ABO IgG antibodytitersofapheresis platelets:Aretrospective single center Egyptian study.

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Abstract: Background: Minor incompatible apheresis platelets (AP) transfusion containing critical anti-A or anti-B titer can cause clinically significant hemolysis. The aim of study was to determine ABO blood groups frequency of AP units, to quantify its titer for anti-A and B IgG by gel method, and evaluated gel card titration method by comparing some of its results with tube testing method. **Methods** blood groups of 270 APs were determined by slide test with anti-A and anti-B reagents. We evaluated 80 group O, 15 group A, 15 group B samples for anti-A and/ or anti-B IgG titer by gel method, to confirm gel card titer results tube testing was performed on eight units of group O and on one sample from each group A, B AP. **Results:** percent frequency of 270 apheresis blood groups were for A, B, O, AB 38.9, 21.9, 29.6 and 9.6% respectively. Group O APs (N80) had significantly higher anti-A and B titers than group A (N 15) or B (N 15) (p<0.00). 17.5% of group O had titers ≥256, no group A or B APs had a titers>256.A highly significant kappa agreement (0.62) was found when comparing gel card technology with tube test (p<0.00**). **Conclusion:** Prevalence of critical anti-A and Btiters in group O was relatively high, and a significant level of diagnostic concordance found between gel and test tube methods.
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1.Introduction

Providing ABO group-identicalplatelets (PLTs) can be problematic to the blood transfusion specialists because only limited supplies are available, PLTs wastage that are not ABO identical, and alsoshelf life shortages of PLT products**. (Shehata *et al.,* 2009)** So, it has been an accepted practice for PLTs to be transfused out of the ABO group. (**Romphruk *et al.,* 2012).**

The apheresis platelet (AP)has a plasma volume about 300 mlcontains the donor's naturally occurring antibodies (anti-A, anti-B, or both according to the donor's ABO group). **(Jeffus & Wehrli, 2012**). There are two types of ABO-incompatibility,a major ABO-incompatibility occurs when donor platelets containing antigens are incompatible with antibodies in the recipient's plasma, causing accelerated destruction of the recipient platelet. In contrast, a minor ABO-incompatibility occurs when donor plasma contains antibodies against the patient's ABO antigen, transfusion of platelets with high titers antibodies to minor ABO-incompatible patient red blood cells antigens can cause clinically significant hemolytic reaction (**Romphruk *et al.,* 2012**), a decrease in the post transfusion increment and may also have adverse effects on the course of the recipient's disease**.(Lozano *et al.,* 2010).**

Although antibody titration method has been considered the poorest one regarding standardization, automation, and reproducibility, it is still used in transfusion medicine to monitor blood group antibody titers to evaluate levels of incompatible blood group antibodies before transfusion of either blood or blood components**.** Titration of anti-A and anti-B levels have been measured by several different techniques, of which the test tube indirect anti globulin test (IAT), and the microcolumn gel card test (MG) **(Cheng & Hao, 2011)**, however, other studies have used flow cytometry. **(De França *et al.,* 2011).**

Performing population estimates for anti-A and anti-B levels in blood donors determine the frequency of high titers and help estimate the cost of implementing a nationalized testing policy. In fact there were major challenges in having the ability to drive policy and develop guidelines methodology of which, lack of a standardized test used direct agglutination, absence of a recognized reference method, and the impact on blood product availability donors **(De França *et al.,* 2011)**, additional problem is the lack of studies needed to correlate these methods with and thus an agreement on the critical end titer for non-ABO identical platelet transfusions. **(Denomme, 2011).** However, there is broad recommendation to titer all O group unitsto choose units with low anti-A and anti-B titer. **(Berséus *et al.,* 2011),** a generally accepted cutoff for high anti-A IgG at 1:256 or IgM at 1:64 when performed with saline. Anti-B is an even more rare cause of hemolysis and is not routinely measured. These policies are based on some evidence but rely as much on expert opinion. **(Holland 2006)**

(Surowiecka *et al.*, 2014) stated that all O units showed be titrated and restrict those with high isohemagglutinin levels to within grouppatients and as the cutoff level for this practice varies, proposed cutpoffrange from 128 to 250.

The aim of this study was to determine the percent frequency of ABO blood groups of apheresis platelet units, to quantify its titer for anti-A and anti-B IgG by gel method technique, and lastly evaluated gel card titration method by comparing some of its results with tube testing method.

