

Vitamin D Receptor Activity, Vitamin D Status, and Development of De-novo Donor-specific Antibody after Renal Transplantation

Abstract

Introduction: Vitamin D has immunomodulatory properties and could have a role in allograft outcome. **Methods:** Fifty-two chronic kidney disease patients going for transplantation were studied for vitamin-D receptor (VDR) activity, 25(OH)D, estimated glomerular filtration rate (e-GFR), and de-novo donor-specific antibody (d-DSA). **Results:** Vitamin D deficiency was seen in 25% of recipients before transplant (26.09 ± 12.19 ng/ml), in 48.1% at 6 months posttransplant (23.36 ± 15.11 ng/ml). VDR activity before the transplant was 15.41 ± 31.41 ng/ml, which was similar to control group (13.24 ± 9.78 ng/ml), and after transplantation showed an increase at 3 months to 21.91 ± 38.80 ng/ml and at 6 months to 26.03 ± 53.90 ng/ml. d-DSA developed in 27.3% and 6.7% patients of vitamin D-deficient patients (levels <20 ng/ml) and non-deficient (levels ≥ 20 ng/ml) patients respectively ($P < 0.042$). Low VDR activity at 3 months posttransplant was associated with significantly higher d-DSA positivity (33.3%) as compared to the group with normal VDR activity where d-DSA developed only in 5.9% of patients ($P < 0.009$). Patients with vitamin D levels <20 ng/ml and the group with low VDR activity at 3 months had significantly less e-GFR at 1 year after transplant. **Conclusion:** d-DSA was associated with vitamin D deficiency and low VDR activity with decreased graft GFR at 12 months posttransplant.

Keywords: De-novo DSA, estimated GFR, kidney transplantation, VDR activity, vitamin D deficiency

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Background

Observational studies have demonstrated that vitamin D deficiency, defined as low serum total 25-hydroxyvitamin-D concentration, is associated with increased risks of death and diseases such as cardiovascular diseases, malignancies, infectious diseases, diabetes, autoimmune diseases, and kidney diseases.^[1,2] The prevalence of vitamin D deficiency or insufficiency is high among patients with chronic kidney disease (CKD), especially patients with end-stage renal disease (ESRD) and kidney transplant recipients.^[3,4] The vitamin D receptor (VDR) is a nuclear protein responsible for mediating the biological actions of vitamin D.^[5]

Vitamin D deficiency has been defined as 25(OH)D concentration <20 ng/ml, and insufficiency as 25(OH)D concentration between ≥ 20 and <30 ng/ml. As defined in KDIGO guidelines, about 97% of patients in CKD stage 5 have vitamin D deficiency

or insufficiency.^[6] In renal transplantation, vitamin D could have an additional role in immune modulation. VDRs are present on various immune cells, including T cells, B cells, monocytes, and antigen-presenting cells.^[7,8] In T cells, calcitriol suppresses helper T-cell proliferation and differentiation, alters cytokine production, and causes a shift from a proinflammatory Th1 response to a tolerogenic Th2 response.^[9-11] Calcitriol also inhibits dendritic cell (DC) differentiation and maturation into antigen-presenting cells, which may be protective in transplantation.^[12] In a rat model of chronic allograft nephropathy, administration of 1,25 dihydroxyvitamin-D prolonged allograft survival, decreased episodes of acute rejection, reduced proteinuria, and prevented histological changes associated with chronic allograft nephropathy.^[13] This suggests that vitamin D supplementation may reduce acute rejection and chronic allograft nephropathy in humans through its interactions with the immune system.

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Methods

Fifty-two CKD patients on dialysis going for transplantation were prospectively studied before and after renal transplantation. VDR activity, 25(OH)D, and estimated glomerular filtration rate (e-GFR) were evaluated and correlated with the development of de-novo donor-specific antibody (d-DSA). VDR activity was measured in serum by human vitamin D3 receptor (VDR) ELISA kit (http://www.eiaab.com/entries/f_vitamin%20dCatalog No: E0475h). 25(OH)D (<http://www.laboratoireduquaivalliere.fr/notices%20analyses/FicheTechniqueVITD.pdf>) in serum was estimated by chemiluminescence microparticle immunoassay (Architect i-1000 STAT ABBOTT).

