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ÖZGÜN ARAŞTIRMA / ORIGINAL RESEARCH

Detection of Anti-HLA Antibodies Produced After Transplantation in Renal Transplants and Evaluation of Its Association with the Other Parameters

Böbrek Nakli Olan Hastalarda Nakil Sonrasında Oluşan Anti-HLA Antikorlarının Saptanması ve Çeşitli Parametrelerle İlişkisinin Değerlendirilmesi

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Öz

Amaç: Bu çalışmada İzmir Tepecik Eğitim ve Araştırma Hastanesi'nde kadavra veya canlı donörden böbrek nakli olmuş hastaların nakil sonrasında periyodik olarak toplanan serum örneklerinde grefte karşı gelişen immünolojik cevabın ve ilgili faktörlerle arasındaki ilişkisinin değerlendirilmesi amaçlanmıştır. **Gereç ve Yöntem:** 31 hasta serumunun anti-HLA antikor tarama ve tanımlama testleri flow sitometrik yöntemle yapılmıştır. Bu serum örnekleri, hastanemizde nakilden sonra periyodik olarak (1. gün, 1. hafta, 2. hafta, 4. hafta, 12. hafta, 24. hafta ve 52. hafta) toplanmıştır. Bütün örneklere tarama testi yapılırken, sadece PRA pozitif olan serum örneklerine anti-HLA antikor tipini belirlemek amacıyla tanımlama testi uygulanmıştır. Bütün prosedürler üretici firmanın talimatlarına göre yapılmıştır. Diğer parametreler Pearson korelasyon testiyle istatistiksel olarak değerlendirilmiştir. **Bulgular:** Nakilden sonra 52. haftadan sonra hastaların sırasıyla %12,9 ile %6,45'i anti-HLA sınıf I ve II antikorları bakımından pozitif bulunmuştur. Hastalarda 1. gün, 1., 2., ve 4. haftalarda mismatch antijenlere karşı antikor oluşumu gözlenmemiştir. Bir hastada antikorlar 12. haftadan itibaren oluşmuştur. Yapılan korelasyon analizlerinde hastaların nakil sonrası son kreatinin değerleriyle donör yaşları ve GFR değerleriyle hasta yaşları arasında istatistiksel olarak anlamlı sonuçlar elde edilmiştir (sırasıyla $p<0.001$, $p<0.01$). Nakil sonrasında de novo antikor üreten hastaların kreatinin değerleriyle antikor üretimi arasında ilişki bulunmamıştır ($p>0.05$). **Sonuç:** Nakil öncesinde kan transfüzyonu olan bir hastada 12. haftada anti-HLA antikoru oluştuğu görülmüştür. 5 hastada 24. haftadan sonra antikor oluşmuştur. Dolayısıyla nakil sonrası 12. haftadan itibaren rutinde yapılacak anti-HLA antikoru tarama testleri tedavi protokolü için önemli olabilir.

Anahtar Kelimeler: Böbrek nakli, doku uyumu, HLA

Abstract

Object: The purpose of the study was to evaluate the immunological response to graft and its association with related factors in the sera samples collected periodically from patients transplanted from deceased or alive donors in Izmir Tepecik Education and Research Hospital. **Material and Method:** Anti-HLA antibody screening and identification tests of 31 patient sera samples were tested by flow cytometric method. The sera samples were collected periodically (1st day, 1st week, 2nd week, 4th week, 12th week, 24th week and 52nd week) after transplantations in our hospital. After the screening

of all samples, anti-HLA antibodies of PRA positive samples were identified. All of procedures were performed according to the manufacturer's instructions. The other parameters were statistically evaluated by Pearson correlation test. **Results:** Of the patients, 12.9% and 6.45% were only class I and only class II positive in the post-transplant 52nd week, respectively. The patients were not positive for class I and II antibodies at the same time. It was observed that the patients did not produce donor specific antibodies on the 1st day, 1st, 2nd, and 4th week after transplantation. The antibodies were produced after the 12th week in a patient. There were statistically significant correlations between last creatinine levels-donor ages and GFR values-patient ages ($p<0.001$, $p<0.01$, respectively), whereas there was no significant association between antibody production and creatinine levels. **Conclusion:** It was observed that anti-HLA antibodies were produced after 12th week in a patient with pre-transplant blood transfusion. The antibodies were produced in 24th week in five patients. Therefore, anti-HLA antibody screening tests to be performed on the 12th week after transplantation may be important for the treatment protocol.

