Osmangazi Tıp Dergisi
Osmangazi Journal of Medicine 2022;

Research Article / Araştırma Makalesi

Vitamin D Receptor Contents and Receptor Expression Rates of CD4+ and CD8+ T Lymphocytes in Renal Transplant Recipients

Böbrek Nakli Alıcılarında CD4+ Ve CD8+ T Lenfositlerin Vitamin D Reseptör İçerikleri ve Reseptör Ekspresyon Hızları

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Abstract

This study aims to evaluate the vitamin D receptor (VDR) expression and intracellular amounts of VDRs in CD4+ and CD8+ lymphocytes of renal transplant (RT) recipients with chronic allograft dysfunction (CAD). A total of 43 patients (Group 1:RT patients=29 patients, 15 patients CAD proven by renal biopsy (Group 1a), 14 patients stable renal function (Group 1b), Group 2:Control group=14 healthy individuals) have been enrolled in this study. 25-hydroxycholecalciferol, 1.25 dihydroxycholecalciferol levels were measured. The number of cells expressing VDR among the CD4+ and CD8+ type T lymphocytes of the subjects was determined as % of those cell groups. The mean VDR molecule contents per cell have been measured and expressed as mean fluorescence intensities (MFI). No difference was found between Group 1 and Group 2 in terms of their 25-hydroxycholecalciferol, 1.25 dihydroxycholecalciferol levels, and the percentages of the cells expressing VDR in CD4+ and CD8+ cells ($p>0.05$). CD4+/VDR(MFI) and CD8+/VDR(MFI) values were higher in RT patients than healthy subjects ($p<0.001$). When the RT patient subgroups compared, there were no statistically significant differences regarding CD4+/VDR(%), CD8+/VDR(%), CD4+/VDR(MFI) and CD8+/VDR(MFI) values ($p>0.05$). This study showed VDR in T lymphocytes of patients who had RT did not change, but the VDR content in the cells increased due to reasons independent of serum 25-hydroxycholecalciferol and 1.25 dihydroxycholecalciferol levels.

Keywords: CD4+ lymphocytes, CD8+ lymphocytes, 25(OH)D3, 1.25(OH)2D3, Vitamin D receptor, Renal transplantation

Özet

Bu çalışma, kronik allogreft disfonksiyonu (KAD) olan böbrek nakli (BN) alıcılarının CD4+ ve CD8+ lenfositlerinde D vitamini reseptörü (VDR) ekspresyonunu ve hücre içi VDR miktarlarını değerlendirmeyi amaçlamaktadır.Toplam 43 hasta (Grup 1:BN hastaları=29 hasta, böbrek biyopsisi ile KAD kanıtlanmış 15 hasta (Grup 1a), böbrek fonksiyonu stabil 14 hasta (Grup 1b), Grup 2:Kontrol grubu=14 sağlıklı birey) bu çalışmaya dahil edilmiştir. 25-hidroksikolekalsiferol, 1.25 dihidroksikolekalsiferol seviyeleri ölçüldü. Deneklerin CD4+ ve CD8+ tip T lenfositleri arasında VDR eksprese eden hücre sayısı, bu hücre gruplarının %'si olarak belirlendi. Hücre başına ortalama VDR molekülü içeriği ölçülmüş ve ortalama floresan yoğunlukları (MFI) olarak ifade edilmiştir. Grup 1 ve Grup 2 arasında 25-hidroksikolekalsiferol, 1.25 dihidroksikolekalsiferol düzeyleri ve CD4+ ve CD8+ hücrelerinde VDR eksprese eden hücrelerin yüzdeleri açısından fark bulunmadı ($p>0.05$). BN hastalarında CD4+/VDR(MFI) ve CD8+/VDR(MFI) değerleri sağlıklı kişilere göre daha yüksekti ($p<0,001$). BN hasta alt grupları karşılaştırıldığında, CD4+/VDR(%), CD8+/VDR(%), CD4+/VDR(MFI) ve CD8+/VDR(MFI) değerleri açısından istatistiksel olarak anlamlı fark yoktu ($p>0.05$).Bu çalışma, BN uygulanan hastaların T lenfositlerinde VDR'nin değişmediğini, ancak hücrelerdeki VDR içeriğinin serum 25-hidroksikolekalsiferol ve 1.25 dihidroksikolekalsiferol düzeylerinden bağımsız nedenlerle arttığını gösterdi.