HLA typing was done on Luminex platform using Thermo-fisher kits. d-DSA was evaluated by two different solid phase assays by Luminex technology, using donor lysate DSA assay (Immucor), and single antigen bead assay (Thermo-fisher).^[14] d-DSA was considered positive with Mean Fluorescence Intensity (MFI) of 1000 or above. e-GFR was measured using the CKD-Epidemiology Collaboration (CKD-EPI) equation, which confers less underestimation of GFR in subjects with normal renal function. As there is no consensus regarding normal VDR activity parameters in transplant recipients. We used 25th percentile of normal control as cutoff for lower limit to define normal levels of VDR receptor activity.^[15]

Statistical analysis

Data was expressed as mean values \pm standard deviation and as absolute and relative frequency for categorical variables. Statistical analysis was performed using SPSS software (Version 20.0). Graft function at 3 months, 6 months, and 1 year was compared between the groups by analysis of covariance, taking into account covariates like e-GFR

value at 1 year and 25-hydroxyvitamin D3 at baseline, at 3 months and 6 months. Various categorical variables were analyzed using Pearson's correlation, Chi-square test, and Fisher's exact test. Multiple linear regression statistics was used with e-GFR as dependent variable and other factors as independent variables. Other statistical tests as appropriate were applied to various factors and variables.

Results

Fifty-two CKD (82.7% male) patients on dialysis going for transplantation were prospectively studied before and after renal transplantation. Patient characteristics and demographic data are given in Table 1. Various parameters like VDR activity, 25(OH)D levels and e-GFR were analysed in pre and post transplant period. 25(OH)D and VDR status were correlated with the development of d-DSA at 3 months of transplantation. Patients were evaluated at 3, 6, and 12 months intervals. 25(OH)D levels indicated vitamin D deficiency in 25% of patients before transplant (26.09 ± 12.19 ng/ml). In posttransplant period, vitamin D deficiency was seen at 3 months in 43.1% (23.44 ± 10.64 ng/ml), whereas at 6 months, in 48.1% (23.36 ± 15.11 ng/ml). VDR activity before transplant (15.41 ± 31.41 ng/ml), was similar to control group (13.24 ± 19.30 ng/ml). After transplantation, VDR activity showed an increase to 21.91 ± 38.80 ng/ml at 3 months and further increased at 6 months to 26.03 ± 53.90 ng/ml (P value <0.010 as compared to control). Comparison between groups with e-GFR <60 and ≥ 60 ml/min/1.73 m² showed that posttransplant GFR at 12 months correlated positively with pretransplant VDR activity at baseline ($P = 0.005$), at 3 months (P value = 0.035), and VDR activity at 6 months ($P = 0.043$) posttransplant [Table 2]. d-DSA developed more significantly in patients with vitamin D deficiency

Table 1: Patient demographics

Characteristic	All patients (n=52)	3-month 25(OH)D level <20 ng/ml (n=22)	3-month 25(OH)D level ≥ 20 ng/ml (n=30)	P*
Patient's age (years)	34.85 \pm 9.95	37.50 \pm 9.79	32.90 \pm 9.78	0.97
Sex				
Male	43 (82.7%)	15 (68.2%)	28 (93.3%)	0.00
Induction				
Basiliximab	41 (78.8%)	15 (68.2%)	26 (86.7%)	0.00
ATG	6 (11.5%)	5 (31.8%)	1 (3.3%)	
Others	5 (9.7%)	2 (9.1%)	3 (10%)	
DSA positive	8 (15.4%)	06 (27.3%)	02 (6.7%)	0.00
CNI				
Tacrolimus (ng/ml)	13.47 \pm 6.06 (n=51)	15.02 \pm 5.94	12.29 \pm 5.99	0.99
Rejection	5 (9.6%)	2 (9.1%)	3 (10%)	0.83
3 months post-KT vitamin D (ng/ml)	23.44 \pm 10.64	15.28 \pm 3.08	29.45 \pm 10.03	0.00
3 months post-KT VDR (ng/ml)	21.91 \pm 38.80	18.05 \pm 22.96	24.74 \pm 47.40	0.17
Donor's age (years)	45.36 \pm 11.83	44.84 \pm 9.58	46.70 \pm 16.93	0.03