Keywords: Kidney transplantation, histocompatibility, HLA

Introduction

The significance of anti-human leukocyte (anti-HLA) antibodies in renal transplantation has been known for more than four decades. These antibodies may be produced due to pregnancy, blood transfusions, and previous transplantations. However, recent studies have revealed that donor specific anti-HLA antibodies do not always prevent organ transplantation, and therefore, it is very important to understand the subtypes of anti-HLA antibodies and their activity. Anti-HLA immunoglobulin G (IgG) antibodies produced after transplantation may lead to hyperacute, acute, and chronic rejections via a number of mechanisms including complement cascade activation and immune cell migration to allograft (1,2). Humoral immune response is occurred in a pathway in which a number of antibodies including anti-HLA antibodies mediate. It has been known that HLA-specific alloantibodies produced after kidney transplantation lead to allograft dysfunction and failure (3). Evaluation of kidney transplants is very important to prevent graft failures.

Recently, solid phase based techniques such as flow cytometer and Luminex technologies have been used in order to identify the antibodies before and after transplantation (4,5). Bead based techniques are revealed as more sensitive and specific tests. Pre- and post-transplantation PRA screening and identification tests can be performed by using flow cytometric methods (6).

In this study, it was aimed to evaluate the periodically collected sera of the kidney patients who were transplanted from alive or deceased donor in Izmir Tepecik Education and Research Hospital in order to determine de novo produced anti-HLA antibodies and to assess mismatch HLA antigenicity inducing anti-HLA antibody production in early stage of post-transplantation. In addition, we looked for the factors that could be affected by post-transplant antibody production.

Material and Methods

In this study, patients with negative pre-transplant PRA and donor specific antibody (DSA) who were kidney transplanted and whose sera samples were collected regularly were included. The patients who did not give blood samples on the periodical times regularly were excluded. The patients were followed up to 12 months after kidney transplantation from alive or deceased donor in 2012-2014. After transplantation, sera of these patients were collected periodically (1st day, 1st week, 2nd week, 4th week, 12th week, 24th week and 52nd week). All of the samples were collected in our hospital. The samples were screened for PRA by flow cytometric method. Positive sera were also tested by flow cytometric PRA identification test in order to determine the specifity of anti-HLA antibodies.

FlowPRA Screening Kit (OneLambda, Hannover, Germany) and FlowPRA Class I and II Identification Kits (OneLambda, Hannover, Germany) were used for flow cytometric PRA screening and identification methods, respectively (3,16). Protocols were performed according to manufacturer's instructions. The screening and identification methods were performed similarly. Firstly, the sera samples, including negative and positive control sera, were incubated with screening/identification beads for 30 minutes at room temperature. Subsequently, two washing steps were performed using 1 ml 1X Wash Buffer. After washing step, the bead/serum mixture were incubated with secondary antibody (anti-human IgG-FITC, 100X), whi-

ch was present in the commercial kit, for 30 minutes in the dark at room temperature. After two washing steps, 300 µl wash buffer was added into the tubes. All of the tests were analyzed by FacsCalibur Flow Cytometer (BD Biosciences, CA,US) instrument.

Calculation of Glomerular Filtration Rate
Glomerular Filtration Rates (GFRs) of the patients were calculated according to aMDRD (abbreviated Modification of Diet in Renal Disease) with 4 variables [aMDRD = 175 x (serum creatinine)-1,154 x age-0,203 x (0,742, if female)] (7).

Statistical analysis
All of the statistical analyses were performed by using Statistical Package for Social Sciences for Windows Version 21.0 (SPSS 21.0 Inc, Chicago, USA) Software program for Windows 10. Correlations between post-transplant creatinine levels-donor ages, GFR values-patient ages, and post-transplant creatinine level-de novo antibody production were analyzed by Pearson correlation test. p values <0.05 were accepted as significant.

This study was conducted by Ethical Committee of Izmir Katip Celebi University Faculty of Medicine (No. 249, 10.12.2013). Informed consent forms were obtained from patients. This study was performed in accordance with Helsinki Declaration 2008 Principles (http://www.wma.net/en/30publications/10policies/b3/index.html).

