

Effect of vitamin D levels on lipid, glucose, vitamin B₁₂ and C-reactive protein in acute ischemic stroke

 Nalan Kozaci¹,  Cafer Caliskan²,  Mustafa Avci³,  Gulsum Caliskan³,  Ilhan Uysal⁴

¹Department of Emergency Medicine, Alanya Alaaddin Keykubat University, Alanya Education and Research Hospital, Antalya, Turkey

²Department of Computer Engineering, Antalya Bilim University, Antalya, Turkey

³Department of Emergency Medicine, University of Health Sciences, Antalya Education and Research Hospital, Antalya, Turkey

⁴Department of Computer Technologies, Bucak Emin Gulmez Vocational School of Technical Sciences, Mehmet Akif Ersoy University, Burdur, Turkey

Copyright@Author(s) - Available online at www.annalsmedres.org

Content of this journal is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.



Abstract

Aim: Stroke ranks second among the causes of death world wide and related disability and death rates are high. Recent studies have shown that vitamin D deficiency is higher in patients with ischemic stroke compared to other patients and vitamin D deficiency has emerged as a new risk factor. The aims of the study are to investigate the relation between levels of vitamin D in patients diagnosed with acute ischemic stroke with levels of vitamin B₁₂, glucose, HbA1c, blood urea nitrogen (BUN), creatinine, C-reactive protein (CRP) and lipids, and to determine the effect of vitamin D deficiency on other factors that increase the risk of acute ischemic stroke.

Materials and Methods: Two thousand seven hundred thirty-four patients diagnosed with acute ischemic stroke were included in the study. Results of laboratory tests for levels of vitamin D, vitamin B₁₂, HDL cholesterol, LDL cholesterol, VLDL cholesterol, triglycerides, glucose, HbA1c, BUN, creatinine and CRP were recorded.

Results: As for correlation between vitamin D and other parameters, positive correlations between vitamin D and age, vitamin B₁₂, BUN and creatinine, and negative correlations between vitamin D and triglycerides, LDL cholesterol, VLDL cholesterol, glucose, HbA1c and CRP were identified. Vitamin D and HDL cholesterol showed no correlation in normal HDL values, but there was positive correlation in patients with HDL cholesterol levels <40 mg/dL.

Conclusion: Vitamin D levels in patients that suffered ischemic stroke were significantly correlated with lipid metabolism, glucose metabolism, inflammation, kidney function and vitamin B₁₂ levels.

Keywords: Cholesterol; glucose; HDL; lipid; stroke; vitamin B₁₂; vitamin D

INTRODUCTION

Vitamin D (VitD) is a fat-soluble vitamin and a hormone/hormone-precursor group of sterols, since it is endogenously synthesized under appropriate biological conditions. The major effects of VitD involve calcium and phosphorus metabolism, and bone mineralization. VitD deficiency is the least diagnosed and treated form of malnourishment globally. Despite the abundance of sunlight, VitD deficiency is widespread in Turkey (1).

Stroke ranks second among the causes of death worldwide, and related disability and death rates are high. Stroke accounts for 9% of all deaths. The number of patients is expected to increase each year. Occlusive type cerebrovascular disease constitutes 80-85% of all strokes. Stroke is among the most common causes of epilepsy in elderly patients. Acute stroke patients have high intensive care needs and the length of hospital stay is long (2).

Hypertension, hyperlipidemia, smoking, diabetes mellitus (DM), obesity and atrial fibrillation are among the proven risk factors of ischemic stroke (IS). Recent research have indicated that VitD deficiency is more frequent in IS patients compared to other patients, which prompted the idea that VitD deficiency might be another risk factor. Moreover, there are studies that have shown that VitD deficiency is associated with hypertension, hyperlipidemia and DM, which are risk factors for IS (3-5).

The aims of this study are to investigate the relation between levels of VitD in patients diagnosed with acute ischemic stroke (AIS) in the emergency department (ED) with levels of vitamin B₁₂ (VitB₁₂), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, very-low-density lipoprotein (VLDL) cholesterol, triglycerides (TG), glucose, HbA1c, blood urea nitrogen (BUN), creatinine (Cr) and C-reactive protein (CRP), and to determine the effect of vitamin D deficiency on other factors that increase the risk of AIS.

