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Does Vitamin D Affect Chronic Renal Allograft Function in Pediatric Transplant Patients?

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Background: Correction of hypovitaminosis D is simple, but it is unclear whether it is associated with an accelerated decline of renal allograft function in pediatric renal transplantation patients. This retrospective single center cohort study aimed at analyzing the effect of vitamin D and covariates on the slope of 1/creatinine after the first year.

Material/Methods: After ethics committee approval, 37 (14 male) pediatric renal transplant recipients on mycophenolate mofetil, who were followed between 2006 and 2014, were included in this study. We analyzed the slope of 1/creatinine, length of follow-up, average vitamin D levels, calcium, phosphate, alkaline phosphatase levels, intact parathyroid hormone (PTH) levels, and therapeutic drug monitoring parameters.

Results: Median slope of 1/creatinine was -2.587×10^{-6} L/ μ mol. We divided the 37 patients into two groups based on slope: 18 patients with a poorer slope and 19 patients with a good slope, with the median slope of 1/creatinine being significantly different between the two groups. Creatinine and cystatin C at one-year post-transplantation did not differ between the two groups. Average vitamin D levels were 71.4 ± 31.01 pmol/L and identical in each group (averages 71.67 and 69.23 pmol/L, respectively). Only the mycophenolic acid coefficient of variation (MPA CV), which may promote formation of donor-specific antibodies, and PTH levels were significantly associated with 1/creatinine slope.

Conclusions: Our data suggest that the impact of mild and moderate decreased levels of vitamin D can have a mild impact on the progression of allograft dysfunction in transplant recipients. However, given the medication burden and adherence challenges in adolescents, correction of mildly decreased vitamin D levels may not be necessary.

MeSH Keywords: Calcifediol • Creatinine • Cystatin C • Glomerular Filtration Rate • Medication Adherence • Parathyroid Hormone

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Background

Ideally, every child with end-stage renal disease should receive a functioning renal transplant. Unfortunately, even a perfectly matched allograft will experience deteriorating graft function over time, and eventually the graft will stop working due to nephron under dosing, interstitial fibrosis, tubular atrophy, and many other factors [1]. In native kidney disease, multiple strategies are used to slow the progression to chronic kidney disease (CKD) such as treatment of metabolic acidosis, renal anemia, renal osteodystrophy, and optimal blood pressure control [2,3]. Although every patient who receives a renal transplant by definition has CKD, the treatment of CKD complications is not implemented with the same rigor for all renal transplant patients [4]. One of the modifiable risk factors is the correction of vitamin D deficiency, which is highly prevalent among pediatric renal transplant recipients [5,6].

Several studies have indicated that there is a relationship between vitamin D and its anti-inflammatory effect, decreased vascular calcification, and the protective effect on the renal tubular epithelium [7]. While there is good evidence to support normalization of 25-hydroxy cholecalciferol levels in patients with CKD in their native kidneys [8], the importance of vitamin D supplementation in pediatric renal transplant recipients has been understudied and the results have been inconsistent. In one prospective cohort study, vitamin D deficiency was associated with a more rapid decline of renal function within the first decade of renal transplantation [9]. Another study suggested that a low vitamin D status at 3-months post-transplantation was associated with lower glomerular filtration rate (GFR) and an accelerated development of tubulointerstitial fibrosis [10]. However, these studies that demonstrated this association did so in patients with particularly severe hypovitaminosis D, with levels as low as less than 12 ng/mL. Most transplantation patients would be treated before their vitamin D approached such a low level. On the other hand, a recent study found no significant effect of vitamin D level and progression of CKD in renal transplantation patients with less severe hypovitaminosis D [11].

Vitamin D could be a potentially cheap and modifiable therapeutic option, if there is a demonstrated benefit. As the topic is not well studied, we conducted a retrospective cohort study to determine the significance of vitamin D, in the context of other parameters, in the prediction of renal allograft function in pediatric patients. We hypothesized that the impact of vitamin D on chronic allograft dysfunction would be less significant compared to patient compliance with immunosuppressive medications, which is a well-known risk factor for poor outcome after pediatric renal transplantation [12]. Patient compliance can be determined using the coefficient of variation (CV) of tacrolimus levels [12,13], and potentially

mycophenolic acid (MPA) CV, which has been proposed as an additional marker of non-adherence [14].

