Endothelial Dysfunction and Vitamin D Levels in Kidney Allograft Recipients

Böbrek Nakli Alıcılarında Vitamin D Düzeyi ve Endotel İşlev Bozukluğu

ABSTRACT

There have been several studies demonstrating a relationship between vitamin D and cardiovascular disease. Vitamin D Deficiency/Insufficiency (D/I) is a common problem in chronic renal disease and also renal transplant recipients, however, there is a variation between the summer and winter periods.

We investigated whether there is a relationship between vitamin D D/I and endothelial dysfunction in renal transplant recipients (RTR). We also evaluated vitamin D levels in summer and winter. Flow-mediated dilatation (FMD), soluble endothelial protein receptor C (sEPRC) and soluble thrombomodulin (sTM) were chosen for markers of endothelial function.

Fifty-five patients were recruited to the study. Winter measurements were performed on March, summer measurements were performed in September.

In the winter, methe an vitamin D level was 18.8±7.5, compared with 34.3±13.0 ng/ml in the summer period (p<0.0001). There were 38 (84%) and 20 (44%) patients in the winter and summer periods with vitamin D D/I, respectively. We did not find any significant association between vitamin D D/I and FMD, sTM or sEPCR in either period.

While vitamin D D/I is a common finding during winter in RTR, it recovers in the summer. Moreover, vitamin D D/I seems not to be associated with endothelial dysfunction.

KEY WORDS: Endothelial function, Flow-mediated dilatation, Kidney disease, Renal transplantation, Vitamin D

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ÖZ

Vitamin D düzeyi ile kardiyovasküler hastalıklar arasında ilişkiyi gösteren birçok çalışma vardır. Vitamin D yetersizliği/eksikliği kronik böbrek hastalıklarında ve böbrek nakli alıcılarında sık gözlenmekte ancak vitamin D düzeyi yaz ve kış aylarında değişiklikler göstermektedir. Biz bu çalışmada vitamin D yetersizlik/eksiklik ile endotel işlev bozukluğu arasında ilişkiyi değerlendirmeyi amaçladık. Yaz ve kış aylarında vitamin D düzeyi değerlendirildi. Endotel işlevleri akım aracılı vazodilatasyon ve çözünür endotel protein veseptörü C (sEPCR) ve çözünür thrombomodulin düzeyleri ile değerlendirildi.

Çalışmaya 45 böbrek nakli alıcısı alındı ve kış ölçümü mart ayında ve yaz ölçümleri eylül ayında yapıldı.

Kış çıkışı ortalama vitamin D düzeyi 18,8±7,5, yaz çıkışı ise 34,3±13,0 ng/ml olarak saptandı (p<0,0001). Kış ve yaz aylarında sırası ile 38 (%84) ve 20 (%44) vitamin D yetersiz/eksik hasta vardı. Her iki periyodda vitamin D yetersizlik/eksiklik ile akım aracılı vazodilatasyon, sTM veya sEPCR arasında bir ilişki yoktu.

Sonuç olarak vitamin D yetersizlik/eksiklik kış aylarında böbrek nakli alıcılarında yaygın olarak gözlenirken yazın bu durum çoğu olguda düzelmektedir. Vitamin D yetersizlik/eksiklik ile endotel işlev bozukluğu arasında bir ilişki ortaya konulamamıştır.

ANAHTAR SÖZCÜKLER: Endotel işlev bozukluğu, Akım aracılı vazodilatasyon, Böbrek hastalığı, Renal transplantasyon, Vitamin D, Böbrek nakli

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INTRODUCTION

In addition to its well-known role in the bone mineral metabolism ,vitamin D has gained special interest in recent studies due to its pleomorphic effects in several tissues. It acts through vitamin D receptors which have been shown in many systems including kidney, intestine, bone, parathyroid gland, colon, vascular, and myocardium (1-3).

In experimental studies, vitamin D D/I is not only associated with myocardial hypertrophy but also progression of renal disease, anemia and activation of the renin-angiotensin-aldosteron esystem (4). Moreover, in rats, myocardial hypertrophy has improved with vitamin D treatment (5).

