EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 7
309-317
Year: 2017

THE COMPARISON OF ANTIBODY TITRATIONS BY USING CONVENTIONAL TUBE METHOD AND COLUMN AGGLUTINATION TECHNOLOGY

Dr. Subhashish Das*, Shabbir Ahmed Khan and Phebe G.

[1]Professor of Pathology, Sri Devaraj Urs Medical College, Tamaka, Kolar, Karnataka-563101, India.
[2]Lab Technologist, Sri Devaraj Urs Medical College, Tamaka, Kolar, Karnataka-563101, India.
[3]Lab Technologist, Sri Devaraj Urs Medical College, Tamaka, Kolar, Karnataka-563101, India.

***Corresponding Author: Prof. Dr. Subhashish Das**
Professor of Pathology, Sri Devaraj Urs Medical College, Tamaka, Kolar, Karnataka-563101, India.

Article Received on 22/04/2017 **Article Revised on 14/05/2017** **Article Accepted on 05/06/2017**

ABSTRACT

Introduction: The titre of an antibody represents the strength of that antibody's response to a specific antigen. Several different techniques have been employed for titration, of which the conventional tube test (CTT) and the column agglutination technology (CLAT) are the most frequently used. Further, ABO antibody titration has been used to determine treatment strategies and to evaluate ABO-incompatible transfusion responses. **Aim**: To study this comparison of Antibody titrations by using conventional tube method and column agglutination technology. **Materials and Methods: Study design**: Comparative study. **Methodology**: A minimum of about 100 blood sample (30 samples of A group, 30 samples of B group and 40 samples of O group) collected from donors were subjected to antibody titrations using conventional tube method (CTT) as well as column agglutination technology (CLAT) using Ortho Biovue system. CTT antibody titration was done by both immediate spin (IS) tube methods as well as in Anti Human Globulin (AHG), Immuno gama Globulin (IgG) phase. **Results**: The results obtained display higher titers in the CLAT (p =0.0095) and better reproducibility. When the antibody titer was higher, there was a more discernible difference between the precision of the two methods, which further justifies the use of CLAT for use in alloantibody titration. **Conclusion:** CLAT card is more sensitive and specific than conventional tube methods and also is less time consuming with a better turn over time (TAT) but more expensive. Our study suggests that routine blood bank activities such as grouping, cross-matching and antibody titration CLAT should be promoted with proper validation.

KEY WORDS: antibody titration, tube, column agglutination, ABO group, Rh type.

INTRODUCTION

Alloimmunization consists of the induction of immunity in response to foreign antigen (s), encountered through exposure to cells or tissues from a genetically different member of the same species. It is one of the major complications of regular blood transfusions, particularly in patients who are repeatedly transfused.

Irregular red blood cell alloantbodies produced as a result of alloimmunization have clinical importance because they cause hemolysis in the fetus and newborn and in transfused patients. Significant morbidity in patients is due to accelerated destruction of transfused red cells. Transfusion may stimulate production of unexpected antibodies through either a primary or secondary immune response.[1]

Alloimmunization occurs when a foreign antigen introduced in an immune-competent host evokes an immune response. This commonly occurs following transfusion of blood or in pregnancy, when red cells that bear antigens absent from the individual's own blood enter the circulation. The development of alloantibodies can significantly complicate transfusion therapy and result in difficulties in cross-matching of blood. Pre-transfusion antibody screening of patients' samples prior to cross-matching is an essential component of compatibility testing.[2]

Most literature on alloimmunization is limited to multi transfused individuals, with a few studies on the general hospital patients. This study was carried out to do a comparative assessment of alloantibody titration by using CTT verses CLAT.

MATERIALS AND METHODS

100 donor blood samples which were sero-negative for HIV, HbsAg, HCV, VDRL (Ortho Clinical Diagnostic, OCD, USA) and MP (Tulip Diagnostics Ltd) was taken up for the study which consisted of A +VE group, (30 samples), B group (30 samples) and O group (40 samples). There were 51 males and 49 females (M:F

