Assessment of the Relationship between Vitamin D Status and Graft Function in Living-Related Kidney Transplant Recipients

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Abstract

Background: Kidney transplantation enhances survival rates and quality of life for patients with end-stage renal disease (ESRD). However, the long-term function of graft can be compromised. A common issue among transplant recipients is vitamin D deficiency, which arises due to the use of immunosuppressants and sun protection measures, potentially contributing to the deterioration of the allograft.

Objectives: To determine the incidence of vitamin D deficiency in kidney transplant recipients and its relationship with graft function.

Patients and methods: A prospective observational study, including 50 CKD patients undergoing kidney transplant collected from Internal Medicine department, Benha university and National Institute of Urology and Nephrology. Patients were grouped based on serum 25(OH) vitamin D levels into sufficient (≥30 ng/ml) and insufficient (<30 ng/ml) groups. Comprehensive evaluations were performed, including clinical exams and laboratory tests (CBC, CRP, blood urea, creatinine, eGFR, tacrolimus levels, and serum 25(OH)D levels) before and at 6 weeks, 3 months, and 6 months post-transplant.

Results: The study participants recipients with 36.13 ± 12.33 years in the sufficient vitamin D group and 26.76 ± 9.16 years in the insufficient group. There was no statistically significant difference between the two groups regarding serum urea, creatinine and eGFR over time. Serum urea significantly decreased at three and six months in the insufficient group (P = 0.003).

Conclusion: Vitamin D deficiency is prevalent among kidney transplant recipients and affects graft function over time. Managing vitamin D levels could improve long-term graft outcomes, emphasizing the need for regular monitoring and supplementation.

Keywords: Kidney transplantation; Vitamin D deficiency; Graft function; eGFR, Immunosuppressants; Chronic kidney disease.

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Introduction

Compared to dialysis, kidney transplantation significantly enhances the lives of end-stage renal disease (ESRD) patients by increasing their lifespan, reducing the risk of health problems, and improving their overall well-being. Despite this, kidney transplants deteriorate over time due to factors like nephron underdosing and fibrosis. Identifying modifiable factors, such as serum vitamin D levels, could help prevent allograft deterioration **(Wile et al., 2018; Buyukdemirci et al., 2022).**

Vitamin D is essential for managing the levels of calcium and phosphorus in the body. It also offers protective benefits to the kidneys and can help manage conditions like diabetes and hypertension. However, individuals with chronic kidney disease, especially those who have undergone kidney transplantation, often experience deficiency in vitamin D levels due to a combination of factors **(Filipov and Dimitrov 2017)**.

Vitamin D level deficiency is very common among kidney transplant recipients, with up to 80% of patients affected. This condition is exacerbated by sun avoidance practices and the use of immunosuppressive medications, particularly calcineurin inhibitors. Managing vitamin D levels is vital due to its immunomodulatory and renoprotective properties **(Koimtzis et al., 2022)**.

Vitamin D affects immune regulation in transplantation, with calcitriol receptors in immune cells like T and B cells. Calcitriol may protect transplants by suppressing T cell proliferation **(Erten et al., 2016)**. This study aims to evaluate the impact of vitamin D levels on kidney graft function both before and after transplantation.

This study aimed to determine the incidence of vitamin D deficiency in kidney transplant recipients and to evaluate the correlation between this deficiency and graft function. Graft function was assessed by measuring serum creatinine levels and estimating the glomerular filtration rate (eGFR) using the MDRD equation at 6 weeks, 3 months, and 6 months post-transplantation.

Patients and methods

Study Design and Participant

A prospective observational study took place at the National Institute of Nephrology and Urology, enrolling 50 CKD patients (either pre-emptive or on dialysis) scheduled for kidney transplantation. Participants were then categorized into 2 groups based on their 25(OH) vitamin D levels: those with sufficient value (≥ 30 ng/ml, group 1) and those with insufficient value (< 30 ng/ml, group 2) and follow-up was conducted for 6 months after kidney transplant.

Inclusion criteria: Both genders aged over than 18 years and eligible for renal transplantation were included in the study.

