5%)	129 (64.5%)
Positive	177 (89.5%)	148 (74%)	117 (58.5%)	71 (35.5%)
*P-value**	-	< 0.001	< 0.001	< 0.001

* Calculated using paired-samples *t*-test

Receiver operating characteristic (ROCs) curve was applied to assess the accuracy of IgM anti-A, IgG (DTT), and IgG (2-ME) tests concerning the detection of high-titer antibody. The sensitivity plotted on the γ-axis, whereas the χ-axis represented 1-specificity. The ROC curves plotted based on the χ- and γ-axis and the area under the curve (AUC) of the ROC curve was estimated to evaluate the accuracy. As shown in Figures 1 and 2, the diagonal green curve based on IgM anti-A results and the AUC for IgG (2-ME) test was 0.45 and for IgG (DTT) test was 0.529.

At 100 titer, the prevalence of donors with low titer anti-A IgM antibody and anti-AB IgG antibody measured by DTT and 2-ME was increased with the higher mean of age of donors as demonstrated in Table 6.

Discussion

In this study, a group of 200 blood Group O Iraqi donors with 96% male and 4% female (male to female ratio is 24:1) evaluated for ABO antibodies titer. The lower proportion of female donors is contributing to donor selection criteria which excludes women during pregnancy, abortion, lactation, and menstruation[14] and women with hemoglobin level less than 12.5 g/dl or hematocrit less than 38% for allogeneic blood donations.[15] This was similar to Kagu, *et al*. study.[16]

The titers of antibodies in this study considered as high or low titer depending on two cutoff point (1:50 and 1:100) dilutions. This study showed that the prevalence of blood Group O donors with high titer for anti-A and anti-B IgM of at least 50 was 98.5% and 97.5%, respectively, and of at least 100 was 83.5% and 84%, respectively, this is higher than that found in Bazigou *et al*. study[17] which measure the IgM antibodies by direct agglutination

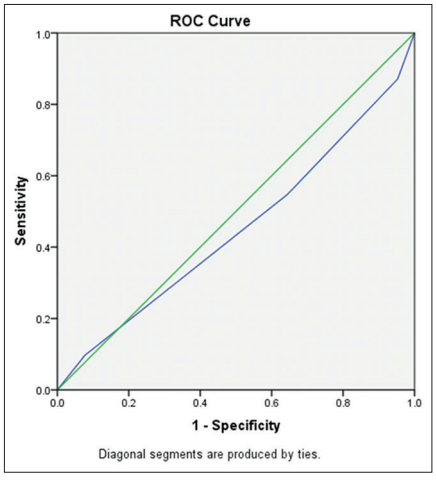

Figure 1: Receiver operating characteristic curves showing the areas under the curves for both IgM anti-A and IgG (2-ME) tests

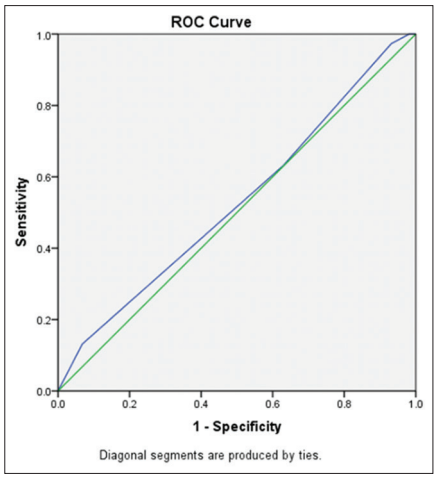

Figure 2: Receiver operating characteristic curves showing the areas under the curves for both IgM anti-A and IgG (DTT) tests

using the manual gel method and consider the high titer of at least 64 and took a smaller sample size that might explain the lower prevalence of anti-A and anti-B IgM antibodies in Bazigou *et al*. study which was 55.8% and 47.2%, respectively.

The prevalence of participants in this study whose serum showed a high titer for IgG antibody after DTT treatment (of at least 50 titer) was 63% that is higher than that concluded in Oyedeyl, *et al*. study[18] who found that 18% of O donors had a visual titer of 8 and above for IgG hemolysins of anti-A and or anti-B, this could be related to differences in titration methods, as Oyedeyl, *et al*. used a 37°C incubation time and read the titers suspension on a spectrophotometer.

The titration after 2-ME treatment in this study revealed a higher prevalence of IgG antibody than when it was estimated by DTT treatment; however, DTT is preferred to 2-ME as it is more efficient in maintaining reduction and it is more resistant to oxidation than 2-ME, DTT cleaves disulfide bonds of pentameric IgM, abolishing their agglutinating and complement binding activities, and permits detection of IgG antibodies in the serum.[19] Yet, the sensitivity and specificity of 2-ME and DTT tests are not superior to IgM anti-A test (the AUC was <0.6 in both the tests indicating poor discrimination[20]) for screening of high antibody titer in donors with blood Group O, so using IgM anti-A test alone in practice is recommended as it does not require additional cost or incubation time.

Regardless the antibody classes, low titers of antibodies (<100) were found more frequently with the increasing age, this is similar to Godin *et al*. study[21] who concluded that the prevalence of dangerous O donors was reduced with increasing the age of donors. The major age groups of population study were 28–37 years and 18–27 years, and ages

Table 5: Relationship between IgM and IgG antibodies titers in details at (1:50) dilution

IgM	IgM	IgG (DTT) (1:50)				
		Positive		Negative		
anti-A	anti-B	IgG(2-ME) (1:50)		IgG (2-ME) (1:50)		Total
(1:50)	(1:50)	Positive	Negative	Positive	Negative	
Positive	Positive	125	-	19	50	194
		(64.4%)		(9.8%)	(25.8%)	(100%)
	Negative	1	-	1	1	3
		(33.3%)		(33.3%)	(33.3%)	(100%)
Negative	Positive	-	-	1	-	1
				(100%)		(100%)
	Negative	-	-	1	1	2
				(50%)	(50%)	(100%)
Total		126	-	22	52	200
		(63%)		(11%)	(26%)	(100%)

Fisher exact P-value = 0.012 (Significant at P < 0.05)

Table 6: Relationship between study variables and age at (1:100) dilution

Variable	Age (years) Mean ± SD		Student's *t*-test	*P*-value*
	Positive	Negative		
I IgM anti-A (1:100)	34±9	40.1±10.7	3.38	0.004*
IgM anti-B (1:100)	34.6±9.5	37.3±10.7	1.44	0.152
IgG DTT (1:100)	31.9±9.4	35.9±9.7	2.44	0.016*
IgG 2-ME (1:100)	32.75±8.5	36.3±10.2	2.49	0.009*

* Significant at *P* < 0.05

of donors with high titers of antibodies, in general, were among these major age groups than other age groups, this was similar to Godin *et al*. study[21] and de França *et al*. study.[22]

Conclusions

There is a high prevalence of high-titer anti-A and anti-B IgM and IgG antibodies among blood Group O Iraqi donors. The detection of anti-A and anti-B IgG antibodies is more practical by using dithiothreitol method, as it needs much shorter incubation time; however, 2-ME is more sensitive. Detection of high titer anti-A IgM antibody in dangerous universal donors, by immediate spin at 1: 50 dilution, can be used alone in practice, replacing the need to use all portfolio of tests of anti-ABO antibodies.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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