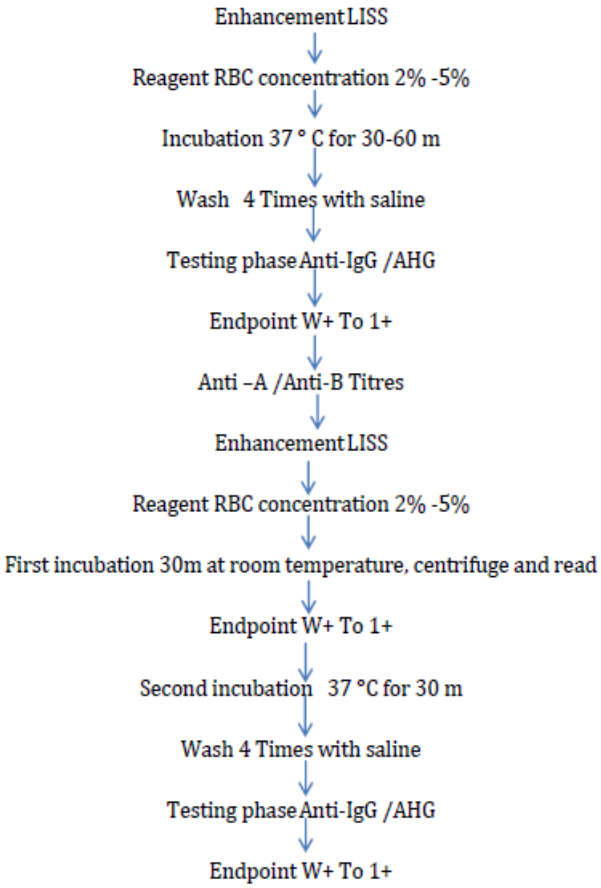
=1:1) with age ranging from 18 to 58 years. Informed donor consent was taken from the donors and approval for Institutional Ethics Committee (IEC) was obtained.

Serial two-fold dilutions were made in normal saline. The first dilution was made by mixing 200 µL of saline, which represents a 1:2 dilution. Subsequent serial dilutions are made by adding 200 µL of diluted plasma to a tube containing 200 µL of saline. The contents of the tube were mixed, and 200 µL was transferred to the next tube containing 200 µL of saline. Pipette tips were changed after the transfer of each dilution. From each master dilution, 100 µL was used fvor tube testing and 25 µL for gel testing.

The grading of the agglutination reactions of both techniques were scored as 4+, 3+,2+,1+ and negative respectively. The antibody agglutination scores were calculated from the summation of scored agglutination reaction results in each dilution. To increase the validity and reliability of the outcomes the laboratory technicians were blinded for test results.

Test Tube Titration for Immunoglobulin M and Immunoglobulin G Initially 200 µl of the test serum is taken of which 50 µl is pipetted into 10 test tubes along with 50 µl is pipetted into 10 test tubes along with 50 µl of 2.5%. RBC suspension and kept for 15 minutes in room temperature followed by centrifugation at 1000 RPM for 1 minute next the remaining 100 ml of diluted serum is included at 37 ^{o}C for 15 minutes with 100 µl of 2.5% RBC cell suspension followed by cell washing for 3 times at 3000 RPM and the supernatant discarded.

Conventional Tube Methods for Antibody Titres is done in the following steps



AHG indicates antihuman globulin; IgG, immunoglobulin G; LISS, low ionic saline solution; RBC, red blood cell;and m, minutes (s).