In recent studies, vitamin D D/I is reported to be common in chronic kidney disease and it is defined by 25OH vitamin D or 1,25 OH vitamin D levels. Up to 80% of the dialysis patients have been reported to have vitamin D D/I and there was a seasonal variation of vitamin D levels (6, 7). In the dialysis population, it is known that the most common cause of mortality is due to cardiovascular complications (8). While mortality improves with renal transplantation, cardiovascular complications remain the most common cause of mortality (9). Although, endothelial dysfunction and vitamin D D/I have been studied previously, there is no study evaluating endothelial dysfunction and vitamin D D/I in renal transplant recipients (RTR).

In this study, we aimed to investigate whether there is a relation between VDD/I and endothelial function and also to see the frequency of vitamin D D/I in RTR in the winter and summer seasons.

MATERIAL and METHODS

This is a single center cross-sectional study including adult RTR who received their first renal transplantation from 1998 to 2007. The patients were evaluated between January 2008 and March 2009. A total of 144 patients were recorded; among them, 31 patients were unwilling to participate, and 21 patients were lost to follow up. Five patients were excluded due to age <18; 17 patients due to high creatinine level (>2 mg/dl); 7 patients due to use of vitamin D supplement; 5 patients in whom flow-mediated dilatation could not be performed technically due to presence of bilateral arterio-venous fistula operation in upper arm; 13 patients who were evaluated only in winter but not in summer period. Forty-five patients were included in the final evaluation. The local Ethics Committee approved the study, and all patients gave informed consent prior to all procedures.

At baseline in the winter and at the end of summer period, fasting blood samples from all subjects were drawn in early morning between 8:00 and 10:00 am., sent to our routine laboratory without delay. Serum samples were separated at +4°C and stored at –85°C until analysis of the soluble. Thrombomodulin (sTM), C-reactive protein (CRP), and soluble endothelial protein C receptor (sEPCR). The demographic data was obtained from the patients' charts. CRP was measured by nephelometric method (Dade Behring). Blood lipids, uric acid, and hemoglobin were measured by an autoanalyser (Beckman LX 20). Estimated glomerular filtration rate (e-GFR) was calculated using the Modification of Diet in Renal Disease (MDRD) equation. Body mass index (BMI) was calculated as the ratio of weight to $(height)^2 (kg/m^2)$.

Measurement of Vitamin D

The winter measurements took place in the March and the summer measurements in September. The 25OH vitamin D levels were measured by HPLC (Spectra System, GmbH, Munich, Germany). Vitamin D deficiency was defined as a 25OH vitamin D level below 10 ng/ml, and insufficiency as below <30 ng/ml.

sEPCR assay

sEPCR levels were determined in the plasma using sEPCR Asserachrom enzyme-linked immunosorbent assay (ELISA) kits from Diagnostica Stago (Asnieres-France), according to the manufacturer's instructions. Samples were assayed in duplicate.

sTM assay

As with sEPCR, sTM was measured quantitatively by using the ELISA technique (Diagnostica Stago-France).

Measurement of Endothelium-Dependent Flow-Mediated Dilatation (FMD)

Endothelium-dependent FMD was performed by using a 13.0 MHz linear array transducer (Vivid 7, Wipro GE Healtcare, GE Medical Systems Inc, Chicago, U.S.A) in two periods including March and September. After 12 hours of fasting (including caffeine, nicotine and alcohol), vascular measurements were performed in a quiet, temperature-controlled (22–24°C) room in the morning (8 am-11 am). Vasoactive medications were not stopped before FMD, but the morning doses were skipped on the examination day. After a 15-minute resting period in the supine position, the transducer was placed 4–5 cm above the elbow in the longitudinal section for the scanning of right brachial artery, and then the basal diameter of the brachial artery (from the anterior intima to posterior intima) and flow velocity were measured. Afterwards, a sphygomanometer cuff was placed on the upper arm, inflated to 250 mmHg for 4–5 min, and deflated abruptly. The flow velocity was recorded within 15 seconds, and post occlusion artery diameters were taken at 60–120 seconds. Brachial artery images were taken by two experienced observers (A.O and C.T) who were blinded to the vitamin D levels of the patients. Diameter measurements were taken at the end of the diastole (timed by peak of R wave on electrocardiogram), at least three times, and the average of these measurements were determined. Endothelium-dependent vasodilatation was defined as the percent change in diameter compared with baseline (maximum diameter-baseline diameter/baseline diameterx100). Intra- and inter-observer variability of FMD measurements was

