의학석사 학위논문

소아 신장 이식환자에서 엡스타인-바 바이러스 혈증과 이식 후 림프증식성 질환에 관한 연구

Post-Transplant Lymphoproliferative Diseases in Pediatric Kidney Allograft Recipients with Epstein-Barr Virus Viremia

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서울대학교 대학원

의학과 중개의학전공

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Abstract

Post-Transplant Lymphoproliferative Diseases in Pediatric Kidney Allograft Recipients with Epstein-Barr Virus Viremia

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Background: Post-transplant lymphoproliferative disease (PTLD) is one of the major complications of organ transplantation, especially in children with Epstein-Barr virus (EBV) viremia (EV). We performed a retrospective study to evaluate risk factors for PTLD in children with EV.

Methods: Among 199 pediatric kidney transplantation (KT) recipients at our center from January 2001 to October 2015, records of those with EBV viral loads of >1,000 copies/mL and/or PTLD were reviewed.

Results: Diagnosis of PTLD was made in seven patients (PTLD group), and 39 patients had EV only (EV only group). The median time from KT to EV and PTLD diagnosis was 6.7 (range 0.4-47.8) months and 8.2 (range, 2.8-98.9) months, respectively. There were no significant differences between the

groups in terms of gender, age at transplantation, donor type, EBV viral load, or EV-free duration after KT. Higher tacrolimus levels before EV (hazard ratio: 44.5, $P = 0.003$) were independent risk factors for PTLD in multivariate Cox regression analysis. Six patients with a high EBV load (median 171,639 copies/mL) were treated with preemptive rituximab therapy, resulting in transient reduction of EBV load. None of these patients developed PTLD (median follow-up 51.5 months); however, two had neutropenia and two developed infection requiring hospital admission.

Conclusion: In pediatric KT recipients, higher tacrolimus levels were associated with a higher incidence of PTLD. Conversely, those who received preemptive rituximab for EV did not develop PTLD.

Keywords: Post-transplant lymphoproliferative disease, Kidney transplantation, Epstein-Barr virus, Rituximab

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LIST OF ABBREVIATIONS

PTLD, post-transplant lymphoproliferative disease

EBV, Epstein-Barr virus

EV, Epstein-Barr virus viremia

CMV, cytomegalovirus

VCA, viral capside antigen

MMF , mycophenolate

AZA, azathioprine

FSGS, focal segmental glomerulosclerosis

MCDK, multicystic dysplastic kidney.

ESRD, end stage renal disease

CKD, chronic kidney disease

INTRODUCTION

Post-transplant lymphoproliferative disease (PTLD) is one of the major complications of organ transplantation, with an incidence ranging from 1% to 16% depending on the allograft organ.[1] The incidence of PTLD was highest in intestinal and multiple-organ transplants, followed by lung and heart transplants and the lowest in renal and liver transplants. The incidence of liver and kidney rages from 0.2%to 2.5%, which is relatively lower than that in other organ transplant cases.[2,3,4] However, since the most frequently transplanted two organs were kidney (65%) and liver (23%)[5], there are many studies about PTLD after kidney and liver transplantation. Furthermore, incidence rates of PTLD are higher in pediatric kidney transplant recipients than in adult kidney transplant recipients, with ranging from 1.2% to 10%.[7-8] In a large registry study of 23,477 (92% adult, 8% child) kidney transplant recipients, the 25-year cumulative incidence of PTLD was 3.6% for childhood recipients.[9] Childhood recipients had a 6.4-fold increased risk of early onset PTLD compared with adult recipients in another large cohort study.[10] More than 90% of pediatric PTLDs are due to Epstein-Barr virus (EBV)-positive B-cell proliferation. Previously reported risk factors for EBV-associated PTLD include recipient EBV seronegativity, degree of immunosuppression, acute rejection episode, use of OKT3 or tacrolimus, recipient age and race, allograft type, host genetic variations, and especially Epstein-Barr virus viremia (EV). Among these risk factors, the Epstein-Barr Virus (EBV) is the main cause of PTLD, particularly those occurring early after transplantation.[11-16] While adult allograft recipients usually have acquired-immunity to EBV at the time of transplantation,

pediatric allograft recipients often experience primary EBV infection after transplantation. Although EBV-infected transformed B cells are highly immunogenic and rapidly eliminated by EBV-specific T cells in healthy hosts, if immunosuppressed pediatric patients have primary EBV infection, then an inadequate immune response may result in massive infection of B cells. Primary EBV infection increases the chance of developing PTLD 6–76 fold.[17-20]

EBV infection and/or reactivation, which can be detected as increasing copy numbers of EBV DNA in the peripheral blood, usually precede PTLD. Therefore, it is recommended that EBV titer be regularly monitored after solid-organ transplantation in patients at a higher risk for PTLD. The Kidney Disease Improving Global Outcomes clinical practice guideline for the care of kidney transplant recipients recommended the following monitoring regimen for EBV viral load monitoring in EBV-seronegative patients who received an allograft from a seropositive donor: every 1 week in the first 3 months after transplantation, at least monthly for 3–6 months, and then every 3 months for the rest of the first year.[21] Once EBV infection and/or reactivation is noted, transplantation physicians reduce immunosuppression to prevent EBV-associated PTLD. However, EBV infection often persists and progresses despite reduction in immunosuppressive drug regimen, and there is no anti-viral agent with proven efficacy against EBV.[12] In contrast, during stem cell transplantation, pre-emptive treatment with rituximab is often used to eradicate B lymphocytes, the reservoir of EBV, and to prevent PTLD.[22,23] A similar approach has been attempted in solid organ transplantation in patients at high-risk for EBV-associated PTLD.[20]

In this study, the risk factors for PTLD were assessed in pediatric kidney allograft recipients with EV, including the effect of pre-emptive rituximab treatment to prevent PTLD.