Exclusion criteria: Patients excluded if they did not provide informed consent, underwent combined organ transplantation, experienced surgical graft failure, had elevated panel reactive antibody levels, were receiving a second kidney transplant, or were currently on vitamin D supplementation.

Methods

All cases were subjected to comprehensive evaluation, including demographic data, cause of renal failure, dialysis duration, medical history, family history, lifestyle habits, and a system review. Clinical examinations cover vital signs, body mass index, and general renal disease signs. Laboratory analyses are conducted before transplantation and at 6 weeks, 3 months, and 6 months post-transplant, including complete blood count, C-reactive protein, blood urea and creatinine, eGFR (using MDRD formula) (mL/min/1.73 m2) =186× (Scr)-1.154× (Age)-0.203× (0.742 if female) × (1.120 if African-American), tacrolimus level, and serum 25(OH)D levels. The studied cases were subdivided into two groups according

to serum level of 25(OH) vitamin D sampled pre-kidney transplant (Basal vitamin D level) into; group 1 with sufficient vitamin D (≥ 30 ng/ml) and group 2 with insufficient vitamin D (< 30 ng/ml).

Sample collection and preparation

Blood samples (10 ml) were obtained from patients after 12-hours fasting state via vascular access. One ml was used for a complete blood count (CBC) in an EDTA tube. The remaining 9 ml was placed in a plain tube, allowed to clot, and then prepared serum using a 3000-rpm centrifuge for 15 minutes.

The serum levels of 25(OH) D (ng/ml) were determined with the following automated immunoassay:25(OH)D Roche Cobas e411 (Roche Diagnostics, Mannheim, Germany)), a fully automated analyzer that uses a patented ElectroChemiLuminescence (ECL) technology for immunoassay analysis. Based on 25OHD levels, patients were categorized as deficient (<15 ng/mL), insufficient (15–30 ng/mL), or sufficient (>30 ng/mL) **(Chon et al., 2017)**. Follow-up was conducted over a 6-month period unless graft failure, defined as the need for re-transplantation or a permanent return to dialysis, or death, occurred earlier.

Ethical considerations: The study was accepted by the Research Ethics Committee, Benha University (Approval code: MS 21-6-2023). All patients (or their first-degree relatives) provided written informed consents prior to their enrolment to participate in the study and for the publication of data. The consent form explicitly outlined their agreement to be enrolled in the study and for the publish of data, ensuring protection of their confidentiality and privacy, and the explanation of the benefits for maintaining graft function.

Statistical analysis

SPSS 27.0 was used for all statistical analyses. Continuous data were summarized as mean ± SD or median (IQR), as appropriate, and categorical data as frequencies and percentages. Statistical comparisons were made using Pearson's chi-square or Monte Carlo tests for categorical data, and t-tests (independent or paired), Mann-Whitney U tests, or Wilcoxon signed-rank tests for continuous data. Statistical significance was set at $p < 0.05$, with $p < 0.01$ indicating high significance.

Results

The study included 50 living-related kidney transplant recipients who were collected from internal medicine department, Benha university and National Institute of Urology and Nephrology and they underwent kidney transplants at the National Institute of Urology and Nephrology (**Table.1 and 2**).

Table 1. Basic demographic, clinical data, and causes of ESRD in the studied cases

Variables		**Study cases**
		No. = 50
Age (Years)	**Mean ± SD**	28.26 ± 10.20
	Range	18 – 54
BMI (Kg/m2)	**Mean ± SD**	24.15 ± 4.48
	Range	17.21 – 35.29
Sex	**Male**	32 (64%)
	Female	18 (36%)
Relation to donor	**Related**	50 (100%)
HLA Typing Mismatch	**2//6**	14 (28%)
	3//6	32 (64%)
	4//6	3 (6%)
	6//6	1 (2%)

