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Correlation between Vitamin D, homocysteine and brain-derived neurotrophic factor levels in patients with ischemic stroke

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Abstract. – OBJECTIVE: Ischemic stroke risk increase is in relation to low serum vitamin D levels in some studies; however, the uncertainty about its relationship to serum homocysteine and brain-derived neurotrophic factor (BDNF) concentration remains. The aim of this study is to investigate the correlation between vitamin D, homocysteine and BDNF levels in patients with acute stroke.

PATIENTS AND METHODS: A total of 126 patients admitted to the emergency department with stroke and 64 healthy controls were included in this study. All patients received 150,000 U of vitamin D as part of a stroke treatment protocol during hospitalization. The patients were divided into two groups: the blood samples of the patients in the group-I (n=54) were taken at the time of admission to the hospital, and the samples of the patients in the group-II (n=72) were taken 24 hours after admission. Serum vitamin D, homocysteine, BDNF and routine biochemical analyses were performed from these samples.

RESULTS: Compared to healthy controls, stroke patients had lower serum vitamin D (p<0.0001) and BDNF (p<0.0001) levels, and higher homocysteine levels (p<0.0001). It was found that the homocysteine level was constant and the BDNF level increased (p=0.01) 24 hours after the 150,000 U vitamin D supplementation. Furthermore, there was a negative significant correlation between vitamin D levels, homocysteine and BDNF in patients who had experienced a stroke.

CONCLUSIONS: Vitamin D supplementation was used as a therapeutic agent in patients with acute ischemic stroke that affects homocysteine and BDNF levels. In this study, it was demonstrated for the first time that there is a correlation between serum vitamin D levels and BDNF levels in patients who have experienced a stroke.

Key Words:
Ischemic stroke, Vitamin D, Homocysteine, BDNF.

Introduction

Stroke is the first cause of disability in the world, as well as the second leading cause of death. Today, stroke-related injuries are less common due to advances in the treatment of acute ischemic stroke (IS). Furthermore, it is predicted that longer lifespans and increased prevalence of stroke risk factors may lead to increased rates of disability in stroke patients who have survived after treatment[1]. There is an increasing need for a powerful, non-invasive biomarker that can be used in the diagnosis of stroke and to predict post-stroke complications[1]. There are many risk factors associated with ischemic stroke, one of which is considered to be vitamin D (25-hydroxyvitamin D) deficiency. The main physiological role of vitamin D is to balance phosphorus and calcium homeostasis and to protect bone health[2]. Studies[2,3] have revealed that vitamin D deficiency increases the risk of IS provided vitamin D therapy gives positive results in recovery.

An increase in homocysteine level increases the risk of vascular diseases such as stroke[4]. Also, reduce in serum homocysteine is associated with a decreased risk of IS[4]. At the cellular level, increased homocysteine levels lead to decreased nitric oxide bioavailability levels. This causes changes in endothelial-mediated dilation, resulting in vascular damage because of the free radical production, as well as lipid peroxidation[4,5].

There have been reports[1] about low serum concentration from brain-derived neurotrophic factor (BDNF) increasing the risk of stroke/transient ischemic attack. BDNF has been widely used in ischemia as an indicator of neural regeneration and recovery[1]; however, BDNF levels were found to be remarkably lower in acute IS patients compared to healthy individuals[1]. The role of BDNF in serum in IS is still unclear. MacLellan et al[6] reported that BDNF mediated the recovery in their study on rats with an IS model.

Although there is a limited number of studies[4] examining the effects of vitamin D on homocysteine in IS patients, there have been no studies present examining the effects of BDNF that could

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be reported. This study is the first to reveal the effects of vitamin D supplementation on homocysteine and BDNF levels. The aim of this clinical academic study was to investigate the relationship between serum vitamin D, homocysteine and BDNF in acute ischemic stroke.

Patients and Methods

This study was conducted on patients with acute cerebrovascular ischemia and a healthy control group. Patients that are present in the study were divided into three groups:

- Group-I (at hospital admission time): 54 patients at the time of admission to the hospital;
- Group-II (24 hours after admission): 72 patients 48 hours after admission to the hospital;
- 64 healthy patients.

As a routine treatment protocol in both groups (group-I and group-II), 150,000 U of vitamin D supplementation was administered at the first admission to the hospital.

Inclusion Criteria

Male and female patients over 18 years of age, patients with acute IS, stroke patients admitted to the emergency room within the first 12 hours, and patients with IS compatible with the clinic in cranial diffusion MRI were included in the study.