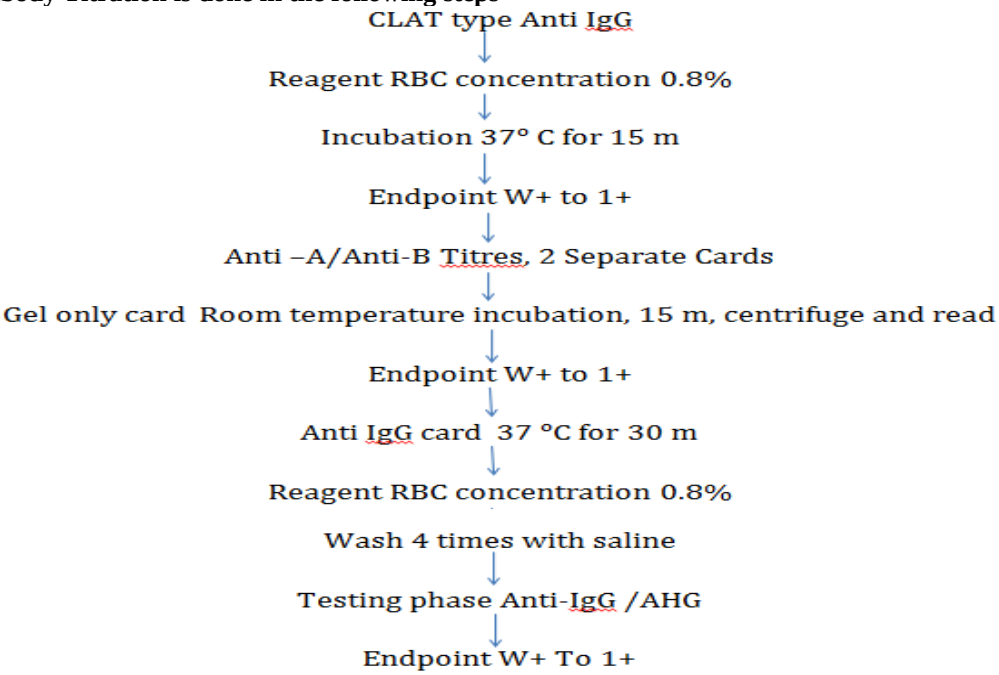
CLAT Method for Titration of IgG and IgM

2 CLAT cards of IgM (having Nacl) and IgG (having AHG haemoglobin) are selected respectively and micro column are labeled correspondingly for dilution from 1:2 to 1:4 to 1:1024.

50 µl of 0.8% donor RBC suspension is added followed by addition of 25 µl of serially diluted serum mixture into corresponding column of gel cards.

CLAT for IgM are kept at room temperature for 15 minutes and AHG CLAT for IgG are incubated at 37 ^{o} C for 15 minutes followed by centrifugation and the result are noted.

CLAT Antibody Titration is done in the following steps



AHG indicates antihuman globulin; IgG, immunoglobulin G; Liss, low ionic saline solution; RBC, red blood cell; m, minutes (s).

Statistical analysis: Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. Chi-square was used as test of significance. Continuous data was represented as mean and standard deviation. ANOVA (Analysis of Variance) was the test of significance to identify the mean difference between more than two groups. Pearson correlation was done to find the correlation between two quantitative variables. p value <0.05 was considered as statistically significant.

Correlation coefficient (r)	Interpretation
0 - 0.3	Positive Weak correlation
0.3-0.6	Positive Moderate correlation
0.6-1.0	Positive Strong correlation
0 to (-0.3)	Negative Weak correlation
(-0.3) to (-0.6)	Negative Moderate Correlation
(-0.6) to – (1)	Negative Strong Correlation

RESULTS

Table 1: Blood Group distribution of subjects: 46.2% of subjects were A positive, 51.2% were B positive and 2.5% were O positive.

		Count	%
Blood Group	A Positive	37	46.2%
	B Positive	41	51.2%
	O Positive	2	2.5%

Table 2: Antibody titers in different methods: A comparative statement on antibody titers in different methods showing both the Mean and SD values

	Total	
	Mean	SD
AHG CAT	106.0	81.6
AHG Tube	71.4	56.0
Rev Dil CAT	82.8	72.1
Saline Tube	45.7	37.4

Table 3: Association between Blood group and titers in different methods: Stastical analysis between blood group and titres in different methods along with respective p values

		Blood Group								P value
		A Positive		B Positive		O Positive		Total		
		Count	%	Count	%	Count	%	Count	%	
AHG CAT	32	11	29.7%	8	19.5%	0	0.0%	19	23.8%	0.249
	64	12	32.4%	11	26.8%	0	0.0%	23	28.7%	
	128	8	21.6%	19	46.3%	1	50.0%	28	35.0%	
	256	5	13.5%	3	7.3%	1	50.0%	9	11.2%	
	512	1	2.7%	0	0.0%	0	0.0%	1	1.2%	
AHG Tube	16	1	2.7%	0	0.0%	0	0.0%	1	1.2%	0.059
	32	21	56.8%	14	34.1%	1	50.0%	36	45.0%	
	64	6	16.2%	17	41.5%	0	0.0%	23	28.7%	
	128	5	13.5%	10	24.4%	1	50.0%	16	20.0%	
	256	4	10.8%	0	0.0%	0	0.0%	4	5.0%	
Rev Dil CAT	16	0	0.0%	2	4.9%	0	0.0%	2	2.5%	0.689
	32	9	24.3%	10	24.4%	1	50.0%	20	25.0%	
	64	20	54.1%	17	41.5%	0	0.0%	37	46.2%	
	128	5	13.5%	10	24.4%	1	50.0%	16	20.0%	
	256	2	5.4%	2	4.9%	0	0.0%	4	5.0%	
	512	1	2.7%	0	0.0%	0	0.0%	1	1.2%	
Saline Tube	8	0	0.0%	1	2.4%	0	0.0%	1	1.2%	0.696
	16	9	24.3%	10	24.4%	1	50.0%	20	25.0%	
	31	1	2.7%	0	0.0%	0	0.0%	1	1.2%	
	32	17	45.9%	12	29.3%	0	0.0%	29	36.2%	
	64	8	21.6%	14	34.1%	1	50.0%	23	28.7%	
	128	1	2.7%	4	9.8%	0	0.0%	5	6.2%	
	256	1	2.7%	0	0.0%	0	0.0%	1	1.2%	