2.Material and methods

Preparation of apheresis platelet units

A total of 270AP units were collected from healthy blood donors who met standard donation criteria and gave their informed consent according to institutional guidelines by Zagazig University Hospital Blood Transfusion Center between March – May 2014. They were from donors aged between18 and 45 years and only four were females. A TrimaAccel® automated blood collection system (TerumoBCT, Software Version 5.1, Lakewood, CO, USA) was used for the apheresis procedure.

Two to threeinch segments, each had 300 to 1000 µl plasma, were prepared for testing from each AP unit studied, centrifuged for five minutes and stored the supernatant between 1–6°C. ABO determination of the donors' blood groups was initially determined by slide test with monoclonal anti-A and anti-B reagents (Agappe diagnostics Ltd., Switzerland GmbH) and repeated using gel card grouping (Diagnostic Grifols, S.A. DG Gel® Confirm, Barcelona, Spain). After excluding AB blood group, we were evaluated retrospectively 80 group O, random selected 15 group A and15 group B platelet units to quantify anti A_1, B IgG titers. All plasma samples were collected to determine antibody titers, stored at ≤20°C until tested.

Antibody titer methodology

Testing was performed for plasma dilutions of 1:32, 1:64, 1:128, 1:256, 1:512, and 1:1024 in 0·9% saline. Undiluted donor serum and serial dilutions from each group AP unit were prepared; the titers were tested with A_1 and/or B cells depending on the ABO type of the unit**.(AABB, 2002)**Our titration procedure did not include the determination of IgM titer levels.

Gel card titration method:

Test consisted of a LISS/Coombs card with six microtubes; each microtube contained a wide reaction chamber in the upper part and a gel and anti human globulin (AHG) in the lower part, in which agglutinates are trapped and free cells pass through to the base. Agglutination intensity varying from a well-defined pellet indicating no agglutination (agglutination score 0), predominantly localized in the lower half of the tube (1+), dispersed throughout the tube (2+), predominantly localized in the upper half of the tube (3+), solid band of RBC on the top of the gel (4+) Fig. 1.

According to manufacturer's instructions 50µl of 0.8% A1, 0.8% B, and 0.8% pooled A1and B RBCs were added to the gel cards (LISS /Coombs anti-IgG+C3d, Bio-Rad Laboratories ID-System diamed GmbH, Switzerland), then 25µl of the appropriate sample dilution were added, cards were incubated at 37.0°C for 15 minutes and then centrifuged for 10 minutes. Reactions were scored from 0 to 4+, we recorded the highest dilution giving a 1+ or higher reaction. When positive results were found at 1:1024, higher dilutions were done. Known titer levels run as control samples with test samples. Titers ≥ 256 for IgG were considered high for the aim of current study, based on levels most commonly cited in previous literature**. (Josephson *et al.*, 2004), (Pietersz *et al.*, 2005)**

Preparation percentof $A_1$and B cells:

The A_1 cells, B cells were prepared by pooling known fresh A group cells from three donors, B group cells from one donor, washed three times with normal 0.9% saline. A 5% cell suspension (one ml of ID-Diluent 2 (Bio-Rad Laboratories -diamed GmbH, Switzerland) and 50µl of washed red cells) was prepared, and then a 0.8% cell suspension was prepared (one ml of ID- Diluent 2 (Bio-Rad Laboratories -diamed GmbH, Switzerland) and 10µl of washed red cells). **(AABB, 2002).** Pooled $A_1$and B RBC were obtained by mixing equal volumes of freshly prepared 0.8% A1 and 0.8% B. The cell suspension must be freshly prepared and tested by corresponding monoclonal antibody before use.

Tube test titration method:

Tube testing was done on 10% of the studied samples (eight units) of the group O AP and on one sample from each group A, B to evaluate gel card titer results. For these studies, pooled 3% A_1, 3% B, and with 3% A_1 and 3% B RBCs separately, was prepared in 0.9% saline. 100µl of titrated plasma from each dilution was mixed with 100µl of A_1 and/or B cells, then incubated at 37°C for 60 minutes, after washing four times with saline, two drops of (AHG) (Biotic Laboratories Ltd, AHG COOMBS (anti IgG + C3d), Cambridge) were added, tubes were centrifuged and read for macroscopic agglutination, one drop of 5% suspension of Coombs control cells was added to all negative results, Coombs control cells must be agglutinated to validate negative test results.(**AABB, 2011).**

