

# CALCINEURIN INHIBITOR WITHDRAWAL AND CONVERSION TO MAMMALIAN TARGET OF RAPAMYCIN INHIBITOR (EVEROLIMUS) IN PEDIATRIC KIDNEY TRANSPLANT PATIENTS AT A TERTIARY HOSPITAL IN THAILAND

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**Abstract.** Due to the potential toxicity of calcineurin inhibitors (CNIs), CNI sparing immunosuppressive regimens were developed to reduce CNI exposure and to improve long-term outcomes in pediatric kidney transplant patients. This study retrospectively reviewed the clinical outcomes of pediatric kidney transplant recipients that were switched from standard regimen (a CNI, a mycophenolic acid agent, and corticosteroids) by CNI withdrawal and conversion to everolimus [a mammalian target of rapamycin (mTOR) inhibitor]. Ten transplant recipients (7 males) were included. Mean age at conversion was  $14.0 \pm 2.2$  years; median time to conversion after transplantation was 0.48 years (range: 0.06-10.5); and, mean duration of CNI-free interval was  $2.5 \pm 1.1$  years. The major indication for CNI elimination in recipients with early conversion ( $\leq 6$  months;  $n=5$ ) and late conversion ( $> 6$  months;  $n=5$ ) was early adverse effects related to the CNI and chronic allograft nephropathy, respectively. After becoming CNI-free, most recipients had improved ( $n=5$ ) or stable ( $n=4$ ) kidney graft function. Three recipients had biopsy-proven acute rejection, and CNI therapy was resumed in 2 of those patients. No grafts were lost in this study. CNI withdrawal and conversion to everolimus is an alternative regimen for pediatric kidney transplant recipients, especially in those requiring early conversion due to adverse effects of CNI.

**Keywords:** calcineurin inhibitor toxicity, acute antibody mediated rejection, CNI sparing, CNI elimination failure

## INTRODUCTION

Kidney transplantation (KT) is the most effective renal replacement modality and is the treatment of choice in patients with end-stage kidney disease (Abecassis *et al*, 2008). Short-term graft survival in pediatric kidney transplantation (ped-

KT) has increased due to improvements in care, and advances in immunosuppressive therapy, infection surveillance, and case management (Van Arendonk *et al*, 2014). Long-term graft survival, however, is less certain due to accumulations of immune and non-immune insults to the kidney, including chronic calcineurin inhibitor (CNI) nephrotoxicity (Naesens *et al*, 2009).

The CNI-based regimen (*ie*, tacrolimus or cyclosporine) is a standard immunosuppressant strategy that has decreased the incidence of acute rejection and has improved early outcomes in pedKT. However, CNI therapy is associated with nephrotoxicity, decreased graft function, post-transplant diabetes mellitus

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(PTDM), dyslipidemia, and immunosuppression with malignancies and infections (Holmberg and Jalanko, 2016). A CNI minimization or CNI-free (avoidance or withdrawal) strategy with supplementation or replacement with another immunosuppressant regimen has been widely studied in an effort to improve long-term outcomes (Hocker and Tonshoff, 2011). A mammalian target of rapamycin (mTOR) inhibitor has been used as an alternative CNI-sparing treatment with beneficial effects in pedKT. Everolimus is an mTOR inhibitor that has been widely used for conversion therapy from CNI in adult KT, but very few studies have been conducted regarding its effectiveness in children (Pape and Ahlenstiel, 2014).

The major concern relative to CNI-minimization or CNI-free regimen is the increased incidence of acute rejection. In contrast, the prolonged use of CNI may induce chronic CNI nephrotoxicity. Both graft rejection and nephrotoxicity will lead to worse long-term outcomes. Accordingly, the benefits and risks of prolonged use of CNIs must be carefully considered.

This aim of this study was to evaluate the outcomes of early and late CNI withdrawal and conversion to everolimus in pedKT recipients that were previously treated with CNI plus mycophenolic acid (MPA) and corticosteroids-based regimen.