determined from 30 randomly selected brachial artery images (Kappa values were 0.92 for intra- and 0.83 for inter-observer agreements; $p < 0.001$ for both of them).

Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences for Windows version 11.5 (SPSS Inc., Chicago, IL, USA). Data were analyzed first for normality of distribution using the Kolmogorov–Smirnov test. The results are reported as the mean ± SD for normally distributed variables. Student's t-test was used to determine the significance of differences between the groups. Non-numerical variables were compared by Fischer's exact test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

The mean age of the 45 RTR (20 Female, 25 Male) was 39±13 years, the dialysis vintage was 32±24 months, and the mean post-transplant follow-up period was 42±40 months. The causes of end stage renal failure were chronic glomerulonephritis in 16 patients, chronic tubulointerstitial nephritis in 19 patients, hypertension in 4, and diabetes mellitus in 2, unknown in 4 patients. There were 8 patients on statin and 12 patients were using anti-hypertensive treatment. There was no patient on angiotensin converting enzyme inhibitor or angiotensin receptor blocker in the group. All patients were on triple immunosuppressive treatment including cyclosporine/tacrolimus-mycophenolate mofetil/azathioprine-prednisolone and they had functional allograft, (the mean estimated glomerular filtration rate was 73±20 ml/min). There were 2 patients with known coronary artery disease in the groupEndothelial function and 25OH vitamin D were measured in both at the end of winter and summer period. In the winter, 4 patients (9%) had vitamin D deficiency and 34 (75%) had vitamin D insufficiency, (84% vitamin D D/I). The result of endothelial function, demographic and laboratory features compared in vitamin D D/I and vitamin D sufficient group in the winter period, are shown in Table I.

As shown in Table I, we did not find any impact of vitamin D D/I on endothelial function as evaluated by FMD, sTM and sEPCR.

Table I: Demographic and laboratory features of vitamin D D/I and Vitamin D normal Groups (In Winter).

	Vitamin D D/I N=38	Vitamin D Normal N=7	P
Age	38±10	42±14	NS
Gender (F/M)	17/21	3/4	NS
Deceased/Living Donor	8/30	2/5	NS
SBP (mmHg)	118±13	121±10	NS
DBP (mmHg)	78±7	74±5	NS
25OH Vitamin D (ng/ml)	17.7±6.4	40.2±11.4	<0.0001
sEPCR (ng/ml)	145±88	140±82	NS
sTM (ng/ml)	59±19	61±24	NS
FMD (%)	8.7±5.2	9.1±4.3	NS
e-GFR (ml/min/1.73m^2)	69±17	71±19	NS
CRP (mg/l)	3.6±3.8	5.3±4.8	NS
LDL (mg/dl)	103±28	102±32	NS
HDL (mg/dl)	51±12	51±14	NS
Triglyceride (mg/dl)	137±57	125±43	NS
BMI (kg/m^2)	26±5	24±4	NS
Duration of RTR (months)	42±57	42±21	NS
Dialysis time (months)	32±26	32±29	NS
Hypertension	11	2	NS
Smoking	2	1	NS

F: Female, **M:** Male, **SBP:** Systolic blood pressure, **DBP:** Diastolic blood pressure, **e-GFR:** estimated glomerular filtration rate, **CRP:** C-reactive protein, **LDL:** Low density lipoprotein, **HDL:** High density lipoprotein, **BMI:** body mass index, **sTM:** Soluble thrombomodulin, **sEPCR:** Soluble endothelial protein C receptor

Table II: Demographic and laboratory features of Vitamin D D/I and Vitamin D normal Groups (In Summer).