Preparation of Coombs control cells Mix equal volumes of weak IgG anti-D reagent (1:50 in saline) and 5% suspension of group 'O' D positive cells,

incubate at 370C for 15 minutes, wash twice. **(AABB, 2011)**

Statistical analysis

Statistical analyses were performed using (SPSS version 20.0) (SPSS Inc., Chicago, IL, USA). Differences between frequencies and percents in each titer in different groups studied were compared by Chi-square test. Median and range of each antibody titer was analyzed. Kappa agreement test used to test the agreement of the diagnostic concordance of the gel and test tube methods, and a $\kappa > 0.75$ was considered to be perfect concordance. **(Cheng & Hao 2011)** *P* value was set at <0.05 for significant results &<0.001 for high significant results.

3. Results

In about three months study period; a retrospective study was initiated in 270 APs units received in our transfusion center. Samples from 110 AP were evaluated for anti-A_1/or anti-B IgG titer study, divided according to groups as followed 80 group O, random selected 15 group A and15 group B platelet units.

The frequency of ABO blood groups of 270 apheresis units detected were for A, B, O, AB 105, 59, 80, 26 with percent of 38.9%, 21.9%, 29.6%, 9.6% respectively.

Median group O anti- A_1titers were 64 (range: 0 – 1024), median group O anti-B titers were 32 (range: 0 – 512), median group O anti- A_1, B were 128 (range: 0 – 1024) median group A anti-B titers were 64 (range: 32-128) and median group B anti-Atiters were 32 (range: 32- 64).

As shown in tables (1,2) the prevalence of critical high anti-A_1/or anti-B IgG titers (defined as ≥ 256) in 80 samples group O were (35%) (28/80) found at 1:256 (17.5%), 512 (15%), 1024 (2.5%), and 2048 (0%) respectively. Fig. 1 showed a one group O unit had anti A_1 with 1+ agglutination at a dilution of 1024,titer study was repeated on this sample to confirm the titer of 1024. 65% (52/80) of IgG titers were ≤ 256.Some group O samples showed mixed field reactivity. All samples that were positive when tested with A_1 and B RBCs separately were also positive when tested with the pooled RBCs, in both gel and tube method.Anti-A_1 was found more frequently (30%) at higher concentrations at titers of 1:265 of group O donors which were considered to have critical high anti-A_1 compared with 13.8% for critical high anti-B titers.

The highest IgG titer found for units group A and group B were128 (4/15) (26.7%), 64 (1/15) (6.7%) respectively, group O had significantly higher IgG anti-A_1 and anti-B titers than group A or B AP products (p<0.00**).

Table3 showed anti-A_1/A_1, B IgG titers by comparing gel card technology to tube test methods in group O, A and B AP units, a highly significant kappa agreement (0.62) was found when comparing tube test with gel card technology (p<0.00**) as shown in table 4.

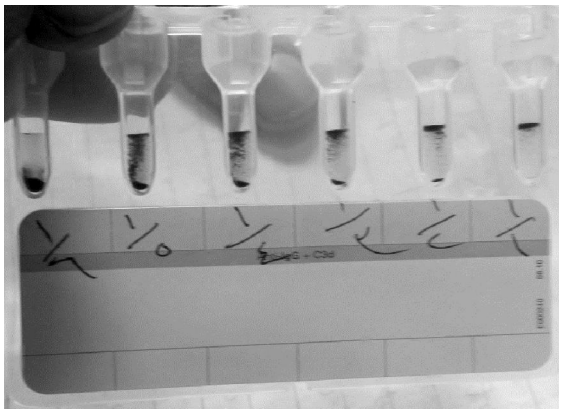

Figure 1: Showed Gel card indirect agglutination test (anti-IgG+c3d)of group O AP of Serum dilutions 1:32, 1:64, 1:128, 1:256, 1:512, and 1:1024 started from most right to most left with anti A agglutination +1 at titer of 1/ 1024.Agglutination scores indicated agglutination strength, from +1 (grade1)most left column to +4 (strongest) most right one. The middle four columns showed example of mixed field reaction.

Table 1. The IgG titer frequency and percent of anti-A_1/or anti-B in 80 group O plasma samples tested by gel card.