Results

In this study, totally 31 patients who were kidney transplanted from alive (54.8%, n:17) or deceased donor (45.2%, n:14) in Izmir Tepecik Education and Research Hospital were included. The mean age of patients and donors were 37.9±14.7 and 45.8±17.3, respectively. Of the patients, 22.6% (n=7) were female and 77.4% (n=24) male. While 51.6% of the patients were preemptive, 45.2% and 3.2% of the patients received hemodialysis and peritoneal dialysis treatments, respectively (Table 1).

Of the patients, 45.2% (n=14) and 54.8% (n=17) were transplanted from deceased and alive donor, respectively (alive donors: 29.4% (n=5) siblings, 23.5% (n=4) mothers, 23.5% (n=4) fathers, 11.8% (n=2) spouses and 11.8% (n=2) other [son and cousin]). Of the patients, 41.9% (n=13) had blood transfusion, 9.7% (n=3) had pregnancy, 3.2% (n=1) had previous transplantation and blood transfusion, and 45.2% (n=14) had no alloimmunizations.

All of the patients were PRA negative before transplantation. Of the sera samples obtained in post-transplant 52nd week, 12.9% (n=4) were only PRA class I positive, 6.4% (n=2) were only class II positive, 87.1% (n=27) were PRA class I negative, and 93.5% (n=29) were PRA class II negative. There was no PRA class I and II positive serum. Of the patients, 80.6% (n=25) were PRA negative. There was no proinflammatory event in which immunosuppression protocol were changed (Table 2).

It was determined that 3 of 4 HLA class I positive patients became positive in 24th week after transplantation, while one patient became positive in 12th week after transplantation (Figure 1). One of HLA class II positive patients became positive in 24th week after transplantation (Figure 2). Thus, the antibodies were mostly produced 24 weeks after transplantation. However, the results could not be evaluated statistically because patient number was lower than five. Graft rejection episode was not observed in our patients.

The highest HLA compatibility was haploidentical (1A1B1DR) in transplantations from alive donors (41.8%; n=7), while 1B1DR, 1B2DR, and 1A1B1DR compatibilities were the most frequently observed compatibilities in transplantations from deceased donors (28.6%; n=4). There was a significant correlation between the last post-transplant creatinine levels of patients and donor ages (r= .644, p<0.001).

The patients were divided into 4 groups according to their ages: 0-18 ages (n=3), 19-40 ages (n=19), 41-60 ages (n=7), and 61-80 ages (n=2). The highest GFR was observed in 0-18 ages, while the lowest GFR was observed in 41-60 ages. In this study, estimated GFR of patients were approximately 62.4±27.5 (ml/min/1.73m2), and mean creatinine levels were 1.4±0.5 (mg/dL). There was a statistically significant association between GFR and patient ages (r= -.584, p<0.01). Creatinine levels were also analyzed in six patients with de novo antibodies. The relation was not significant (r=.145, p>0.05) and this may be due to small number of patients. There was no significant association between PRA results and creatinine levels of the patients (p>0.05).

Table 1 Demographic and clinic characteristics of patients and donors.

