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Original Article

ABO 부적합 신 이식 전 치료적 혈장교환술 횟수의 예측 변수로서의 초기 ABO 항체 역가

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Initial ABO Antibody Titer as a Variable for Estimating Number of Therapeutic Plasma Exchange prior to ABO Incompatible Kidney Transplantation

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Background: Therapeutic plasma exchange (TPE) for desensitization in ABO incompatible kidney transplantation (KT) has raised concerns regarding efficiency and safety. The purpose of this study was to determine the number of TPE prior to KT required to reach target titer for KT according to ABO blood groups.

Methods: The distribution of ABO antibody (Ab) titer of 117 patients was investigated. The relationship between initial ABO Ab and number of TPEs required to reach target titer to ≤1:8 prior to KT was evaluated retrospectively according to blood groups and ABO Ab classes.

Results: The initial IgG ABO Ab titers were the highest in blood O group recipients, and the average number± standard deviations (range) of TPEs performed prior to ABO incompatible KT was 3.0±1.1 (0～5) in blood group A, 3.7±1.5 (0～8) in blood group B, and 5.3±1.9 (2～13) in blood group O, respectively. The best correlation was observed in the linear relationship between initial ABO Ab titer and number of TPEs required ($y=0.6829x+0.0523$, $R^2=0.946$, $x=\log_2$ initial ABO Ab titer, y=number of TPE required), regardless of the specific ABO blood group.

Conclusion: The number of TPEs can be highly deduced from initial ABO Ab titer and our developed equation in desensitization programs would help increase the efficiency of TPE and patient safety. **(Korean J Blood Transfus 2016;27:22-30)**

Key words: Plasma exchange, Blood group incompatibility, Kidney transplantation, ABO antibodies

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Introduction

ABO-incompatible kidney transplantation (iABO KT) has expanded the donor pool by increasing the availability of transplantable organs, decreasing the waiting period. The blood type barrier is a major obstacle to the naturally occurring anti-A or anti-B, which cause hyperacute rejection in preliminary cases, by not removing them from the circulation at the time of transplantation.[1] Therapeutic plasma exchange (TPE) before an iABO KT may be used to prevent hyperacute rejection, and posttransplantation TPE is often used to treat antibody-mediated rejection that occurs in this setting.[2]

Currently, most courses of desensitization include TPE followed by intravenous immunoglobulin (IVIG) as a preconditioning and medication of thymoglobulin, tacrolimus, mycophenolate mofetil, and steroids as an immunosuppressant worldwide.[3] TPE, one of several immunomodulating techniques, plays an important role by removing IgG and IgM ABO antibody (Ab) in iABO KT.

TPE to permit iABO KT has been successfully performed at several centers in Korea and is currently an American Society for Apheresis Category II indication.[4] Albumin is the most commonly used replacement fluid for TPE. The procedure cost of TPE including the charge of albumin as replacement fluid is 740,000 Korean won per one exchange in Korea. However, only a limited number of plasma exchanges is covered by Korean medical insurance.[5] In the United States, the cost is $5,925 per one exchange, only including base inputs without facility and side effect costs.[6] Although the major adverse effects of TPE, such as symptomatic hypocalcemia, hypotension, urticaria, and nausea, occur in approximately 4% of procedures, long-lasting immunosuppression may lead to hazardous clinical consequences, such as persistent lymphopenia, increased risk of infection, or viral reactivation.[7-9]

A review of our TPE program for iABO KT is necessary to determine whether it is efficient and reasonable because individual institutions use their own innate Ab removal programs. The Johns Hopkins group uses a schedule for the number of planned pre- and posttransplant TPE treatments based on pre-TPE IgG titer based on a goal ABO Ab titer of $\leq$16, whereas the Royal Melbourne Hospital group uses a goal ABO Ab titer of 8～32 depending on the titration method.[10,11]

The substance removal kinetics of TPE is known to be exponential based on a several compartment models, and post-exchange events with optimal intervals between the procedures have been developed.[12] The objective of our study was to define the number of TPEs according to initial ABO Ab titers to guide clinicians in the design of ideal strategies for patients preparing iABO KT.