Table 4: Pearson correlation between AHG CAT and AHG Tube: Significant positive correlation was observed between AHG CAT, AHG Tube, Rev Dil CAT and Saline Tube. I.e. with increase in AHG CAT titers, there was increase in AHG Tube values, Rev Dil CAT values and saline tube values and vice versa.

Correlations					
		AHG CAT	AHG Tube	Rev Dil CAT	Saline Tube
AHG CAT	Pearson Correlation	1	0.795**	0.851**	0.530**
	P value		<0.001*	<0.001*	<0.001*
	N	80	80	80	80
AHG Tube	Pearson Correlation	0.795**	1	0.639**	0.620**
	P value	<0.001*		<0.001*	<0.001*
	N	80	80	80	80
Rev Dil CAT	Pearson Correlation	0.851**	0.639**	1	0.474**
	P value	<0.001*	<0.001*		<0.001*
	N	80	80	80	80
Saline Tube	Pearson Correlation	0.530**	0.620**	0.474**	1
	P value	<0.001*	<0.001*	<0.001*	
	N	80	80	80	80
**. Correlation is significant at the 0.01 level (2-tailed).					

Table 5: Comparison of Titers at 32 between Tube and CAT method: Significant association was observed between CAT method and Tube method at cut off of 32. Higher specificity was observed for CAT method.

		Tube Method			
		<32		>32	
		Count	%	Count	%
CAT Method	<32	18	48.6%	1	2.3%
	>32	19	51.4%	42	97.7%

$\chi 2 = 23.56$, df = 1, p <0.001*

Parameter	Estimate	Lower - Upper 95% CIs
Sensitivity	48.65%	(33.45, 64.11[1])
Specificity	97.67%	(87.94, 99.59[1])
Positive Predictive Value	94.74%	(75.36, 99.06[1])
Negative Predictive Value	68.85%	(56.41, 79.06[1])
Diagnostic Accuracy	75%	(64.52, 83.19[1])

Table 6: Comparison of Titers at 64 between Tube and CAT method: Significant association was observed between CAT method and Tube method at cutoff of 64. Higher specificity was observed for CAT method.

		Tube Method			
		<64		>64	
		Count	%	Count	%
CAT Method	<64	41	68.3%	1	5.0%
	>64	19	31.7%	19	95.0%

χ 2 = 24.12, df = 1, p <0.001*

Parameter	Estimate	Lower - Upper 95% CIs
Sensitivity	68.33%	(55.77, 78.69[1])
Specificity	95%	(76.39, 99.11[1])
Positive Predictive Value	97.62%	(87.68, 99.58[1])
Negative Predictive Value	50%	(34.85, 65.15[1])
Diagnostic Accuracy	75%	(64.52, 83.19[1])

Table 7: Comparison of Titers at 128 between Tube and CAT method: Significant association was observed between CAT method and Tube method at cutoff of 128. Higher specificity was observed for CAT method. At 128 cutoffs CAT method had 92.1% specificity, 100% specificity and 92.41% diagnostic accuracy

		Tube128			
		<128		>128	
		Count	%	Count	%
CAT128	<128	70	92.1%	0	0.0%
	>128	6	7.9%	3	100.0%

χ 2 = 24.25, df = 1, p <0.001*

Parameter	Estimate	Lower - Upper 95% CIs
Sensitivity	92.11%	(83.83, 96.33[1])
Specificity	100%	(43.85, 100[1])
Positive Predictive Value	100%	(94.8, 100[1])
Negative Predictive Value	33.33%	(12.06, 64.58[1])
Diagnostic Accuracy	92.41%	(84.4, 96.47[1])

Table 8: Antibody titer by CAT compared to CTT at Room temperature: Analysis of the given data reveals that at RT, 39 samples had identical titer values by both methods. The CAT titers were higher than CTT titer for 39 samples (one dilution higher in 32 samples and two dilutions higher in 7 samples). One dilution lower was seen in CAT.