Patient ID	Birth of patient	Blood type of patient	Gender	Dialysis type	Alloimmunization	Donor	Birth of donor	Blood type of donor
TH-1	2007	0 Rh (+)	M	PD	Blood transfusion	**D-1**	2008	0 Rh (+)
TH-2	1976	B Rh (+)	M	Preemptive	NA	**D-2**	1952	B Rh (+)
TH-3	1976	A Rh (+)	M	Preemptive	Blood transfusion	**D-3**	1981	A Rh (-)
TH-4	1992	A Rh (+)	F	Preemptive	Blood Transfusion and previous transplantation	**D-4**	1973	0 Rh (+)
TH-5	1953	A Rh (+)	M	Preemptive	NA	**D-5**	1954	A Rh (+)
TH-6	1955	0 Rh (+)	M	Preemptive	NA	**D-6**	1937	0 Rh (+)
TH-7	1981	0 Rh (+)	M	Preemptive	Blood transfusion	**D-7**	1937	0 Rh (+)
TH-8	1955	AB Rh (-)	M	HD	Blood transfusion	**D-8**	1969	AB Rh(+)
TH-9	1963	B Rh (+)	F	HD	Blood transfusion	**D-9**	1959	B Rh (+)
TH-10	1990	AB Rh (+)	M	Preemptive	Blood transfusion	**D-10**	1963	AB Rh (+)
TH-11	1994	A Rh(+)	M	Preemptive	NA	**D-11**	1967	0 Rh (+)
TH-12	1986	0 Rh (+)	M	Preemptive	NA	**D-12**	1958	0 Rh (+)
TH-13	1985	A Rh(+)	F	Preemptive	NA	**D-13**	1957	0 Rh (+)
TH-14	1977	B Rh (+)	F	Preemptive	Blood transfusion	**D-14**	1985	B Rh (+)
TH-15	1997	A Rh (+)	M	HD	NA	**D-15**	2000	A Rh (-)
TH-16	1969	B Rh (+)	M	HD	NA	**D-16**	1969	B Rh (+)
TH-17	1985	B Rh (+)	M	Preemptive	Blood transfusion	**D-17**	1989	0 Rh (+)
TH-18	1990	A Rh (-)	M	HD	NA	**D-18**	1989	A Rh (+)
TH-19	1979	AB Rh(+)	M	Preemptive	Blood transfusion	**D-19**	1966	AB Rh(+)
TH-20	1952	0 Rh (+)	F	HD	Pregnancy and curettage	**D-20**	1950	0 Rh (+)
TH-21	1981	A Rh(+)	M	HD	Blood transfusion	**D-21**	1978	0 Rh (+)
TH-22	1974	AB Rh(-)	M	Preemptive	Blood transfusion	**D-22**	1983	A Rh (+)
TH-23	1977	B Rh (+)	F	HD	Pregnancy	**D-23**	1972	0 Rh (+)
TH-24	1989	0 Rh (+)	M	Preemptive	NA	**D-24**	1992	0 Rh (+)
TH-25	1994	A Rh (+)	M	HD	Blood transfusion	**D-25**	1965	A Rh(+)
TH-26	1959	0 Rh (+)	F	Preemptive	Pregnancy	**D-26**	1984	0 Rh (+)
TH-27	2000	0 Rh (+)	M	HD	Blood transfusion	**D-27**	1972	0 Rh (+)
TH-28	1987	A Rh (+)	M	HD	NA	**D-28**	1964	A Rh (+)
TH-29	1988	A Rh (+)	M	HD	NA	**D-29**	1955	A Rh (+)
TH-30	1961	A Rh (+)	M	HD	NA	**D-30**	1943	A Rh (+)
TH-31	1978	0 Rh (+)	M	Preemptive	NA	**D-31**	1973	0 Rh (+)

TH: A code for patient, M: Male, F: female, PD: Peritoneal dialysis,
HD: Hemodialysis, NA: No Alloimmunization

Table 2 PRA test results, specifity of anti-HLA antibodies, and clinical features of patients after transplantation.

