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Serum Vitamin D Levels in Kidney Transplant Recipients

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Abstract

Introduction. Vitamin D deficiency is very common, and in kidney transplant recipients (KTRs) it has a high prevalence of up to 80%. The classical and non-classical effects of vitamin D deficiency are complicated by the use of steroids and calcineurin inhibitors (CNIs) in the KTRs.
Methods. This cross-sectional study has been performed at Clinic of Nephrology, Clinical Center University of Sarajevo. The total of 106 KTRs has participated in the study. Based on serum vitamin D values they were divided into 3 groups: deficiency, insufficiency and sufficiency of vitamin D.
Results. Vitamin D deficiency was diagnosed in 32.2% of patients, vitamin D insufficiency in 60% of patients, while only 7.7% had sufficient serum vitamin D values. The Vitamin D deficiency was associated with CNIs and mycophenolate treatment, while no association was seen with oral or pulse steroid treatment. Other variables included in analysis: proteinuria, eGFR, the time elapsed after transplantation, and kidney transplantation diseased or living donors were significantly associated neither with vitamin D insufficiency, nor vitamin D deficiency.
Conclusion. Our study showed a high prevalence of hypovitaminosis D in kidney transplant recipients. The vitamin D status of the patients in our transplant center was influenced by a broad spectrum of factors. In addition to the well-known determinants of vitamin D, a significant influence of calcineurin inhibitor and mycophenolate treatment on vitamin D was observed. Further studies still need to investigate and explicitly clarify the possible link between immunosuppressive therapy and vitamin D in kidney transplant recipients.

Keywords: kidney transplant recipients, vitamin D, insufficiency, deficiency

Introduction

Vitamin D deficiency results in increased risk for osteoporosis in adults. Also, the deficiency is associated with myopathies, autoimmune and cardiovascular diseases, and an increased prevalence of various cancers. Vitamin D is produced de novo following sun exposure, and 10-20% of the recommended daily intake is typically obtained from dietary sources. Vitamin D deficiency is prevalent worldwide, particularly among patients with chronic kidney disease [CKD] [1]. It is difficult to predict vitamin D levels in the kidney recipient population. Deficiency can be expected for several reasons. Hypovitaminosis D appears in kidney transplant patients as a result of immunosupression therapy and low sun exposure, as well as prefiguring kidney disease. Some degree of CKD exists in most of the recipients, and patients are advised to avoid sun exposure because of an increased skin cancer risk. Also, corticosteroids commonly used against rejection, increase vitamin D catabolism. However, compared to CKD patients, transplant recipients can maintain an active lifestyle with possibly more sun exposure and can consume a more diverse diet that could be richer in vitamin D [2]. Several studies examined vitamin D deficiency prevalence in kidney transplant recipients, mostly finding it to be common. Inadequately low levels were found in 80–97% of kidney recipients [KTRs] examined in various countries in Europe [3-6]. In our country, the exact prevalence of nutritional vitamin D deficiency in this population is unknown. The aim of the present study was, therefore, to explore, in a cross-sectional design, the prevalence of vitamin D deficiency in kidney recipients in Clinical Center University [CCU] of Sarajevo, Bosnia and Herzegovina, and to identify possible associated factors.

Material and methods

This cross-sectional study has been performed at Clinic of Nephrology, CCU Sarajevo, Bosnia and Herzegovina. The total of 106 kidney transplant patients participated into the study. Based on serum vitamin D values they were divided into 3 groups: deficiency, insufficiency,

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and sufficiency of vitamin D. The only exclusion criterion was the refusal or inability of the patient to sign the informed consent.

Each patient was asked to complete a questionnaire on demographic details, the cause of end-stage kidney disease, transplant type, or time when dialysis begun. The questionnaire also included questions of patients' consumption of vitamin supplements, food additives, and "natural" supplements.