Material and Methods

Patients

A post-hoc analysis of a single-center cohort study that was approved by the Western University Research Ethics Board (HSREB File Number 105148) was conducted retrospectively [14–16]. We analyzed all existing data on 37 pediatric renal transplant recipients who were followed between January 1, 2006, and March 31, 2014. This study cohort included all pediatric patients who had undergone renal transplantation including re-transplants ($n=2$), and who had a functioning graft and were treated with mycophenolate mofetil (MMF). They were consistently followed at the Children's Hospital at the London Health Sciences Centre during the study period. Since non-adherence to immunosuppressive medication was assessed by tacrolimus and MMF pharmacokinetic monitoring (trough levels and CV), two patients who did not receive MMF monitoring were not included in our cohort.

Clinical information

Information, such as gender, age at transplantation, follow-up time after transplantation, daily concomitant medication, and anthropometric data (height, weight, blood pressure) were obtained from the patient's paper and electronic charts. Vitamin D was measured as per protocol every 3 months. We used our electronic health record to obtain the following data: vitamin D (25 hydroxy-cholecalciferol), cystatin C, creatinine, calcium, phosphate, alkaline phosphatase, intact parathyroid hormone (PTH), MPA CV, tacrolimus trough concentration, sirolimus trough concentration, length of follow-up, and eGFR at 12 months as a measure of quality of the allograft. Creatinine was measured throughout the study period using the enzymatic method and was IDMS traceable throughout the study period [17]. eGFR was calculated using the Filler formula using cystatin C [18]. We also recorded all concomitant medication.

The slope of $1/\text{creatinine}$ is an established tool for describing the severity of the loss of renal allograft function [19] and was determined for this study using simple regression analysis. The slope of the linear regression line was our primary outcome variable, with measurements beginning day 366 post-transplantation. The first year was not considered because rejection episodes may heavily influence graft outcomes. We then grouped the patients by their slope of $1/\text{creatinine}$ and defined the 19 patients with the shallowest slopes as the good slope group, and the remaining 18 patients with the steepest slopes as the poor slope group, which was supported by

significantly different 1/creatinine levels between the groups ($P < 0.0001$, Mann-Whitney U test).

Statistical analysis

Clinical data were collected using a paper-based data entry form that was approved by the ethics board. Available electronic laboratory data (Cerner, PowerChart) was transferred to an Excel database and stored on a secure hospital drive) and then formatted. Intra-patient CVMPA, and tacrolimus and sirolimus trough level variability were calculated as previously described [14]. The data on patients' medication dosages (MMF, tacrolimus, or sirolimus), all laboratory parameters, and concomitant medication doses were expressed using appropriate parametric or non-parametric methods based on the normality assessment computed using the Kolmogorov Smirnov test. Subgroup analyses were performed for patients with poor slope and patients with good slope, and the subgroups were compared using appropriate parametric or non-parametric methods (unpaired *t*-test or Mann-Whitney U test). All analyses were performed in Excel (Office for Mac 2011, version 14.3.8) or GraphPad Prism for Mac OS X version 5 (GraphPad Software, San Diego, CA, USA) or STATA for Mac version 11.2 (StataCorp, College Station, TX, USA).

Results

The patient characteristics are summarized in Table 1: patients with a good slope in the second column, and patients with a poor slope in the third column. With the exception of slope and duration of follow-up, there was no significant differences in patient characteristics. The good-slope patients were followed for a longer duration than the poor-slope patients. Importantly, renal function at one year was identical in both groups. The median cystatin C, eGFR at one-year post-transplantation was 79.1 mL/min/1.73 m² (interquartile range (IQR) 90.6, 44.3 mL/min/1.73 m²) in all 37 patients, and was not significantly different between the group with a poor slope (median 81.5, IQR 93.7, 48.0) and the group with good slope (median 71.3, IQR 90.6, 22.7, $P = 0.193$, Mann-Whitney U test).

A total of 234 vitamin D samples were drawn from patients and analyzed (median 2/patient/year). Of these, 53 (mean vitamin D level 63±45 nmol/L) were drawn in the winter, 59 (mean 75±37 nmol/L) in the spring, 72 (mean 77±40 nmol/L) in the summer, and 50 (mean 60 ± 30 nmol/L) in the fall. While the number of samples per season did not differ, the concentrations differed significantly by season ($P = 0.0393$, ANOVA). Overall, the average vitamin D (25-OH) level per patient was 70.4±30.3 nmol/L. In each 1/creatinine slope group, there was only one patient with an average vitamin D level that was severely deficient (<10 nmol/L), with the rest of the patients categorized

as either insufficient (30–50 nmol/L) or sufficient (>50 nmol/L). The mean vitamin D level was 71.7±34.6 nmol/L in the patient group with a poor slope, which was not significantly different from the patient group with a good slope (69.2±26.5 nmol/L, $P = 0.408$, Student's *t*-test, Figure 1).