		AHG CAT Room Temperature				
		1:32	1:64	1:128	1:256	1:512
		Count	Count	Count	Count	Count
AHG Tube	1:16	1				
	1:32	17	12	7		
	1:64	1	10	12		
	1:128		1	9	6	
	1:256				3	1
Total		19	23	28	9	1

Table 9: Antibody titer by CAT compared to CTT at 32°C AHG phase: Similarly, analysis of the given data reveals that, 7 samples had identical titer values by both methods. The CAT titers were higher than CTT for 66 samples (one dilution higher in 55 samples and two dilutions higher in 11 samples). However, the CAT titers showed one dilution lower in 7 samples as compared to CTT and two dilutions lower in 1 sample.

ATT		Reverse Dilution CAT					
		1:16	1:32	1:64	1:128	1:256	1:512
		Count	Count	Count	Count	Count	Count
Saline Tube	1:8	1					
	1:16		15	5			
	1:32	1	1	27	1		
	1:64		3	4	12	4	
	1:128		1	1	2	0	1
	1:256				1		
	Total	2	20	37	16	4	1

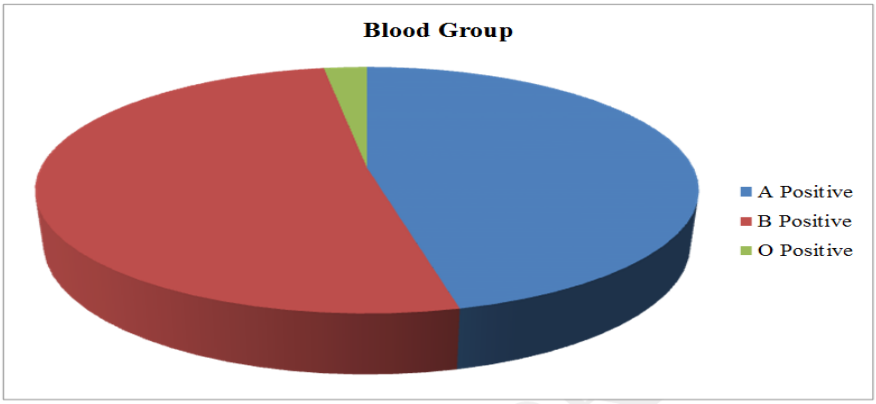


Figure 1: Pie diagram showing Blood Group distribution of subjects

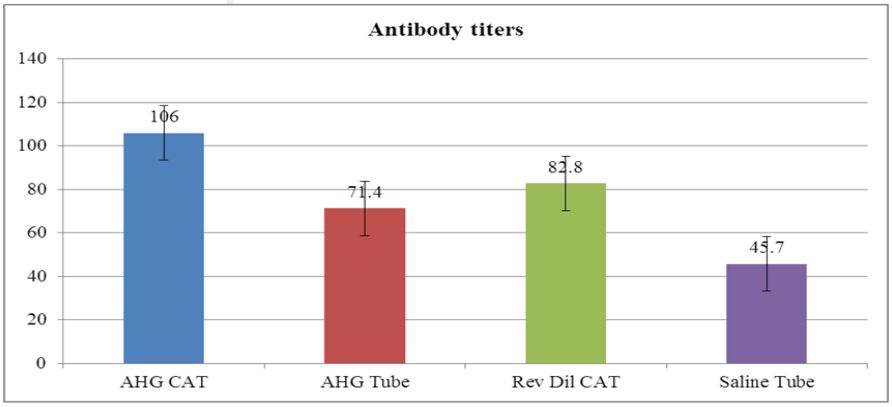


Figure 2: Bar diagram showing Antibody titers in different methods

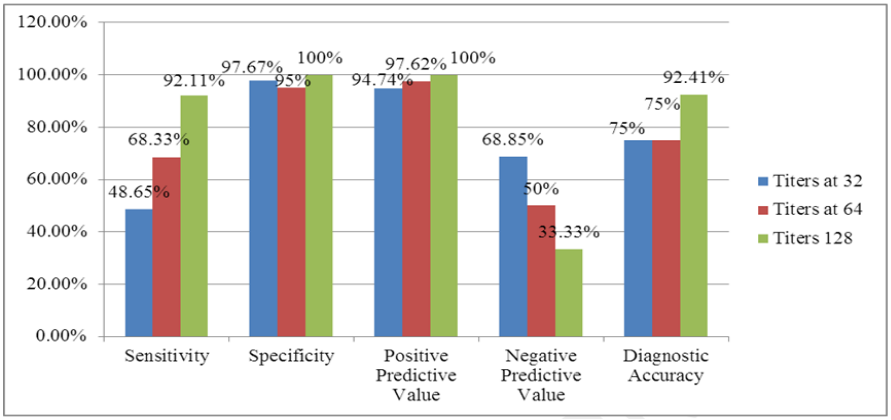


Figure 3: Validity of CAT Method at different titers of antibodies

DISCUSSION

According to the requirements of the Standards for Blood Banks and Transfusion Services, it is recommended that the blood bank have a policy concerning transfusion of components, which contain significant amounts of ABO incompatible antibodies.[3]

Removing ABO barrier expands the donor pool, increases availability of organs for transplantation, decrease time on organ waiting list, and ultimately facilities transplantation. Another important condition includes transfusion of ABO-compatible PC, it is an acceptable practice to transfuse other blood group PCs. Intravascular hemolysis due passively transferred antibodies in a minor ABO-incompatible PC transfusion can cause severe morbidity and mortality. These antibodies are naturally occurring anti-A and anti-B of IgM type and can readily activate complement leading to hemolysis. Many recent studies have focused on the role of antibody titers in the causation of hemolytic transfusion reaction (HTRs).[4]

There has been a growing interest in defining safe or critical titer levels for the purpose of issuing a minor incompatible PC. However, there is a lack of consensus over what constitutes a safe titer level because of differences in methodologies adapted to quality tiers, type and class of antibody detected, and the lack of studies necessary to correlate these methods. Antibody titrations