Patients' records were used to complete the reported data and prescribed treatment. The immunosuppressive regimen, vitamin supplements, and cholecalciferol were recorded for dosage, whereas other medications were documented as taken or not. Serum vitamin D levels were tested using an electrochemiluminescence immunoassay on Elecsys [Roche Diagnostics, Mannheim, Germany]. Vitamin D status was defined according to the K/DOQI guidelines for kidney disease patients, considering serum concentrations ≥30 ng/ml as adequacy, 16-30 ng/ml as insufficiency, and ≤15 as a deficiency. Blood urea nitrogen, serum creatinine, and urine for 24 hours protein and creatinine secretion were measured using a Cobas system (Roche Diagnostics). Serum calcineurin [CNIs] trough levels were measured: tacrolimus, cyclosporine using CMIA assays on an Architect i1000SR system (Abbott Diagnostics, Abbott Park, IL, USA).

Statistical analysis was performed using the three above mentioned categories of vitamin D sufficiency state as well as a two-category set contrasting patients with a proper deficiency from patients with insufficiency or adequacy pooled together. Statistical analyses were performed using SPSS 21 Windows (version 21.0, SPSS Inc, Chicago, Illinois, USA). Analyses of variance or Student's t-test were performed for normally distributed variables, and Mann-Whitney tests for variables that were not. Categorical variables were analyzed using Pearson's chi-square test, as appropriate. Correlations between continuous variables were assessed using Pearson's correlation or Spearman's rho. Multiple regression analysis was applied to examine the relationship between vitamin D levels and a set of immunosuppressive regimen and laboratory parameters. All tests were two-sided, and P values <0.05 or at a confidence level of 95% were considered significant.

Results

We enrolled 106 patients who met our inclusion criteria. Table 1 shows characteristics of the patients, including their vitamin D and proteinuria results (provided as means ± standard deviation). Majority of patients were treated with triple immunosuppressive regimen (CNIs, mycophenolate, and steroids).

Table 1. Characteristics of the kidney transplant recipients

	All patients	Vitamin D deficiency	Vitamin D insufficiency	Vitamin D adequacy
No. of patients (%)	106 (100)	31 (32.3)	67 (60)	8 (7.7)
Gender (M/F)	76/30	24/7	47/20	5/3
Age (years)	48.03±13.19	49.22±9.18	47.12±12.21	48.84±12.22
Creatinine (μmol/L)	114.5(93-164)	122.0 (97-157)	115.0 (98-150)	133 (125.5-189)
eGFR (ml/min/1,73m^2)	64.6 (44.7-86.3)	59.8 (49.1-86.3)	76.5 (55.7-106.7)	62,8 (53.15-76,4)
Parathyreoid hormone (pg/ml)	117 (78.2-227)	132 (107-228)	127.5 (98-216)	113.5 (70.5-239)
Proteinuria (g/l)	0.125 (0.05-0.34)	0.13 (0.05-0.22)	0.15 (0.07-0.36)	0.45 (0.35-0.85)
Time after Tx (years)	5.3 (2.7-9.96)	4.75 (2.7-6.25)	5.5 (3.8-6.65)	5.92 (4.23-10.16)
Tx Live related (No.)	59 (55.7%)	18	37	4
Tx Live unrelated (No.)	27 (25.5%)	8	18	1
Tx deceased donor (No.)	20 (18.9%)	5	12	3
Tacrolimus QD (No.)	67 (63.2%)	8	41	18
Tacrolimus BID (No.)	30 (28.3%)	16	11	3
Cyclosporin A (No.)	9 (8.5%)	5	4	
Mycophenolate mofetil (No.)	17 (16.04%)	10	5	2
Mycophenolate sodium (No.)	86 (81.1%)	9	67	10
Glucocorticoids (No.)	49 (46.2%)	21	20	8
Sirolimus (No.)	1 (0.9%)	1	0	
Everolimus (No.)	2 (1.9%)		2	
Vitamin D(ng/ml)	19.45±8,5	12.15±4.5	19.35±10.5	30.5±9.5
Supplement vit.D (Yes/ No)	47 (44.3%)	24	13	10

The mean value of vitamin D in patients after kidney transplantation was 19.45+8.5 ng/ml. Out of the total number of patients, 32.3% were in the vitamin D deficiency group, 60% belonged to the group with the insufficiency of vitamin D, and 7.7% of patients had sufficient values of vitamin D (Figure 1).