In Table 1, we list other laboratory parameters, including calcium, phosphate, calcium phosphate product, intact PTH, magnesium, microalbumin/creatinine ratio, hemoglobin, and anion gap (all as mean for the duration of follow-up) for all study participants, and for the group with the poor and the good slope. Average MPA and average tacrolimus level were not significantly different between the groups, except for PTH values and the MPA CV, which were significantly different (Figure 2).

Discussion

In this small single-center retrospective cohort study of all eligible 37 pediatric renal transplant recipients, we were unable to demonstrate an effect by vitamin D levels on the slope of 1/creatinine or on the loss of allograft function. Of note, the patients within the good-slope group were followed for a significantly longer duration. Although the difference in length of follow-up between the two groups could potentially have led to an increased accuracy of the 1/creatinine slopes in the good-slope group, the graft function of both the good-slope and poor-slope groups at one-year post-transplantation was not affected by the average vitamin D levels over time. Thus, between the two groups, with significantly different 1/creatinine slopes and similar levels of vitamin D, significant differences were only observed for MPA CV and intact PTH. We observed a trend for the average MPA trough level, minimum tacrolimus trough level, anion gap, and average phosphate levels. Despite the small sample size, the significant association with the MPA CV was surprising and important, as many centers do not utilize therapeutic drug monitoring of MMF therapy, although there is compelling evidence to support its use [20].

While studies in pediatric transplant recipients were scant [5,13,21], our study contrasted with the findings of Obi et al. [9] and Bienaime et al. [10], who demonstrated an impact of vitamin D levels on the decline of allograft function in adult renal transplant recipients. In the Obi et al. study, there were 264 adult renal transplant recipients, and in the Bienaime et al. study, there were 634 adult renal transplant recipients. Of note, in both studies there was a high prevalence of vitamin D deficiency with many patients had values less than 12.5 nmol/L. However, in the study by Marcen et al. [11], there were 189 adult renal transplant recipients who were treated with vitamin D (38.3% deficient, 46.9% insufficient, and 14.7% normal), and they showed no effect of vitamin D on allograft function, which was in accordance with our results.

Table 1. Patient characteristics and evaluated parameters for all patients, patients with a good slope, and patients with a poor slope.

Parameter	All patients [IQR]	Poor slope (n=18) [IQR]	Good slope (n=19) [IQR]	Statistical evaluation
Gender	23 F, 14 M	11 F, 8 M	12F, 6M	P=1.000 Fisher's exact test
Age at transplantation [years]	8.2±4.6	7.9±4.4	8.5±4.9	P=0.6983 unpaired t-test
1/creatinine slope	-2.59E-0.006 [-8.752E-0.006, -1.000E-0.006]	-8.75E-0.006 [-1.48E-0.005, -5.705E-0.006]	-1.00E-0.006 [-1.934E-0.006, 0.000]	P<0.0001 Mann-Whitney test**
Duration of follow-up [years]	8.7±5.9	5.3±5.0	12.1±4.8	P=0.0002 Student's t-test**
Creatinine at one year [μmol/L]	62 [31,39]	62 [44, 93]	90 [62, 113]	P=0.0772 Mann-Whitney test*
CysC at one year [mL/min/1.73 m ²]	1.14 [1.01, 1.91]	1.11 [0.98, 1.78]	1.25 [1.01, 3.47]	P=0.1930 Mann-Whitney test
Average calcium [mmol/L]	2.32 [2.26, 2.42]	2.34 [2.25, 2.42]	2.31 [2.28, 2.43]	P=0.9636 Mann-Whitney test
Average phosphate [mmol/L]	1.37 [1.26, 1.46]	1.4 [1.33, 1.56]	1.3 [1.20, 1.45]	P=0.0829 Mann-Whitney test*
Average calcium phosphate product [mmol ² /L ²]	3.23 [2.97, 3.37]	3.27 [3.13, 3.43]	3.15 [2.87, 3.32]	P=0.1406 Mann-Whitney test
Average intact PTH [pmol/L]	10.63 [5.40, 19.11]	12 [6.30, 25.95]	10.32 [4.96, 14.97]	P=0.0435 Mann-Whitney test**
Average magnesium [mmol/L]	0.73 [0.72, 0.79]	0.74 [0.71, 0.79]	0.73 [0.72, 0.82]	P=0.5419 Mann-Whitney test
Average microalbumin/creatinine ratio [g/mol]	7.56 [2.84, 36.58]	15.5 [3.85, 35.16]	4.1 [2.20, 41.50]	P=0.1939 Mann-Whitney test
Average hemoglobin [g/L]	111.2±12.6	108.5±10.8	113.7±14.0	P=0.1052 Student's t-test
Anion gap [mmol/L]	10.84 [9.60, 12.28]	11.37 [9.98, 13.07]	10.77 [8.88, 11.88]	P=0.575 Mann-Whitney test
Min tacrolimus trough level [ng/mL]	2.5 [2.00, 3.40]	2.0 [2.00, 3.05]	2.7 [2.00, 3.50]	P=0.0893 Mann-Whitney test*
Tacrolimus CV [%]	0.43±0.13	0.45±0.15	0.53±0.30	P=0.4468 unpaired t-test
Average tacrolimus trough level [ng/mL]	7.6±2.0	7.8±2.0	7.5±2.0	P=0.2412 unpaired t-test
Min. MPA trough level [mg/L]	0.34±0.05 (SEM)	0.30±0.05 (SEM)	0.39±0.09 (SEM)	P=0.1849 unpaired t-test
MPA CV [%]	0.62±0.29	0.72±0.25	0.53±0.30	P=0.0241 unpaired t-test**
Med. MPA trough level [mg/L]	2.76 [2.13, 3.45]	2.59 [1.95, 3.16]	3.05 [2.67, 4.03]	P=0.0935 Mann-Whitney test*