Values are reported as number of patients (percentage), mean \pm SD, as appropriate. *P-value represents tests of significance from t-test, Chi-squared test, or Fisher's exact test, as appropriate

Table 2: Descriptive statistics of vitamin D (pre- and post-kidney transplant) compared with graft e-GFR category at 12 months (post-KT)

Parameters (n=52)	e-GFR 12 months (post-KT) <60 ml/min/1.73 m ² (n=20)	e-GFR 12 months (post-KT) ≥60 ml/min/1.73 m ² (n=32)	P
Pre-KT VDR (ng/ml)	5.53±13.89	20.43±37.46	0.005
Pre-KT vitamin D (ng/ml)	22.95±8.47	28.00±13.89	0.342
3 months post-KT VDR (ng/ml)	12.63±18.04	28.02±46.74	0.035
3 months post-KT vitamin D (ng/ml)	21.09±8.74	25.23±11.27	0.570
6 months post-KT VDR (ng/ml)	10.065±9.53	35.960±67.02	0.043
6 months post-KT vitamin D (ng/ml)	20.72±15.35	25.55±14.73	0.908
3 months post-KT Sr creatinine (mg/dl)	1.240±0.30	1.01±0.26	0.563
6 months post-KT creatinine (mg/dl)	1.42±0.26	1.11±0.20	0.175
12 months post-KT creatinine (mg/dl)	1.97±1.33	1.11±0.18	0.016

(30.7%) as compared to patients without vitamin D deficiency (5.5%) (Chi-square test, $P < 0.042$) (Pearson's correlation $P = 0.043$).

Low VDR activity at 3 months posttransplant was associated with significantly higher d-DSA positivity (33.3%) as compared to the group with normal VDR activity where d-DSA developed only in 5.9% ($P < 0.009$, Pearson's correlation $P = 0.008$) [Table 3]. In our study, DSA positivity was associated with increased rejection episodes (25%) as compared to d-DSA negative (6.8% rejections). All the patients except one were on tacrolimus-based immunosuppression. Tacrolimus levels at 1 month were 13.47 ± 6.06 ng/ml (normal target range of 10–15 ng/ml at 1 month).

Multivariate analysis revealed that 2 weeks post transplant GFR, 3 months VDR, and 6 months VDR (considered as continuous variables) were independent predictors of 12-month GFR of allograft [Table 4].

Discussion

Our study shows that there is a high prevalence of vitamin D deficiency and insufficiency in patients with ESRD going for transplantation and it persists even after kidney transplantation in majority of patients as compared to control population.^[16] This is also supported by our results, which show that pretransplant VDR activity was low. After the transplant, VDR activity became normal at 3 months. VDR activity was higher as compared to the control group at 6 months posttransplant. In our study, we used vitamin D supplements only in pretransplant period. No vitamin D analog was used in the posttransplant period. Transplant patients had low 25(OH)D. Patients after transplant though achieved good graft function (as indicated by serum creatinine levels), but the e-GFR was still suboptimal in posttransplant period.

