Original Article

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Pathology Section

Detection of Anti-ABO Antibodies Titres Prior ABO Incompatible Renal Transplantation using Indirect Antihuman Globulin Test via Tube Incubation and Gel Column Technique

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ABSTRACT

Introduction: Renal transplantation is the ideal treatment for patients with End Stage Renal Disease (ESRD). Nowadays, ABO incompatible renal transplant give encouraging results. It reduces the waiting time of patients of ESRD waiting for renal transplant. Antibody titre estimation plays a major role in deciding the donor for ABO incompatible transplant. There are two conventional methods for antibody titre estimation tube incubation technique and gel method. We have compared both the methods so it may help the clinicians in deciding the donor.

Aim: ABO incompatible transplantation is practiced to increase donor pool for Renal Transplant (RT) patients. Indirect Antihuman Globulin (IAT) test by Tube Incubation Technique (TIT) and by gel column technique (IAT-Gel) were compared for detection of anti-ABO antibodies for potential ABO incompatible RT.

Materials and Methods: This was retrospective study of 100 patient sera and corresponding donor red cells used for estimation of anti-ABO antibody titres. Anti-B-antibody titre was performed in group-"A", anti-A-antibody titre in group "B" and either anti-A or anti-B titre was performed in group-"O" patients. Anti-human globulin was employed for both the techniques. Mean and standard deviation was calculated to measure the amount of variation in the titre values by both the methods in all the blood groups.

Results: "O positive", "O negative", "A positive", and "B positive" blood groups were noted in 75, 2, 12 and 11 patients respectively. Anti-A antibody titre was performed in 30 "O positive", 1 "O negative" and 11 "B positive" patients. Anti-B antibody titre was performed in 45 "O positive", 1 "O negative" and 12 "A positive" patients.

By IAT-gel technique mean anti-A titres were 339.35 and anti-B titres were 212 in group "O", anti-B titres were 31.83 in group "A", and anti-A titres were 29 in group "B" patients.

By IAT-TIT, mean anti-A titres were 149.42 and anti-B titre were 122.04 in group "O", anti-B titres were 64.17 in group "A", and anti-A titres were 21.78 in group "B" patients. Mean titres were higher by IAT-gel technique versus IAT-TIT

Conclusion: IAT technique is more sensitive method than IAT TIT for ABO antibody titre measurement.

Keywords: ABO incompatible transplant, Antibody titre, Tube incubation technique, Gel card technique

INTRODUCTION

Patients on ESRD without ABO compatible donors can opt for ABO incompatible (ABOi) RT and thus reduce the waiting time for transplantation [1]. Apart from red blood cells, ABO antigens are present on the spleen, liver and in the glomerular capillary endothelium and the distal tubular cells [2]. Hence, ABO antigens can cause rejection of the graft. Antibody titration plays a major role in deciding the potential donor for ABOi RT. Antibody titration determines the concentration of a specific antibody in the patient's serum [3].

Although, the antibody titre is very important there are variations in the results by different methods. No consensus has been arrived at for an acceptable method. Anti-IgG titre is significant for ABOi RT [4]. Estimation of IgG antibody titre is usually performed by indirect Coomb's test/ indirect agglutination test (IAT). IAT is used to detect in-vitro antigen-antibody reactions for antibody identification. There are two conventional methods; column agglutination method (IAT-gel) and tube incubation method (IAT-TIT).

We compared the two conventional methods for detection of anti-ABO antibodies; IAT-TIT and IAT-gel, in potential

ABOi living related donor RT (LDRT) patients suffering from ESRDs.

MATERIALS AND METHODS

This was a retrospective open labeled observational study carried out at Institute of Kidney Diseases and Transplantation Sciences. It was approved by the ethical committee as well as institutional review board (Reference: IKDRC/LAB/620/2017-18) 100 ESRD patients were studied. The study was carried out from June 2016 to June 2017. Patients with incompatible ABO group donors were included in the study.

Adult blood related family member in good mental and physical health, free from any chronic and organ specific diseases were selected by clinicians for further evaluation as a potential donors.

Adults who were not in good physical or mental health were excluded from the study.

For patients, 4 mL of blood was collected in serum separator vacutainers and for donors, the same amount was collected in Ethylene Diamine Tetra Acetic Acid (EDTA) vacutainer and mixed well. Grouping of all patient/donor samples was performed on fully automated Immunohematology analyzer (Immucor Gamma-Galileo, Nor cross, USA).

Out of 100 patients, 83 were males and 17 were females, with mean age 30.83±2.68 years. Seventy seven patients had "O" blood group, 12 had "A" and 11 had "B" blood group. Their respective donors had blood group "A" in 42 and "B" in 58 patients. None of the donors had "O" and "AB" blood groups. Among 77 patients with "O" blood group, 75 were Rh positive and 2 were Rh Negative. Out of 75 "O Positive" blood group patients, 60 were males and 15 were females, in "O Negative" blood group both were males. In "A Positive" blood group there were 12 patients and all were males. In "B Positive" blood group there were 9 males and 2 females.

