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ORIGINAL ARTICLE

The Relationship between Serum Vitamin D Level, Ischemic Stroke Severity and Lesion Volume in Ischemic Stroke Patients in Iran, 2019: An Analytic Cross-sectional Study

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KEYWORDS
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ABSTRACT: Vitamin D deficiency is a major global problem. The relationship between serum vitamin D level, ischemic stroke severity, and the resulted lesion volume is controversial. This study is aimed at investigating the relationship between serum vitamin D level, severity of ischemic stroke, and lesion volume compared to the control group. This analytic cross-sectional study was performed on 93 ischemic stroke patients compared to the control group during 2019. Brain imaging, lesion volume measuring, and record were performed for all patients. Severity of stroke was assessed by MRS score at the time of admission and discharge. Serum 25(OH) D levels were measured by enzyme-linked immunosorbent assay (ELISA). In the end, the data were analyzed by SPSS19 software. The mean ±standard deviation of vitamin D was 28.78±9.5 $ng.ml^{-1}$ with the range of (10-49) in patients, and 29.11±8.7 with range of (12-51) in control group. Vitamin D level significantly decreased depending on age (P=0.003). The mean vitamin D levels had a significant negative relationship with the first and second MRS scores (P<0.001). Mean vitamin D levels had a significant negative relationship with the severity of stroke and lesion volume (P<0.001). Reduced vitamin D serum level is associated with higher severity of stroke and lesion volume.

INTRODUCTION

Stroke is the third major death cause in developed societies after heart disease and cancer [1]. Due to motor impairment, inability to perform social activities, and the caused depression, it is an important problem in the general population, especially among the elderly, [2-5].

There are about 33 million stroke patients worldwide today most of whom suffer from moderate or severe disabilities [6]. As the second major death cause after ischemic heart disease in Iran, stroke was the cause of 41,600 deaths in 2012. In the United States, about 795,000 people are affected by stroke each year of whom, 610,000 people experience that for the first time. On average, stroke occurs in one person every 40 seconds and one dies of stroke every 4 minutes in the United States [7].

Eighty percent of strokes are ischemic [8], which is actually the onset of a neurological disorder that can be explained by vascular cause. Cerebral ischemia lasting for more than a few second results in ischemic stroke, and glycogen depletion leads to the appearance of neurological symptoms. If the decrease in blood flow lasts for more than a few minutes, infarction or brain cell death will occur [9].

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Stroke is a complex and heterogeneous disorder with a strong genetic component [10]. This genetic component has been the subject of several studies, and various genes have been investigated based on the major physiopathological pathways of diseases such as coagulation, lipid metabolism, renin-angiotensin-aldosterone system, endothelial dysfunction, inflammation, and abnormal homocysteine metabolism[11, 12]. However, there are several more functional genes that may be involved in the risk of stroke occurrence with the vitamin D receptor being one of the potential candidates for cardiovascular disorders [13].

Vitamin D is the most important steroid hormone involved in the homeostatic regulation of inorganic ions. In fact, vitamin D and its metabolites are hormones and hormonal precursors rather than vitamins; because they can be synthesized in body under appropriate biological conditions [9]. Vitamin D exerts its biological effects through binding to a member of the nuclear receptor super family called vitamin D receptor (VDR)[9].The discovery that most tissues in the body have a VDR has provided new insights into the widespread functions of vitamin D and the non-osseous effects of its deficiency[14, 15].Although the role of vitamin D in bone metabolism and calcium homeostasis is well known, there is a lot of evidence about the relationship between vitamin D deficiency and conditions such as hypertension, insulin resistance, diabetes mellitus, cancers, infections, autoimmune diseases, heart failure, cardiovascular disease and stroke [15-17].

Decreased 25-OH vitamin D plasma level which is a diagnostic marker of vitamin D deficiency, seems to be associated with several ischemic stroke risk factors such as hypertension, thrombosis, atherosclerosis and inflammation [18]. Although some research has shown a significant relationship between vitamin D serum levels and stroke severity [19-27], this relationship was not significant in some other studies [28, 29].

Given the importance of this issue, with regard to the fact that stroke and vitamin D deficiency are both major health problems in Iran and the very limited number of studies conducted in this field in this country, the present research investigated vitamin D serum levels and their relationship with stroke severity and extent of the lesions occurred in patients referring to Kowsar Hospital in 2019.