Received: 27.04.2020 **Accepted:** 27.07.2020 **Available online:** 18.05.2021

Corresponding Author: Mustafa Avci, Department of Emergency Medicine, University of Health Sciences, Antalya Education and Research Hospital, Antalya, Turkey **E-mail:** dravcimustafa@gmail.com

MATERIALS and METHODS

This retrospective study was initiated after it was approved by the Ethics Committee of Antalya Education and Research Hospital with the issue number 27/21 on December 26, 2019. The data of the patients that were diagnosed with stroke (searched via ICD codes) were obtained from the hospital automation system database and hospital archives. Patients in the adult age group (age ≥ 18 years) that were admitted to the neurology clinic or the intensive care unit of the neurology department from ED between the dates of 01 January 2018 and 31 December 2019 were included in this study. Patients in the children age group (age < 18 years) that were discharged from ED or referred to another hospital were excluded from the study. Patients whose data were not fully available, those who had hemorrhagic stroke, and those with VitD and VitB₁₂ levels higher than the normal ranges were excluded from the study. Searches on the hospital automation system database and hospital archives, selection of patients, and application of inclusion and exclusion criteria were performed by the coordinator emergency physician of the study. A data record form was prepared for the study, on which patients' age, gender, pre-diagnosis, and results of imaging and laboratory tests were recorded. Results of laboratory tests for levels of VitD, VitB₁₂, HDL cholesterol, LDL cholesterol, VLDL cholesterol, TG, glucose, HbA1c, BUN, Cr and CRP were recorded.

In total, 4,343 patients were screened for the study. A total of 1,609 patients were excluded from the study, including 1,303 patients whose data were not fully available, 23 patients with levels of VitD higher than 100 $\mu\text{g/L}$, 77 patients with levels of VitB₁₂ higher than 500 ng/L and 206 patients diagnosed with hemorrhagic stroke.

Blood Samples and Laboratory Analysis

Blood samples were drawn within half an hour following the admission of the patients to the clinic or intensive care unit of the neurology department. The approximate duration of centrifugation was 10 minutes at 4,100 rpm for the laboratory tests. VitD, VitB₁₂, HDL cholesterol, LDL cholesterol, TG and CRP measurements were made with a Beckman Coulter UniCel Dxl 800 (CA, USA) autoanalyzer. Results were reported in $\mu\text{g/L}$ for VitD, and ng/L for VitB₁₂, HDL cholesterol, LDL cholesterol, TG and CRP. VLDL cholesterol was calculated automatically by a Beckman Coulter UniCel Dxl 800 (CA, USA) autoanalyzer using the formula $\text{TG}/5 = \text{VLDL}$. Results were reported in mg/dL. HbA1c measurements were made with an Chromsystem autoanalyzer (München, Germany). Results were reported in %. Glucose, BUN and Cr measurements were made with a Beckman Coulter AU 680 (CA, USA) autoanalyzer. Results were reported in mg/dL. Measurement of serum/plasma levels took an hour for VitD and VitB₁₂, 30 minutes for HDL cholesterol, LDL cholesterol, VLDL cholesterol and TG, and 45 minutes for glucose, BUN, Cr, CRP and HbA1c.

Statistical Analysis

Data of 2,734 patients were used for statistical analysis, which was performed with the SPSS 21 package. Firstly,

demographic analysis was carried out using frequency distribution and the χ^2 test. In order to determine the distribution of groups, the Shapiro-Wilk test was utilized. For group comparisons of paired samples, the student t-test was used for parametric distributions and the Mann-Whitney U test was used for non-parametric ones. Correlations between the level of VitD and levels of VitB₁₂, HDL cholesterol, LDL cholesterol, VLDL cholesterol, TG, glucose, HbA1c, BUN, Cr and CRP were assessed using the Pearson bivariate correlation test. Then, linear regression method was performed in order to formulize the relations between VitD levels and other parameters. To validate these formularizations, an advanced statistical modeling tool, the generalized linear model (GLM), was used. A p value of < 0.05 was accepted as statistically significant.