Sample Preparation

Patient serum was diluted serially by doubling dilution with Normal Saline (NS). Limitation of our study was that sera was not treated with Dithiothreitol (DTT). Neat (undiluted) serum 100 µL, was taken in the first tube, 100 µL NS was added to each of the following dilution tubes. An equal volume of 100 µL of serum was added to NS in the first dilution tube (1 in 2). The contents were mixed thoroughly at each dilution step up to 1:1024. RBCs of the potential donors were washed three times and 1% suspension prepared for Gel IAT and 5% suspension prepared for tube method.

ABO Antibody Titration Methods

Gel IAT method: Manufacturer's instructions were followed. Total 50 µL of 1% cell suspension was added to 25 µL of serially diluted serum in ID Matrix-Tulip (Goa India) Gel card having Anti Human Globulin C3d incorporated in it. After incubation at 37ºC for 15 minutes, the gel cards were centrifuged and titres were determined as the highest dilution showing 1+ agglutination.

Tube IAT method [3]: About 37 µL 5% cell suspension was added to 70 µL of serially diluted serum remaining in the tube, incubated at 37ºC for 30 minutes and washed three times. Monospecific AHG (Tulip) was added and the tubes were centrifuged. Titre was determined as the highest dilution showing 1+ agglutination to the naked eye.

Antibody titre was performed according to the patient and their respective donor's blood group. In "O" group patient, if the donor blood group was "A", anti-A antibody titre was performed, if the donor group was "B", anti-B antibody titre was performed. In blood group "A", patient's anti-B antibody titre was performed and in blood group "B" patients, anti-A antibody titre was performed.

Anti-A titre was performed in 42 patients with blood group "O Positive", "O Negative" and "B Positive". Anti-B titre was performed in 58 patients with blood group "O Positive", "O Negative" and "A Positive".

In blood group "O Positive", anti-A titre was performed in 30 patients and anti-B titre was performed in 45 patients. For "O Negative" blood group, anti-A titre was performed in 1 patient and anti-B titre was performed in 1 patient. In blood group "A Positive", anti-B titre was performed in 12 patients. In blood group "B Positive" anti-A titre was performed in 11 patients. None of the patients or the donors' blood group were "AB" [Table/Fig-1].

Blood Group Patient	Number of Patients	Patient M/F	Donor Blood Group A (Anti A)	M:F	Donor Blood Group B (Anti B)	M:F
"O"+	75	60/15	30	10/20	45	10/35
"O"-	2	2/0	1	F	1	F
"A"+	12	12/0	NA	NA	12	2/10
"B"+	11	9/2	11	3/8	NA	NA

[Table/Fig-1]: Group-wise distribution of the patients.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS version-16 (Statistical Package of Social Sciences). Continuous data are presented as the mean±SD. Student's t tests were used to compare two groups. P<0.05 was considered statistically significant.

RESULTS

In "O" group patients [Table/Fig-2], the mean anti-A titre by IAT gel was 339.35±368.65 and by IAT-TIT the titre was 149.42±220.9. Median titre by IAT Gel was 256 and by IAT TIT was 64. The mean anti-B titre in group O group patients was 212±276.4 in IAT gel and 122.04 ±186.24 in IAT TIT. The median anti-B titre was 128 and 64 respectively. In blood group "A" patients, the mean anti-B titre by IAT Gel was 31.83±37.16

and by IAT TIT it was 64.17±142.62. The median titre was 16 in both the methods. In blood group "B" patients, anti-A titre was 29±40.78 by IAT Gel and 21.78±24.26 by IAT TIT .The median titre was 16 and 8 respectively.

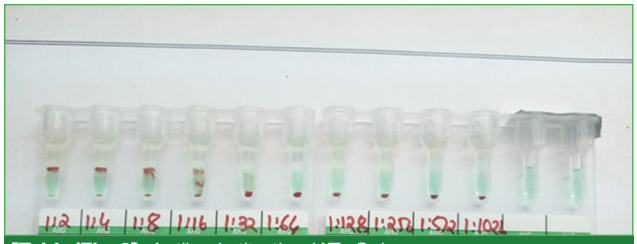
The mean titre in blood group "O" patients (n=77) was higher by the IAT Gel method as compared to IAT TIT method in both anti-A titre and anti-B titre (p<0.01).The median titre was higher by IAT gel method compared to IAT TIT in both anti-A titre and anti-B titre. The median titre by IAT TIT was same in anti-A and anti-B titre.

In blood group "A" patients (n=12) mean anti-B titre was less by IAT gel method compared to IAT TIT method (p=0.46).The median titre was same by both the methods.