Anahtar Kelimeler: CD4+ lenfositler, CD8+ lenfositler, 25(OH)D3, 1.25(OH)2D3, Vitamin D reseptörü, Böbrek nakli

Received 27.07.2021 Accepted 11.08.2021 Online published 16.08.2021

Ural Edebali S, Koksoy S, Yilmaz VT, Ozdem S, Yilmaz F, Sozel H, Ersoy FF, Vitamin D Receptor Contents and Receptor Expression Rates of CD4+ and CD8+ T Lymphocytes in Renal Transplant Recipients, Osmangazi Journal of Medicine, 2022,44(1):25-36
Doi: 10.20515/otd.974905

1. Introduction

Renal transplantation (RT) is the most prominent treatment method for patients developing stage 5 chronic renal disease. Acute/chronic rejections are the main immunological problems that occur after RT.

Active vitamin D level in RT recipients is associated with kidney allograft function. Vitamin D and its analogs, reduce intraglomerular hypertension and proteinuria, limit glomerular and tubulointerstitial damage (1). Vitamin D may also reduce renal fibrosis by inhibiting mesangial cell proliferation, podocyte loss, and prevent podocyte hypertrophy (2). It is suggested to prevent profibrotic cytokine synthesis and renal inflammation (3,4).

Vitamin D receptor (VDR) belongs to the nuclear hormone receptor family and is bound to chromosomal DNA in the nucleus and is found in the cytoplasm. Vitamin D receptors are found in antigen-presenting cells as well as in various immune cells such as T and B lymphocytes, monocytes, macrophages, and mast cells. Active vitamin D prevents the differentiation of dendritic cells and causes their apoptosis (5). It is involved in immune tolerance by preventing antigen presentation to T lymphocytes and thereby the differentiation of antigen-specific T lymphocytes (5). It also inhibits Thelper1 (Th1) differentiation by inhibiting interleukin-12 (6). Vitamin D also inhibits the release of interleukin-2, interleukin -3, interferon-gamma and, tumour necrosis factor α (TNF-a) from the Th1 cells and exhibits anti-inflammatory and anti-rejection activity by stimulating the release of anti-inflammatory cytokines. Correlation between transforming growth factor beta 1 (TGF-beta1) expression and interstitial fibrosis and glomerulosclerosis and its relationship with the development of chronic allograft dysfunction (CAD) has also been shown (7). Active vitamin D analogs have been shown to inhibit the expression of proinflammatory cytokines by inhibiting the nuclear factor kappa B (NF-KB) pathway (4).

CAD is characterized by progressive kidney dysfunction which is manifested by slowly and continuously increasing serum creatinine and proteinuria and frequently, hypertension. The etiopathogenesis of CAD is still uncertain and no definitive therapy and special preventive methods have been established yet (7). The etiology of CAD includes immunological factors such as previous acute rejections, subclinical rejection, antibody-mediated chronic rejection, human leucocyte antigen incompatibility, inadequate immunosuppression, or non-immunological factors associated with viral infections, hyperlipidemia, hypertension, nephrotoxic effects of calcineurin inhibitors (8). Definitive diagnosis requires a biopsy for the exclusion of other factors such as acute rejection, recurrent glomerulonephritis, drug toxicity, and infections.

This study aims to compare serum $25(OH)D_3$ and $1.25(OH)_2D_3$ levels and CD4+ and CD8+ lymphocyte VDR levels in RT patients with healthy people and to evaluate their relationship with some demographic and clinical parameters.

2. Material and Methods

A total of 43 people were enrolled in the study, including 29 RT patients older than 18 years of age who received an RT at Akdeniz University, Faculty of Medicine, Prof. Dr. Tuncer Karpuzoglu Transplantation Center, with a posttransplant period longer than 6 months (Group 1) and age and gender-matched healthy control subjects without any known chronic or acute diseases (Group 2: n=14). RT recipients were divided into two subgroups: patients with chronic allograft dysfunction (Group 1_a:n=15 patients) and patients with stable renal function (Group 1_b:n=14 patients). All demographics such as age, gender, body mass index (BMI), etiology of CKD, duration, and type of dialysis, type of donor, number of rejections and their treatment, immunosuppressive drug use, and clinical,

laboratory results were obtained from the patient files. Glomerular filtration rate (GFR) level was calculated by using the CKD-EPI formula. BMI was calculated by dividing body weight (kg) by the square of the height in meters.