Exclusion Criteria

Patients with rheumatological disease, patients with impaired liver function, patients with impaired renal function, and patients with trans ischemic attack were excluded. Patients who were not initially treated with 150,000 U of vitamin D were also not included in this study. In addition, the control group in which vitamin D supplements were taken in the last 30 days, was excluded from this study.

Blood samples were obtained at the first admission and 24 hours after the treatment. Blood samples were centrifuged at 3,000 g for 20 minutes in 1 hour and stored below -80°C till the measurements were recorded. Serum glucose and HbA1C levels, which are among biochemical parameters, were measured in the Abbott Architect C 4000 device (Architect c4000, Abbott Laboratories, IL, USA) using the calorimetric method and commercial kits. Serum homocysteine levels were investigated by high-performance liquid chromatography (HPLC) in Agilent 1200 device (Agilent Technologies, Waldbronn, Germany) using commercial kits (Chromsystems Instruments & Chemicals GmbH, München, Germany). Serum vitamin D levels were investigated with Roche Cobas e 411 devices (Roche Diagnostics GmbH, Germany) using commercial kits. The electrochemiluminescence method was used. The Total BDNF Quantikine ELISA Kit (MyBioSource, San Diego, CA, USA) was also used to quantitatively determine the BDNF level.

Statistical Analysis

Evaluation of the data was made with the GraphPad Prism 9.0. Data were tested for normality using the Kolmogorov-Smirnov test. Data were given as number of units (n), percentage (%) and mean±standard deviations. Statistical analyses were performed using the Chi-square test, ANOVA test and Unpaired *t*-test, depending on the appropriate variable. Correlation analysis was done with Spearman correlation analysis. Correlation coefficient was performed as described by Koo and Li[7]. A p-value < 0.05 was considered significant.

Results

The ages of the patients in the group-I sampled at the time of admission to the hospital (61.07±6.28 years), the patients who were sampled 24 hours after hospitalization (59.25±7.14 years), and the control group (58.71±5.66 years) were compared. There was no significant difference between the groups according to age and gender (p>0.05). No statistical difference was detected between groups according to prognosis, hypertension, dyslipidemia, and cardiac disease (p=0.71, p=0.28, p=0.77, p=0.85, respectively) (Table I).

When the serum vitamin D value was compared with the control, it appeared to have decreased in group-I (p<0.0001) and increased significantly in group-II (p<0.0001). When the patient groups were compared within themselves, it was found that vitamin D was higher in group-II than in group-I (p<0.0001) (Table I and figure 1A).

Serum homocysteine levels were higher in both patient groups compared to the control group (p<0.0001 for both). When the patient groups were compared within themselves, it was found that vitamin D was significantly higher in group-I than group-II (p=0.003) (Table I and Figure 1B).

When the serum BDNF value was compared with the control group, it seemed to be significantly lower in group-I and group-II (p<0.0001 and p=0.036, respectively). When the patient groups

Table I. Comparison of patient and control group in terms of various variables.

		Control	Patients (n)		Total (n)	*p*-value
			Group-I*	Group-II*		
Gender	Female	36 (56.3%)	38 (70.4%)	40 (55.6%)	114 (60.0%)	0.43**
	Male	28 (43.8%)	16 (29.6%)	32 (44.4%)	76 (40.0%)	
State of improvement	Poor Prognosis	-	6 (11.1 %)	6 (8.3%)	12 (9.5%)	0.71**
	Improvement	-	48 (88.9%)	66 (91.7%)	114 (90.5%)	
Hypertension	Absent	-	2 (3.7%)	8 (11.1%)	10 (7.9%)	0.28**
	Present	-	52 (96.3%)	64 (88.9%)	116 (92.1%)	
Dyslipidemia	Absent	-	26 (48.1%)	32 (44.4%)	58 (46.0%)	0.77**
	Present	-	28 (51.9%)	10 (55.6%)	68 (54.0%)	
Cardiac disease	Absent	-	44 (81.5%)	60 (83.3%)	104 (82.5%)	0.85**
	Present	-	10 (18.5%)	12 (16.7%)	22 (17.5%)	
Hgb (g/dL)		13.24±1.9	13.03±1.0	12.56±1.3	-	0.089***
WBC (10^3/μL)		6.97±2.06	6.78±1.4	6.52±1.28	-	0.706***
Glucose (mg/dL)		98.0±24.0	198.8±69.1	92.8±8.7	-	p<0.001***
Plt (10^3/μL)		277.8±70.9	163.1±39.9	147.9±31.4	-	p<0.001***
HbA1c (%)		-	8.2±0.92	5.0±4.5	-	p<0.001#
Systolic Pressure (mm/Hg)		12.2±0.4	15.5±1.4	15.4±1.4	-	p<0.001***
Diastolic Pressure (mm/Hg)		8.0±0.3	8.6±0.5	8.7±0.4	-	p<0.001***
Modified Rankin		-	2.7±1.0	2.8±0.9	-	0.879#
25-OH Vitamin D (ng/mL)		15.8±5.5	8.4±5.7	33.5±7.7	-	##
Homocysteine (μmol/L)		12.83±6.8	29.28±10.9	25.14±8.4	-	##
BDNF (pg/mL)		1,307±425.6	906.6±306.4	1,194±385.3	-	##