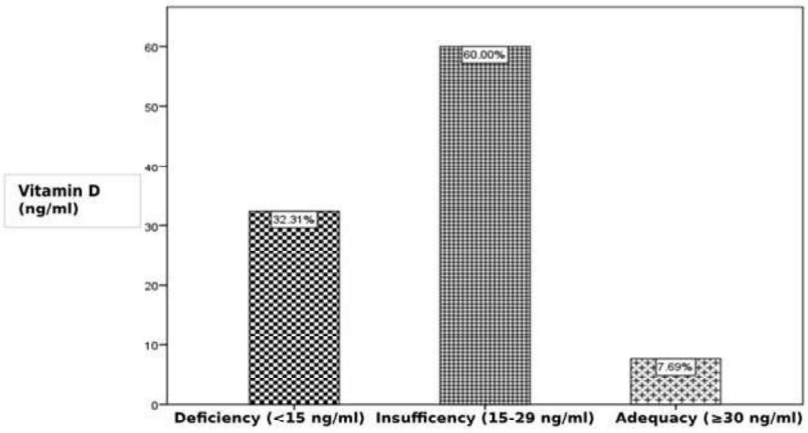

Fig. 1. Distribution of Vitamin D in kidney transplant patients

Of the total number of patients, 47 patients used therapy with vitamin D substituents. No significant statistical differences between the values of vitamin D were observed in the group of patients treated with supplement vitamin D in comparison to the group od patients without vitamin D substituent treatment [19,95 (14,78-25,53) vs. 15,55 (9,42-24,33)] ng/ml (Figure 2).

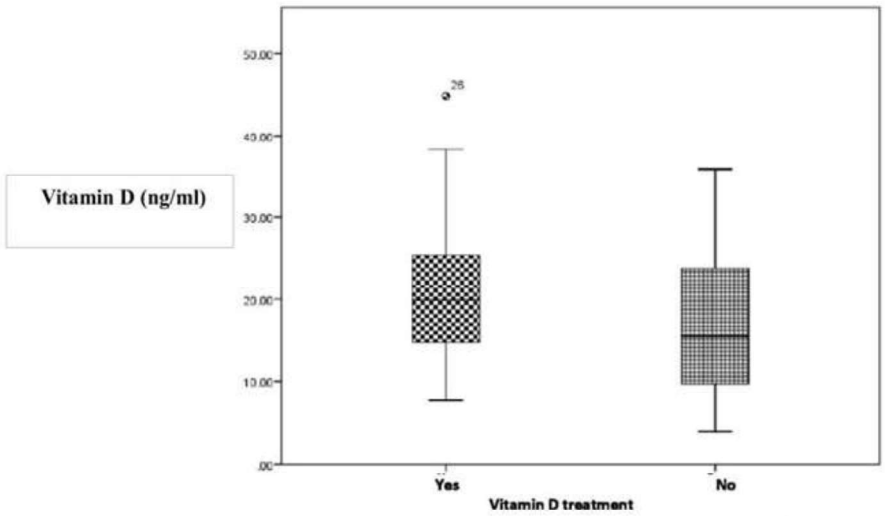

Fig. 2. The difference between the values of vitamin D depending on vitamin D treatment