RESULTS

A total of 2,734 patients diagnosed with AIS were included in the study. The mean age of the patients was 66 ± 12 years. 1,758 (64%) patients were male and 976 (36%) were female. The mean level of VitD in female patients was 23 ± 11 (min:3, max:91) $\mu\text{g/L}$, while that in male patients was 21 ± 12 (min:2, max:88) $\mu\text{g/L}$ ($p < 0.001$). Reference ranges of the laboratory tests and mean values of patients' results are shown in Table 1. Accordingly, there were 2,190 patients (80%) with VitD levels $< 30 \mu\text{g/L}$, 258 patients (9%) with VitB₁₂ levels $< 126 \text{ ng/L}$, 1,489 patients (54%) with LDL levels $> 100 \text{ mg/dL}$, 938 patients (34%) with VLDL levels $> 30 \text{ mg/dL}$, 1,965 patients (35%) with TG levels $> 150 \text{ mg/dL}$, 950 patients (71%) with HbA1c levels $> 5.6\%$, 866 patients (32%) with CRP levels $> 5 \text{ mg/L}$, 682 patients (25%) with BUN levels $> 20 \text{ mg/dL}$, 801 patients (29%) with Cr levels $> 1.09 \text{ mg/dL}$ and 230 patients (8%) with HDL levels $< 60 \text{ mg/dL}$ (Table 1).

Table 1. Mean laboratory test results of the patients and reference ranges

Value	Laboratory results of the patients (Mean \pm SD)	Reference ranges
Vitamin D ($\mu\text{g/L}$)	22.13 \pm 11.45	30-100
Vitamin B12 (ng/L)	240 \pm 99	126-505
Triglyceride (mg/dL)	148 \pm 92	10-150
HDL Cholesterol (mg/dL)	45 \pm 10	40-60
LDL Cholesterol (mg/dL)	109 \pm 38	10-100
VLDL Cholesterol (mg/dL)	30 \pm 19	2-30
Glucose (mg/dL)	116 \pm 49	74-106
BUN (mg/dL)	18 \pm 7	8-20
Creatinine (mg/dL)	1.04 \pm 0.33	0.66-1.09
CRP (mg/L)	8 \pm 8	0-5
HbA1c (%)	6.5 \pm 1.4	3.5-5.6

HDL: High-Density Lipoprotein, LDL: Low-Density Lipoprotein, VLDL: Very-Low-Density Lipoprotein, BUN: Blood Urea Nitrogen, CRP: C-Reactive Protein, HbA1c: Hemoglobin A1c

When correlation between VitB₁₂ and other parameters was assessed, correlations between levels of VitB₁₂ and VitD ($r=0.122$, $p<0.001$), VitB₁₂ and BUN ($r=0.043$, $p=0.025$), and VitB₁₂ and Cr ($r=0.045$, $p=0.018$) were identified. As for correlation between VitD and other parameters, positive correlations between VitD and age, VitB₁₂, BUN and Cr, and negative correlations between VitD and TG, LDL cholesterol, VLDL cholesterol, glucose, HbA1c and CRP were identified (Table 2). VitD and HDL cholesterol showed no correlation in normal HDL values. However, there was

positive correlation between VitD and HDL cholesterol ($r=0.740$, $p=0.025$) in patients with HDL cholesterol levels <40 mg/dL (Table 2).

In order to formulize the relation between VitD levels and other laboratory test results, linear regression method was used (Figure 1). According to this analysis, for prediction of VitD levels, gender, age, and levels of VitB₁₂, TG, HDL cholesterol and BUN exhibit positive coefficients, while levels of LDL cholesterol, VLDL cholesterol, glucose (fasting), CRP and HbA1c exhibit negative coefficients.

Table 2. Correlations between vitamin D levels and other laboratory parameters

	Age	VitB12	TG	HDL	LDL	VLDL
r	0.084**	0.122**	-0.110**	0.030	-0.057**	-0.110**
p	0.000	0.000	0.000	0.114	0.003	0.000
	Glucose	BUN	Creatinine	CRP	HbA1c	
r	-0.108**	0.047*	0.052**	-0.082**	-0.105**	
p	0.000	0.013	0.006	0.000	0.000	

r: Correlation Coefficient, p: Probability value, **: $p<0.001$, *: $p<0.05$, VitB12: Vitamin B12, TG: Triglycerides, HDL: High-Density Lipoprotein, LDL: Low-Density Lipoprotein, VLDL: Very-Low-Density Lipoprotein, BUN: Blood Urea Nitrogen, CRP: C-Reactive Protein, HbA1c: Hemoglobin A1c

$$\text{Vitamin D} = [2.360 \times \text{gender (female:0, male:1)}] + [0.055 \times \text{age}] + [0.014 \times \text{VitB}_{12}] + [0.037 \times \text{TG}] + [0.031 \times \text{HDL cholesterol}] - [0.009 \times \text{LDL cholesterol}] - [0.231 \times \text{VLDL cholesterol}] - [0.012 \times \text{glucose (hunger)}] + [0.075 \times \text{BUN}] - [0.055 \times \text{CRP}] - [0.389 \times \text{HbA1c}] + 17.659$$