PATIENTS AND METHODS

Patients and data collection

We performed a retrospective study that included all patients aged 0–19 years who underwent kidney transplantation in Seoul National University Children's Hospital from January 2001 to October 2015. Patients were enrolled if their EBV load in whole blood was greater than 1,000 copies/mL for 2 consecutive tests. The EBV Q-PCR Alert kit (ELITech Group, Puteaux, France) was used to quantify the amount of Epstein-Barr virus nuclear antigen (EBNA)-1 in whole blood. The diagnosis of PTLD was made histologically after biopsy, and the association with EBV was assessed in tissue specimens by in-situ hybridization of Epstein-Barr virus-encoded RNA (EBER). Patients who received another solid organ transplantation before or after kidney transplantation were excluded. Data were obtained from electronic medical records. Data reviewed included underlying disease, sex, age at transplantation, EBV serologic status of the donor and the recipient at transplantation, donor source, type of induction therapy, maintenance immunosuppressive medication, rejection episodes, and tacrolimus level at EV onset and median levels before and after onset of EV. The median levels of tacrolimus before the onset of EV were calculated using all values measured from transplantation to the appearance of EV. EBV serostatus at the time of transplant was determined by viral capsid antigen (VCA)-IgM, VCA-IgG, and EBNA. Cytomegalovirus (CMV) viremia was defined as positive antigenemia or detection of CMV DNA determined by whole-blood PCR.

Immunosuppression

In our center, induction immunosuppression therapy for kidney transplantation consisted of methylprednisolone with or without basiliximab or antithymocyte globulin. Steroids, tacrolimus, and mycophenolate mofetil were started perioperatively and continued as maintenance therapy. Methylprednisolone was administered as 10 mg/kg intravenous bolus dose at the time of surgery and was tapered gradually to a maintenance dose of prednisolone 0.3 mg/kg by 1 month after transplantation. The target tacrolimus trough level was 8–12 ng/mL for up to 3 months, 6–8 ng/mL between 3 and 6 months, and 4–6 ng/mL thereafter. Tacrolimus levels were monitored weekly for a month after discharge from operation, biweekly for the next 3 months, and monthly thereafter. From 2001 to 2008, basiliximab was used as induction therapy for high-risk patients with deceased-donor kidney transplant or higher number of human leukocyte antigen (HLA) mismatches. After 2008, most patients received basiliximab induction therapy.

EV monitoring

Principally, EBV was monitored every month for the first three months following transplant, then every 3 months for the rest of the first year, and then yearly. For recipients who were positive for EBV VCA IgG, EBV was monitored every three months. When EV was detected, EBV was monitored again in 2 weeks, and if the titer was still high, immunosuppression was reduced, usually by reducing antimetabolites and then tacrolimus according to the judgment of the physicians.

And these patients were closely followed for signs of PTLD such as fever, lymphadenopathy, gastrointestinal pain, etc. they were also monitored for allograft rejection by regular serum creatinine tests every 1~2 weeks.

Pre-emptive rituximab therapy

Some of the patients who exhibited persistent high titers of EV (more than 1 × 104 copies/mL in whole blood for two consecutive weeks) despite immunosuppression reduction were treated with rituximab. These patients had prolonged high viremia over 1 year, a 3-fold or greater increase in EBV titer, or higher risk of malignancy (WT1 mutation). After confirming that the patients did not have active infection or neutropenia, a single dose of rituximab therapy of 375 mg/m2 body surface area was administered.

Statistical analysis

To determine statistical differences between groups, we used the chi-square test or Fischer' s exact test for categorical variables and the t test or Mann-Whitney test for continuous variables. Cox regression analysis was performed to identify risk factors for PTLD following EV. We performed the univariate Cox regression test to identify significant independent variables, and used independent variables with a univariate P value < 0.2 for multivariate Cox regression analysis A P value <0.05 was considered statistically significant. The statistical analysis was performed using IBM SPSS Statistics version 22.0 (IBM cooperation, Armonk, NY).

Ethics statement

The study was approved by the Institutional Review Board (IRB) of our center (IRB No.H-1312-068-541). The informed consent requirement was waived by the board.