Patient ID	FC-PRA Screening[a] CI	CII	FC-PRA Identification[a] CI	CII	Immunosuppresive therapy	The last creatinine (mg/dl)	HLA type of patient	HLA type of donor	HLA mismatches	Donor type
TH-1	NEG	NEG	NEG	NEG	Mycophenolate mofetil Prednisolone Tacrolimus	0.7	A*02, A*03, B*35, B*35, DRB1*01, DRB1*11	A*02, A*23, B*35, B*50, DRB1*03, DRB1*11	1A 1B 1DR	deceased
TH-2	NEG	NEG	NEG	NEG	Mycophenolate mofetil Prednisolone Tacrolimus	1.5	A*23, A*31, B*38, B*49, DRB1*01, DRB1*04	A*31, A*32, B*38, B*55, DRB1*04, DRB1*14	1A 1B 1DR	father
TH-3	NEG	NEG	NEG	NEG	Mycophenolate mofetil Prednisolone Cyclosporine A	1.2	A*11, A*24, B*35, B*38, DRB1*04, DRB1*14	A*02, A*02, B*15, B*35, DRB1*04, DRB1*14	2A 1B	deceased
TH-4	POS	NEG	%6 A23	NEG	Mycophenolate natrium Prednisolone Tacrolimus	0.8	A*11, A*68, B*08, B*51, DRB1*03, DRB1*13	A*32, A*68, B*08, B*35, DRB1*03, DRB1*15	1A 1B 1DR	mother
TH-5	NEG	NEG	NEG	NEG	Mycophenolate mofetil Prednisolone Tacrolimus	1.4	A*03, A*11, B*35, B*51, DRB1*01, DRB1*07	A*24, A*26, B*18, B*18, DRB1*04, DRB1*14	2A 2B 2DR	spouse
TH-6	NEG	NEG	NEG	NEG	Mycophenolate mofetil Prednisolone Tacrolimus	3.3	A*02, A*26, B*39, B*52, DRB1*15, DRB1*16	A*24, A*30, B*27, B*39, DRB1*15, DRB1*16	2A 1B	deceased
TH-7	NEG	NEG	NEG	NEG	Mycophenolate mofetil Prednisolone Tacrolimus	2.1	A*02, A*31, B*44, B*60, DRB1*15, DRB1*15	A*24, A*30, B*27, B*39, DRB1*15, DRB1*16	2A 2B 1DR	deceased
TH-8	NEG	NEG	NEG	NEG	Mycophenolate mofetil Prednisolone Tacrolimus	1.2	A*01, A*23, B*07, B*35, DRB1*11, DRB1*15	A*02, A*02, B*35, B*35, DRB1*11, DRB1*11	2A 1B 1DR	deceased
TH-9	POS	NEG	%6 B46, B62	NEG	Mycophenolate mofetil Prednisolone Sirolimus	1.5	A*24, A*31, B*35, B*38, DRB1*11	A*03, A*24, B*18, B*35, DRB1*11, DRB1*11	1A 1B 1DR	deceased
TH-10	NEG	NEG	NEG	NEG	Mycophenolate natrium Everolimus Prednisolone	1.9	A2, A24, B51, DRB1*11, DRB1*14	A2, A26, B35,B51, DR11, DR4	1A 1B 1DR	deceased
TH-11	NEG	NEG	NEG	NEG	Mycophenolate natrium Prednisolone Tacrolimus	1.7	A*03, A*24, B*07, B*35, DRB1*11, DRB1*15	A*01, A*03, B*07, B*08, DRB1*03, DRB1*15	1A 1B 1DR	mother
TH-12	NEG	NEG	NEG	NEG	Mycophenolate mofetil Prednisolone Sirolimus	1.6	A*01, A*03, B*35, B*40, DRB1*04, DRB1*11	A*03, A*26, B*35, B*38, DRB1*04, DRB1*15	1A 1B 1DR	mother
TH-13	NEG	NEG	NEG	NEG	Mycophenolate natrium Prednisolone Tacrolimus	1.1	A*03, A*68, B*08, B*44, DRB1*03, DRB1*04	A*03, A*26, B*08, B*08, DRB1*03, DRB1*03	1A 1B 1DR	father
TH-14	NEG	NEG	NEG	NEG	Mycophenolate mofetil Prednisolone Cyclosporine A	0.9	A*23, A*29, B*44, B*52, DRB1*11, DRB1*15	A*24, A*24, B*18, B*35, DRB1*11, DRB1*15	2A 2B	deceased
TH-15	NEG	NEG	NEG	NEG	Mycophenolate mofetil Prednisolone Tacrolimus	1	A1, A3, B35, DRB1*04, DRB1*04	A*03, A*26, B*35, B*50, DRB1*04, DRB1*15	1A 1B 1DR	deceased
TH-16	POS	NEG	%6 B8	NEG	Mycophenolate mofetil Prednisolone Tacrolimus	1.3	A11, A28, B35, DRB1*01, DRB1*14	A*23, A*24, B*40, B*49, DRB1*11, DRB1*14	2A 2B 1DR	deceased
TH-17	NEG	NEG	NEG	NEG	Mycophenolate natrium Prednisolone Tacrolimus	1.4	A*02, A*33, B*44, B*04, DRB1*13, DRB1*16	A*26, A*31, B*07, B*27, DRB1*01, DRB1*15	2A 2B 2DR	sibling
TH-18	NEG	NEG	NEG	NEG	Mycophenolate mofetil Prednisolone Tacrolimus	1.1	A*02, A*24, B*41, B*51, DRB1*03, DRB1*08	A*29, A*30, B*07, B*51, DRB1*08, DRB1*13	2A 1B 1DR	deceased
TH-19	NEG	NEG	NEG	NEG	Mycophenolate natrium Prednisolone Tacrolimus	2.1	A*11, A*11, B*51, B*55, DRB1*11, DRB1*13	A*02, A*30, B*18, B*51, DRB1*11, DRB1*13	2A 1B	deceased
TH-20	NEG	NEG	NEG	NEG	Mycophenolate mofetil Prednisolone Tacrolimus	1.2	A*02, A*24, B*07, B*35, DRB1*11, DRB1*15	A*02, A*24, B*07, B*35, DRB1*11, DRB1*15	No Mismatch	sibling
TH-21	NEG	POS	NEG	%27 DQ2	Mycophenolate natrium Prednisolone Tacrolimus	1.2	A*29, A*68, B*35, B*42, DRB1*03, DRB1*14	A*03, A*03, B*07, B*35, DRB1*01, DRB1*15	2A 1B 2DR	sibling
TH-22	NEG	NEG	NEG	NEG	Mycophenolate mofetil Prednisolone Tacrolimus	1.2	A*24, A*29, B*13, B*35, DRB1*04, DRB1*07	A*24, A*29, B*13, B*51, DRB1*04, DRB1*07	1B	cousin
TH-23	NEG	NEG	NEG	NEG	Mycophenolate mofetil Prednisolone Tacrolimus	0.9	A*02, A*26, B*38, B*52, DRB1*14, DRB1*15	A*33, A*66, B*14, B*52, DRB1*01, DRB1*15	2A 1B 1DR	spouse
TH-24	NEG	NEG	NEG	NEG	Mycophenolate natrium Everolimus Prednisolone	1.1	A*30, A*68, B*13, B*51, DRB1*07, DRB1*13	A*30, A*68, B*13, B*51, DRB1*07, DRB1*13, DQB1*02, DQB1*06	No Mismatch	sibling
TH-25	POS	NEG	%12 A23, A24	NEG	Mycophenolate natrium Prednisolone Cyclosporine A	1	A*03, A*26, B*08, B*38, DRB1*03, DRB1*07	A*01, A*03, B*08, B*37, DRB1*03, DRB1*15	1A 1B 1DR	father
TH-26	NEG	NEG	NEG	NEG	Mycophenolate mofetil Prednisolone Cyclosporine A	1	A*24, A*32, B*18, B*52, DRB1*03, DRB1*15	A*03, A*24, B*47, B*52, DRB1*07, DRB1*15	1A 1B 1DR	son
TH-27	NEG	NEG	NEG	NEG	Mycophenolate natrium Prednisolone Tacrolimus	1.6	A*02, A*24, B*35, B*41, DRB1*14, DRB1*07	A*02, A*24, B*07, B*35, DRB1*14, DRB1*15	1B 1DR	mother