have been difficult to standardize due to multiple factor which includes variable pipetting techniques, quality and concentration of reagents used and lack of consistence with regard to centrifugation speed and time along with the absence of optimal incubation time and temperature. In addition, inter laboratory titration methods vary significantly in methodology, incubation time, temperature, testing phase, and endpoint to interpret the results.[5]

The AABB Technical Manual states that titration is a semi-quantitative method which is quite technique dependent and is used to determine the concentration of antibody in a serum sample or to compare the strength of antigen expression on different red cell samples.[4]

By using a detailed procedure and a weak positive endpoint, the CAP Transfusion Medicine Resources Committee has been able to reduce the inter laboratory variation. The committee also concluded that using detailed and consistent laboratory methods for antibody titres may render the current practices of repeating titres from a previous sample with the current sample unnecessary, because the variation of titres reduces over time in each laboratory. Applying this weak positive endpoint reduced the variance of results. The investigation also found that the gel-card technique generated less variance between laboratories than tube testing, although fewer laboratories used the gel technique than tube method.[6]

Blood banks routinely detect and identify antibodies; in some clinical situations, however, it is important to semi quantitate the concentration of an antibody. Antibodies are titrated by making serial, two-fold dilutions of the patient's plasma and grading the strength of reactivity with selected red blood cells (RBCs) that possess the corresponding antigen. The results of a titer are reported as the reciprocal of the highest dilution of plasma demonstrating macroscopic agglutination.[7]

A review of literature indicates that titer between 16 and 600 may be clinically relevant. The United Kingdom have addressed the issue by implementing a policy to screen all O group donors for IgM titers and label those with titers $\geq$ 100 as "high-titer" and allowed to be transfused only to O group patients. However, there is no recommended standard in the blood bank community. In India, there is a dearth of national standards of practice to guide ABO-incompatible PLT transfusions. This is coupled with a paucity of data on titer levels in Indian population with only a few reports about them.[8]