Also, we tested whether the time elapsed from the transplantation was associated with vitamin D deficiency (Table 2). Vitamin D deficiency was not associated with the type of transplantation, history of previous kidney transplantations, or cause of the kidney failure. Interestingly, the deficiency was significantly associated with a shorter time from transplantation. Most of the kidney recipients at our clinic are treated with a combination of a corticosteroid, an antimetabolite, and a CNIs. We explored the effect of prescribed doses of these medications on the vitamin D levels (Table 2). Increased prednisone doses were associated with lower vitamin D levels, both by correlation to measured levels [P=0.04, correlation coefficient -0.201] and by the association to deficiency state categories [P=0.004]. Treatment with mycophenolate mofetil irrespective of dosing was significantly associated with vitamin D deficiency (P= 0.04). Interestingly, no correlation was found between the dosing of mycophenolate sodium and vitamin D levels. When considered irrespective of preparation (as sodium or mofetil), mycophenolate dose was signifycantly inversely correlated with vitamin D levels (P= 0.054, correlation coefficient -0.130), but was not associated with deficiency. Treatment with tacrolimus irrespective of dosing was also associated with vitamin D deficiency (P=0.02) (Table 2). Also, we found a significant association of higher vitamin D level with tacrolimus QD treatment in comparison to tacrolimus BID and cyclosporine treatment (P=0.015) (Figure 3).

Table 2. Transplantation characteristics, cholecalciferol and immunosuppression regimen in kidney transplant patients according to the status of vitamin D

	All patients (106)	Vitamin D Deficiency (%)	Vitamin D Insufficiency (%)	Vitamin D Adequacy (%)	P †*
Time elapsed from transplantation (yr)	5.3(2.7-9.96)	3.8 (3.1-5.4)	5.5(2.7-8,8)	7.1(3.3-9.9)	0.03
Prednisone daily dose (mg)	6.1 ± 5.8	8.0 ± 9.3	6.0 ± 4.8	4.6 ± 1.8	0.004
Treatment with prednisone (%)	89(98)	23(22.8)	54(53.5)	24(23.8)	NS
Treatment with mycophenolate mofetil (%)	17(16.04)	4(23.52)	13(17.47)	1(5.8)	0.042
Treatment with mycophenolate sodium(%)	86(81.1)	16(18.6)	63(73.3)	7(8.1)	NS
Treatment with tacrolimus (%)	92(86.8)	26(28.3)	61(66.3)	5(5.4)	0.022
Treatment with cyclosporine (%)	9(8.5)	2(22.2)	6(66.7)	1(11.1)	0.197
Treatment with everolimus (%)	3(2.83)	1	1	1	-
Treatment with sirolimus (%)	2(1.9)	-	2	-	-
Tacrolimus trough level (ng/ml)	5.53 ± 3.9	5.2 ± 2.2	5.7 ± 2.7	6.7 ± 3.0	NS
Cyclospoirne trough level(ng/ml)	66.3 ± 19	60 ± 16	69 ± 19	70 ± 21	NS
Everolimus trough level (ng/ml)	4.5 ± 1.4	-	5.4 ± 1.1	3.5 ± 0.8	NS
Sirolimus trough level (ng/ml)	12.4 ± 5.1	-	12.4 ± 5.1	-	-
Cholecalciferol (daily dose (IU))	407 ± 646	248 ± 443	454 ± 625	600 ± 865	0.056

Legend: †Two-sided P values for comparisons between the three categories of vitamin D sufficiency state; *P values < 0.05 were considered significant; NS not significant

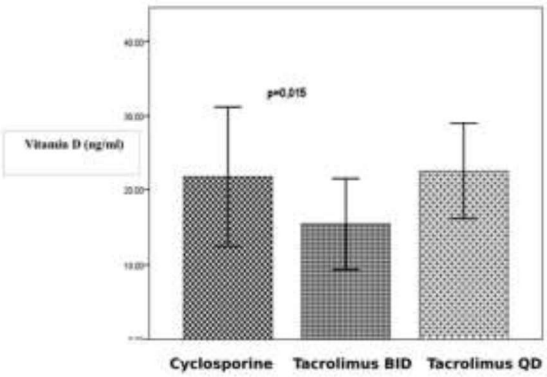

Fig. 3. Vitamin D values depending on the type of calcineurin inhibitor treatment