RESULTS

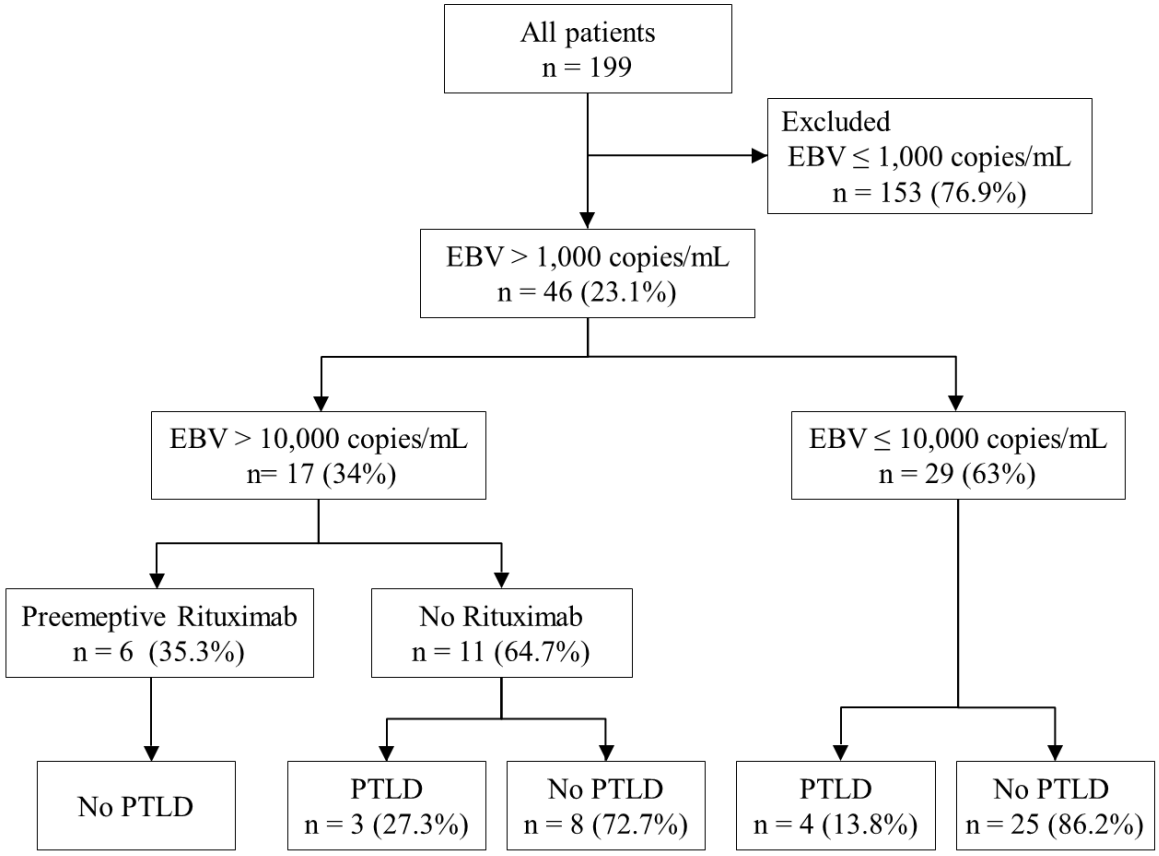
During the study period, 199 children underwent kidney transplantation in our center. Of these, 46 (23.1%) had viremia defined as an EBV load greater than 1,000 copies/mL in whole blood for 2 consecutive tests during a median follow-up period of 5.3 years. Among 17 (34%) patients who had high viremia(EBV > 10,000 copies/mL), only 6 patients were treated with pre-emptive rituximab. 7 patients had a diagnosis of PTLD; 3 patients with EBV > 10,000 copies/mL and 4 patients with EBV ≤ 10,000 copies/mL (Fig. 1). Viremia of all patients (EBV > 1,000 copies/mL) was first detected at a median of 6.7 months (range, 0.4–47.8 months) after kidney transplantation. The diagnosis of PTLD was made in seven patients (PTLD group) at a median of 8.2 months (2.8–98.9 months) after transplantation. The other 39 patients had EV only (EV only group).

Clinical course of PTLD

Patients with PTLD presented with fever, lymph node enlargement, or gastrointestinal symptoms such as abdominal pain, vomiting, and diarrhea (Table 1). Any gastrointestinal symptoms and/or lymph node enlargement raised suspicion for PTLD and prompted the clinician to perform further work-up to rule out PTLD. 3 patients had gastrointestinal organ involvement, including small bowel and intraperitoneal lymph nodes. The majority of patients (n=6) were diagnosed with PTLD in the 1st year after transplantation. Pathologic diagnosis of PTLD revealed one case of early lesion, two cases of polymorphic PTLD, one case of Burkitt

lymphoma, and three cases of diffuse large B-cell lymphoma. There was no extranodal PTLD. Three patients (patients 1, 3, 4) were diagnosed with PTLD within one month after the onset of EBV viremia. The other three patients (patient 2, 5, 6) were diagnosed 4 to 8 months, and patient 7 were diagnosed 51 month after the onset of viremia. Upon diagnosis of PTLD, immunosuppressive medications were reduced, and rituximab and/or chemotherapy were administered as appropriate. All patients achieved complete remission of PTLD after treatments. While one patient lost her allograft kidney due to complications of chemotherapy, six patients retained renal function after follow-up for 2.5-10.5 years.