Vitamin D deficiency has been reported to be associated with risk of acute rejections in posttransplant period. Vitamin D as an immunomodulator has potential for reducing and preventing allograft rejection after transplantation.^[17-19] At 3 months posttransplant,

Table 3: Correlation between 25(OH)D levels, VDR activity, and d-DSA at 3-month posttransplant

n=52	<20 ng/ml 25(OH)D levels	≥20 ng/ml 25(OH)D levels	P
d-DSA negative	72.7% (n=16)	93.3% (n=28)	0.042
d-DSA positive	27.3% (n=6)	6.6% (n=2)	
n=52	<2.4 ng/ml VDR activity	≥2.4 ng/ml VDR activity	P
d-DSA negative	66.7% (n=12)	94.1% (n=32)	0.009
d-DSA positive	33.3% (n=6)	5.9% (n=2)	

Table 4: Model summary of multivariate linear regression analysis

	Coefficients±SD	t	P	
Full model ^a (constant)	19.415±7.57	2.56	0.015	
2 week post-transplant	2.800±0.69	4.045	0.000	
e-GFR ^b				
3-month VDR ^b	0.274±0.10	2.662	0.012	
6-month VDR ^b	0.114±0.04	2.620	0.013	
Dependent variable: GFR (predictor variables: 3-month VDR, 6-month VDR)				
Model summary	R	R-square	Adjusted R-square	Std. error of the estimate
	0.771	0.595	0.549	15.400

Only the variables independently associated with 12 months e-GFR are presented (final models after forward selection procedure).

^aThe full model includes all the variables used in this study. ^bFor an increase of 1 unit of the variable

development of d-DSA in our patients was significantly associated with vitamin D deficiency ($P < 0.042$) and low VDR activity ($P < 0.009$). Vitamin D has been shown to control both innate and adaptive immune responses^[20,21] and could modulate allogeneic response. Our results indicate that lower levels of 25(OH)D and low VDR activity are linked to development of d-DSA. This effect of vitamin D deficiency on development of d-DSA could adversely affect allograft function, consequence of the associated stronger alloimmune response.^[22] Prevalence of 25(OH)D deficiency at the time of renal transplantation has been reported to be quite high.^[23]

We correlated d-DSA development with vitamin D status after transplantation. Studies in CYP27B1 knockout mice suggest that VDR activity correlates with number of mature DCs and is associated with aberrant DC trafficking.^[9] VDR expression in human B cells may be upregulated by activated B cells. *In vitro* studies show that B cells are capable of intracrine response to bioactive metabolite of vitamin D. The antiproliferative effects of 1,25(OH)₂D₃ (i.e., stimulation of apoptosis, suppression of proliferation and differentiation, decreased production of immunoglobulin) on B cells have been reported to be indirectly driven by T-helper cells.^[24] In our study, development of d-DSA was associated with low VDR activity at 3 months after transplantation (33.3% d-DSA positivity in low VDR activity group and 5.9% d-DSA positivity in group with normal VDR activity, $P < 0.009$). Despite normal levels of 1,25-(OH)₂D, the insufficient expression of VDR has been reported to be responsible for an impaired translation of vitamin D-induced signaling, which can contribute to a sustained inflammatory reaction.^[25]

VDR activity can modulate immune response. VDR activity has been reported to affect and inhibit progression of CKD in animal models of nonimmunological CKD diseases. VDR activity also helps to preserve podocyte function.^[26] VDR activity and signaling reduces glomerular inflammation and tubular cell proliferation and interferes with the renin-angiotensin system, epidermal growth factor receptor activity, and transforming growth factor (TGF)- β signaling.^[16,27,28]

In our study, VDR, gender, immunosuppression, tacrolimus levels, proteinuria, rejection, and induction were studied by binary and linear logistic regression models. Low GFR correlated negatively with induction and VDR receptor activity. On backward selection (binary logistic regression), only induction with basiliximab or thymoglobulin showed significant correlation, whereas in linear regression model, 3- and 6-month VDR activity was predictor of graft GFR. Other parameters studied did not predict GFR at 1 year posttransplant. In our study, all patients except one were on tacrolimus-based triple immunosuppression. No patient was on steroid-free immunosuppression [Table 1].