Due to the large number of possible factors influencing vitamin D status in KTRs, linear regression analysis was performed. The model was statistically significant and could explain between 52% (R2 Cox and Snell) and 77% (R2 Nagelkerkea) variance results and correctly classified 60% of cases (Table 3). Variables included in this statistical method were proteinuria, eGFR, time after kidney transplantation, kidney transplantation diseased or living donors, and treatment with CNIs, mycophenolates treatment, as well as oral and pulse steroid treatment. We evaluated which factors were independent predictors of vitamin D deficiency and insufficiency in KTRs during the monitoring period. Among all factors tested, several statistically significant predictors were identified, with a negative or positive influence on vitamin D values. Treatment with CNIs, mycophenolates treatment, and pulse steroid treatment were significantly associated with vitamin D insufficiency, while oral steroids intake was not. Furthermore, CNIs treatment and mycophenolates treatment were significantly associated with vitamin D deficiency, while oral steroid treatment and pulse steroid treatment were not. Other variables included in linear regression analysis: proteinuria, eGFR, the time elapsed after transplantation, and kidney transplantation diseased or living donors were significantly associated neither with vitamin D insufficiency, nor vitamin D deficiency. A logistic regression model for vitamin D deficiency as a dependent variable, taking as independent variables the va-

Table 3. Effect of immunosuppressive regimen on vitamin D levels in KTRs

Model	B	SE	p-value	Exp(B)	95% CI
Vitamin D insufficiency					
CNIs	-0.173	0.080	0.038	0.033	0.002-0.898
Oral steroids	0.019	0.076	0.642	0.877	0.795-0.967
Pulse steroids	-1.402	0.652	0.032	4.063	1.131-14.591
Mycophenolates	0.171	0.078	0.029	1.186	1.018-1.383
Vitamin D deficiency					
CNI	-0.434	0.204	0.033	1.544	1.035-2.304
Oral steroids	-0.096	0.066	0.264	0.658	0.485-0.893
Pulse steroids	-0.159	0.086	0.821	0.733	0.611-0.880
Mycophenolates	-3.137	1.546	0.042	0.043	0.002-0.898
Dependent variable: Vitamin D level					

Legend: SE- Standard error; CI- Confidence Interval; CNIs- calcineurin inhibitors

riables found associated independently with deficiency, revealed that the use of tacrolimus or mycophenolate were both associated with vitamin D deficiency, quasi-R^2 being 58%.

Discussion

This study was undertaken to explore the prevalence of vitamin D deficiency in KTRs in CCU of Sarajevo and to assess possible factors affecting the vitamin D status of these patients.

Adequate vitamin D levels were found in only 7.7% of the kidney recipients, 60% had insufficient levels and 32.3% showed a definite deficiency. This distribution is substantially better than demonstrated in most previous studies of kidney transplant patients [7,8].

Stavroulopoulos *et al*. [6] found 90% of hypovitaminosis D in renal transplant recipients in England, Unger *et al*. observed 77.4% rate of hypovitaminosis in healthy individuals [9], and Jean *et al*. [10] has reported it in approximatively 90% of CKD and dialysis patients.

In this study, the prevalence of hypovitaminosis D in renal transplant recipients was similar to those found in other geographic regions of the world [7]. The factors responsible for this high prevalence of hypovitaminosis D after the kidney transplant, even in high sun exposure areas, are unclear.

Although hypovitaminosis D was the aim of many studies, still there is not a consensus about the cholecalciferol dosage, especially in renal transplant recipienta. The Kidney Disease Outcome Quality Initiative gave recommendations concerning the treatment of vitamin D deficiency in both CKD and renal transplant recipients, suggesting treatment strategies applied to the general population [11].