Patients with a pathological diagnosis of chronic allograft dysfunction were included in the study. The diagnosis of CAD was based on the BANFF-2013 classification (9). Patients who have been diagnosed with acute rejection, infection, primary disease, recurrence/glomerulonephritis, etc. were excluded from the study. Furthermore, although the pathological diagnosis was CAD, patients using vitamin D and its metabolites, patients with parathyroidectomy/idiopathic hypoparathyroidism, those with active malignancy were also not included in the study. Those with an active infection, chronic liver disease, non-CKD calcium-phosphorus metabolism disease, and those who did not accept biopsy were excluded from the study. Biopsy results were evaluated by the same expert pathologist in the pathology department of our center. Local ethics committee from XXX University clinical research ethics committee (date/number: 31.07.2013/86) approval was received for the study. The study was performed by the Helsinki 2013 Brasil version. Written informed consent to participate in the study was obtained from the participants.

Blood samples were collected from all healthy participants and RT recipients included in the study and the sera were separated by centrifugation at 3000 rpm for 5 minutes to be kept at -80 C°. Lymphocytes were isolated from the blood cells and the number of cells expressing VDR was given as VDR(%).Determination of the percentage of peripheral lymphocytes expressing VDR was made by flow cytometry. Subsequently, the VDR content in the VDR carrier cells was measured and reported as VDR(MFI) (mean fluorescence intensity).

Based on the Kidney Disease Outcomes Quality Initiative guidelines, levels of $25(OH)D_3$ lower than 5 ng/ml were considered as severe; 5-15 ng/ml as mild vitamin D deficiency and 15-29 mg/ml as vitamin D deficiency. Levels higher than 30 mg/ml were considered as normal and levels higher than 150 mg/ml as vitamin D intoxication.(10) $1.25(OH)_2D_3$ and $25(OH)D_3$ levels were tested to measure the serum vitamin D activity.

Serum creatinine was measured using the Jaffe method; BUN, calcium, phosphorus, albumin were all measured using the enzymatic colorimetric method; Serum intact parathyroid hormone (iPTH) and $25(OH)D_3$ analysis was performed using the electrochemiluminescence immunoassay method using a Cobas 8000 autoanalyzer (Roche Diagnostics, Mannheim, Germany) in the central biochemistry laboratory of our hospital.

Serum 1.25 $(OH)_2D_3$ Analysis

Serum $1.25(OH)_2D_3$ Analysis was carried out using a solid-phase sandwich ELISA method using Cusabio branded kit (Cusabio, Human-1.25- Dihydroxy vitamin D3 (DHVD3), Cat. No: CSB-E05120H). The amounts of $1.25(OH)_2D_3$ in serum samples were calculated from the curve plotted using standards. The results are given in pg / mL.

Statistical analysis

"Statistical Package for Social Sciences (SPSS) v.18.0" package program was used for statistical analysis. The continuous variables were expressed as arithmetic mean ± standard deviation (x ± SD). For numerical parameters with normal distribution properties, unpaired student t-test was used for comparison of two independent groups, while one-way analysis of variance (ANOVA) and Scheffe test as a post-doc test were used for comparison of three independent groups; categorical parameters were compared with the chi-square test. Correlations between vitamin D and VDR levels in various cells were determined by

Pearson correlation analysis. $p<0.05$ was considered statistically significant.

3. Results

There were no statistically significant differences in age, gender, and BMI values between RT recipients and the healthy control groups, as well as RT subgroups 1a and 1b ($p> 0.05$) (Table 1). There was no significant difference between RT patients with and without CAD in terms of dialysis duration, donor types, number of previous acute rejections, age at transplantation date and transplantation vintage, immunosuppressive drug protocols, mismatch numbers, diabetes mellitus and preemptive transplant rate ($p>0.05$) (Table 2).

Table 1. Basic demographics, clinical and laboratory data of patients.