Platelet (PLT) transfusion need to take into account the issue that arise due to significant amounts of ABO antigen being present on the platelets surface and anti-ABO alloisogglutinins being present in the donor's plasma. Although relatively rare, acute intravascular haemolytic transfusion reactions (AHTRs) have been caused by passive transfer of anti-A and anti-B antibodies, present in apheresis platelets (APs) of group O donors, across a minor ABO incompatibility (group A, B and AB recipients). Transfusion Service Personnel and Clinicians should be aware of the potential risk and they should always be alert and vigilant when it comes to ABO-incompatible platelet transfusions.[9]

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 inter laboratory variation in antibody titrations.[9]

Although ABO incompatible transplant are becoming popular owing to improved outcomes, there are no standard protocols or external survey program for measuring ABO titres. Various techniques like tube, Column agglutination technique (CAT) by gel or bead and flow cytometry are in used. To reduce isoagglutinin titers prior to ABO-1 kidney transplantation, various preparative regimens are available. Standardization of ABO titration coupled with antibody reduction methods like plasmapheresis and Immuno-adsorption have made ABO-1 renal transplant as safe as compatible donor transplants. Further research is needed to ensure optimal antibody titration method, its reproducibility and interpretation.[10]

Similar to our studies, recent studies by various authors have been shown a high isoagglutinin titer in the Asian and Black populations have been attributed to increase the incidences of mosquito bites and intestinal parasitic infections. Among O group individuals, high titers of ABO antibodies (IgM) and hemolysins (IgG) can be due to vaccination and other antigen exposures such as pregnancy, and transfusion. The levels of naturally occurring antibodies depend on the ethnic background of the donors and also on environmental factors. These titers levels can change over a period as cited in a study on Japanese population. The reasons cited for titer reduction were betterment of environmental hygiene leading to lesser parasitic and enteric infections along with higher consumption of processed food by the Japanese donors as compared to other Asiatic population.[11]

Similar to our studies have also shown the gender-wise distribution of titres and found the titers to be higher in females as compared to males across all age groups. The study highlighted a rising trend for titers of anti-A in females after 40 years of age. This can be attributed to the immune exposure such as pregnancy and vaccination. An opposite trend is revealed in males, with titer level progressively reducing in men across all age groups. Similar findings have been reported from Brazilian population.[12]

In addition to the titre estimation our study also highlights the numerous advantages of the CLAT over the CTT. Very few Indians are available regarding estimation of antibody titer in blood donor. Our study will help in estimation of antibody titers and its distribution among the various blood groups along with its clinical significance.

Our study highlights the facts that CLAT is an easy and sensitive technique that require no red cell washing and uses gel filtration media impregnated with an AHG reagent to induce agglutination. The simplicity factor of CLAT is further enhanced with its ability to provide for a stable end point for testing and has encouraged many blood banks and transfusion department to gradually adopt this technique because of ease of performance, better standardization of cells, and more importantly, stable results and reproducibility.[13]

Similarly to the studies conducted by Sawroop et al[14], Kaur et al[15], our study also conclusively proves that CLAT is a better technique then conventional spin tube method because of its simplicity stability of results, dispensation of controls, absence of wash phase with comparable sensitivity and specificity. The average time required for single compatibility test by CLAT was approx. 15-20 minutes while that for conventional spin method was approx. 90 minutes including use of AHG (IAT).

CONCLUSIONS

Although the titers and scores in CLAT are higher than tubes, performing antibody titration using CLAT technology is worth serious consideration because of its inherent advantages.

CLAT is an easy and sensitive technique that requires no red cell washing and uses gel filtration media impregnated with an AHG reagent to induce agglutination. The simplicity factor of CLAT is further enhanced with its ability to provide for a stable end point for testing and has encouraged many blood banks and transfusion department to gradually adopt this technique.[16]

Our study also highlights the clinical significance of ABO titration with regard to incompatible ABO organ transplantation and also for the development of our universal platelet screening programme with particular reference to ABO incompatible platelet transfusion as it will help in the determination of a threshold for critical antibody titers.[17]

To reduce isoagglutinin titers prior to ABO–I kidney transplantation, various preparative regimens are available. Standardization of ABO titration coupled with antibody reduction methods like plasmaapheresis and Immuno-adsorption have made ABO-I renal transplant as safe a compatible donor transplant. Further research is needed to ensure optimal antibody titration method, its reproducibility and interpretation.[18]

The level of information generated by the study regarding antibody titers level and the distribution will help us in minimizing the risk associated with the transfusion of ABO incompatible platelets. Very few Indian studies are available regarding estimation of antibody titers in blood donor, particularly of O group donors.