Serum levels of vitamin D were directly correlated with vitamin D supplementation, though only weakly, and vitamin D deficiency was associated with lower vitamin D supplementation doses. Vitamin D supplementation at doses of 400 IU/day led to no significant change in vitamin D levels of kidney recipients, while supplementation doses in the order of 7000 IU/day produced a dramatic increase [12]. Supplementation doses in our study were low, which might account for the partially observed effect of supplementation. We found no association between vitamin D and kidney function or proteinuria, being in line with previous reports [13]. Our findings confirmed the data from previous studies that have shown low doses of vitamin D supplements did not improve vitamin D deficiency [14]. There were not more treated patients in the group with normal vitamin D levels, and there were no differences in vitamin D concentrations between treated and untreated patients.

Prevalence rates of vitamin D deficiency and insufficiency found in our study were similar to those of the general population [15]. This might be explained by the intensity of medical follow-up of the transplant patients, counteracting their multifactorial stronger predisposition toward hypovitaminosis D. Schreiber *et al*. [16] found the lack of difference in hypovitaminosis D between kidney and liver transplant patients, despite limitations of the comparison. Those results suggest that the type of organ transplanted is not of paramount importance to the risk of vitamin D deficiency. The lack of difference in vitamin D deficiency prevalence between KTRs with organs from different donors in our study implies that kidney function is not a major risk factor for deficiency in this population, concurring with our finding of no association between eGFR and vitamin D deficiency within the kidney transplant group (data not shown).

Vitamin D deficiency was associated with several aspects of the immunosuppressive treatment, partially contrasting with the results of previous studies of these agents in autoimmune diseases [17]. Higher prednisone doses were associated with lower vitamin D concentrations and a greater tendency towards deficiency. This might be explained by the stimulatory effect of glucocorticoids on vitamin D catabolism [14], or related to the reason necessitating the higher steroid dose. Tretment with mycophenolate sodium, irrespective of dose, was found to be associated with vitamin D insufficiency. Crucial for the study, in our institution the choice between mycophenolate preparations is arbitrary; hence, a confounding factor related to the choice of preparation seems less plausible.

The relationship between calcineurin inhibitors [CNIs] and vitamin D metabolism has been studied with conflicting reports. Grenet *et al*. [18] reported increased 1,25-(OH)2D levels, decreased calbindin-D28k, decreased vitamin D receptor and 24-hydroxylase expression in Wistar rats treated with cyclosporine A. Our results indicate that CNIs intake is associated with lower 25 (OH)D concentrations, while treatment with mTORI and their affection on vitamin D status after kidney transplantation could not be estimated because of the very small number of patients on that treatment. Eyal *et al*. [19] found a negative influence of tacrolimus and other immunosuppressive medications on 25(OH)D in KTRs. A possible explanation for these findings may be the fact that liver CYP3A4 has 25-hydroxylase activity which is suppressed by CNIs resulting in lower 25(OH)D [18].

Our study demonstrated that tacrolimus QD and cyclosporine treatment is superior to the tacrolimus BID treatment in maintaining better vitamin D levels in KTRs.

The fact that CNIs and mycophenolate intake, but not steroids dosage, showed a significant association with vitamin D deficiency, suggests that the association reported here might result from a factor associated with both vitamin D levels and the need for greater tacrolimus doses to reach goal concentrations, such as metabolism rates or intestinal lipid absorption [20]. Due to the results of our study, we have concluded that CNIs and

mycophenolate intake are an independent predictor of vitamin D deficiency in KTRs.
Our study is a cross-sectional retrospective one, which is its major disadvantage. However, most reports on vitamin D after kidney transplant share this limitation. Further prospectively designed research would be needed for more accurate assessment of the link between CNIs and vitamin D status.

Conclusion

Our study revealed high prevalence of hypovitaminosis D in kidney transplant recipients. The vitamin D status of the patients in our transplant center was influenced by a broad spectrum of factors. In addition to the well-known determinants of vitamin D, a significant influence of calcineurin inhibitor and mycophenolate treatment on vitamin D was observed. As calcineurin inhibitors are currently the backbone of immunosuppressive treatment after renal transplantation, further studies still need to investigate to explicitly clarify the possible link between immunosuppressive therapy and vitamin D in kidney transplant recipients.

Conflict of interest statement: None declared

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