Panel Reactive Antibodies (PRA) Class I	**2 %**	2 (4%)
	3 %	1 (2%)
	4 %	1 (2%)
	5 %	1 (2%)
	7 %	2 (4%)
	zero %	43 (86%)
Panel Reactive Antibodies (PRA) Class II	**1 %**	1 (2%)
	3 %	1 (2%)
	4 %	2 (4%)
	zero %	46 (92%)
Causes of ESRD		**Study cases**
		No. = 50
2ry Amyloidosis		3 (6%)
Alport's syndrome		1 (2%)
Analgesic Nephropathy		3 (6%)
Chronic interstitial nephritis		1 (2%)
Diabetic Kidney Disease		3 (6%)
FSGS		3 (6%)
HTN nephrosclerosis		3 (6%)
Lupus Nephritis		4 (8%)
MPGN		2 (4%)
polycystic kidney		1 (2%)
Reflux Nephropathy		5 (10%)
stones		1 (2%)
Unknown Cause		20 (40%)

Table 2. Basal vitamin D level, status, IS medications, and induction in the studies cases

Variables		**Study cases**
		No. = 50
Vitamin D (ng/dl)	**Mean ± SD**	19.27 ± 9.58
	Range	5.80 – 37.38
Vitamin D categories	**Sufficient vitamin d (≥ 30 ng/dl)**	8 (16%)
	Insufficient vitamin d (< 30 ng/dl)	17 (34%)
	Vitamin d deficiency (< 20 ng/dl)	25 (50%)
IS Medication	**Steroids**	50 (100%)
	Tacrolimus	50 (100%)
	Cellcept	30 (60%)
	Myfortic	20 (40%)
Induction	**ATG**	12 (24%)
	Basiliximab	7 (14%)
	No Induction	31 (62%)

While BMI, sex, and HLA mismatch did not differ significantly between the vitamin D groups, patients with sufficient vitamin D were significantly older (36.13 ± 12.33 years) than those with insufficient vitamin D (26.76 ± 9.16 years). The causes of ESRD were also similar between the groups (**Table.3**).

Table 3. Comparison of the demographic, clinical data, and causes of ESRD according to basal vitamin D status

Variables		Group 1 No. = 8	Group 2 No. = 42	Test value	P-value	Sig.
Age (Years)		36.13 ± 12.33	26.76 ± 9.16	T= 2.506	**0.016**	**S**
BMI (Kg/m^2)		26.11 ± 3.41	23.78 ± 4.59	T= 1.361	0.180	NS
Sex	**Male**	6 (75%)	26 (61.9%)	FET = 0.500	0.479	NS
	Female	2 (25%)	6 (38.1%)			
HLA Mismatch	**2//6**	2 (25%)	12 (28.6%)	MC= 0.895	0.827	NS
	3//6	5 (62.5%)	27 (64.3%)			
	4//6	1 (12.5%)	2 (4.8%)			
	6//6	0 (0%)	1 (2.4%)			
2ry Amyloidosis		0 (0%)	3 (7.1%)	MC= 21.726	0.060	NS
Alport's syndrome		0 (0%)	1 (2.4%)			
Analgesic Nephropathy		1 (12.5%)	2 (4.8%)			
Chronic interstitial nephritis		0 (0%)	1 (2.4%)			
Diabetic Kidney Disease		1 (12.5%)	2 (4.8%)			
FSGS		2 (25%)	1 (2.4%)			
HTN nephrosclerosis		2 (25%)	1 (2.4%)			
Lupus Nephritis		0 (0%)	4 (9.5%)			
MPGN		0 (0%)	2 (4.8%)			
polycystic kidney		0 (0%)	1 (2.4%)			
Reflux Nephropathy		0 (0%)	5 (11.9%)			
stones		0 (0%)	1 (2.4%)			
Unknown Cause		2 (25%)	18 (42.9%)			

NS: Non-significant; P-value < 0.05: Significant; FET: Fischer's exact test; MC : Monte-Carlo test, t: Independent t-test

There was no statistically significant difference between the two groups in hemoglobin levels, WBC count, platelet count, and eGFR at six weeks, three months, and six months. Both groups showed a significant increase in hemoglobin levels over time. WBC count significantly increased at three months compared to six weeks only in the insufficient vitamin D group. Platelet count and eGFR remained unchanged over the follow-up period in both groups (**Table.4**).

