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Comparison of antibody titers using conventional tube technique versus column agglutination technique in ABO blood group incompatible renal transplant

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Abstract:

INTRODUCTION: Measurement of alloantibody titer to a red cell antigen (ABO titers) is an integral part of management of ABO incompatible kidney transplants (ABOiKT).

MATERIAL AND METHODS: There are different methods of titer estimation. Alloantibody detection by tube titration and Gel agglutination columns are accepted methodologies. It is essential to find the difference in titers between the two methods so as to set the 'cut-off' titer accordingly, depending upon the method used.

RESULTS: We did a prospective observational study to compare and correlate the ABO titers using these two different techniques – conventional tube technique (CTT) and the newer column agglutination technique (CAT). A total of 67 samples were processed in parallel for anti-A/B antibodies by both tube dilution and column agglutination methods. The mean titer by conventional tube method was 38.5 + 96.6 and by the column agglutination test was 96.4 + 225. The samples correlated well with Spearman rho correlation coefficient of 0.94 ($P = 0.01$).

CONCLUSION: The column agglutination method for anti A/B titer estimation in an ABO incompatible kidney transplant is more sensitive, with the column agglutination results being approximately two and half fold higher (one more dilution) than that of tube method.

Key words:

ABO incompatible kidney transplants, column agglutination technique, conventional tube technique

Introduction

Measurement of alloantibody titer to a red cell antigen is an essential semi-quantitative tool in ABO blood group incompatible renal transplants (ABOiRTs). Titers are usually determined by serial double-fold dilution method. Different centers have their "cutoff" titers pretransplant, which they target with plasma exchange (PEX) or antibody adsorption. These cutoff titers were estimated earlier by the conventional tube dilution method. However, with the availability of column agglutination technique (CAT) test, which is more sensitive, reproducible, less time-consuming, and easier method,[1,2] it is essential to find whether the two methods correlate. In addition, it is essential to find the difference in titers between the two methods so as to set the "cutoff" titer accordingly, depending on the method used. The purpose of our study was to evaluate prospectively the alloantibody titers in ABOiRTs by both conventional tube and newer column agglutination methods and to determine whether they correlate.

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Aim

The aim of this study is to compare and correlate the alloantibody titers against red cell antigen by conventional tube technique (CTT) and CAT in ABOiRTs.

Materials and Methods

This was a single-center, prospective, observational study done between June 2014 and June 2015. During workup of ABOiRTs, the "cutoff" titer of 1:16 by the conventional tube method (CTT) was targeted for renal transplant. All patients underwent series of alloantibody titer estimation against donor antigens at baseline and then after every second PEX or after every session of antibody adsorption. Antibody titers of sequential recipient's serum were done in parallel by both tube and gel methods.

Immunoglobulin G (IgG) titers were determined by indirect hemagglutination method – by tube dilution method and by DiaMed Coombs anti-IgG gel cards (DiaMed GmbH, Cressier, Switzerland). Initially, neutralization of the IgM present in recipient's serum/plasma was done by heat inactivation at 63°C for 10 min.[3] Titration of immune anti-A1 or anti-B IgG antibodies in the recipient's serum was carried out in parallel against standard red blood cell (RBC) at 0.8% (ID-Diacell ABO 0.8%). A dilution series was prepared using the previously neutralized serum/plasma.[4,5] In this method, ten tubes were labeled for the appropriate dilutions and serial dilutions or a series of doubling dilutions (1:2, 1:4, 1:8, etc.,) of 200 µL recipient's serum in low ionic strength solution (LISS) was prepared. After having filled a set of test tubes with 200 µL of LISS, 200 µL of neutralized serum/plasma was placed in the first test tube and 200 µL of the mixture was transferred from one dilution test tube to the next and so on. From every dilution in the test tube, 50 µL of serum mixture was transferred to the respective microcolumns (labeled according to the test tubes) of the DiaMed Coombs anti-IgG gel cards, together with 25 µL of standard 0.8% Group A1 RBC or Group B RBC (ID-Diacell ABO 0.8%). The cards were then incubated at 37°C for 15 min and then centrifuged and read. The reading of the titer was given by the inverse value of the highest dilution of the serum/plasma which gives an agglutination reaction of 1+. Polyclonal cards (IgG + C3d) were not used since they can increase the titer inappropriately.

Further, 150 µL of serum/plasma which remained in the set of ten test tubes (left after transferring 50 µL in microcolumns of the DiaMed Coombs anti-IgG gel cards) was incubated at 37°C for 15 min after adding 5% LISS-suspended Group A1 or Group B RBCs. After incubation, the above set of test tubes was washed with 0.9% normal saline thrice at 3000 g for 5 min in centrifuge. After washing, 1–2 drops of anti-human globulin/Coombs sera was added in precipitate of all the above test tubes. All the test tubes were mixed well and centrifuged at 1000 g for 30 s to see the agglutination, following which results were taken macroscopically (taken 1+ as a cutoff) by two technicians and a blood bank physician.

Titers by both methods were then compared for correlation by correlation coefficient and concordance by Lin's concordance.