Low 25(OH) vitamin D levels have been reported to be associated with poorer graft function and faster GFR decline. There is no report about vitamin D levels and DSA.^[16,29] Low 25(OH) vitamin D levels have been reported to be associated with inferior kidney function on the long term.^[30] Vitamin D supplementation in randomized controlled (RCT) and retrospective trials has not shown renoprotective effects.^[31,32] While supplementation with paricalcitol has been shown to reduce proteinuria as renoprotective mechanism, paricalcitol has been reported to be a VDR activator in animal models.^[33]

It is possible that VDR activation may be more effectively mediated by paricalcitol than by 25(OH) vitamin D.

This may explain the result of RCTs of vitamin D supplementation. These RCTs have also not looked at VDR activity and DSA.

In the present study, VDR levels and induction have been shown to be significant predictors of graft function. The main new finding of this study is that better VDR activity is associated with better graft function.

Conclusion

In the present study, vitamin D deficiency and low VDR activity was associated with the d-DSA development and decreased graft e-GFR at 12 months posttransplant. Future studies are required to test whether vitamin D supplementation after kidney transplantation could improve allograft outcomes.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

- Weinstock MA, Moses AM. Skin cancer meets vitamin D: The way forward for dermatology and public health. *J Am Acad Dermatol* 2009;61:720-4.
- Christie FT, Mason L. Knowledge, attitude and practice regarding vitamin D deficiency among female students in Saudi Arabia: A qualitative exploration. *Int J Rheum Dis* 2011;14:e22-9.
- González EA, Sachdeva A, Oliver DA, and Martin KJ, Vitamin D insufficiency and deficiency in chronic kidney disease: A single center observational study, *Am J Nephrol* 2004;24:503-10.
- Stavroulopoulos A, Cassidy MJD, Porter CJ, Hosking DJ, Roe SD, Vitamin D status in renal transplant recipients, *Am J Transplant* 2007;7:2546-52.
- Strugnell SA, DeLuca HF. The vitamin D receptor-structure and transcriptional activation. *Proc Soc Exp Biol Med* 1997;215:223.
- National Kidney Foundation: K/DOQI clinical practice guidelines for bone metabolism and disease in chronic kidney disease. *Am J Kidney Dis* 2003;42(Suppl 3):S1-201.
- Priehl B, Treiber G, Pieber TR, Amrein K. Vitamin D and immune function. *Nutrients* 2013;5:2502-21.
- Mora JR, Iwata M, von Andrian UH. Vitamin effects on the immune system: Vitamins A and D take centre stage. *Nat Rev Immunol* 2008;8:685-98.
- Hewison M. An update on vitamin D and human immunity. *Clin Endocrinol* 2012;76:315-25.
- Di Rosa M, Malaguarrera M, Nicoletti F, Malaguarrera L. Vitamin D₃: A helpful immuno-modulator. *Immunology* 2011;134:123-39.
- Van Belle TL, Gysemans C, Mathieu C. Vitamin D in autoimmune, infectious and allergic diseases: A vital player? *Best Pract Res Clin Endocrinol Metab* 2011;25:617-32.
- Boonstra A, Barrat FJ, Crain C, Heath VL, Savelkoul HF, O'Garra A. 1 α ,25-dihydroxyvitamin d₃ has a direct effect on naive CD4+ T cells to enhance the development of Th2 cells. *J Immunol* 2001;167:4974-80.
- Hullett DA, Laeseke PF, Malin G, Nessel R, Sollinger HW, Becker BN. Prevention of chronic allograft nephropathy with