## MATERIALS AND METHODS

### Study subjects

From May 1996 to April 2015, 39 children aged less than 18 years underwent kidney transplantation at the Department of Pediatrics, Siriraj Hospital – a national tertiary care center located in Bangkok, Thailand. CNI withdrawal and conversion to everolimus in pedKT recipients is a protocol that was started at our center in January 2011. This retrospective chart review followed these transplant recipients through April 2015. All pedKT recipients who were

converted from CNI-based regimen (a CNI plus an MPA and corticosteroids) to an everolimus-based regimen (everolimus plus an MPA and corticosteroids) during the study period were recruited. This study was approved by the Siriraj Institutional Review Board (SIRB), Faculty of Medicine Siriraj Hospital, Mahidol University (COA Si 173/2013).

The initial immunosuppression protocol for pedKT patients at our center was the conventional regimen (Halloran, 2004). Anti-IL-2 antibody, a biologic agent, was prescribed for induction treatment in cases of deceased donor kidney transplant with prolonged cold ischemic time, high human leukocyte antigen (HLA) mismatch, slightly high panel reactive antibody (PRA), or in patients who could afford the medication. The maintenance triple drug regimen included corticosteroids, MPA agent [mycophenolate mofetil (MMF) or enteric-coated mycophenolate sodium (EC-MPS)], and a CNI [cyclosporine (CSA) or tacrolimus (TAC)] (Kasiske *et al*, 2010). Dosages of MPA were prescribed, as follows: MMF 600 mg/m<sup>2</sup>/day or EC-MPS 430 mg/m<sup>2</sup>/day divided into 2 doses given every 12 hours, and then gradually increased, as tolerated, to a maximum of 1,200 mg/m<sup>2</sup>/day of MMF or 860 mg/m<sup>2</sup>/day of EC-MPS. The dose of CSA or TAC was adjusted to maintain the target trough blood level (Weber, 2015). The initial prednisolone dosage was 2 mg/kg/day, which was then gradually reduced to 0.15 mg/kg/day over a 6-month period. Once everolimus was initiated, the dosage was adjusted to achieve trough blood levels of 3-8 ng/ml. To improve adherence, drug administration education was provided to pedKT recipients by a pharmacist.

Clinical parameters, including patient demographic data and baseline transplant data, such as donor type, human leukocyte antigen (HLA) mismatch, panel reactive antibody (PRA) percentage, anti-cytomegalovirus (CMV) antibody status of donor and recipient, and initial immunosuppressive regimen, were recorded. The

following clinical parameters were also reviewed: indications for conversion, graft function before and after conversion, history of biopsy-proven rejection before and after conversion, CNI elimination failure with reinstitution of CNI, and graft loss following CNI withdrawal. Glomerular filtration rate (eGFR) was estimated by Schwartz equation (Schwartz *et al*, 1976; Schwartz *et al*, 2009) or CKD-EPI depending on the creatinine measurement method and age of each patient (Levey *et al*, 2009). Biopsy-proven rejection was defined by Banff classification of kidney allograft pathology (Solez *et al*, 2008; Haas *et al*, 2014). Early conversion and late conversion were defined as CNI withdrawal  $\leq 6$  months and  $>6$  months after kidney transplant, respectively. Potential side effects of everolimus, such as dyslipidemia and proteinuria, were evaluated and recorded.

### Statistical analysis

Demographic and clinical information was presented using descriptive statistics (mean  $\pm$  standard deviation or median and range). GFR was compared between before and after conversion using paired *t*-test. Statistical analyses were performed using PASW Statistics version 18.0 (IBM, Armonk, NY). A *p*-value  $< 0.05$  was considered to be statistically significant.

## RESULTS

Ten of 39 kidney transplant recipients completed CNI withdrawal and conversion to everolimus during January 2011 to December 2014. Mean age at transplantation, conversion, and last follow-up was  $11.5 \pm 2.8$ ,  $14.0 \pm 2.2$ , and  $16.7 \pm 1.8$  years, respectively. Clinical characteristics of each transplant recipient are shown in Table 1. Most recipients (7/10; 70%) were male; 8 patients underwent kidney transplant with a graft from a deceased donor; 8 had HLA mismatch that ranged from 2-3; 9 had PRA less than 5%; 9 had anti-CMV IgG antibody status of donor (D)/recipient (R) as D+/R+; 9 received TAC; and, only 1 patient received biologic agent.

The median duration after transplantation to everolimus conversion was 5.8 months (range: 0.7-126). The 2 reasons for CNI withdrawal in recipients with early conversion (patients 1-5) were: 1) to resolve effects associated to the CNI; and, 2) in response to early evidence of declining renal function. The 2 indications for CNI withdrawal in recipients with late conversion (patients 6-10) were: 1) insults from chronic allograft nephropathy (CAN); and, 2) chronic CNI nephrotoxicity.