Materials and Methods

1. Patients

One hundred seventeen end-stage renal disease patients, excluding two outlier patients among 119 patients, with negative pre-transplant lymphocyte cross match test who had undergone iABO KT at Severance Hospital between June 2010 and July 2015 were included in this study. The relationship of initial ABO Ab titer and the number of TPEs re-

quired to reach post-TPE Ab titer to ≤1:8 prior to kidney transplantation was retrospectively evaluated according to their blood groups and ABO Ab classes. Clinical and laboratory data were extracted from our electronic database and the patient's medical records.

2. Pre-transplantation conditioning

All patients underwent the pre-transplantation conditioning protocol that consists of TPE followed by IVIG (100 mg/kg) and immunosuppressants (0.1 mg/day tacrolimus, 1500 mg/day mycophenolate, 20 mg/day prednisone, 375 mg/m^2 rituximab) administration. All patients received pre-transplantation conditioning prior to the operation. TPE was conducted using the COBE spectra system (Terumo BCT, Lakewood, CO, USA) mostly for patients who had ABO Ab titers greater than 1:8. One plasma volume was removed from each patient and 100% replacement was provided using a 5% albumin solution (Green Cross, Yongin, Korea) or fresh frozen plasma (FFP) of the AB blood group. TPE and IVIG treatments were conducted every other day before transplantation until both IgM and IgG titers were no greater than 1:8 in most cases. TPE was performed using 5% albumin solution for the initial sessions, and the last 2 sessions of TPE were carried out with the AB blood group FFP to prevent bleeding before transplantation. Immunosuppressive drugs were used before transplantation to prevent graft rejection. Administration of tacrolimus, mycophenolate, and prednisone was initiated 7 days prior to transplantation, and rituximab was administered 2 days before transplantation after performing TPE.[13,14]

3. Measurement of ABO antibody titers

ABO Ab titers were determined by the tube method, testing two-fold serial dilutions of the patients' serum with commercially available A/B indicator red cells using 3.0% Affirmagen (Ortho Clinical Diagnostics, Raritan, NJ, USA). After incubation at room temperature for 30 min and centrifugation at

Table 1. Distribution of recipient-donor ABO type and ABO antibody titers

Blood group		Anti-A or Anti-B titer				No. of cases (%)
Recipient	Donor	IgG		IgM		
		Anti-A	Anti-B	Anti-A	Anti-B	
O	A	4～512	-	8～512	-	18 (15.1)*
O	AB	32～128	16～64	32	16～64	3 (2.5)
O	B	-	8～1,024	-	8～128	18 (15.1)*
A	AB	-	≤2～128	-	4～256	14 (11.8)
A	B	-	≤2～64	-	4～64	21 (17.6)
B	AB	≤2～64	-	4～128	-	24 (20.2)
B	A	2～128	-	4～128	-	21 (17.6)
Total						119 (100.0)

*One outlier patient was included.

3400 rpm for 15 seconds, the highest serum dilution ratio showing 1+ reactivity by indicated the ABO Ab titers was identified. IgG titers were measured using serum samples treated with 0.01 M dithiothreitol solution (Sigma-Aldrich, St. Louis, MO, USA), while IgM titers were determined from untreated samples.[2] Antibody titers were evaluated every day after initiation of the conditioning protocol while preparing for transplantation. The initial ABO Ab variable used in the correlation analysis is defined as an IgG, IgM and higher Ab titer between IgG and IgM classes before the initiation of Ab depletion.