*Group-I: at hospital admission time; Group-II: 24 hours after admission. Statistical analyses were performed with **Chi-square test, ***ANOVA test and #Unpaired *t*-test. ## Tukey's multiple comparisons test (*p*-values are given in Figure 1).

were compared within themselves, BDNF levels in group-II were higher than in group-I (p=0.015) (Table I and Figure 1C).

Investigations were made on whether there was a correlation between serum vitamin D levels and changes in homocysteine and BDNF levels in the control and patient groups. No correlation between serum vitamin D levels with homocysteine (r=0.03, p=0.75) and BDNF (r=0.17, p=0.08) levels in the control group (Figure 2A and 2B) was detected. In group-I, a weak negative correlation had been detected between serum vitamin D levels with homocysteine (r=-0.44, p<0.0001) and BDNF (r=-0.44, p<0.0001) level (Figure 2C and 2D). In group-II, a weak negative correlation was found between serum vitamin D levels with homocysteine (r=-0.56, p<0.0001) and BDNF (r=-0.55, p<0.0001) levels (r=-0.55, p<0.0001) (Figure 2E and 2F).

Discussion

This is the first study revealing the relationship between serum vitamin D levels and homocysteine and BDNF in stroke patients. In stroke patients, serum vitamin D levels were found to be lower than those of healthy individuals at the time of admission to the first hospital. We observed that the homocysteine level was high and the BDNF level was low. Stroke, which is a cerebrovascular disease, is one of the main causes of disability, as well as the third cause of death[8]. It has been reported that low vitamin D levels are among the risk factors for ischemic stroke[9]. It has also been reported that vitamin D is effective in preventing stroke and reducing post-stroke mortality and morbidity[10].

Kilkkinen et al[10] reported a significant relationship between vitamin D levels, the development

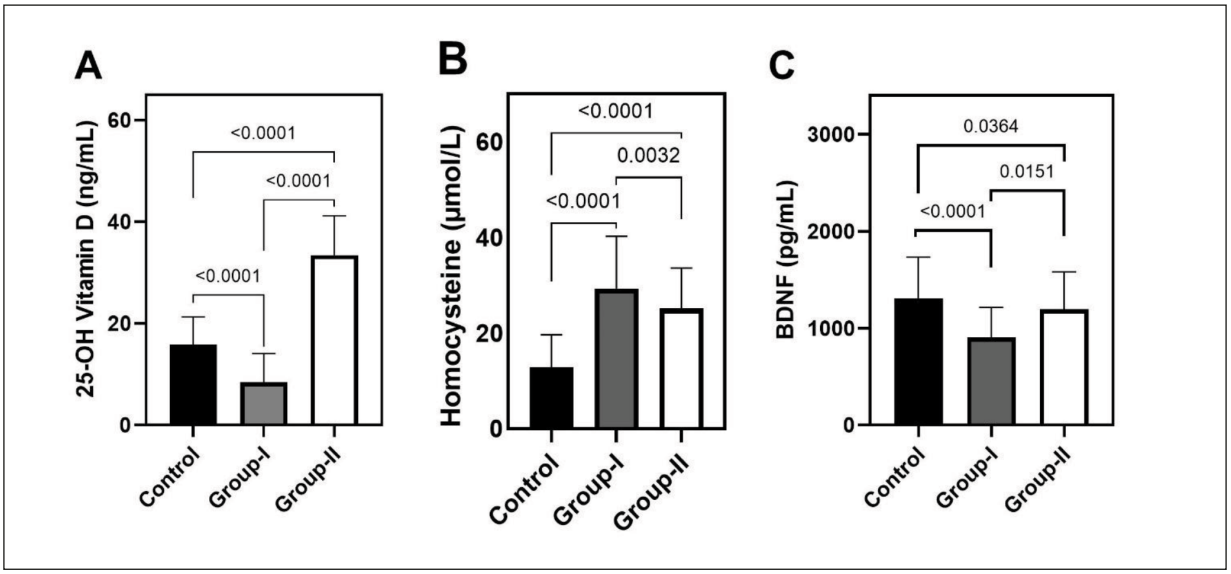

Figure 1. Comparison of serum vitamin D (**A**), homocysteine (**B**), BDNF (**C**) in control and patient groups (Group-I: at hospital admission time; Group-II: 24 hours after admission. Statistical analysis was done with Tukey's multiple comparison test).