Parameter	Subgroup 1a(CAD+) n=15	Subgroup 1b(CAD-) n=14	Group 2 (HG) n=14	p
Age[t]	45.67±11.67	41.29± 12.89	37.64± 9.43	0.298
BMI[+]	24.80±4.66	24.86±4.02	25±3.23	0.991
BUN	33.20±14.76	13.79±3.79	13.79±4.90	**<0.001***
Creatinine	2.25±0.78	0.88±0.18	0.72±0.19	**<0.001***
GFR	33.75±12.88	93.43±16.0	99.97±13.17	**<0.001***
Calcium	9.09±0.78	10.0±0.65	9.44±0.32	**0.004***
Phosphorus	3.62±1.0	3.41±0.92	3.64±0.58	0.740
Albumin	3.58±0.62	4.35±0.44	4.58±0.25	**<0.001***
iPTH	167.10±164.24	73.38±46.67	49.20±20.55	**0.001***
$25(OH)D_3$	18.98±8.16	23.04±12.58	22.77±11.08	0.593
$1.25(OH)_2D_3$	15.70±9.44	16.50±11.82	12.21±6.73	0.544
CD4+/VDR(%)	65.46±20.0	70.24±9.52	64.01±13.52	0.468
CD8+/VDR(%)	66.83±16.36	72.29±82.9	63.84±12.71	0.224
CD4+/VDR(MFI)	1091.40±331.82	810.86±203.88	627.0±72.0	**<0.001***
CD8+/VDR(MFI)	1013.53±281.14	770.64±183.79	595.43±52.06	**<0.001***

[t]*Kruskal Wallis test;* [+] *One-way analysis of variance (ANOVA)*

Abbreviations: CAD: Chronic allograph dysfunction, HG; Healthy group Tx: Transplantation, CKD: chronic kidney disease, BUN: Blood urea nitrogen, iPTH:intact parathyroid hormone, BMI: body mass index, MFI: MFI: Mean Fluorescence Intensity,GFR: glomerular filtration rate, CD8+/VDR(%): Percentage of VDR-expressing CD8+ lymphocytes, CD4+/VDR(%): Percentage of VDR-expressing CD4+ lymphocytes, CD8+/VDR(MFI): Vitamin D receptor amount in CD8+ lymphocytes, CD4+/VDR(MFI): Vitamin D receptor amount in CD4+ lymphocyte

Table 2. Demographic and clinical characteristics of patients with and without chronic allograft dysfunction

	Subgroup 1a (CAD positive)	Subgroup 1b (CAD negative)	p
Gender[#]			0.858
Women (n,%)	7 (46.7%)	7 (50.0%)	
Men (n,%)	8 (53.3%)	7 (50.0%)	
Etiology of CKD			
Hypertension	13.3%	35.7%	
Of unknown primary cause	26.7%	14.3%	
Urological	33.3%	21.4%	
Other	26.7%	28.6%	

Relatives[#]			0.858
First degree (n,%)	8 (53.3%)	7 (50.0%)	
Fourth degree (n,%)	7 (46.7%)	7 (50.0%)	
Dialysis modality[$]			0.999
Preemptive (n,%)	5 (33.3%)	5 (35.7%)	
HD-PD (n,%)	10 (66.7%)	9 (64.3%)	
Medication[$]			0.999
TAC (n,%)	11 (73.3%)	10 (71.4%)	
Cyclosporin-A(n,%)	4 (26.7%)	4 (28.6%)	
Tx type[$]			0.999
Live (n,%)	12 (80%)	12 (85.7%)	
Cadaver (n,%)	3 (20%)	2 (14.3%)	
Acute rejection episode during follow-up[$]			0.330
No (n,%)	11 (73.3%)	13 (92.9%)	
Yes (n = 16)	4 (26.7%)	1 (7.1%)	
Donor age[+] **(year)**	46.47±11.35	42.36±10.49	0.321
Tx age [ι] **(year)**	43.07±11.86	39.07±12.74	0.484
Tx duration [ι] **(month)**	32.67±18.01	28.21±9.22	0.662
Dialysis duration [ι] **(month)**	20.90±17.12	25.78±27.06	0.902
Number of Missmatches[ι]	3.83±1.64	3.82±2.18	0.682

[#] The chi-square value in the Pearson chi-square test; [$] Fisher's exact test (this test has no test statistic value); [ι] The z value in the Mann-Whitney U test; [+] The t value in the Student t test.

Abbreviations: CAD: Chronic allograph dysfunction, Tx: Transplantation, CKD: chronic kidney disease.

In RT patients with CAD, BUN and creatinine values, eGFR, iPTH, albumin, and calcium were found to be significantly different than patients without CAD ($p < 0.05$). There were no significant differences in $25(OH)D_3$ and $1.25(OH)_2D_3$ and CD4+/VDR(%), CD8+/ VDR(%), CD4+/VDR(MFI), CD8+/VDR(MFI) levels between these two groups ($p > 0.05$) (Table 3).