Fig. 1. Distribution of patients with kidney transplantation by Epstein–Barr virus status and post–transplant lymphoproliferative disease.



EBV= Epstein–Barr virus, PTLD= post–transplant lymphoproliferative disease,

Table 1. Characteristics of patients with post-transplant lymphoproliferative disease

Case No.	Sex/age at time of PTLD (years)	Primary disease	Time from KT to PTLD (months)	Time from Viremia to PTLD (months)	Presentation	Involved site[a]	Histologic diagnosis	EBV serostatus	Treatment	EBV titer (copies/mL)			Outcome
										Peak	Median	PTLD	Graft
1	F/5	FSGS	10	<1	Fever, cervical LNE	Neck mass, jugular LNs, portocaval LN	Diffuse large B-cell lymphoma[b]	D^{UK}/R^{UK}	rituximab, chemotherapy	81,700	13,852	Remission	Function
2	F/7	FSGS	6	3	Vomiting, abdominal pain	Cervical, axillary, inguinal LNs, stomach, small bowel, umbilicus, vagina	Diffuse large B-cell lymphoma[b]	D^{UK}/R^{-}	rituximab , chemotherapy	166,571	587	Remission	Function
3	F/14	FSGS	5	<1	Epigastric pain, Poor oral intake	Stomach, duodenum, mesenteric nodules	Diffuse large B-cell lymphoma[b]	D^{+}/R^{-}	rituximab , chemotherapy	152,987	1,133	Remission	Lost
4	M/18	FSGS	3	<1	Head and neck LNE	Cervical LNs	Polymorphic type[b]	D^{UK}/R^{-}	rituximab	555	555	Remission	Function
5	F/7	Congenital NS	8	7	Tonsillar hypertrophy	Bilateral tonsils, cervical LNs	Early lesion[b]	D^{UK}/R^{-}	rituximab	52,135	6,081	Remission	Function
6	M/4	Bilateral MCDK	10	3	Fever, cervical LNE	Cervical LNs	Polymorphic type[b]	D^{+}/R^{-}	rituximab	134,159	208,947	Remission	Function
7	F/15	Fraiser syndrome[c]	99	52	Diarrhea, abdominal mass with tenderness	Perigastric, mesenteric LNs, stomach, small bowel	Burkitt lymphoma[b]	D^{UK}/R^{-}	rituximab , chemotherapy	315,080	86,642	Remission	Function

PTLD = post-transplant lymphoproliferative disease, KT = kidney transplantation, EBV = Epstein-Barr virus, F = female, FSGS = focal segmental glomerulosclerosis, LNE = lymph node enlargement, LN = lymph node, D/R = donor/recipient, UK = unknown, M = male, NS = nephrotic syndrome, MCDK = multicystic dysplastic kidney. [a]Lesion detected by imaging study (computed tomography or positron emission tomography). [b]EBV in-situ hybridization positive. [c]Genetic disorder caused by *WT1* mutation.

Risk factors for PTLD

Table 2 shows the comparison of clinical variables between the PTLD and EV only group by univariate analysis. There were no significant differences between the two groups in terms of sex, age at transplantation, donor type, interval between transplantation, and first appearance of EV. Although the peak median EBV titer was higher in the PTLD group (152,987 EBV copies/mL whole blood) than the EV only group (17,305 copies/mL whole blood), there was no statistical significance. There were also no significant differences between groups in terms of median EBV viral load and EV-free duration after kidney transplant. At the time of transplantation, six patients (85.7%) in the PTLD group and 14 patients (35.9%) in the EV only group were seronegative for EBV (P = 0.009). Data of donor EBV status before transplantation were available only in a few cases, with no statistically significant difference observed between the groups.

Tacrolimus levels before EV tended to be higher in the PTLD group (9.5 ng/mL) than in the EV only group (7.7 ng/mL, P = 0.039). Maintenance immunosuppression regimen or history of rejection was not significantly different between the two groups. Six patients were treated with pre-emptive rituximab, none of whom developed PTLD, while the number of rituximab -treated patients was too small to be statistically significant.

The Cox proportional-hazard model was used to identify factors associated with an increased risk of developing PTLD after EV (Table 3). Values of 8.9 ng/mL for tacrolimus level and 35,900 copies/μL for peak EBV titer were determined as