Patient ID	FC-PRA Screening[a] CI	CII	FC-PRA Identification[a] CI	CII	Immunosuppresive therapy	The last creatinine (mg/dl)	HLA type of patient	HLA type of donor	HLA mismatches	Donor type
TH-28	NEG	NEG	NEG	NEG	Mycophenolate natrium Prednisolone Tacrolimus	1.8	A*01, A*23, B*49, B*58, DRB1*03, DRB1*11	A*24, A*24, B*07, B*49, DRB1*03, DRB1*11	2A 1B	deceased
TH-29	NEG	NEG	NEG	NEG	Mycophenolate mofetil Prednisolone Tacrolimus	1.2	A*02, A*03, B*35, B*50, DRB1*15, DRB1*07	A*02, A*03, B*35, B*51, DRB1*09, DRB1*15	1B 1DR	father
TH-30	NEG	NEG	NEG	NEG	Mycophenolate mofetil Prednisolone Tacrolimus	1.9	A11, A30, B35, B53, DRB1*07, DRB1*07	A*01, A*31, B*35, B*51, DRB1*07, DRB1*16	1B 1DR	deceased
TH-31	NEG	POS	NEG	%27 DQ2	Mycophenolate mofetil Prednisolone Tacrolimus	1.8	A*01, A*24, B*08, B*08, DRB1*03, DRB1*03	A*03, A*32, B*35, B*51, DRB1*01, DRB1*04	2A 2B 2DR	sibling

TH: A code for patient, CI: Class I, CII: Class II, POS: Positive, NEG: Negative;
[a]The results were obtained in 52nd week after transplantation

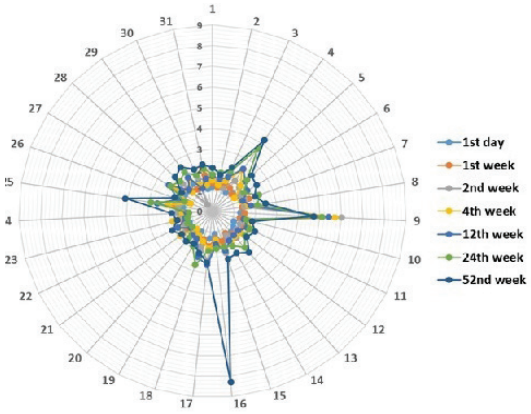

Figure 1. Comparison of the duration in which HLA class I positive sera samples became positive after transplantation. 1-31: patients, lines: sample median/ negative median ratios found by flow cytometry.