CD4+/VDR(MFI), CD8+/VDR(MFI) values of patients with CAD were significantly higher, and GFR and albumin values were lower than normal healthy subjects ($p < 0.05$). There were no statistically significant differences between the groups in terms of CD8+/VDR(%), CD4+/ VDR(%).

CD4+/VDR(MFI) and CD8+/VDR(MFI) values of patients with stable renal function without CAD were higher than healthy controls ($p < 0.05$). However, there was no statistically significant difference between the groups in terms of BUN and creatinine, eGFR, albumin, calcium, and iPTH levels (Table 3).

Table 3. Intergroup paired comparisons

	With and without CAD	With CAD and HG	Without CAD and HG
BUN [ι]	**<0.001***	**<0.001***	0.999
Creatinine [ι]	**<0.001***	**<0.001***	0.394
GFR[+]	**<0.001***	**<0.001***	0.443
Calcium [ι]	**0.004***	0.058	0.999
Albumin [ι]	**0.004***	**<0.001***	0.602
iPTH [ι]	0.128	**0.001***	0.312

CD8+VDR(MFI)ᵗ	0.177	**<0.001***	**0.014***
CD4+/VDR(MFI)ᵗ	0.156	**<0.001***	**0.018***
CD8+VDR(%)	0.743	0.520	0.471
CD4+/VDR(%)	0.556	0.494	0.176

*ᵗBonferroni-Dun Test; ⁺ Tukey's post-hoc test; * p<0.05*

Abbreviations: CAD: Chronic allograph dysfunction, HG; Healthy group Tx: Transplantation, CKD: chronic kidney disease, BUN: Blood urea nitrogen, iPTH:intact parathyroid hormone, BMI: body mass index, MFI: MFI: Mean Fluorescence Intensity,GFR: glomerular filtration rate, CD8+/VDR(%): Percentage of VDR-expressing CD8+ lymphocytes, CD4+/VDR(%): Percentage of VDR-expressing CD4+ lymphocytes, CD8+/VDR(MFI): Vitamin D receptor amount in CD8+ lymphocytes, CD4+/VDR(MFI): Vitamin D receptor amount in CD4+ lymphocytes

When 29 RT patients were evaluated as a single group, $25(OH)D_3$, $1.25(OH)_2D_3$, CD4 +/VDR(%) and CD8+/VDR(%) levelswere not different than healthy control group (p> 0.05), while CD4+/VDR(MFI) and CD8+/VDR(MFI) values were higher in RT recipients (p <0.05) (Table 4).

In the CAD group, $25(OH)D_3$ levels indicated a mild deficiency in 26.6%, deficiency in 66.6%, and were within normal limits in 6.6% of participants. In the group of patients without CAD, 21.42% had a mild deficiency, 64.2% had a deficiency, 14.2% were normal, while in the healthy controls 28.6% had a mild deficiency, 50% had a deficiency and 21.4% were normal. There was no statistically significant difference in $25(OH)D_3$, $1.25(OH)_2D_3$ levels between all three groups (p> 0.05).

Table 4. Comparison of $25(OH)D_3$, $1.25(OH)_2D_3$ and VDR in renal transplant recipients and healthy group

	Renal transplant recipients	Healthy group	p
$25(OH)D_3$ᵗ	20.94±10.54	22.77±11.08	0.392
$1.25(OH)_2D_3$ᵗ	16.08±10.47	12.21±6.73	0.271
CD8+/VDR % ᵗ	67.77±15.75	64.01±13.52	0.228
CD8+/VDR(MFI) ᵗ	896.28±265.44	595.43±52.06	**<0.001***
CD4+/VDR(%)ᵗ	69.47±13.17	63.84±12.71	0.108
CD4+/VDR(MFI)ᵗ	955.97±307.74	627.0±72.01	**<0.001***

*ᵗ z statistic in the Mann-Whitney U test; * p<0.05*

*Abbreviations: **CD8+/VDR(%):** Percentage of VDR-expressing CD8+ lymphocytes, **CD4+/VDR(%):** Percentage of VDR-expressing CD4+ lymphocytes, **CD8+/VDR(MFI):** Vitamin D receptor amount in CD8+ lymphocytes, **CD4+/VDR(MFI):** Vitamin D receptor amount in CD4+ lymphocytes*

When the correlation of GFR with $25(OH)D_3$, $1.25(OH)_2D_3$ and VDR was evaluated with Spearman correlation test, there was a significant negative correlation with CD4+/VDR (MFI), CD8+/VDR(MFI) values in RT patients and the healthy group (p <0.05). In patients with CAD, there was a positive correlation between C4+/VDR(%) and CD8+/VDR(%) values and eGFR (p <0.05). In patients without CAD, there was no correlation between eGFR and$25(OH)D_3$, $1.25(OH)_2D_3$, and VDR values (p> 0.05) (Table 5).