Table 2. Characteristics of patients

	PTLD (n = 7)	EV only (n = 39)	*P* value
Sex, M:F	2:5	18:21	0.446
Age at transplantation, years	6 (3–18)	7 (1–16)	0.811
Donor type			1.000
Deceased	4 (57.1)	21 (53.8)	
Living related	3 (42.9)	18 (46.2)	
EBV recipient serostatus			0.009
Positive	0	23 (59.0)	
Negative	6 (85.7)	14 (35.9)	
Unknown	1 (14.3)	2 (5.1)	
EBV donor serostatus			1.000
Positive	2 (28.6)	10 (25.6)	
Negative	0	3 (7.7)	
Unknown	5 (71.4)	26 (66.7)	
Time to EV, months	5 (1–48)	8 (0–47)	0.946
Peak EBV level, copies/mL	152,987 (555–1,341,159)	17,305 (1,198–1,279,841)	0.278
Median EBV level, copies/mL	6,081 (555–208,947)	4,250 (485–326,880)	0.834
CMV viremia	2 (28.6)	12 (30.8)	1.000
Induction therapy			
Basiliximab	6 (85.7)	22 (56.4)	0.220
Thymoglobulin	0	2 (5)	1.000
Maintenance medication			0.496
Steroid + tacrolimus + MMF	6 (85.7)	36 (92.3)	
Steroid + tacrolimus + AZA	1 (14.3)	1 (2.6)	
Steroid + tacrolimus	0	2 (5.1)	
Immunosuppressant after EV			0.423
Monotherapy	2 (28.6)	5 (12.8)	
Double immunotherapy	3 (42.9)	26 (66.7)	
Triple immunotherapy	2 (28.6)	8 (20.5)	
Tacrolimus level, ng/mL			
Pre EV diagnosis	9.5 (6.2–10.3)	7.7 (5.4–12.7)	0.039
At EV diagnosis	6.5 (2.5–9.6)	5.9 (2.2–14.3)	0.549
Post EV diagnosis	4.1 (0–5.8)	4.5 (2.4–7.2)	0.278
Rejection history	5 (71.4)	15 (38.5)	0.213
Preemptive rituximab	0	6 (15.4)	0.266

Values are expressed as numbers (%) and median (range).

PTLD = post-transplant lymphoproliferative disease, EV = Epstein-Barr virus viremia, EBV = Epstein-Barr virus, CMV = cytomegalovirus, MMF = mycophenolate, AZA = azathioprine..

cutoff values based on the receiver operating characteristic curve analysis. The areas under curve of tacrolimus and peak EBV titer were 0.745 (95% CI 0.522–0.969, sensitivity 85.7%, and specificity 79.5%) and 0.634 (95% CI 0.399–0.869, sensitivity 85.7%, and specificity 59.0%), respectively. A higher tacrolimus level (hazard ratio [HR] 13.7; 95% confidence interval [CI]: 1.6–117.9, P = 0.017) was associated with PTLD. Basiliximab induction therapy, higher EBV titer, EBV seronegativity of recipients, and rejection history were not significant in multivariate Cox regression analysis.

Table 3. Risk factors for post-transplant lymphoproliferative disease in patients with Epstein-Barr virus viremia

	Univariate			Multivariate[a]		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Tacrolimus level ≥8.9 ng/mL before EV	16.783	2.013–139.950	0.009	13.737	1.601–117.892	0.017
Basiliximab induction therapy	4.480	0.537–37.374	0.166	–	–	0.438
Peak EBV titer ≥35,900 copies/μL	7.304	0.877–60.832	0.066	–	–	0.149
Recipient EBV serostatus negative	87.887	0.112–69135.034	0.188	–	–	0.054
Rejection history	3.651	0.707–18.852	0.122	–	–	0.285
Age <5 years	reference					
6 to <12 years	0.580	0.097–3.471	0.550	–	–	–
≥12 years	1.518	0.213–10.788	0.677	–	–	–

HR = hazard ratio, CI = confidence interval, EV = Epstein-Barr virus viremia, EBV = Epstein-Barr virus.

[a]Factors with a value of $P < 0.2$ in the univariate Cox regression analysis were included in the multivariate analysis.

Efficacy and safety of pre-emptive rituximab treatment in pediatric kidney transplant

Six patients in the EV only group (male: female, 2:4) who had a high EBV load received preemptive rituximab therapy. Their median age at transplant was 4 years (1–14 years), and EBV infection was first detected at 4.3 months (3.9–9.9 months) after kidney transplantation. Administration of rituximab was carried out at a median of 29.2 months (5.1–69.6 months) after transplantation. These six patients did not exhibit any symptoms such as fever, lymph node enlargement, or gastrointestinal problems; and no imaging studies were performed. Two patients were EBV seropositive and four patients were EBV seronegative (Table 4). The two EBV-seropositive patients were at low risk for the development of PTLD. However, rituximab was administered to these patients based on the clinician's decision; one patient had a mutation of WT1, and was therefore prone to tumor development, while the other had persistently high EBV load for 46 months despite the reduction of immunosuppression. Median EBV viral loads at the time of rituximab treatment were 171,639 copies/mL (6,181–783,504 copies/mL). After a single dose of rituximab therapy, a concordant decrease in EBV load and B lymphocytes was observed (Figure 2). In five patients, EV disappeared within months; the other patient showed reduction of EBV titer but persistence of EV despite rituximab treatment. Unfortunately, the patient lost the allograft due to rejection and concomitant infection within 8 months after rituximab therapy, and EBV titer was not monitored after this adverse event. In the remaining five patients, EBV load rebounded along with recovery of B cells in a median 8 months. However,

none of these five patients developed PTLD over a median follow-up of 51.5 months.