Figure 1. Calculation of vitamin D levels using linear regression method

GLM was performed to validate the results obtained by the linear regression method. Accordingly, VitD levels were found to be positively correlated with VitB₁₂ and BUN levels. On the other hand, VitD levels negatively correlated with levels of CRP, LDL cholesterol, VLDL cholesterol, glucose and HbA1c (Figure 2).

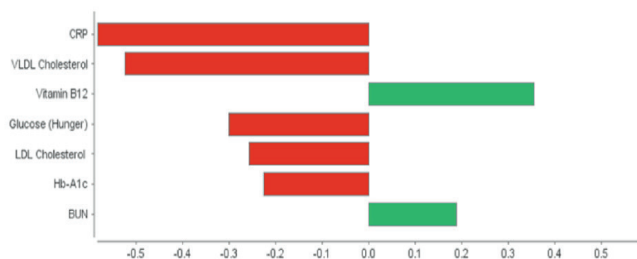


Figure 2. Relationship between vitamin D levels and other parameters according to the generalized linear model

DISCUSSION

AIS is a major cause of long-term disability globally. IS has a heterogeneous etiology with changeable and unchangeable risk factors. VitD deficiency, which is lately considered an independent risk factor, has been found to elevate the risk of vascular disease and IS in healthy individuals, and has been reported to be an independent risk factor for poor outcomes in AIS patients (6,7). In this

study, in which we investigated the relationship between VitD levels and VitB₁₂, HDL cholesterol, LDL cholesterol, VLDL cholesterol, TG, glucose, HbA1c, BUN, Cr and CRP levels in patients diagnosed with AIS in the emergency department, 80% of the patients had VitD levels, 9% had VitB12 levels and 8% had HDL cholesterol levels below normal limits. Additionally, LDL cholesterol levels in 54%, VLDL cholesterol levels in 34%, TG levels in 35%, HbA1c levels in 71%, CRP levels in 32%, BUN levels in 25% and Cr levels in 29% of the patients were found to be above normal limits.

Studies have shown that VitD deficiency is associated with hypertension, DM and dyslipidemia (8). A study has reported that glycemic control was disrupted in type-II DM patients with low VitD levels and that VitD supplementation helped alleviate the disruption of glycemic control (9). In a study that investigated the relation between VitD and cholesterol levels, VitD deficiency has been shown to be associated with poor clinical outcomes in patients with low HDL cholesterol levels (10). Another study has found that VitD supplementation raised the levels of total cholesterol, TG, VLDL, LDL and HDL significantly, and that VitD had potentially negative effects on lipid metabolism (11).

Pathophysiology of stroke is complex and not fully understood. Subsequent to cerebral ischemia, various inflammatory processes are activated and tissue damage is elevated due to increased inflammation. In studies that investigated the role of the inflammation marker CRP in AIS, high levels of serum CRP was found to be independently associated with stroke, mortality and post-stroke intracerebral hemorrhage (12). A similar study has reported that management of both LDL cholesterol and CRP levels could prevent reoccurrence of stroke and

transient ischemic attack (13). VitD is known to play an important role in regulation of inflammatory processes. Research has shown that high-sensitivity CRP levels are elevated in patients with VitD deficiency (14-17). In a study involving patients that suffered AIS, serum VitD levels were found to be negatively correlated with IL-6 and high-sensitivity CRP levels (18).