The mean CNI-free interval was  $2.5 \pm 1.1$  years. During the CNI-free period, mean eGFR increased from  $46.1 \pm 20.6$  to  $60.6 \pm 18.6$  ml/min/1.73m<sup>2</sup> (*p*=0.064). Most recipients had increased eGFR (*n*=5) or stable eGFR (*n*=4). The early conversion group had improved mean eGFR of approximately 28 ml/min/1.73m<sup>2</sup> after conversion ( $35.9 \pm 17.9$  vs  $63.8 \pm 17.2$  ml/min/1.73m<sup>2</sup>; *p*=0.057); whereas, the mean eGFR in late conversion group was stable ( $56.4 \pm 19.2$  vs  $57.4 \pm 21.4$  ml/min/1.73m<sup>2</sup>; *p*=0.76). Six recipients had hyperlipidemia that was controlled with a lipid lowering agent. No transplant recipients in this study developed de novo proteinuria after everolimus conversion.

Acute antibody-mediated rejection (ABMR) occurred in 3 patients (patients 5, 7, and 8) and was treated with plasmapheresis and intravenous immunoglobulin. Patient 5 received anti IL-2 antibody during KT induction therapy, developed CMV and BK viremia, and then ABMR at 3 months after everolimus conversion. However, his graft function then stabilized. Patient 7 and 8 had impaired graft function coexisting with infectious episodes. Patient 7 had recurrent CMV viremia and patient 8 had recurrent acute diarrhea with acute kidney injury, prior to everolimus conversion. Both patients had acute ABMR in the second year post-conversion and CNI therapy was reinstituted. Patient 8 had pre-existing proteinuria that did not worsen during everolimus administration.

Table 1  
Clinical characteristics of pediatric kidney transplantation recipients with CNI withdrawal and conversion to everolimus.

| No.              | Gender | Donor type | HLA MM | CMV <sup>a</sup> status | Age at KT (yr) | Indication for CNI elimination          | CNI duration post-KT | CNI free duration (yr) | eGFR prior conversion (ml/min/1.73m <sup>2</sup> ) | eGFR as CNI-free at last follow-up (ml/min/1.73m <sup>2</sup> ) | AR after CNI elimination | CNI elimination failure |
|------------------|--------|------------|--------|-------------------------|----------------|-----------------------------------------|----------------------|------------------------|----------------------------------------------------|-----------------------------------------------------------------|--------------------------|-------------------------|
| Early conversion |        |            |        |                         |                |                                         |                      |                        |                                                    |                                                                 |                          |                         |
| 1                | M      | DD         | 3      | D+/R+                   | 10.5           | Hemolytic uremic syndrome               | 23 days              | 4.3                    | 10.66                                              | 66.72                                                           | No                       | No                      |
| 2                | F      | DD         | 2      | D+/R+                   | 10.3           | Post-transplant diabetes mellitus       | 2 months             | 3.1                    | 41.74                                              | 61.78                                                           | No                       | No                      |
| 3                | F      | DD         | 3      | D+/R+                   | 14.0           | DGF and nephrocalcinosis                | 3 months             | 2.7                    | 27.85                                              | 40.81                                                           | No                       | No                      |
| 4                | M      | DD         | 2      | D+/R+                   | 13.9           | AKI with renal artery stenosis of graft | 3 months             | 2.2                    | 40.27                                              | 88.99                                                           | No                       | No                      |
| 5                | M      | DD         | 3      | D+/R+                   | 14.3           | DGF with unfavorable graft function     | 4 months             | 2.7                    | 58.93                                              | 60.57                                                           | Yes                      | No                      |
| Late conversion  |        |            |        |                         |                |                                         |                      |                        |                                                    |                                                                 |                          |                         |
| 6                | F      | DD         | 2      | D+/R+                   | 15.6           | CAN with BK nephropathy                 | 7.5 months           | 3.3                    | 36.44                                              | 36.74                                                           | No                       | No                      |
| 7                | M      | DD         | 4      | D+/R+                   | 11.8           | DGF with unfavorable graft function     | 1 year               | 1.5                    | 41.41                                              | 44.28                                                           | Yes                      | Yes                     |
| 8                | M      | LD         | 3      | D+/R+                   | 9.6            | Recurrent diarrhea with AKI and CAN     | 4.1 years            | 2.1                    | 57.35                                              | 46.8                                                            | Yes                      | Yes                     |
| 9                | M      | LD         | 3      | D+/R+                   | 8.8            | Chronic CNI nephrotoxicity              | 7.3 years            | 2.5                    | 85.08                                              | 87.75                                                           | No                       | No                      |
| 10               | M      | DD         | 4      | D+/R-                   | 6.7            | CAN                                     | 10.5 years           | 0.3                    | 61.48                                              | 71.5                                                            | No                       | No                      |