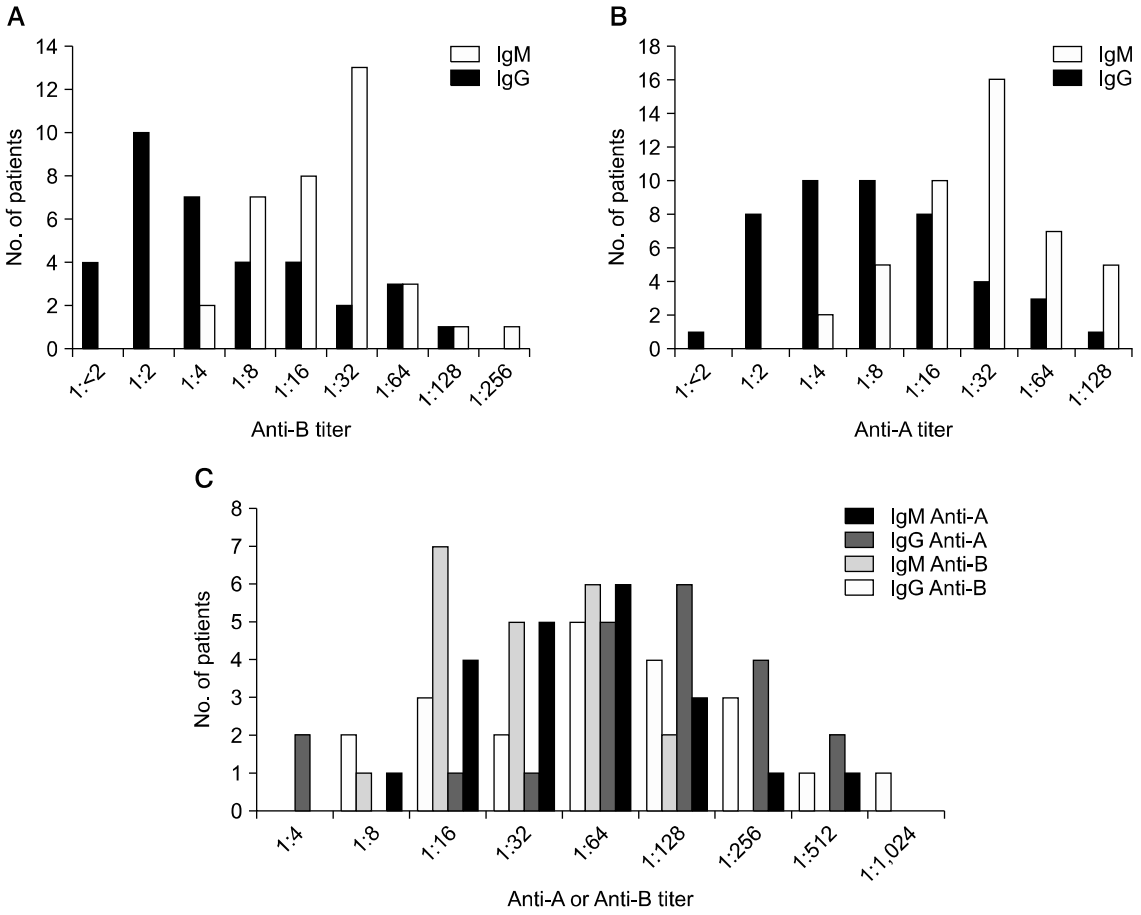

Fig. 1. Distribution of initial ABO antibody titer of recipients according to blood group. (A) Distribution of blood group A recipients, (B) B recipients, and (C) O recipients. The initial ABO Ab titers of blood group O recipients were significantly higher in the case of IgG, compared to both blood group A and blood group B recipients ($P<0.001$), whereas in the case of IgM, only ABO Ab titers of blood group O recipients were significantly higher compared to blood group A recipients ($P=0.040$).

4. Statistical analysis

The two-sample t-test was used to compare ABO Ab titers (log_2 initial ABO Ab titer) among the blood groups with SPSS software (version 23.0; SPSS Inc., Chicago, IL, USA), and a two-tailed value of $P<0.05$ was considered statistically significant. The value of R^2 (coefficient of determination) was calculated by using Microsoft Excel 2010 (Microsoft Corporation, Richmond, WA, USA).

Results

1. Patients

Among the total of 117 patients, 80 patients were male (68.4%) and the median age of the patients was 46 years (interquartile range 37～53 years). Transplantation from AB donors to B recipients (AB→B), A→B, and B→A were majority pair combinations including approximately 19.7%, 17.9%, and 17.1% of the population, respectively, and three cases (2.6%) of AB→O were performed (Table 1).

2. Initial ABO antibody and TPE

The distribution of initial IgG or IgM ABO Ab titer is illustrated according to recipients' blood types (Fig. 1).

The initial ABO Ab titers of blood group O recipients were significantly higher in case of IgG, compared to both blood group A and blood group B recipients ($P<0.001$), whereas in case of IgM, ABO Ab titers of blood group O recipients were only significantly higher compared to blood group A recipients ($P=0.040$).