Table 5. Correlation of glomerular filtration rate with $25(OH)D_3$, $1.25(OH)_2D_3$and VDR

	$25(OH)D_3$	$1.25(OH)_2D_3$	CD8+/VDR %	CD8+ VDR(MFI)	CD4+/VDR %	CD4+/VDR (MFI)
Whole group n=43						
r	.075	-.034	.039	**-.587****	.079	**-.604****
p	.631	.828	.805	**.000**	.615	**.000**
Group 1 (CAD positive) n=15						
r	.222	-.265	**-.754****	.064	**-.737****	.059
p	.427	.340	**.001**	.820	**.002**	.834
Group 2 (CAD negative) n=14						
r	.192	.285	-.029	.108	.117	.166
p	.511	.324	.922	.713	.690	.572
Group 3 (healthy group) n=14						
r	-.440	.279	.000	**-.612***	.057	**-.667****
p	.115	.333	1,000	**.020**	.846	**.009**

*All analyzes were made using Spearman Correlation Test. ** p<0.01; *p<0.05*

Abbreviatios: CAD: Chronic allograph dysfunction, ***CD8+/VDR(%):*** *Percentage of VDR-expressing CD8+ lymphocytes,* ***CD4+/VDR(%):*** *Percentage of VDR-expressing CD4+ lymphocytes,* ***CD8+/VDR(MFI):*** *Vitamin D receptor amount in CD8+ lymphocytes,* ***CD4+/VDR(MFI):*** *Vitamin D receptor amount in CD4+ lymphocytes*

In terms of the correlation of the percentage of VDR expressing CD4+ and CD8+ T lymphocytes with demographic, clinical, and laboratory parameters were evaluated; CD8+/ VDR(%) was negatively correlated with BMI and positively correlated with donor age ($p < 0.05$). CD4+/VDR(%) had a significant negative correlation with BMI ($p < 0.05$) (Table 6). CD4+/VDR(MFI) and CD8+/VDR(MFI) values had a positive correlation with BUN, creatinine, and iPTH and a negative correlation with eGFR and albumin ($p < 0.05$). VDR did not show any difference in gender, kinship, dialysis, and transplantation types between groups ($p > 0.05$). The percentage of CD4+/VDR(%) was significantly higher in patients using cyclosporin-based immunosuppressive drugs compared to those using tacrolimus-based immunosuppressive drug regimens ($p < 0.05$).

In patients with CAD, there was no statistically significant correlation between vitamin $25(OH)D_3$ levels and VDR values, but there was a negative correlation between $1.25(OH)_2D_3$ levels and CD8+/VDR(MFI) and CD4+/VDR(MFI) values and it was statistically significant ($p < 0.05$). No significant correlation was found between $25(OH)D_3$ and $1.25(OH)_2D_3$ levels and VDR values in patients without CAD and in the healthy group ($p > 0.05$).

Table 6. Analysis for the correlation of vitamin D receptor percentage with demographics, clinical and laboratory data.

	CD8+/VDR%	Age	BMI	Tx Age	Tx Duration	Dialysis Duration	Number of Missmatch	Donor age	Leukocyte	Lymphocyte	Bun	Creatinine	GFR	Calcium	Phosphorus	Alb	iPTH
CD8+/VDR%	-.956**	-	-	-	.202	-.179	-.169	.447*	-.125	.177	-.065	.019	.039	.018	-.070	-.091	.081
r		.128	.330*	.142													
p	.000	0.412	0.03	0.462	0.294	0.464	0.441	0.015	0.426	0.257	0.679	0.905	0.805	0.910	0.658	0.564	.606
n	43	43	43	29	29	19	23	29	43	43	43	43	43	43	43	43	43
CD4+/VDR%		-	-397**	-	.180	-.141	-.081	.352	-.167	.156	-.091	.029	.079	.073	-.087	-.086	.061
r		.167		.198													
p	.	0.286	0.008	0.303	0.350	0.565	0.712	0.061	0.284	0.316	0.561	0.853	0.615	0.640	0.579	0.585	.695
n		43	43	29	29	19	23	29	43	43	43	43	43	43	43	43	43