	Vitamin D D/I N=20	**Vitamin D Normal N=25**	**P**
Age	39±10	38±12	NS
Gender (F/M)	8/12	12/13	NS
Deceased/Living Donor	4/16	6/19	NS
SBP (mmHg)	118±14	119±12	NS
DBP (mmHg)	79±6	76±7	NS
25OH Vitamin D (ng/ml)	24.7±4.5	43.9±11.1	<0.0001
sEPCR (ng/ml)	146±96	144±81	NS
sTM (ng/ml)	60±26	64±27	NS
FMD (%)	11.7±6.7	14.0±4.3	NS
e-GFR (ml/min/1.73m^2)	72±18	69±21	NS
CRP (mg/l)	3.8±4.2	3.5±4.7	NS
BMI (kg/m^2)	26±5	25±4	NS

F: Female, **M:** Male, **SBP:** Systolic blood pressure, **DBP:** Diastolic blood pressure, **e-GFR:** estimated glomerular filtration rate, **CRP:** C-reactive protein, **sTM:** Soluble thrombomodulin, **sEPCR:** Soluble endothelial protein C receptor

While the mean 25OH vitamin D level was 18.8±7.5 ng/ml in the winter period, it was 34.3±13.0 ng/ml in the summer period (p<0.0001). At the end of summer, the 25OH vitamin D level increased significantly and there were 20 (44%) patients with vitamin D/I (1 deficient and 19 insufficient). These patients were among the previous vitamin D D/I patients in the winter period.

Table II presents the vitamin D D/I and normal vitamin D groups when we investigated endothelial function in the summer period.

As shown in Table II, FMD was lower at the VDD/I group in comparison to the vitamin ID sufficient group, however, it did not reach the statistical significance (p>0.05).

In correlation analysis, we did not find any association between FMD and vitamin D levels. Also, there was no correlation between vitamin D level and GFR. As it was shown, in summer period, FMD levels increased significantly in our patients, however, we do not have any explanation for this finding.

DISCUSSION

In this study, we found that vitamin D D/I is common in RTR in winter and it improves significantly in summer. Moreover, we did not find association between vitamin D D/I and endothelial dysfunction in winter and summer. Although FMD was slightly better in the vitamin D normal group in both periods, it did not reach statistical significance.

Vitamin D D/I is common in dialysis patients and it was reported that it is also common in kidney allograft recipients (10). In RTR, several studies have been reported that vitamin D D/I might be associated with infection (11), acute rejection (12), chronic rejection (13), as well as bone mineral abnormality (14), however, there is no study evaluating vitamin D D/I and cardiovascular disease in RTR. It is known that RTR have a 50-fold increased cardiovascular risk in comparison to the general population (15). It has been reported in general population and the patients with renal disease, for these complications in this regard the relationship between vitamin D D/I and endothelial dysfunction can be important for RTR.

In recent studies, the pleomorphic effects of vitamin D have been reported and vitamin D reseptor (VDR) was found in different tissues including cardiovascular, epithelium, and immune system (16). In experimental studies, vitamin D has been implicated in the regulatory function of endothelium, immune system, fibrosis, atherosclerosis, myocardial hypertrophy, epithelial cancers, activation of renin angiotensin aldosteron system, vascular calcification, and progression of renal disease (17, 18, 19). Moreover, VDR deficiency leads to enhanced cardiac contractility which results with cardiac failure and hypertrophy, and vitamin D treatment is associated with reduced cardiac hypertrophy and left ventricular diameter in spontaneously hypertensive heart failure-prone (cp/+) rats (20).