Titer	**Group O, anti A_1,B**		**Group O, anti A_1**		**Group O, anti B**		X^2	*P*
	Frequency	%	Frequency	%	Frequency	%		
≤ 32	**14**	**17.5**	**18**	**22.5**	**32**	**40**		
32	**10**	**12.5**	**11**	**13.8**	**12**	**15**		
64	**14**	**17.5**	**15**	**18.8**	**10**	**12.5**	**23.8**	**0.00****
128	**14**	**17.5**	**12**	**15**	**15**	**18.8**		
256	**14**	**17.5**	**14**	**17.5**	**7**	**8.8**		
512	**12**	**15**	**8**	**10**	**4**	**5**		
1024	**2**	**2.5**	**2**	**2.5**	**0**	**0**		
2048	**0**	**0**	**0**	**0**	**0**	**0**		
Total	**80**	**100**	**80**	**100**	**80**	**100**		

** Highly significant.

Table 2. Titers of Ig G anti-A_1 in group O,B and anti-B in group O, A.

Titer	Ig G Anti-A_1 Group O F	%	Group B F	%	Ig G Anti-B Group O F	%	Group A F	%
0	18	22.5	0	0	32	40	0	0
32	11	13.8	14	93.3	12	15	4	26.7
64	15	18.8	1	6.7	10	12.5	7	46.7
128	12	15	0	0	15	18.8	4	26.7
256	14	17.5	0	0	7	8.8	0	0
512	8	10	0	0	4	5	0	0
1024	2	2.5	0	0	0	0	0	0
2048	0	0	0	0	0	0	0	0
total	80	100	15	100	80	100	15	100
X_2	129.6				77.3			
P	0.00**				0.00**			

F = frequency. ** Highly significant.

Table 3. Anti-A_1/orA_1, BIgG titers in group O (from one to eight), group A (nine)and group B(10) AP units: comparison of card gel method to tube testing method titers.

No of units	AP units group	Tube method titer	Gel method titer
1 groupO		1/32	1/32
2 O		1/512	1/512
3 O		1/128	1/256
4 O		1/512	1/1024
5 O		1/256	1/256
6O		1/64	1/64
7 O		1/64	1/128
8 O		1/128	1/128
9 A		1/128	1/128
10 B		1/64	1/32

AP= apharesis platelet. No= number

Table 4. Comparing diagnostic concordance of Anti-A_1/orA_1, B IgG titers by tube testing and gel card technology.

gel	tube									
1/32	1/32	1/64	1/128	1/256	1/512	1/1024	X^2	P	Kappa agreement	*p*
1/64	1	1	0	0	0	0	1277.7	0.00**	0.62	0.00**
1/128	0	1	0	0	0	0				
1/256	0	1	2	0	0	0				
1/512	0	0	1	1	0	0				
1/1024	0	0	0	0	1	0				

** Highly significant.

4. Discussion

AABB Standards for Blood Banks and Transfusion Services 2009 require transfusion services to have a policy concerning transfusion of components containing significant amounts of incompatible ABO antibodies. **British Committee for Standards in Haematology guidelines, 2004** stated that incompatible plasma and platelets should lack high-titer anti-A/ anti-B and avoid transfusion of blood from high-titer anti-A and/or anti-B donors to non-group O without giving data about the testing procedure. So, regarding this issue there is variation in local, national and international policies. (**Sadani *et al.*, 2006).**

The lack of agreement of a critical titer result obliges transfusion services to establish local cutoffs, so we aimed this study to determine the prevalence of critical high anti-A_1/or anti B IgG titers in group O, A and B APs that may be transfused out of group which may only represent a first step in solving this increasing important risk of transfusion.

First we determined the percent of ABO blood groups of 270 apheresis donors studied for A, B, O, AB 38.9%, 21.9%, 29.6%, 9.6% respectively, which when compared with astudy done by **(Franchini & Liumbruno, 2013)** studied current knowledge on ABO blood group, and its role in human diseases pathogenesis, they found frequency of ABO blood groups varies greatly in different populations, in most populations, about 50% are Group O, followed by group A, with groups B and AB showing a much lower incidence**,** we found that our apheresis donors had group A the dominant group then group O but agreed with their finding regarding groups B and AB had a lower incidence. So, we strongly agreed with concept of increasing methods to raise safety of high frequent group O products.