Abbreviations: *CAD: Chronic allograph dysfunction, HG; Healthy group Tx: Transplantation, CKD: chronic kidney disease, BUN: Blood urea nitrogen, iPTH:intact parathyroid hormone, BMI: body mass index, MFI: MFI: Mean Fluorescence Intensity,GFR: glomerular filtration rate, CD8+/VDR(%): Percentage of VDR-expressing CD8+ lymphocytes, CD4+/VDR(%): Percentage of VDR-expressing CD4+ lymphocytes, CD8+/VDR(MFI): Vitamin D receptor amount in CD8+ lymphocytes, CD4+/VDR(MFI): Vitamin D receptor amount in CD4+ lymphocytes*

4. Discussion

In this study, we compared $25(OH)D_3$, $1.25(OH)_2D_3$ levels, CD4+, CD8+ T lymphocyte VDR expressing rates, and the number of VDR per cell in RT recipients with healthy control subjects and investigated their possible relationship with CAD.

A high rate of $25(OH)D_3$ deficiency was found in RT recipients. That result was similar to previous studies and no difference was observed between the healthy group, indicating a high prevalence of vitamin D deficiency in both patients and healthy individuals (11). There was no significant difference between groups with and without CAD regarding their $25(OH)D_3$, $1.25(OH)_2D_3$, and VDR values in T lymphocytes.

The treatment of many factors such as optimal blood pressure control, metabolic acidosis, renal osteodystrophy, and anemia, which slow the progression of CKD in RT recipients whose chronic kidney disease is confirmed by definition, is not that precise.(12) One of the treatable factors is vitamin D deficiency. The current KDIGO guidelines recommend that RT recipients be evaluated and treated for bone and mineral metabolism disorders as in CKD patients not receiving RT (13). Patients having a transplant generally do not receive vitamin D supplements. Wals et al. reported that 800 IU / day of vitamin D given to RT recipients have hardly increased serum $25(OH)D_3$ level (14). In the study of Courbebaisse et al. the monthly treatment of 100.000 IU vitamin D is recommended for adult RT recipients (4). Wissing et al. reported that treatment with 25.000 U/month of vitamin D given to RT recipients could not improve serum vitamin D levels (15). These studies suggest that the need for vitamin D in RT recipients may be higher than the amount recommended by the general guideline.

The role of vitamin D in acute and chronic allograft rejection has been demonstrated by many studies. Vitamin D deficiency triggers chronic inflammatory processes through various mechanisms (oxidative stress, DNA damage, endothelial dysfunction, increased proinflammatory cytokines, decreased antiinflammatory cytokines, etc.). It was demonstrated that vitamin D treatment slows GFR loss and improves graft function in patients with chronic allograft dysfunction who take vitamin D (1,16). This effect is probably due to the inhibition of profibrotic and proinflammatory pathways by vitamin D (6,17).

Sezer et al. reported that patients with low vitamin D before RT had higher creatinine and proteinuria levels in the first posttransplant year (18). Tanacı et al. reported that osteoporotic RT recipients had less rejection after calcitriol treatment (19). In the study of Uyar et al. the evaluation of 3-year data of 59 patients using calcitriol and 52 patients not using calcitriol for osteoporosis after RT have revealed that creatinine and iPTH levels of patients using calcitriol were significantly lower (20). Wesseling-Perry et al. did not find a relationship between $25(OH)D_3$ level and 2-year graft function in 68 pediatric RT patients with stable graft function (21). Animal studies have shown that $1.25(OH)_2D_3$ prolongs the life of the allograft and is effective in maintaining renal graft function with low-dose cyclosporin A (22). In our study, there was no statistically significant difference between the vitamin levels of RT recipients with and without CAD and also the healthy group of participants.

In this study, CD4+/VDR(MFI) and CD8+/VDR(MFI) levels showing the amount of VDR per cell in all the immune cell types assessed in the RT group were found to be statistically and significantly higher compared with the healthy group. These high levels could be similarly determined in CD4+/VDR (MFI) and CD8+/VDR (MFI) values in patients with CAD. Cell percentages expressing VDR in the RT group and subgroups did not differ from healthy subjects. These results suggested that the VDR amount increased significantly, although the number of cells with VDR expression in the immune system cells of the patients who underwent RT did not change.