Numerous human studies have shown the effect of vitamin D D/I on the cardiovascular system including vascular

calcification, myocardial hypertrophy, and hypertension in epidemiological and population studies (21). Recent data including 1739 individuals in the Framingham Offspring Study who had no previous cardiovascular disease demonstrated significantly increased cardiovascular events in patients with low 25OH vitamin D levels (patients with 25OH vitamin D levels below a threshold of 15 ng/mL) (22). In patients with chronic kidney disease, there was a strong evidence that vitamin D or VDR agonists have been associated with better survival (23). Also, there has been an association between vitamin D D/I and anemia in chronic kidney disease (24, 25). London et al., showed an association between vitamin D D/I and endothelial dysfunction and aortic calcification in hemodialysis patients (26). In addition, the Accelerated Mortality on Renal Replacement (ArMORR) study (prospective cross-sectional study) has shown the beneficial effect of VDR agonist treatment on mortality in 1,25 OH vitamin D and 25OH vitamin D D/I hemodialysis patients (27). Therefore, vitamin D treatment has been associated with better survival in hemodialysis patients and extrapolation of experimental studies to humans may explain the pathophysiological consequences of vitamin D D/I in several systems.

Before cardiovascular endpoints occur, the patients should have some evidence of early cardiovascular dysfunction. In this regard, markers for endothelial dysfunction may be used as an early finding of cardiovascular disease (28, 29). Endothelial injury is of paramount importance in the development of subsequent cardiovascular complications, and there have been various methods and markers described for the evaluation of endothelial function, including brachial FMD (30), forearm laser Doppler flow (31), adhesion molecules (ICAM-1, VCAM-1) (32), von Willebrand factor (33), homocysteine (34), interleukins (35), circulating endothelial cells (36), and circulating endothelial progenitor cells (37). In recent reports, sTM and sEPCR, important anti-thrombotic factors that are produced extensively by endothelial cells during injury, seem to be markers of endothelial function and in several diseases in which endothelial damage is prominent (38, 39, 40). After binding to EPCR Protein C is activated and shows anti-thrombotic and anti-inflammatory activity. Also, thrombomodulin is a cell surface glycoprotein produced by vascular endothelial cells, is also an important cofactor for activation of protein C. Activated protein C is one of the best known anticoagulants, and it has anti-apoptotic and anti-inflammatory properties (39). Activated protein C, TM and EPCR can be reflection of the endothelial response to injury, and recent studies have demonstrated that numerous diseases including sepsis, SLE, Wegener's granulomatosis, and renal failure were found to be associated with elevated levels of these markers (39, 40). They might also predict the disease activity in Wegener's granulomatosis. Moreover, renal involvement was associated with higher sEPCR in SLE patients, suggesting that circulating sEPCR may be an indicator of vasculopathy and renal injury in SLE (40). In recent studies, FMD was also

regarded as a marker of endothelial injury. Sugden and et al., have shown that vitamin D D/I was associated with impaired FMD and after treatment with vitamin D, FMD was improved significantly (41). However, in our study, we did not find any association between FMD and vitamin D D/I. Moreover, additional endothelial markers including sEPCR and sTM were not different in vitamin D D/I and the normal vitamin D group. It is well known that vitamin D levels changes significantly in winter versus summer especially in certain geographic areas. Vitamin D /I may be seen in patients with limited sun exposure due to seasonal variation in duration of sunlight. In our study, we observed significant seasonal variation in vitamin D levels of RTR. This was reported in previous studies and seasonal variation in vitamin D levels should be considered in all studies aiming to show a pathophysiological relation between vitamin D status and cardiovascular disease. Vitamin D /I in winter should not be taken as a permanent situation and it improves in most cases in summer period. Therefore, as a cardiovascular risk factor, seasonal variation and temporary vitamin D D/I in winter should be considered for future population studies. In conclusion, vitamin D D/I is a common problem in RTR in winter and it improves in most cases in summer. We did not find any impact of vitamin D D/I on endothelial function which was assessed by FMD, sTM and sEPCR in winter and summer period, however, FMD seems to be better in normal vitamin D group in both periods. Vitamin D substitution in winter period should be considered for RTR and future studies for cardiovascular effects of vitamin D D/I may clarify the relationship between endothelial dysfunction and vitamin D.

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