Our study will help to define a critical titer values in O group donors as they are more responsible for transfusion reactions.

ACKNOWLEDGEMENT

Our sincere thanks to Mr.Prabhagran, Mr. Afsar Pasha C and Mrs. Sarala for their support while preparing the manuscript.

REFERENCES

1. Shukla JS, Chaudhary RK. Red cell alloimmunization in multi-transfused chronic renal failure patients undergoing hemodialysis. Indian J Pathol Microbiol. 1999; 42: 299–302.
2. Patel J, Shukla R, Gupte S. Red cell alloimmunization in multitransfused patients and multiparous women. Indian J Hematol Blood Transfus. 2009; 25: 49–52.
3. Preparation of solution & methods. In: Saran RK, editor. Transfusion Medicine Technical Manual. 2nd

ed. NewDelhi: Directorate General of Services, Ministry of Health and Family Welfare, Government of India, 2003; 305-40
4. Downes KA, Shulman IA. Pretransfusion testing. In: Roback JD, Grossman BJ, Harris T, Hillyer CD, editors. American Association of Blood Banking (AABB) Technical Manual. 17th ed. AABB, Bethesda, 2011; 437–61.
5. Rumsey DH, Ciesielski DJ. New protocols in serological testing: A review of techniques to meet today's challenges immunohaematology 200; 16: 131-7.
6. Weisbach V, Kohnhäuser T, Zimmermann R, Ringwald J, Strasser E, Zingsem J, et al. Comparison of the performance of microtube column systems and solid-phase systems and the tube low-ionic-strength solution additive indirect antiglobulin test in the detection of red cell alloantibodies. Transfus Med. 2006; 16: 276–84.
7. General laboratory methods. In: Roback JD, Grossman BJ, Harris T, Hillyer CD, editors. American Association of Blood Banking Technical Manual. 17th ed. AABB, Bethesda; 2011. p. 863-74.
8. Fluit CR, Kunst VA, Drenthe-Schonk AM. Incidence of red cell antibodies after multiple blood transfusion. Transfusion 1990; 30: 532-5.
9. Cooling LL, Downs TA, Butch SH, Davenport RD. Anti-A and anti-B titers in pooled group O platelets are comparable to apheresis platelets. Transfusion 2008; 48: 2106-13.
10. Shirey RS, Cai W, Montgomery RA, Chhibber V, Ness PM, King KE. Streamlining ABO antibody titrations for monitoring ABO-incompatible kidney transplants. Transfusion 2010; 50: 631-4.
11. Mazda T, Yabe R, Na thalang O, Thammavong T, Tadokoro K. Differences in ABO antibody levels among blood donors: A comparison between past and present Japanese, Laotian and Thai populations.Immunohaematology 2007; 23: 38-41.
12. de Franca ND, Poli MC, Ramos PG, Borsoi CS, Colella R. Titers of ABO antibodies in group O blood donors. Rev Bras Hematol Hemoter 2011; 33: 259-62.
13. Adriaansen MJ, Perry HE. Validation of column agglutination technology for blood group alloantibody titration. N Z J Med Lab Sci 2013; 67: 92-6.
14. Col D Swarup, Brig PS Dhot, Lt Col J Kotwal, Lt Col AK Verma. Comparative Study of Blood Cross Matching Using Conventional Tube and Gel Method. Air force journal india.2008; 129-130.
15. Kaur R,Kakar et al. Use of gel based DiaMed -ID microtyping system for cross matching enhanced sensitivity. Indian J Pathol Microbiol. 2003; 46: 617-20.
16. Novaretti MCZ, Jens ES, et al. Comparison of tube and gel technique for antibody identification. Immuno haematology 2000; 16: 138-41.
17. Rumsey DH, Ciesielski DJ. New protocols in serological testing: A review of techniques to meet today's challenges. Immunohaematology 2000; 16: 131-7.
18. Bromilow IM et al. Evaluation of the ID gel test for antibody screening and identification. Transfusion Medicine 1991; 1: 159-61.