Table 4. Comparison of serum hemoglobin level, WBCs count, platelets count, and eGFR according to the basal vitamin D level throughout the duration of follow-up

Hemoglobin level (gm/dl)		Group 1 No. = 8	Group 2 No. = 42	Test value	P-value	Sig.
Six weeks		9.46 ± 1.04	9.38 ± 1.33	T= 0.174	0.862	NS
Three months		9.94 ± 0.89	9.73 ± 1.07	T= 0.518	0.607	NS
Six months		10.35 ± 0.72	10.20 ± 0.95	T= 0.424	0.674	NS
Comparison between Durations (Paired samples t-test)	P1	0.010*	< 0.001*			
	P2	0.016*	< 0.001*			
	P3	0.083	0.001*			
WBCs count (10^3/fl)						
Six weeks		9.9 (7.9 – 19)	8.65 (5.1 – 45.9)	z= -1.642	0.101	NS

Three months	9.89 (5.9 – 15.8)		9.6 (4.9 – 18.6)	z= - 0.569	0.576	NS
Six months	9.02 (6.5 – 12.5)		8.69 (6.10 – 17.9)	z= - 0.199	0.843	NS
Comparison between Durations (Wlcoxon signed rank test)	P1	0.528	0.041*			
	P2	0.093	0.516			
	P3	0.093	0.099			
Platelets count (10^3/fl)						
Six weeks	269.88 ± 72.43		241.33 ± 71.04	T= 1.038	0.304	NS
Three months	236.88 ± 45.95		258.12 ± 64.25	T= - 0.889	0.378	NS
Six months	237.63 ± 51.46		264.62 ± 63.13	T= -1.137	0.261	NS
Comparison between Durations (Paired samples t-test)	P1	0.200	0.106			
	P2	0.329	0.056			
	P3	0.980	0.359			
eGFR (ml/hr/1.73 m2)						
Six weeks	86.48 ± 19.39		84.13 ± 21.64	T= 0.285	0.777	NS
Three months	79.54 ± 11.62		79.73 ± 14.76	T= - 0.035	0.972	NS
Six months	77.31 ± 11.18		79.65 ± 14.62	T= 0.410	0.671	NS
Comparison between Durations (Paired samples t-test)	P1	0.385	0.075			
	P2	0.241	0.091			
	P3	0.348	0.961			

NS: Not significant (NS); t: Independent t-test; z: Mann-Whitney U test; FET: Fisher's exact test; MC: Monte Carlo test; P1: 6 weeks vs. 3 months; P2: 6 weeks vs. 6 months; P3: 3 months vs. 6 months.

Serum urea, creatinine, CRP, and Tac levels remained similar between the two groups throughout the six-month follow-up period (six weeks, three months, and six months). In the insufficient vitamin D group, serum urea significantly decreased at three and six months compared to six weeks. Serum creatinine and CRP levels showed no significant change over time in both groups. Tac levels significantly decreased at six months compared to six weeks and three months in both groups (**Table.5**).

Table 5. Comparison of serum urea, creatinine, CRP level, and Tacrolimus level according to the basal vitamin D level throughout the duration of follow-up

Serum urea (mg/dl)		**Group 1** **No. = 8**	**Group 2** **No. = 42**	**Test value**	**P-value**	**Sig.**
Six weeks		33.63 ± 7.65	35.81 ± 7.87	T= - 0.723	0.473	NS
Three months		30.38 ± 6.76	31.98 ± 7.21	T= - 0.581	0.564	NS
Six months		31.38 ± 6.89	31.43 ± 6.58	T= - 0.021	0.983	NS
Comparison between Durations (Paired samples t-test)	**P1**	0.394	0.003*			
	P2	0.504	0.003*			
	P3	0.655	0.655			
Serum creatinine (mg/dl)						
Six weeks		0.96 ± 0.21	1.03 ± 0.23	T= - 0.729	0.469	NS