Vitamin D resistance occurs in CKD as there is a disruption in the transcription of VDR-regulated genes and the VDR expression of tissues. Uremic plasma suppresses the enzyme 1-alpha hydroxylase and blocks their VDR's and the sensitivity of VDR to vitamin D decreases (23). Activation of VDR by's reduces the activation of dendritic cells and interleukin-2 transcription, preventing antigen presentation to T lymphocytes and antigenic stimulation. Additionally, fibroblast growth factor 23, which rises in the early stages of CKD, inhibits active vitamin D synthesis by suppressing the enzyme 1-alpha hydroxylase (24). Calcineurin inhibitors in RT patients additionally may cause downregulation of VDRs, leading to vitamin D resistance (25). In the subgroup with CAD, the number of receptors may have increased due to the decreased sensitivity of VDR to vitamin D. However, in the subgroup without CAD, the mean VDR count, MFI, was higher, although not statistically significant, compared with the normal healthy group. As a result, in response to the immunosuppressive effects of immunosuppressive drugs, vitamin D may be tried to be used more effectively by increasing the VDR count to maintain immune activation. However, when VDR exceeds a certain cut-off value, immune activation starts, and the question "May this be the onset of graft rejection?" come to mind. Is VDRactivation in immune cells undesirable in transplantation? We do not know. Perhaps VDR'smay needs to be suppressed. This hypothesis needs to be elucidated.

In the study by Lee.C et al., VDR's were suppressed when calcineurin inhibitors were used in animals that had RT (26). In the study by Grenet et al., when using

cyclosporin-A in RT rats, calcium-binding protein (calbindin) and VDR were decreased (27). In our study, patients using cyclosporin-A had significantly higher CD4+/VDR(%) than those using tacrolimus, but there was no difference between VDR amounts. The reason for this is not clearly understood.

The fact that there was a negative significant correlation between GFR, therefore, the level of uremia and CD4+/VDR(MFI) and CD8+/VDR(MFI) in Group 1, including all patients who underwent RT indicated that uremic toxins have suppressed VDR activity. A positive significant correlation was determined between CD4+/VDR(%) and CD8+/VDR(%) values and GFR only in the patients with CAD in terms of the cell percentages demonstrating VDR expression in the immune cells.

In the present study, no correlation was found between the VDR amount in the immune cells and $25(OH)D_3$ and $1.25(OH)_2D_3$ levels. When all patients with RT were included, a positive correlation was found between the VDR activities determined by CD4+/VDR(MFI), CD8+/VDR(MFI) and BUN, creatinine, and iPTH; on the other hand, a negative correlation was found between CD4+/VDR(MFI), CD8+/VDR(MFI) and GFR, albumin. Those findings have suggested that the uremic environment has decreased the VDR activity in CD4+ and CD8+ lymphocytes. A negative significant correlation was found at both CD4+/VDR(MFI), CD8+/VDR(MFI), and $1.25(OH)_2D_3$ levels only in the subgroup with CAD. Although the mechanism of regulation of VDR expression is not fully elucidated, this mechanism depends on calcitriol synthesis and metabolism. The regulation of VDR expression is specific to cell type and has been demonstrated in different cell lines that include both transcription and posttranscription mechanisms (28,29). Changes in serum calcium and phosphorus levels cause differences in VDR expression in the target tissue (30,31). iPTH plays a role in the regulation of VDR expression (30,32).

In this study, a negative correlation was found between CD4+/VDR(%) and CD8+/VDR(%) and BMI. A positive correlation was found between CD8+/VDR(%) and the age of transplant. In patients with CAD, there was a significant positive correlation between CD4+/VDR(%), CD8+/VDR(%)values, and GFR. Since vitamin D is a fat-soluble vitamin, it may have been sequestered in the fat tissue. In the study by Wortsman et al., there was an inverse correlation between BMI and $25(OH)D_3$ levels, but in our study, CD4+/VDR(%) and CD8+/VDR(%) and BMI were negatively correlated (33).

This study has several limitations. The first limitation is the cross-sectional design of the study and the relatively low number of patients. The second is the seasonal variability affecting vitamin D levels was not evaluated.

5. Conclusion

As a sum up, through our findings, we have seen that vitamin D deficiency is frequently observed in RT patients. There was no difference in vitamin D levels, VDR expression rates, and VDR contents per cell among RT patients with chronic allograft dysfunction and those with normal renal function. The effect of vitamin D supplementation on immune functions and long-term graft functions should be evaluated with randomized controlled studies in RT recipients.

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