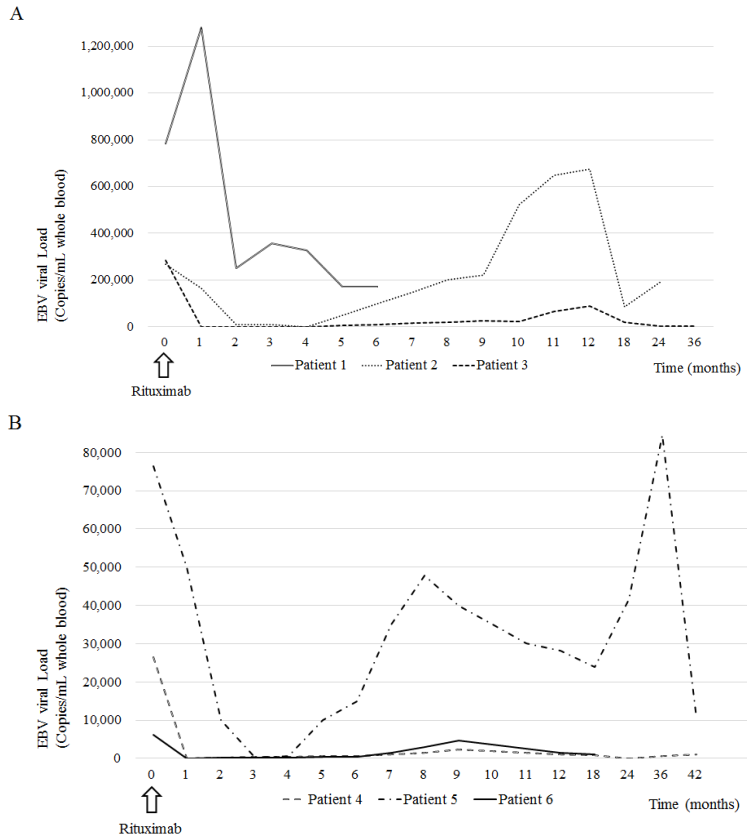
Regarding the safety of rituximab therapy, only one patient complained of chest discomfort during rituximab infusion. Two patients experienced neutropenia at 4 months and 1 month after rituximab treatment (Fig. 3). Two patients were admitted for viral or bacterial infections at 1 month and 2 months after rituximab treatment. In comparison, of 8 patients with high EV (EBV > 10,000 copies/mL) but without rituximab treatment, three patients experienced 6 infection episodes and one patient developed neutropenia during 12 months after the occurrence of high EV. Infectious complications included Citrobacter freundii, CMV, influenza A, adenovirus and Pneumocystis jirovecii infections. There were no significant differences in the infectious complications and neutropenia between the rituximab group and the non- rituximab group in patients with high EV.

Table 4. Description of six patients with preemptive rituximab therapy

Patient	Age at KT, yr	Sex	Underlying disease	EBV Sero-status	Duration of EV before rituximab (months)	Median EBV before rituximab (copies/mL)	EBV at rituximab (copies/mL)	IS at rituximab	EBV at last F/U (copies/mL)	Rejection	Out-come
1	14	F	Lupus Nephritis	D^{UK}/R^{-}	3.3	335,728	783,504	Tacrolimus steroid	172,839	Acute rejection	ESRD
2	1	F	Denys-Drash syndrome[a]	D^{+}/R^{-}	1.2	266,825	266,825	Tacrolimus	192,321	No	Normal
3	10	M	Frasier syndrome[a]	D^{+}/R^{+}	18.3	20,296	283,074	Tacrolimus sirolimus	2,050	No	Normal
4	2	F	Nephronophthisis	D^{UK}/R^{+}	46.1	19,738	26,464	Tacrolimus sirolimus	1,122	No	CKD stage 2
5	2	F	Nephronophthisis	D^{+}/R^{-}	26.0	195,724	76,453	Tacrolimus sirolimus	10,411	No	Normal
6	6	M	Acute tubular necrosis	D^{UK}/R^{-}	63.6	23,813	6,181	Tacrolimus ,sirolimus	981	No	Normal

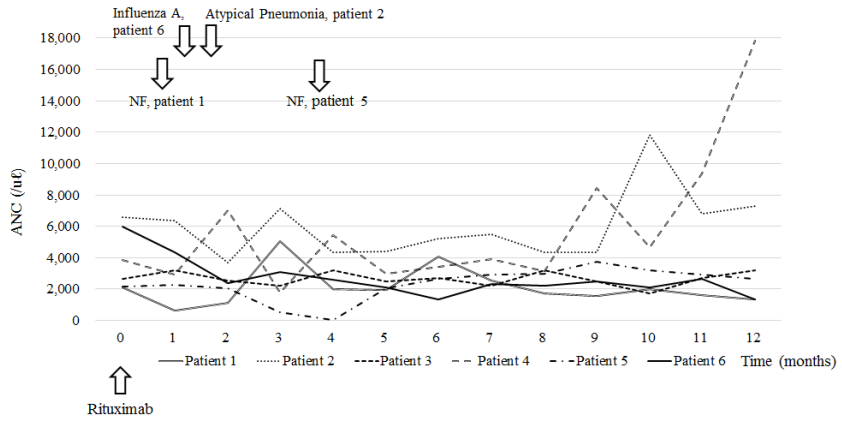
KT = kidney transplantation, EBV = Epstein-Barr virus, EV = Epstein-Barr virus viremia, IS = immunosuppressant, F/U = follow up, F = female, D/R = donor/recipient, UK = unknown, ESRD = end stage renal disease, CKD = chronic kidney disease, M = male, [a]Genetic disorder caused by *WT1* mutation.