- vitamin D. *Transpl Int* 2005;18:1175-86.
14. Mehrotra S, Sharma RK, Mayya M, Gupta A, Prasad N, Kaul A, *et al.* Lumindex solid-phase crossmatch for de novo donor-specific antibodies in living-donor related transplants. *Exp Clin Transplant* 2017;4:394-9.
 15. Pablo UT, Marie M, Jean PH, Alexandre K, Jean JB, Martin F, *et al.* Association of kidney function, vitamin D deficiency, and circulating markers of mineral and bone disorders in CKD. *Am J Kidney Dis* 2011;58:544-53.
 16. Bienaimé F, Girard D, Anglicheau D, Canaud G, Souberbielle JC, Kreis H, *et al.* Vitamin D status and outcomes after renal transplantation. *J Am Soc Nephrol* 2013;24:831-41.
 17. Saber A, Fotuhi F, Rostami Z, Einollahi B, Nemati E. Vitamin D levels after kidney transplantation and the risk of cytomegalovirus infection. *Nephrourol Mon* 2015;7:e29677.
 18. Park YJ, Kim SU, Lee KH, Lee JH, Kwon E, Jung HY, *et al.* Vitamin D deficiency is associated with increased risk of bacterial infections after kidney transplantation. *Korean J Intern Med* 2017;32:505-13.
 19. Stein EM, Shane E. Vitamin D in organ transplantation. *Osteoporos Int* 2011;22:2107-18.
 20. Baeke F, Takiishi T, Korf H, Gysemans C, Mathieu C. Vitamin D: Modulator of the immune system. *Curr Opin Pharmacol* 2010;10:482-96.
 21. Hewison M. Vitamin D and innate and adaptive immunity. *Vitam Horm* 2011;86:23-62.
 22. Filipov JJ, Zlatkov BK, Dimitrov EP, Svinarov D. Relationship between vitamin D status and immunosuppressive therapy in kidney transplant recipients. *Biotechnol Biotechnol Equip* 2015;4:29:331-5.
 23. Sadlier DM, Magee CC. Prevalence of 25(OH) vitamin D (calcidiol) deficiency at time of renal transplantation: A prospective study. *Clin Transplant* 2007;21:683-8.
 24. Lemire JM, Adams JS, Sakai R, Jordan SC. 1 alpha, 25-dihydroxyvitamin D3 suppresses proliferation and immunoglobulin production by normal human peripheral blood mononuclear cells. *J Clin Invest* 1984;74:657-61.
 25. Kempinska-Podhorodecka A, Milkiewicz M, Wasik U, Ligocka J, Zawadzki M, Krawczyk M. Decreased expression of vitamin D receptor affects an immune response in primary biliary cholangitis via the VDR-miRNA155-SOCS1 Pathway. *Int J Mol Sci* 2017;18:E289. doi: 10.3390/ijms18020289.
 26. Li YC. Podocytes as target of vitamin D. *Curr Diabetes Rev* 2011;7:35-40.
 27. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest* 2002;110:229-38.
 28. Mizobuchi M, Morrissey J, Finch JL, Martin DR, Liapis H, Akizawa T, Slatopolsky E. Combination therapy with an angiotensin-converting enzyme inhibitor and a vitamin D analog suppresses the progression of renal insufficiency in uremic rats. *J Am Soc Nephrol* 2007;18:1796-806.
 29. Keyzer CA, Riphagen IJ, Joosten MM, Navis G, Muller Kobold AC, Kema IP, *et al.*; NIGRAM consortium. Associations of 25(OH) and 1,25(OH)₂ vitamin D with long-term outcomes in stable renal transplant recipients. *J Clin Endocrinol Metab* 2015;100:81-9.
 30. Obi Y, Hamano T, Ichimaru N, Tomida K, Matsui I, Fujii N, *et al.* Vitamin D deficiency predicts decline in kidney allograft function: A prospective cohort study. *J Clin Endocrinol Metab* 2014;99:527-35.
 31. Courbebaisse M, Souberbielle JC, Baptiste A, Taieb J, Tsatsaris V, Guibourdenche J, *et al.* Cholecalciferol supplementation does not protect against renal allograft structural and functional deterioration: A retrospective study. *Transplantation* 2011;91:207-12.
 32. Thiem U. VITA-D study: Outcome of a 1-year randomized controlled trial transplant patients. European Renal Association- European Dialysis and Transplant Association (ERA-EDTA) 52nd Congress: Abstract LBA-3564, London, UK, 28-31 May, 2015.
 33. Park JW, Bae EH, Kim IJ, Ma SK, Choi C, Lee J, *et al.* Paricalcitol attenuates cyclosporine-induced kidney injury in rats. *Kidney Int* 2010;77:1076-85.