of fatal ischemic stroke, and a nonsignificant relationship with hemorrhagic stroke. Brondum-Jacobsen et al[11] reported that vitamin D levels were gradually correlated with the increased risk of IS, but not with hemorrhagic stroke. Again, this difference was explained by the relationship between traditional risk factors of ischemic stroke and reduced vitamin D concentration. In a study[12] conducted on 382 stroke patients, it was found that low serum vitamin D levels were associated with a more severe clinical picture at hospital admission and more disability at discharge. Tu et al[13], in their study conducted on 220 ischemic stroke patients, examined the acute phase and the short-term functional status on the 90th day. In the study, serum vitamin D levels of patients with acute IS were reported to be significantly lower than the control group, and they were also found to be independent prognostic markers for predicting stroke severity, functional status at 90 days, and mortality in the early period. In another study[14] conducted on 464 women, a relationship was found between vitamin D level deficiency and ischemic stroke; however, the strongest relationship was found to be with lacunar infarction. In this study, vitamin D levels of the patients admitted to the hospital with the diagnosis of acute IS were found to be significantly lower than those of the control group (Figure 1A). It is thought that this finding supports the idea that low vitamin D levels may be one of the risk factors for IS.

High blood homocysteine concentration was related to the increased risk of stroke[15]. On the other hand, vitamin D supplementation was reducing serum homocysteine level[16]. Our findings support this study; when the patients included in this study applied to the hospital, vitamin D treatment was started. Therefore, as seen in Figure 1A, serum vitamin D levels increased 24 hours after admission to the hospital. In correlation to this, we found that the level of homocysteine increased at the 24th hour (Figure 1B). However, we found that there was no significant difference between the homocysteine level at the first admission to the hospital and at the 24th hour (Figure 1B). This finding indicates that vitamin D supplementation does not effectively increase homocysteine levels in the short term. We found a weak correlation between vitamin D levels and homocysteine levels in both group-I and group-II in this study. High homocysteine level was related to the increased risk of stroke[15], and vitamin D supplementation reduced the level of homocysteine[16]. Therefore, this study reveals the relationship between vitamin D supplementation and homocysteine levels in stroke patients.

BDNF plays an important role in the prognosis, pathogenesis and rehabilitation of stroke. Low circulating levels of BDNF have been reported[17] to be associated with a higher risk of stroke and poor recovery. A meta-analysis[18] of 4 studies involving 499 stroke patients revealed that serum BDNF con-