Another risk factor for AIS is VitB₁₂ deficiency. In a study that investigated the relation between VitB₁₂ deficiency and AIS, low VitB₁₂ levels, especially together with low folate levels, were shown to increase the risk of cerebral ischemia (19). A study involving AIS patients has reported that levels of serum homocysteine, VitB₁₂ and VitD were associated with the severity of first-time stroke, but also contributed to prognosis of AIS to a certain extent (20). In a study that examined the levels of VitB₁₂, folic acid, homocysteine and VitD in children and adolescents with obsessive-compulsive disorder, the levels of VitB₁₂ and VitD were found to be significantly lower in the patient group compared to the control group, while homocysteine levels were higher (21). A study has investigated the correlation between the levels of ischemia VitB₁₂ and VitD, and anti-thyroid peroxidase (anti-TPO) antibodies in patients with autoimmune hypothyroidism. Their results suggest that deficiencies of VitB₁₂ and VitD are associated with autoimmune hypothyroidism, and that a negative correlation exists between VitB₁₂ and VitD levels, and anti-TPO levels in these patients (22).

The risk of cardiovascular disease is high in the presence of concurrent chronic kidney disease (CKD). This elevated risk is associated with arterial hypertension, DM and dyslipidemia, as well as various other factors such as chronic inflammation (23). In CKD, VitD deficiency and related disruption of Ca metabolism pose a serious problem. Moreover, recent research has shown that in patients with VitD deficiency, DM is more prevalent, and levels of non-HDL cholesterol and TG are higher compared to healthy subjects (24,25).

The cited research taken together suggests that in addition to mineral metabolism, VitD affects lipid, glucose, kidney and inflammatory functions in the body, and that it is associated with VitB₁₂. In our study, different analysis methods were used to investigate how VitD levels were related to levels of VitB₁₂, HDL cholesterol, LDL cholesterol, VLDL cholesterol, TG, glucose, HbA1c, BUN, Cr and CRP. The results of these analyses collectively indicated that VitD and VitB₁₂ levels were positively correlated. VitD was also shown to be positively correlated with the kidney function markers BUN and Cr. Furthermore, a positive correlation was found between VitD and HDL cholesterol in patients with low HDL levels. In contrast, VitD negatively correlated with levels of TG, LDL cholesterol and VLDL cholesterol. A negative correlation was also shown between VitD and the inflammatory marker CRP. Similarly, VitD was found to be negatively correlated with levels of glucose and HbA1c. The results of our study support other studies.

LIMITATIONS

Patients with high VitD and VitB₁₂ levels were excluded from the study due to insufficient numbers. Thus, the relation between high levels of VitD and VitB₁₂, and other laboratory test parameters could not be investigated.

CONCLUSION

To conclude, in addition to mineral metabolism in the body, VitD is associated with levels of TG, LDL cholesterol, VLDL cholesterol, HDL cholesterol, glucose, HbA1c, BUN, Cr, CRP and VitB₁₂, which are risk factors for IS. Changes in VitD levels cause changes in lipid metabolism, glucose metabolism, inflammation, kidney function and VitB₁₂ levels. Therefore, by eliminating VitD deficiency, the risk of IS can be reduced. Additionally, TG, LDL cholesterol, VLDL cholesterol, HDL cholesterol, glucose, HbA1c, BUN, Cr, CRP and VitB₁₂ levels can be estimated by measuring the VitD levels of the patient between laboratory tests.

Competing interests: The authors declare that they have no competing interest.

Financial Disclosure: There are no financial supports.

Ethical approval: This study was approved by the Ethics Committee of Antalya Education and Research Hospital. Decision date: December 26, 2019; issue number: 27/21.

REFERENCES

1. Fidan F, Alkan BM, Tosun A. Pandemic Era: Vitamin D Deficiency and Insufficiency. *Turkish J Osteoporosis* 2014;20:71-4,
2. Avci M, Kozaci N, Atik I, et al. Analysis of acute stroke patients admitted to the emergency department. *Ann Med Res* 2018;25:406-9.
3. Ucar F, Taslipinar MY, Soydaş AO, et al. 25-OH Vitamin D Levels in Patients Admitted to Ankara Etlik İhtisas Training and Research Hospital. *Eur J Basic Med Sci* 2012;2:12-5.
4. Narasimhan S, Balasubramanian P. Role of Vitamin D in the Outcome of Ischemic Stroke- A Randomized Controlled Trial. *J Clin Diagn Res* 2017;11:6-10.
5. Wei ZN, Kuang JG. Vitamin D deficiency in relation to the poor functional outcomes in nondiabetic patients with ischemic stroke *Biosci Rep* 2018;38 BSR20171509
6. Turetsky A, Goddeau RP Jr, Henninger N. Vitamin D independently predicts cerebral infarct volume and post-stroke outcome. *J Stroke Cerebrovasc* 2015;24:1555-63.
7. Berghout BP, Fani L, Heshmatollah A, et al. Vitamin D Status and Risk of Stroke: The Rotterdam Study. *Stroke* 2019;50:2293-8.
8. Moretti R, Morelli ME, Caruso P. Vitamin D in Neurological Diseases: A Rationale for a Pathogenic Impact. *Int J Mol Sci* 2018;19:2245.
9. Wu C, Qiu S, Zhu X, et al. Vitamin D supplementation and glycemic control in type 2 diabetes patients: A systematic review and meta-analysis. *Metab* 2017;73:67-76.