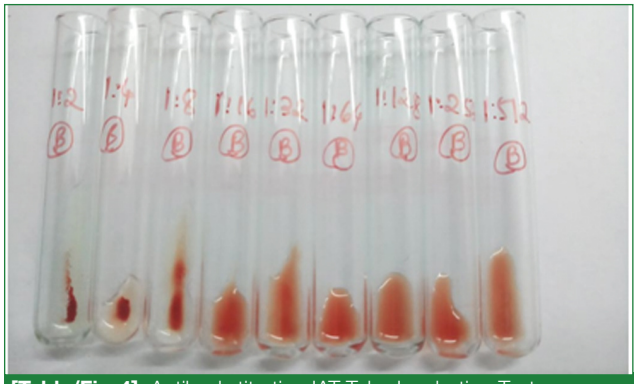
In blood group "B" patients (n=11) mean anti-A titre was more by IAT gel method compared to IAT TIT (p=0.46). The median titre was more by IAT gel method compared to IAT TIT [Table/Fig-2-4].

Antibody titration method	IAT-Gel Mean titre	IAT-TIT Mean titre	IAT-Gel Median	IAT-TIT Median
Blood group O (N=77)				
Anti A	339.35±368.65	149.42±220.9	256	64
Anti B	212±276.4	122.04±186.24	128	64
Blood group A (N=12)				
Anti B	31.83±37.16	64.17±142.62	16	16
Blood group B (N=11)				
Anti A	29±40.78	21.78±24.26	16	8

[Table/Fig-2]: Antibody titre results and comparison of both the methods.



[Table/Fig-3]: Antibody titration IAT -Gel .



[Table/Fig-4]: Antibody titration IAT Tube Incubation Test.

DISCUSSION

In ABOi transplant, ABO incompatibility may cause rejection of the transplanted organ. Hence, precise estimation of ABO antibody titre is important. Anti-A and Anti-B antibodies may be of IgG, IgM or IgA type in blood group "A" or "B". However, sera of group "O" individuals have IgG class of anti-A and anti-B antibodies [5,6]. Although, there is a lot of variation from centre to centre the tube technique is the commonest method used to perform the antibody titration. In tube IAT method, serum required is more and the interpretation has to be done with naked eye. There is no automated method to perform Tube IAT. Gel IAT on the other hand requires less quantity of serum, automation is possible and it gives more consistent results [4]. Hence, we decided to compare the two conventional methods of performing ABO antibody titres in this study.

Our study demonstrates that there is a significant difference in the results by both the methods depending on the blood group of the patient. In blood group "O", anti-A and anti-B antibodies are of IgG class and in blood group "A" and blood group "B" they are of IgM class. "O" group patients therefore show higher titres in Gel IAT method especially in anti-A titre. We have used DTT untreated serum. Hence, IgM type of antibody may interfere with the IgG type of antibody in the tube method. We have used monospecific AHG in tube IAT test while Gel IAT has poly-specific AHG and c3d, so cross reaction with IgA and complement may take place in gel method. Gel card with monospecific AHG was not available with us. Hence, the Gel IAT may show higher titres. Kang SJ et al., have also compared antibody titres on the basis of antibody detection methods and they have also found that in blood group O, the mean titre in Gel IAT was significantly higher than that of Tube IAT for Anti-A which is similar to our results [4]. However, they have compared Hem-agglutination method with Flow Cytometry (FCM) also. FCM with anti-IgM showed the highest titre compared to Tube or Gel method in all of the blood groups.

Doctors who are interpreting the titre results should also keep in mind that the results may vary according to the method used. They should then correlate the results [7]. Our study demonstrates that both the IAT Gel and IAT tube methods show different detection capacity in different blood groups. Gel method or Column Agglutination method is known for reducing inter laboratory variations [8]. Shirey RS et al., have also compared both the methods and observed that Gel method is less time consuming and more consistent [9]. Sood R et al., compared antibody titre by tube method and Gel method in 200 blood group O donors however no significant correlation was found between the two methods [10].

IgG class of antibodies have a more significant role than IgM class, in post transplant antibody mediated rejection.. ABO

antibody titre estimation plays a very critical role in pre transplant and post-transplant period for desensitisation protocols [11,12]. Hence, a method which provides consistent result and estimates the IgG titre should be followed. Gel IAT method estimates the IgG titre, is less time consuming and can be used in automated methods. Gel card technique is better than the saline tube test because it shows better reproducibility [13-16].

LIMITATION

Dithiothreitol (DTT) untreated serum was used hence IgM antibodies may interfere with IgG antibodies. Gel card with mono-specific AHG was not available with us hence gel IAT with poly-specific AHG and c3d was used. This could have caused cross reaction with IgA and complement.

CONCLUSION

ABO antibody titre performed by the Gel IAT technique is better than the Tube IAT method as it is easy to perform, measures the IgG class of antibodies and it can be performed on automated platforms also. According to this study, it is the ideal method for ABO antibody titre measurement and in monitoring the patients of ABOi RT especially in blood group O patients.

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