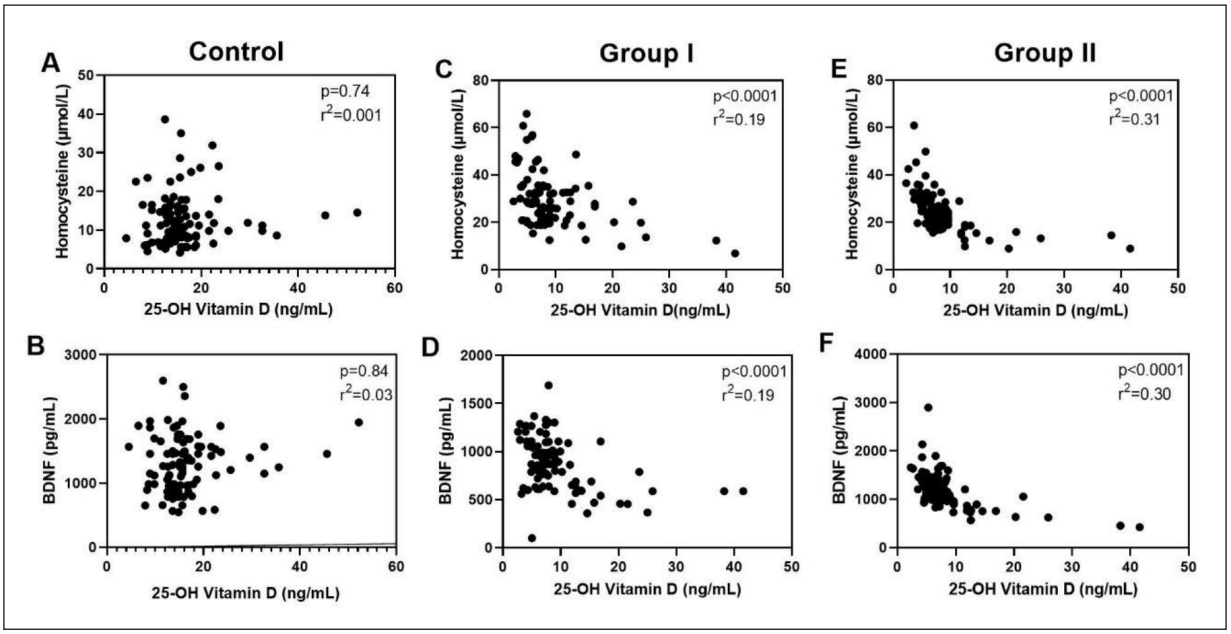

Figure 2. Correlation analyzes; control group vitamin D *vs.* homocysteine (**A**), vitamin D *vs.* BDNF (**B**); Group-I vitamin D *vs.* homocysteine (**C**), vitamin D *vs.* BDNF (**D**); Group-I vitamin D *vs.* homocysteine (**E**), vitamin D *vs.* BDNF (**F**) (Correlation analysis was performed with Spearman correlation test).

centrations were significantly lowered in the early post-stroke period. The level of BDNF has been associated with clinical prognosis in the acute phase of ischemic stroke[19]. Better prognosis has been demonstrated using agents that manipulate BDNF levels in the treatment of stroke[20]. In this study, it was found that BDNF levels in stroke patients were significantly lower than in healthy patients. This result is supported by findings reported by other researchers to further prove its credibility[19,20]. The BDNF level was lower than that of healthy individuals, both after the first admission to the hospital and 24 hours after admission (Figure 1C). On the other hand, it was found that the level of BDNF at the first admission was significantly lower than some time after the 24th hour. We also performed a correlation analysis between vitamin D levels and BDNF levels within the groups (Figure 2). It was found that in both groups (group-I and group-II) BDNF levels showed a weak correlation with vitamin D levels. This suggests that this result may be due to the vitamin D treatment administered to the patients. There is a limited number of studies revealing the relationship between vitamin D levels and BDNF in stroke. For instance, Abiri et al[21] reported that vitamin D supplementation increased the serum level of BDNF in the brain.

Karantali et al[1] reported that stroke severity in the acute stroke phase was negatively correlated with BDNF levels. In the same study, it was reported that serum BDNF levels were lower in patients with acute stroke compared to healthy controls[1]. The findings obtained in this study support their results. As seen in Figure 1C, BDNF level is lower in the acute stroke period compared to the control group consisting of healthy individuals. It was observed that the BDNF level increased significantly when vitamin D supplementation was started. BDNF has been widely used in stroke as an indicator of neural regeneration and recovery. Its role in angiogenesis, neurogenesis, brain repair and synaptic plasticity has been demonstrated through animal experiments and has identified BDNF as an important component of post-stroke recovery[22]. These two studies[1,22] show that vitamin D treatment in the acute stroke period both reduces the severity of the stroke and contributes to the recovery process. Therefore, it is important to reveal the correlation between Vitamin D and BDNF. The findings of this study regarding BDNF are thought to be important in this respect.

IS is an important health problem. The occurrence of IS is more frequent worldwide due to the increasing age of the population. Therefore, the search for effective target molecules in the treatment of IS continues. In recent studies[23,24], homocysteine and BDNF have been proposed as

therapeutic targets in the treatment of IS. In these patients, vitamin D supplementation was found to reduce homocysteine and BDNF levels therapeutically (negative correlation). Considering this aspect of this study, it is thought that it will shed light on new studies.

This study should be tested in an animal ischemic stroke model. The levels of some protein concentrations in the tissue and serum levels differ. Therefore, analyzing the homocysteine and BDNF protein and mRNA expression level of vitamin D supplementation animal model of ischemic stroke will increase the reliability of the results.

Conclusions

In this study, a weak correlation was found between vitamin D levels and homocysteine and BDNF levels in both group-I and group-II. This study demonstrated for the first time the relationship between vitamin D supplementation and homocysteine and BDNF levels in stroke patients. It has been reported that homocysteine and BDNF levels are low in acute stroke patients[1] and that these molecules are an important component of recovery after stroke[22]. Therefore, revealing the correlation between vitamin D, homocysteine and BDNF is thought to be important in the treatment of IS.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Funding

None

Ethics Approval

For this study, permission was obtained from the Harran University Clinical Research Ethics Committee (number: HRU.19.0816).

Informed Consent

The authors declare that the patients included in the study signed informed consent forms to use their medical information in the studies.

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