The average number±standard deviation (range) of TPEs performed prior to iABO KT was 3.0±1.1 (0～5) in blood group A, 3.7±1.5 (0～8) in blood group B, and 5.3±1.9 (2～13) in blood group O recipients, respectively. While most transplantations proceeded at an ABO Ab titer of 1:8, 11 patients (9.4%) with IgG or IgM titers not lower than 1:16 underwent iABO KT, including nine blood group O recipients (five A→O and four B→O cases) and two blood group B recipients (one A→B and one AB→B case).

The correlation between the initial ABO Ab titer of IgG, IgM and higher Ab titer between IgG and

Table 2. Correlations between initial ABO antibody titer and number of TPEs

Blood group		ABO antibody	R^2		
Recipient	Donor		IgG	IgM	IgG or IgM
O	B, AB	Anti-B	0.831*	0.7017	0.8855*
O	A, AB	Anti-A	0.8414*	0.0581	0.8874*
A	B, AB	Anti-B	0.6525	0.8484	0.7919
B	A, AB	Anti-A	0.5044	0.7924	0.65

*The four correlations which showed the best fit in linear relationship.

IgM classes and the number of TPEs observed mostly showed an exponential relationship (x=$\log_2$ initial ABO Ab titer, y=number of TPE required), excluding four circumstances when the recipient was O blood type, calculated with IgG and higher Ab titer between IgG and IgM classes (Table 2). However, the best correlation between the initial ABO Ab titer of the higher Ab titer between IgG and IgM classes and the number of TPEs observed showed an linear relationship (y=0.6829x+0.0523, R^2=0.946, x=$\log_2$ initial ABO Ab titer, y=number of TPE required), if all the 117 cases were integrated in regardless of recipient's blood type or ABO Ab classes. This can be universally applied in practice, for example, if a blood group A recipient had an initial IgG type anti-B with 1:8 and IgM type anti-B with 1:32. The x is 5 (the higher value, 1:32 IgM, transformed to log base 2) and y, the expected number of TPEs for iABO KT, was 3.467 (Fig. 2).

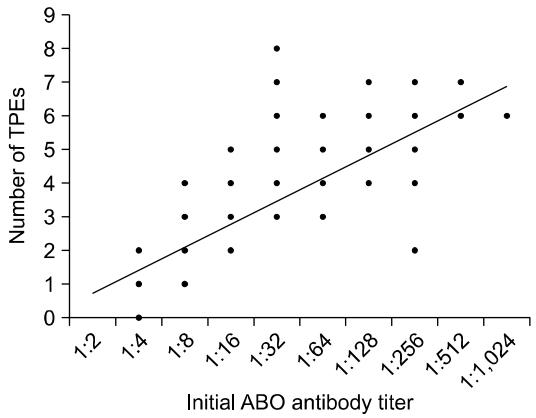

Fig. 2. Relationship between initial ABO antibody titer (Ab) and number of therapeutic plasma exchanges (TPEs). The best correlation between the initial ABO Ab titer of the higher Ab titer between IgG and IgM classes and the number of TPEs observed showed a linear relationship (y=0.6829x+0.0523, R^2=0.946, x=$\log_2$ initial ABO Ab titer, y=number of TPE required), if all 117 cases were integrated regardless of recipient's blood type or ABO Ab classes.

3. Outlier patients

Among the 119 cases, two patients had undergone an unexpected number of TPEs, with both patients showing initial ABO Ab titers of 1:256. One patient was an blood group O 5-year-old boy who was diagnosed with Wilms' tumor, nephrectomy due to renal failure aggravated by localized solid mass, and had received a kidney from his mother, who was A blood type. Immunosuppressant therapy and two rounds of TPE by using replacement fluids including albumin and FFP, respectively, lowered his titers to 1:4, with a good response to Ab removal.

Another patient was an blood group O 64-year-old male diagnosed with end-stage renal disease due to prolonged hypertension and a history of chronic hepatitis C. He received a kidney from his daughter, who had a B blood type. Although the pre-conditioning regimen was adopted, his anti-B titer did not decrease easily. A total of 13 rounds of TPE were performed (9 albumin and 4 FFP episodes) to lower his titers to 1:4, conducted by the addition of eight TPEs to five TPEs, which were more than initially planned.