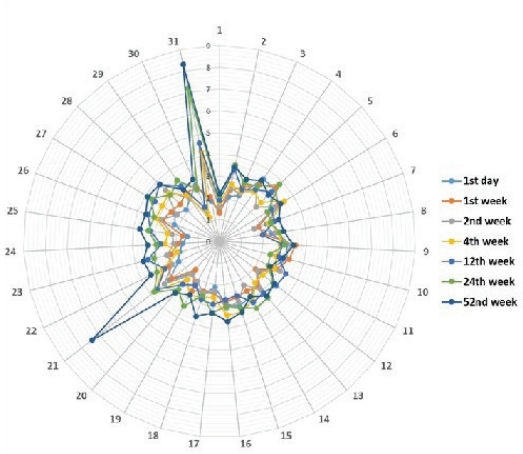

Figure 2. Comparison of the duration in which HLA class II positive sera samples became positive after transplantation. 1-31: patients, lines: sample median/ negative median ratios found by flow cytometry.

Discussion

In this study, the production, the duration of production, and the antigenicity of de novo anti-HLA antibodies were investigated. In addition, the factors that could be affected by post-transplant antibody production were evaluated.

In a review, the alloimmunizations were hierarchized as previous transplants, blood transfusion, and pregnancy, respectively (8). The authors reported that the most effective alloimmunization was previous transplants respectively followed by blood transfusion and pregnancy, on de novo antibody production. There was no significant association between alloimmunizations type and de novo antibodies in our study. In a recent study, it was observed that patients with anti-HLA antibodies had significantly higher creatinine levels (9). In our study, there was no significant relation between post-transplant creatinine levels and de novo antibody production. The difference may be due to the lower number of patients in our study.

In our study, six of 30 patients produced de novo antibody after transplantation. The antibodies were detected mostly in post-transplant 24th week. Only 1 patient developed the antibodies 12 weeks after transplantation. Zhang et al. reported that their patients developed anti-HLA antibodies 12 weeks after transplantation (10). The antibody production du-

rations of our patients were similar to their patients. However, our patient group with de novo antibodies was a very small group for statistical analysis.

It was reported that the effects of de novo antibodies produced after transplantation on graft failure changed due to being DSA or non-DSAs. It was revealed that the patients with DSAs lost the graft in one year (11). In our study, anti-HLA antibodies were non-DSA and the patients had no graft rejection.

It was observed that creatinine levels of patients increased in direct proportion to donor age. In a study, the effect of donor age on graft survival was examined by considering creatinine clearance and glomerular filtration rate (GFR) seven days after transplantation but they could not find significant association (12). Imamovic et al. reported that the association between GFR, age, creatinine, and creatinine clearance was also statistically significant. Mean creatinine levels of patients transplanted from alive and deceased donor were compared (13). Although the results were not significant due to our limited number of patients ($p>0.05$), creatinine levels of patients transplanted from deceased donor were higher than the others. The reason may be long cold ischemia duration. It was revealed that high creatinine levels were related to long ischemia duration (14).

In recent years, DQ mismatches have gained importance due to their relation to graft rejections. Lim et al. evaluated totally 788 patients and he concluded that DQ mismatches were associated with acute rejection (15). In our study, 6.45% (n=2) of the patients produced anti-HLA-DQ antibody and the association between DQ-specific antibodies and GFR, creatinine levels were not significant. This may be due to limited number of patients in our study.

Conclusion

A number of studies examining donor age, patient age, serum creatinine, and GFR levels of patients have been performed. Consistent with these studies, there were significant association between donor age-creatinine levels, mean GFR-patient age in our study. Anti-HLA antibody screening tests to be performed on the 12th week after transplantation may be important for the treatment protocol. However, further investigations including larger number of patients should be performed. A database may be generated by incorporating more patient-donor couples including the other transplantation centers into the study. When we consider that minimum 5 years of graft survival is a success in organ transplantation, follow up after transplantation will contribute to achieving this success.

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