<sup>a</sup>anti-cytomegalovirus IgG antibody status of donor (D) and recipient (R) with the result of positive (+) or negative (-).

AKI, acute kidney injury; AR, acute rejection; BK, history of BK polyoma virus nephropathy; CAN, chronic allograft nephropathy; CMV, cytomegalovirus; DD, deceased donor ; DGF, delayed graft function; eGFR, estimated glomerular filtration rate; HLA/MM, human leucocyte antigen mismatch; KT, kidney transplant; LD, living donor.

## DISCUSSION

Studies in immunosuppressive strategies for CNI sparing in pedKT are limited, with most having been conducted in small study populations and reporting results that vary by study and medical center. For instance, CNI minimization combined with MMF-based regimen (Benz *et al*, 2006) or mTOR inhibitor-based regimen (Pape *et al*, 2007) was shown to be safe for pedKT recipients relative to improvement in or stabilization of GFR and acceptable acute rejection rate. CNI withdrawal and switch to mTOR inhibitor (sirolimus) plus MMF-based therapy was comparable with CNI minimization plus MMF for improvement in GFR and acute rejection after 12 months (Hocker *et al*, 2006). Dincel *et al* (2013) studied 18 pedKT recipients who had CNI withdrawal and conversion to everolimus. They reported that creatinine clearance increased from  $59.4 \pm 11.4$  to  $70 \pm 12$  ml/min/1.73m<sup>2</sup> after conversion, and 3 cases had biopsy-proven acute rejection.

Early or late CNI withdrawal and conversion to mTOR inhibitor may affect kidney outcome by decreasing CNI toxicity, but may increase episodes of rejection. Budde *et al* (2011) studied the effect of everolimus with EC-MPS regimen in both early and late conversion in adult KT patients. The early conversion study, which was open-label, 12-month, randomized, controlled, multicenter study in CSA conversion to everolimus at 4.5 months post-transplant, showed a significant improvement in GFR at 1, 3, and 5 years in the everolimus group, but the incidence of biopsy-proven acute rejection was higher than control group at the 3-year time point. However, the cumulative incidence of biopsy-proven acute rejection, graft loss, mortality, serious adverse events, and neoplasms were not different (Budde *et al*, 2011; Budde *et al*, 2012; Budde *et al*, 2015a). The study in late conversion that took place several years after KT found that conversion from CNI to everolimus preserved renal function and did not compromise

immunosuppressive efficacy (Budde *et al*, 2015b).

In this study, everolimus with MMF/EC-MPS plus corticosteroid regimen improved GFR in early conversion recipients and stabilized GFR in late conversion patients. There was no de novo proteinuria and no significant progression of pre-existing proteinuria among our pedKT patients. However, biopsy-proven ABMR that resulted in CNI elimination failure was found in two patients with late conversion. Impaired graft function coexisting with multiple episodes of infection may have associated with poor outcomes after everolimus conversion. Nevertheless, most recipients did not receive induction treatment with anti-IL-2 antibody, which may prevent acute rejection episode, but may increase the potential for infection. Although there was no graft loss, death, or serious side effects from everolimus therapy in this study, longer follow-up study is needed to elucidate the effectiveness of the everolimus with MMF/EC-MPS plus corticosteroid regimen.

Limitations of this study include its retrospective design and small sample size. That acknowledged, studies in everolimus with MMF/EC-MPS plus corticosteroid regimen in pedKT are scarce. This study demonstrates that this CNI-sparing regimen is an alternative treatment for pedKT recipients, especially early period in those with side effects of CNI.

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## CONFLICTS OF INTEREST

The authors hereby declare no personal or professional conflicts of interest regarding any aspect of this study.

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