In the current study we found the prevalence of critical high anti-A_1/or anti-B IgG titers≥256 in 80 samples group O were (35%) found at 1:256 (17.5%), 512 (15%), 1024 (2.5%), and 2048 (0%) respectively and 65% were ≤256. Our finding was consistent with relatively high titer results when compared with prior studies, **(Josephson *et al.,* 2004)**using both test tube and gel card; found a relatively high prevalence of high-titer anti-A/anti-A, anti-B (≥256 for IgG) in group O AP donors (30 – 40%).Another retrospective analysis by**(Karafin*et al.*,2012)** found IgG anti A and B titersin about 10% of group O APs had a titer ≥512, and 26.3% had titers ≥ 256, defined IgG titers of ≥ 512 as high titer,(**Larsson *et al.*,2000)** found 10-20% of group O donors had high titer anti-A or anti-B,and a study by **(Cooling *et al.,* 2008)** showedthat 60%of group O pooled PLT units had an anti A and / or anti B titer of ≥64 by gel method**.**

In our study we found median group O anti- A and anti-B titers were 64, 32 respectively, which was similar to finding of **(Cooling, 2007)** who tested 124 pools of group O PLT by gel method and found median anti-A and anti-B titer were 64 and 32 respectively. Group O anti-Atiters in our study had critical high anti-A titers of 1:265 (30%) at more frequently higher concentrations compared with (13.8%) for critical high anti-B, which was almost agreed with finding by (**Fung *et al.,* 2007)** who was found anti-A (8– 50%) of group O donors compared with (0.4– 9%) for high anti-B antibodies. The differences in these results might be due to the method and reagents used and the type of platelet units studied.

The prevalence of the highest anti-A_1/or anti-B IgG titers found in our study for units group A were1:128 (26.7%), and group B were1:64 (6.7%), none of the group A or B AP had the critical high titer of study (≥256 for IgG),which was almost close finding with **(Karafin *et al.,* 2012)** showed no group A or B APs had an IgG titer of greater than 128 (0/342), and also we were agreed with their work in that group O had significantly higher IgG anti-A and anti-B titers than group A or B AP products.

In current study antibody titration levels were assessed by using gel technique and its values were be evaluated compared to conventional tube testing (CTT) titration method .We selected gel method to standardize result interpretations, establish a practical method in a step towards applied automation availability, and the high throughput efficiency gained was suitable for the needed titer study in APs.

In our study a highly significant kappa agreement (0.62) was found when testing the diagnostic concordance, comparing gel card to test tube methods in anti-A_1/A_1, B IgG titers in group O, A and B AP units, which was closer to study by **(Cheng & Hao, 2011)** who had been determined whether the critical values of anti-A and anti-B IgG antibodies based on CTT are applicable to MG techniques and found a good correlation between CTT and MG results for titration of both anti-A and B IgG, and found by κ test values (0.79) a level of diagnostic concordance in the perfect range for both antibodies, and **(Cooling *et al.,* 2008)** reported gel titers were higher than tube titers, In contrast, **(Josephson *et al.,* 2004)** showed good concordance in the titer results between tube and gel methods, with slightly increased sensitivity of test tube compared with gel technology. Also, **(Fung *et al.,* 2007)** found gel titers were equivalent or slight lower than tube titers, such difference might be due to the defined high titer of study**,** the used methods and reagents**.**

Our study had limitations may be due to the small sample size studied, difficulty to compare all samples studied by two methods used for better evaluate differences, the retrospective study of anti IgG titration for AP units was evaluated after the transfusion process, so titer results did not influence transfusion decision or transfusion reaction.

Conclusion:

Our study confirm high prevalence of high titer anti-A and anti-B in group O AP. Our challenge is to re-evaluate our practices and help to establish an institutional and national level of safe ABO antibodies titer. Further studies are needed to ensure adequate confirmatory data and to generate an evidence based practices that will lead to optimal transfusion practice. However, Transfusion medicine services and physicians should be aware of increase ABO-identical PLT, which is firmly recommended for neonates, children, and chronic platelet transfusion patients' needs, but less in patients with acute platelet transfusion needs**.**

Our recommendations regarding ABO- non identical PLT transfusions policies to rationalize

limited supply of these products and minimize cost effective issue that, we may test units for anti-A alone to control both anti-A and anti-B as it seems from our study to be more frequently found at more higher titer than anti B, also do single titer test for each donor, not each donation, as titers are stable over time, and to select donor with lower titre results.

Conflict of interest: none of the authors have conflict of interests.

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