Fig. 2. Epstein–Barr viral load after rituximab therapy. (A) Epstein–Barr viral loads in Patients 1,2, and 3. (B) Epstein–Barr viral loads in Patients 4, 5, and 6.



EBV = Epstein–Barr virus

Fig. 3. Late adverse events after rituximab treatment.



ANC = absolute neutrophil count, NF = neutropenic fever.

DISCUSSION

During the last 15 years, among the almost 200 pediatric kidney transplantation recipients at our center, seven developed EBV-associated PTLD (3.5%). In our study, the risk factor for PTLD in pediatric kidney transplant recipients with EV was a high tacrolimus level before EV. Twenty (43.4%) out of 46 recipients who had viremia were EBV seronegative, which is similar to the rates reported in previous studies in North America and Europe (19-57%).[25-27] Pre-transplant recipient EBV seronegativity is a well-known risk factor for PTLD. In adult transplant studies, the rate of developing PTLD is 5-12 times higher in EBV-seronegative patients than in EBV-seropositive patients.[2,3,28] McDonald et al.[8] reported that EBV-seronegative pediatric subjects have a 4.7-fold higher relative hazard ratio than EBV-positive subjects. In our study, recipient EBV seronegativity did not increase the risk of PTLD in multivariate Cox regression analysis. However, EBV-negative recipient serology was a tendency to significance (p=0.053) and could be risk factor if the number of subjects increased. While transplant from an EBV-seropositive donor to a seronegative recipient has been associated with the development of PTLD,[29,30] there was no statistically significant difference in EBV serostatus (donor/recipient) in the present study. This finding was attributed mainly to the fact that there were no EBV seropositive recipients in PTLD group. Although EBV serology data were available for the majority of recipients, EBV donor data were only available for 32% of all cases. Information for EBV serostatus in deceased donor and living donor were 14.3%(3/21) and 48% (12/25), respectively. Recognition of pre-transplant EBV

serostatus helps management the high risk group for close monitoring by EBV viral load and preemptive interventions such as decreased immunosuppression or preemptive rituximab. Therefore, EBV serology tests as part of the donor evaluation should be performed on all donors including deceased donor.

The majority of kidney allograft recipients in this study were given tacrolimus, and mycophenolate was used in more than 80% of patients instead of azathioprine.[31] By the late 2000s, monoclonal interleukin-2 receptor antibodies were used as induction therapy in up to 80% of patients. Basiliximab, a monoclonal antibody which targets activated T lymphocytes, was not related to PTLD risk in our study, as previously reported.[6,32] Several studies have suggested that higher tacrolimus levels are associated with higher risk for PTLD, and others have reported that the net state of immunosuppression, rather than any individual agent, increases the risk for PTLD.[33-35] In our study, all patients received tacrolimus for maintenance immunosuppression, thus allowing us to assess the impact of this medication. We found that a higher pre-EV tacrolimus level in the PTLD group compared with the EV only group was a risk factor for PTLD.

Regular monitoring of EBV viral load and early recognition of recipients at high risk of PTLD have been identified as clinical priorities in recent years.[36] Previous studies have shown that elevated levels of EBV DNA and persistent high EBV loads are risk factors for PTLD,[16,24,37] but no clear cut-off point of EBV viral load for the prediction of PTLD development has been determined. We did not found a significant relationship between EV and PTLD in this study; however, six patients with a high EBV titer were treated with preemptive rituximab and they did not

develop PTLD. Because they were included in analysis, this could have confounded the causality of high EBV titer and PTLD development.

Treatment strategies for organ transplant recipients with EV include reduction of immunosuppression with/without antiviral agents, immunoglobulin, or rituxiamb.[38] These treatments are still undergoing clinical studies. Preemptive administration of rituximab is widely used and has been demonstrated to reduce the incidence of PTLD in stem cell transplant recipients with a high EBV viral load.[22,38,39] Pre-emptive rituximab therapy has been reported in 14.5% of global transplant programs, and in more than 60% of pediatric transplant patients worldwide.[38] However, only one study reported the use of rituximab in five pediatric renal allograft recipients,[24] and there have been no prospective studies on the efficacy of pre-emptive rituximab therapy in solid organ transplantation. Rituximab is a murine/human chimeric monoclonal anti-CD20 antibody and is usually associated with the rapid depletion of circulating B cells, including those infected with EBV. Although rituximab was effective for reducing EBV viral load in our patients, this reduction was not permanent, in line with previously reports.[24] In addition, significant adverse effects, such as infection and neutropenia, accompanied rituximab administration. In patients with stem cell transplant (SCT), preemptive rituximab has been reported as not being associated with an increase in infectious complications.[22,23] This is important because, while SCT recipients are able to discontinue their immunosuppressive agents within 6–9 months after SCT, recipients of solid organ transplantation have to be on immunosuppressive agents

for as long as their allografts are functioning. Therefore, the long-term use of immunosuppressive agents may explain why infection and neutropenia are common clinical findings after rituximab therapy in this patient population.