Table 1: Baseline characteristics of study patients

Parameters	Recipient	Donor
Age (Yrs)	40.8+8.7	51.3+11.2
Sex (Male, %)	85.7	100
Blood group (%)		
O	85	-
A1	15	57
B	-	14
A1B	-	28
Basic disease		
Chronic glomerulonephritis	2	NA
Chronic interstitial nephritis	1	
Crescentic glomerulonephritis	1	
Unknown	3	
HLA matching		
3/6	3	NA
2/6	2	
1/6	1	
0/6	1	

Results

A total of 67 samples in seven consecutive ABOiRTs were included in the study. Baseline characteristics are shown in Table 1. Pretransplant, all recipients received rituximab 500 mg at day 14–28 days, PEX or Glycosorb for antibody removal, tacrolimus, and mycophenolate from day 7, and posttransplant, interleukin 2 antibody induction at day 0 and day 4. Mean titer just before transplant was 7.3 ± 5.3 (0–16). Mean PEX sessions were 4.8 ± 2.5, and two patients (with titer 1024) used Glycosorb for antibody removal. Two doses of intravenous Ig were used after the last two PEX in all patients.

Sera were processed in parallel for anti-A/B antibodies by both tube dilution (CTT) and microcolumn gel (CAT) methods. The mean titer by conventional tube method was 38.5 ± 96.6 and by the microcolumn gel test was 96.4 ± 225 [Table 2]. The samples correlated well with Spearman rho correlation coefficient of 0.94 (P = 0.01). However, Lin's concordance coefficient was 0.58 [Table 2]. The sensitivity of gel method was greater than that of tube method, with the gel results being approximately 2.5-fold higher (one more dilution) than that of tube method.

Table 2: Alloantibody titers in ABOi Renal Transplant

	IgM	IgG (Gel method)*	IgG (Tube method)*	Pearson Correlation	Lin's Concordance
Mean	24.7+77	96.4+225	38.5+96.6	0.849 (*P*=0.01)	0.58 (*P*=ns)
Range	0-512	0-1024	0-512		

Posttransplant, patients were followed for 6 months in the study. Baseline mean creatinine was 0.95 ± 0.1 mg/dl. One patient had prolonged hospital stay because of significant drain postoperative. There were no acute rejections in any study patient. Urinary tract infection was witnessed in 5/7 patients (71%). Finally, follow-up mean creatinine was 1.1 ± 0.2.

Discussion

It has long been known that antigens of the ABO blood group are expressed not only on the surface of RBCs but also on cells of other tissues, such as the renal parenchyma, at the level of the glomerular capillary endothelium and the distal tubule cells.[6] In an ABOiRT , the ABO antigens are the targets not only of the corresponding natural antibodies (IgM) but also of the immune antibodies (IgG) whose titer can increase abruptly during the hours or days, following the transplant. These antibodies, through the activation of complement, are able to trigger the so-called "hyperacute rejection," characterized by hemorrhagic thrombosis of the transplanted organ with irreversible loss of its function. This explains the need for precise, regular monitoring for anti-A/B antibodies in the serum of the recipient.[7] Hence, ABO titers are an integral part of management of ABO incompatible (ABOi) kidney transplants.

An acceptable titer is desired before the transplant surgery as preoperative anti-A/B titers is predictor of results of ABOi kidney transplants.[8] Various methods such as adsorption or removal by PEX are utilized to lower the titers to the acceptable levels. Therapeutic PEX reduces ABO titers and permits incompatible transplant.[9] Titration of an alloantibody to a red cell antigen is a semi-quantitative tool utilized for this purpose. There are different methods of titer estimation. The results are different depending on the method used. Alloantibody detection by tube titration and gel columns are accepted methodologies.

The gel method is easy, reproducible, and less time-consuming.[10] Furthermore, the gel cards can be saved for future reference or review by peers for quality assurance. There are studies comparing the different methods of titer estimation,[11-13] but not many in kidney transplants. Titrations using CAT may result in titers several dilutions higher as compared to tube method. Hence, the cutoff titers earlier approved for tube method may not be valid for gel methods. It is important not just the titers but also the method used accurate interpretation and application of results. There are not many studies addressing the titers by different methods in application to kidney transplants. We analyzed our data to correlate titers by both these methods so as to help establish a cutoff for gel method.

Data from our study prove that the gel technique correlated well with the old tube method of antibody titers in ABOiRTs (correlation coefficient 0.94). It also shows that gel method is more sensitive and about one dilution higher in these cases. Steiner *et al*. also reported antibody titers and scores in gel to be consistently higher than titers and scores in tubes.[10] Our study showed that the gel method has a concordance of 0.58 with that of the tube method, which is not very strong concordance. In another study, sensitivity of the gel was 98% as compared to 92% for LISS tube method.[1]

This study is also useful in defining the "cutoff" titer values to be targeted pretransplant by the gel method. As the gel method is more sensitive, the "cutoff" value pretransplant can be a dilution higher than the tube method.

Conclusion

One should be careful in interpreting the results of anti-A/B titers in ABOi kidney transplants depending on the method used for titer estimation. Gel card or the column agglutination method for anti-A/B titer estimation in an ABOi kidney transplant is more sensitive and easy method. The "cutoff" titers would be at least a dilution higher if estimated by CAT.

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Conflicts of interest
There are no conflicts of interest.

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