** Denotes statistically significant difference and * denotes one-sided trend. CysC – cystatin C; PTH – parathyroid hormone; MPA CV – mycophenolic acid coefficient of variation.

Figure 1. 25(OH) vitamin D levels in two groups of patients: patients with a poor 1/creatinine slope and patients with a good 1/creatinine slope. There were no statistically significant differences between the two groups.

In our study, only two patients were vitamin D deficient. In one of the few pediatric transplantation studies, Brodersen et al. [5] included 35 patients and described a relationship between hypovitaminosis and PTH, but did not analyze the impact of vitamin D levels on progression of allograft dysfunction. A study by Wesseling-Perry et al. [21] included 68 pediatric renal transplant recipients, but the focus of the study was on fibroblast growth factor 23. At the time of our study, we were not aware of any other pediatric studies on this subject.

With mild to moderate levels of hypovitaminosis D, the patients in our study had a significant association between the MPA CV and the progression of allograft dysfunction. The CV of immunosuppressant trough levels may reflect adherence or a risk for development of donor-specific antibodies [12,13]. There is growing evidence for the importance of MPA therapeutic drug monitoring [15,20], as underexposure of MPA may be associated with donor-specific antibody formation [14,15]. The outcomes for adolescent renal transplant recipients remain particularly poor, especially around the time of transition to adult care [13]. With formal MPA therapeutic drug monitoring implementation, this particular challenge can be addressed with better immunosuppressive drug adherence. However, in our study, tacrolimus CV was not associated with poorer slope, which suggests that medication adherence may not have been a factor for a faster decline of allograft function.

The strengths of our study included the frequency of monitoring of vitamin D levels per patient, an even spread of the sampling over all seasons, and a long follow-up period. Unfortunately, our study had some major limitations. First, the sample size was small compared to adult studies. We did not include a

Figure 2. Coefficient of variation of the mycophenolic acid trough level for the patients with a poor 1/creatinine slope and those with a good 1/creatinine slope. The difference was statistically significant ($P=0.0241$, unpaired *t*-test).

control group, and most important, we had very few severely vitamin D deficient patients. The small sample size did not permit the careful evaluation of covariates such as socioeconomic status, nutrition and other determinants of treatment adherence. It is possible that these factors might have played a role in affecting the well-established impact of vitamin D deficiency in patients with CKD in their native kidneys. However, especially in adolescents, where we often see patients struggling with medication adherence, it appears adding vitamin D supplementation to the already significant medication burden may not be critical. We did not see steeper slopes of 1/creatinine in patients with a mild to moderate decrease of vitamin D levels, which supports our hypothesis. From a clinical standpoint, for those patients who are vitamin D insufficient with known medication adherence issues, supplementing may not be as important as focusing on increasing adherence to immunosuppressive medications.

Conclusions

Only MPA CV and MPA exposure were significantly associated with 1/creatinine slope, which is a measure of renal allograft dysfunction. This suggested that mild or moderate vitamin D levels in renal transplantation patients might not affect the deterioration of graft function as much as other factors. Routine vitamin D supplementation in addition to the already significant medication burden appears to have a limited effect on graft survival. Hence, it might be of greater benefit to address adherence to immunosuppressive medications to ameliorate renal allograft functional decline in these pediatric transplant recipients.

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