10. Xu T, Zhong C, Peng Y, et al. Serum 25-hydroxyvitamin D deficiency predicts poor outcome amongst acute ischaemic stroke patients with low high density lipoprotein cholesterol. *Eur J Neurol* 2016;23:1763-8.
11. Schwetz V, Scharnagl H, Trummer C, et al. Vitamin D supplementation and lipoprotein metabolism: A randomized controlled trial. *J Clin Lipidol* 2018;12:588-96.
12. Ahangar AA. Predictive ability of C-reactive protein for stroke. *Caspian J Intern Med* 2016;7:151-2.
13. Kitagawa K, Hosomi N, Nagai Y, et al. Cumulative Effects of LDL Cholesterol and CRP Levels on Recurrent Stroke and TIA. *J Atheroscler Thromb* 2019;26:432-41.
14. Danielsson C, Nayeri S, Wiesinger H, et al. Potent gene regulatory and antiproliferative activities of 20-methyl analogues of 1,25dihydroxyvitamin D3. *J Cell Biochem* 1996;63:199-206.
15. Boonstra A, Barrat FJ, Crain C, et al. 1alpha,25-Dihydroxyvitamin d3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells. *J Immunol* 2001;167:4974-80.
16. Jablonski KL, Chonchol M, Pierce GL, et al. 25-Hydroxyvitamin D deficiency is associated with inflammation-linked vascular endothelial dysfunction in middle-aged and older adults. *Hypertension*, 2011;57:63-9.
17. Cirillo P, Golino P, Calabrò P, et al. C-reactive protein induces tissue factor expression and promotes smooth muscle and endothelial cell proliferation. *Cardiovasc Res*. 2005;68:47-55.
18. Wang Q, Zhu Z, Liu Y, et al. Relationship between serum vitamin D levels and inflammatory markers in acute stroke patients. *Brain Behav* 2018;8:e00885.
19. Weikert C, Dierkes J, Hoffmann K, et al. B vitamin plasma levels and the risk of ischemic stroke and transient ischemic attack in a German cohort. *Stroke*. 2007;38:2912-8.
20. Markišić M, Pavlović AM, Pavlović DM. The Impact of Homocysteine, Vitamin B12, and Vitamin D Levels on Functional Outcome after First Ever Ischaemic Stroke. *Biomed Res Int*. 2017;2017:5489057.
21. Esnafoglu E, Yaman E. Vitamin B12, folic acid, homocysteine and vitamin D levels in children and adolescents with obsessive compulsive disorder. *Psychiatry Res* 2017;254:232-7.
22. Aktas S. Vitamin B12 And Vitamin D Levels In Patients with Autoimmune Hypothyroidism And Their Correlation With The Antithyroid Peroxidase Antibodies. *Med Prin Pract* 2019.
23. Visconti L, Benvenga S, Lacquaniti A, et al. Lipid disorders in patients with renal failure: Role in cardiovascular events and progression of chronic kidney disease. *J Clin Transl Endocrinol*. 2016;6:8-14.
24. Echida Y, Mochizuki T, Uchida K, et al. Risk factors for vitamin D deficiency in patients with chronic kidney disease. *Intern Med* 2012;51:845-50.
25. Rodríguez VI, Ortega O, Gallar P, et al. Clinical and biochemical characteristics of predialysis patients in terms of 25 hydroxy vitamin D levels]. *Nefrologia* 2011;31:185-91.