Discussion

The findings of our retrospective study confirm that the number of TPEs required to proceed to iABO KT shows an exponential relationship be-

tween initial ABO Ab, which was previously reported by Lawrence et al.[15] Although his study focused on IgG Ab titer, we considered all aspects in IgG, IgM, and either higher Ab titer of IgG or IgM classes, because there is currently a debate regarding which immunoglobulin class is more potent in the course of rejection.[16] In addition, we focused on the correlation according to the recipients' blood group and ABO Ab titer, which is critical for graft survival.[17] This is the first study to suggest a number of TPEs based on the investigation considering the recipients' specific blood types and ABO Ab class.

As TPE removes substances present intravascularly, it can be expected that removal efficacy is superior in IgM compared to that in IgG or IgA, as the extravascular distribution was found to be 55%, 58%, and 22%, respectively.[18] The findings of our study in cases of recipient blood groups A and B were consistent with this principle; however, in cases of recipient blood group O, the best correlation was observed when a higher Ab titer of either IgG or IgM classes was applied.[19] In addition, as ABO IgM isoagglutinins exist in a greater amount than IgG, the number of TPE may have been affected by this cause.[20]

Blood group O recipients had higher Ab titers than other blood group recipients, especially in IgG isoagglutinin; the maximum levels of IgG anti-A and anti-B were 1:512 and 1:1024, respectively. This finding is consistent with the observations of previous studies in Korea in that blood group O individuals generally have high titers against A or B blood antigens.[21,22]

The use of replacement fluid in TPE differs among institutions based on the physiological impact of such fluids. FFP, which contains all coagulation factors in normal concentrations, can minimize bleeding risk prior to operation. In the protocol used by the Johns Hopkins Medical Center, FFP compatible with the donor and recipient is used as the last portion of the replacement fluids after the use of albumin when the patient is at risk of bleeding.[10] FFP is associated with a higher risk of hypersensitivity reactions and transmission of viral infections with the supplementation of coagulation factors; however, its use as a replacement fluid is preferred compared to albumin in many cases.[23]

The strength of this study is that the TPE protocol is universally applied among most cases, as AB type FFP is used in the two last sessions before transplantation after several TPEs with albumin. Therefore, the number of TPEs acquired from our equation can be easily adopted. The clinical importance of immunoglobulin classes is unclear; however, our approach is safe for preserving good allograft outcomes. The Ab titer can be quantified using advanced methodologies, such as flow cytometry or gel techniques, which can provide more accurate correlations.[24,25]

In conclusion, we suggest that a number of TPEs can be highly deduced from initial ABO Ab titers regardless of the specific blood type or ABO Ab classes; therefore, can contribute to the reduction in patients' side effects as well as financial costs.

요 약

배경: ABO 부적합 신이식시 탈감작을 위해 시행하고 있는 치료적 혈장교환술의 효율성과 안전성에 대한 관심이 증가되고 있다. 본 연구에서는

신이식 전 목표 역가에 도달하는데 필요한 ABO 혈액형별 치료적 혈장교환술의 횟수에 대해 결정해보고자 하였다.

방법: ABO 부적합 신이식을 받은 117명의 환자를 대상으로 ABO 항체의 역가 분포를 조사하였다. 또한 초기 ABO 항체의 역가와 이식전 1:8 이하의 목표 역가를 달성하기 위한 치료적 혈장교환술의 횟수를 각 혈액형 및 ABO 항체 종류별로 후향적으로 분석하였다.

결과: 초기 ABO 항체의 역가는 O형 혈액형 수여자의 IgG에서 가장 높은 값을 보였으며, ABO 부적합 신이식시 치료적 혈장교환술 횟수의 평균±표준편차(분포)는 A형 3.0±1.1 (0～5), B형 3.7±1.5 (0～8), 및 O형 5.3±1.9 (2～13) 회의 치료적 혈장교환술이 시행되었다. 또한 초기 ABO 항체의 역가와 치료적 혈장교환술의 횟수는 117명의 ABO 혈액형에 상관없이 모두 취합하여 계산한 경우 가장 적합한 직선성 관계를 보였다($y=0.6829x+0.0523$, $R^2= 0.946$, $x=\log_2$ 초기 ABO 항체 역가, y= 치료적 혈장교환술의 횟수).

결론: 초기 ABO 항체의 역가를 바탕으로 치료적 혈장교환술의 횟수를 추정할 수 있었으며 향후 탈감작 프로그램에서의 본 공식은 치료적 혈장교환술의 효율성 제고와 환자 안전에 기여하는데 도움을 줄 것으로 판단된다.

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