Although the small sample size of the current study precludes us from drawing any definite conclusions, our observations suggest that preemptive rituximab treatment may effectively reduce high EBV viral load in pediatric recipients of solid organ transplants. Because rituximab therapy eradicates B-lymphocytes including transformed lymphocytes, the risk of PTLD might be reduced at least during the period of B cell depletion. However, one should take into account that this treatment significantly increases the risk of neutropenia and infection. More research into the influence of preemptive rituximab therapy on PTLD development is needed.

The occurrence of PTLD in kidney transplant recipients follows a bimodal distribution, with one peak in the first year and the second in the later post-transplantation period. Early PTLD, occurring within the first year of transplantation, is associated with EBV infection and tends to occur more commonly in children than adults.[16,41,42] In this study, while six patients in the PTLD group were diagnosed with PTLD within the first year of renal transplantation, one patient with *WT1* mutation developed PTLD later than 8.2 years after renal transplantation. We suspect that in this patient, the *WT1* mutation of a tumor suppressor gene might have increased the risk of PTLD, and especially that of late-onset. The occurrence of PTLD in patients with *WT1* mutation has been reported previously, within the first year in two cases and later than the first

year in another.[43-45] The role of WT1 mutation in PTLD is less certain. As the understanding of this risk factor has expanded, the association between *WT1* mutation and development of PTLD requires further investigation in future clinical studies and is beyond the scope of the current study.

There are several limitations of this study. First, this is a retrospective observational study of a single center and the number of the patients observed was therefore small. The limitations of this small sample size might have affected the outcome of multivariate analysis. In addition, data of donor EBV serology were not uniformly available in our study. Data on donor serology status was only available for 33% of participants. Therefore, our study did not show any association between PTLD and EBV-donor/recipient serostatus. Finally, the number of patients who were treated with rituximab was not large enough to draw any definitive conclusions.

In summary, this study demonstrates that a higher tacrolimus level before EBV viremia is correlated with the development of PTLD. Preemptive rituximab appears to be effective for reducing EBV viral load in pediatric kidney transplant recipients. Those who received preemptive rituximab for EBV viremia did not develop PTLD. However, the reduction of EBV viral load was not persistent, and adverse effects of rituximab, namely infection and neutropenia, were clinically significant.

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Disclosure

No potential conflict of interest relevant to this article was reported.

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초 록

배경: 이식 후 림프증식성 질환(Post-transplant lymphoproliferative disease;PTLD)은 엡스타인-바 바이러스 혈증(Epstein-Barr virus viremia; EV)이 있는 소아 장기이식 환자에서 주요한 합병증 중에 하나이다. 엡스타인-바 바이러스 혈증이 있는 환자에서 림프증식성 질환에 대한 위험요인을 평가하기 위해 후향적 연구를 수행하였다.

방법: 2001년 1월부터 2015년 10월까지 199명의 소아신장 이식환자 중, 엡스타인-바 바이러스의 양이 > 1000 copies/mL 및 이식 후 림프증식성 질환을 가진 환자의 기록을 검토하였다.

결과: 7명의 환자가 이식 후 림프증식성 질환을 진단 받았고 (PTLD 군), 39명의 환자는 엡스타인-바 바이러스 혈증만 있었다 (EV군). 신장 이식 시행 받은 날에서 엡스타인-바 바이러스 혈증과 이식 후 림프증식성 질환을 진단까지의 평균 시간은 각각 6.7(범위 0.4–47.8) 개월과 8.2 (범위, 2.8-98.9) 개월이었다. 성별, 이식 연령, 기증자 유형, 엡스타인-바 바이러스러의 정량 또는 신장 이식후 엡스타인-바 바이러스 혈증이 없는 기간에 있어서 그룹간에 유의 한 차이는 없었다. 엡스타인-바 바이러스가 검출되기 전 높은 타크롤리무스의 약물농도는 (위험률: 44.5, P = 0.003)은 다변량 콕스 회귀분석에서 PTLD에 대한 독립적인 위험 인자였다. 높은 엡스타인-바 바이러스 혈증(중앙값, 171,639 copies / mL)를 가진 6 명의 환자는 선제적 리툭시맙 치료를 받았고 엡스타인-바 바이러스 양을 일시적으로 감소시켰다. 이들 환자 중에서 PTLD는 아무도 발생하지 않았다 (추적 관찰기간의 중앙값, 51.5 개월). 그러나 2명은 호중구 감소증이 있었고 2명은 병원 입원이 필요한 감염이 발생했다.

결론: 소아 신장 이식환자에서, 타크롤리무스 농도가 높을수록 이식 후 림프증식성 질환의 발생률이 높았다. 엡스타인 바-바이러스 혈증을 가진 환자에서 선제적 리툭시맙 투여를 받은 경우 이식 후 림프증식성 질환은 발생하지 않았다.

주요어: 이식 후 림프증식성 질환, 신장 이식, 엡스타인-바 바이러스, 리툭시맙

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