Three months		1.01 ± 0.14	1.05 ± 0.15	T= -0.568	0.573	NS
Six months		1.04 ± 0.13	1.05 ± 0.15	T= -0.140	0.889	NS
Comparison between Durations (Paired samples t-test)	**P1**	0.516	0.416			
	P2	0.320	0.456			
	P3	0.351	0.999			
CRP level (mg/dl)						
Six weeks						
Less than 6		7 (87.5%)	36 (85.7%)	MC = 1.435	0.838	NS
10		0 (0%)	1 (2.4%)			
12		1 (12.5%)	2 (4.8%)			
18		0 (0%)	2 (4.8%)			
24		0 (0%)	1 (2.4%)			
Three months						
Less than 6		7 (87.5%)	37 (88.1%)	MC = 0.622	0.733	NS
6		1 (12.5%)	3 (7.1%)			
12		0 (0%)	2 (4.8%)			
Six months						
Less than 6		7 (87.5%)	37 (88.1%)	MC = 2.482	0.779	NS
10		0 (0%)	1 (2.4%)			
16		0 (0%)	1 (2.4%)			
18		0 (0%)	1 (2.4%)			
24		1 (12.5%)	1 (2.4%)			
30		0 (0%)	1 (2.4%)			
Comparison between Durations (Paired samples t-test)	**P1**	0.652	0.642			
	P2	0.487	0.570			
	P3	0.522	0.64			
Tac trough level (ng/ml)						
Six weeks		11.53 ± 1.04	11.24 ± 0.98	T= 0.764	0.449	NS
Three months		10.76 ± 0.92	11.11 ± 0.76	T= 0.652	0.253	NS
Six months		8.76 ± 0.77	8.83 ± 0.81	T= -0.242	0.810	NS
Comparison between Durations (Paired samples t-test)	**P1**	0.231	0.522			
	P2	0.001*	< 0.001*			
	P3	0.003*	< 0.001*			

NS: Not significant; t: Independent t-test; P1: 6 weeks vs. 3 months; P2: 6 weeks vs. 6 months; P3: 3 months vs. 6 months.

Discussion

Kidney transplantation is the treatment of choice for patients with end-stage kidney disease (ESKD), markedly enhancing both survival rates and quality of life **(The et al., 2024)**. Despite substantial improvements in short-term outcomes, with one-year patient and graft survival rates surpassing 90% **Banas et al. (2020)**, the challenge of achieving long-term graft survival persists.

Graft deterioration in kidney transplant recipients can be attributed to various immunological and non-immunological factors, including vitamin D deficiency **(Hesketh et al., 2014; Andrian et al., 2023).** This study investigates the high prevalence of vitamin D deficiency among kidney transplant

recipients and its potential impact on graft function, emphasizing the crucial need for routine monitoring and appropriate management of vitamin D levels to optimize post-transplant outcomes. The demographic characteristics of the current study align with those observed by **Iqbal et al. (2024)**, who examined a cohort of 102 adult kidney transplant recipients with a mean age of 35 ± 8 years and a predominantly male population (89% male, 11% female).

Our study population shared similar demographic characteristics with that of **Buyukdemirci et al. (2022)**, whose study included 52 of 130 kidney transplant recipients with a mean age of 41 ± 11.9 years, a predominantly male composition (73.1% male, 26.9% female), and a mean HLA mismatch of 3 ± 1.

The distribution of ESRD causes in our study was consistent with Erdem et al., who reported unknown causes as the most frequent (30%), followed by glomerulonephritis (16%), diabetes mellitus (13.33%), and hypertension, vesicoureteral reflux, polycystic kidney disease, and other causes (10% each). Similarly, our baseline vitamin D levels were comparable to those reported by **Zimmerman et al. (2017)**: sufficient (34%), insufficient (30%), and deficient (36%)

In our study steroids and Tacrolimus were used in all the cases while cellcept was used in 60% and myfortic in 40%. There were 31 cases with no induction therapy while ATG was used in 24% and Basiliximab in 14%.

Similarly, **Mehrotra et al., 2020** in a study conducted on 52 CKD patients on dialysis going for transplantation that were prospectively studied before and after renal transplantation, reported that all patients used Tacrolimus except for one, while Basiliximab was used in 41 (78.8%) and ATG was used in 6 (11.5%) patients.

Regarding basic demographics according to basal vitamin D status, our results agreed with a study by **Thorsen et al., 2019** which found that age as a significant influencer on vitamin D, while sex and BMI did not significantly influence vitamin D level.

Regarding causes of ESRD according to basal vitamin D status, our study was in alignment with **Thorsen et al., 2019** who found that between the groups of patients there was no statistically significant difference between ESRD and vitamin d levels.

Concerning serum hemoglobin level according to the basal vitamin D level along the duration of follow up, our results agreed with **Buyukdemirci et al., 2022** who noted that there was no there was no significant difference between two groups regarding hemoglobin 10 weeks post-transplant with an increase in baseline hemoglobin level.

Regarding the WBCs count according to the basal vitamin D level along the duration of follow up, our study was in line with **Bienaimé et al., 2013** found no statistically significant difference between the studied groups regarding the WBCs count 3 months post-transplant.

Regarding the platelets count, our results were in coincidence with the previous result by **Stavroulopoulos et al., 2007** who found no statistically significant difference between the two studied groups regarding the platelets count post-transplant.

Our findings regarding eGFR align with **Erdem et al. (2018)**, who found no significant correlation between vitamin D levels (both baseline and post-transplant) and eGFR, and also reported no significant change in vitamin D levels over time.

In contrast, **Mehrotra et al. (2020)** reported that vitamin D deficiency (<20 ng/mL) at 3 months post-transplant was associated with lower eGFR compared to higher vitamin D levels (>20 ng/mL).

Similarly, **Thorsen et al. (2019)** found that sufficient vitamin D at 10 weeks post-transplant was associated with lower serum creatinine. Regarding serum urea, our study is consistent with

Buyukdemirci et al. (2022), who found no significant difference between groups, but observed a decrease in urea levels in the group with vitamin D >15 ng/mL.

Analysis of serum creatinine levels based on baseline vitamin D status showed no significant differences between the two groups at any time point (6 weeks, 3 months, and 6 months). Moreover, creatinine levels remained stable within both groups throughout the 6-month follow-up.

Regarding renal function, **Mehrotra et al. (2020)** observed significantly higher serum creatinine levels at 3-, 6-, and 12-months post-transplant in recipients with vitamin D deficiency (<20 ng/mL) compared to those with sufficient vitamin D levels (>20 ng/mL). In contrast, neither our study nor **Buyukdemirci et al. (2022)** found any significant differences in C-reactive protein (CRP) levels between vitamin D-deficient and sufficient recipients at any time point. Furthermore, we observed no significant changes in CRP levels within either group throughout the follow-up period. Similarly, for tacrolimus (Tac) levels, we did not find any significant differences between vitamin D-deficient and sufficient groups at 6 weeks, 3 months, or 6 months. However, within both groups, Tac levels were significantly lower at 6 months compared to both 6 weeks and 3 months.

About 85% of urea is eliminated through the kidneys, whereas the rest is excreted through the gastrointestinal (GI) tract. Serum urea levels increase in conditions where renal clearance decreases, such as acute and chronic renal failure or impairment. Urea may also increase in other conditions not related to renal diseases, such as upper gastrointestinal bleeding, dehydration, catabolic states, and high protein diets. Urea may be decreased in starvation, low-protein diet, and severe liver disease. Compared to urea, creatinine is less affected by diet and more suitable as an indicator of renal function **(Salazar 2014)**.

Consistent with our findings, **Mehrotra et al. (2020)** reported no significant difference in serum Tac levels between groups at 3 months post-transplant. They did, however, observe a trend towards lower Tac levels at 3 months in patients with vitamin D levels ≥20 ng/ml, though this difference was not statistically significant. This study is limited by its single-center design, which restricts generalizability, and its small sample size, which may have limited the ability to establish causality.

Study limitations: This study has several limitations. Its single-center nature limits the generalizability of the findings to a broader population. Additionally, the study population was small, which might have been insufficient for establishing definitive cause-and-effect relationships.

Conclusions

Due to the high prevalence of vitamin D deficiency in kidney transplant recipients and its association with impaired long-term graft function, regular monitoring and supplementation to optimize vitamin D levels may be a clinically relevant strategy to improve long-term graft outcomes and patient health.

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