MATERIALS AND METHODS

The present analytic cross-sectional study was performed on patients admitted in neurology ward of Kowsar Hospital of Semnan in 2019 for stroke. The 93 patients with ischemic stroke in the patient group and the 93 patients from the same ward in the control group were selected by convenience sampling. Inclusion criteria included: patients hospitalized in the neurology ward for the first experience of ischemic stroke based on neurological diagnosis and confirmation of the disease by clinical symptoms and brain images. Exclusion criteria included: stroke record, vitamin D intake in the past 12 months, malignant tumors, cerebral hemorrhage, head trauma, severe edema and autoimmune diseases. Before conducting the study under the project number 1189 and Ethics Code IR-SEMUMS.REC.1395.214, and after explaining the study process and objective to the participants, informed consent was obtained from all of them.

The patients hospitalized with ischemic stroke for whom the diagnosis was confirmed by a neurologist based on clinical examination and brain imaging (CT scan or MRI) were studied within the first 24 hours after hospitalization. Demographic data (age and gender) and risk factors (diabetes mellitus, atrial fibrillation, hypercholesterolemia, coronary artery disease, positive family history and hypertension) were extracted from the patients' medical files. In the patient group, the stroke severity was determined based on modified Rankin Scale (MRS) by a neurologist at the time of admission and discharge. The values of this scale ranged from 0 to 6 (with higher scores indicating more severe stroke); 0-2, 3-4 and 5-6 respectively indicated mild, moderate and severe stroke[30]. In addition, the infarction volume in the patient group was calculated by a neurologist using the formula $0.5\times a\times b\times c$ in which, a is the largest longitudinal lesion diameter, b is the largest transverse lesion diameter, and c is the number of 10-mm slices in which infarction is seen [31]. Vitamin D 25-OH serum levels were measured in both groups by ELISA assay at baseline, and the results were recorded in medical files.

The data were analyzed in SPSS 16 by ANOVA, independent *t*-test, chi-square and Pearson's and Spearman's correlation tests at the significant level of 0.05.

RESULTS

In this study, 93 patients without ischemic stroke in the control group and 93 patients in the ischemic stroke group were evaluated in terms of vitamin D levels, stroke risk factors, lesion volume, and MRS score (in the patient group). The subjects' age was 72.27±12 (mean ±SD) with the range of (51-98) years in the patient group, and 71.48±11 (mean ±SD) with the range of (53-92) years in the control group; there was no significant difference between the subjects in terms of age. In both groups, 56 patients (60.2%) were male and thirty-seven (39.8%) were female. The frequency of the risk factors in both groups is presented in Table 1.

Table1. Frequencies of different risk factors in the patient and control groups

Underlying disease		Patient group Number (%)	Control group Number (%)
Hypertension	+	71 (76.3)	69 (74.1)
	_	22(23.7)	24(25.8)
Hyperlipidemia	+	11(11.8)	15(16.1)
	_	82(23.7)	78(83.8)
Ischemic heart disease	+	21(22.6)	18(19.3)
	_	72(77.4)	75(80.6)
Diabetes Mellitus	+	29(31.2)	28(30.1)
	_	64(68.8)	65(69.8)
Congestive Heart Failure	+	9(9.8)	10(10.7)
	_	84(90.3)	83(89.2)
Smoking	+	10(10.8)	13(13.9)
	_	83(89.2)	80(86.02)

Table 2 presents the frequency distribution of patients in terms of the (MRS) criterion in the patient group at the time of admission and discharge. The scores of 0-2, 3-4,and 5-6 were respectively considered as mild, moderate, and severe stroke[30].

Table 2. Frequency distribution of the patients in terms of MRS score sat the time of admission and discharge.

MRS	First time No. / %	Second time No. / %
0	0 / 0	1 / 1.1
1	1 / 1.1	11 / 11.8
2	10 / 10.8	22 / 23.7
3	20 / 21.5	17 / 18.3
4	32 / 34.4	20 / 21.5
5	30 / 32.2	18 / 19.4
6	0 / 0	4 / 4.2
Total	93 / 100	93 / 100

The frequency distribution of stroke severity at admission and discharge according to the MRS score are shown in Table 3.

Table 3. Frequency distribution of patients in terms of stroke severity at the time of admission and discharge based on MRS

MRS Score	Admission(%)	Discharge(%)
Mild	12	36
Moderate	56	40
Severe	32	24

Vitamin D levels were 28.78±9.5 $ng.ml^{-1}$ (mean ±SD) with the range of 10-49in the patient group and 29.11±8.7 $ng.ml^{-1}$ (mean ±SD) with the range of12-51in the control group with no significant difference between the subjects. In addition, the mean lesion volume was 10.51±12.4mm^3 (mean ±SD) ranging in0.02-40 mm^3in the patient group. According to the results, vitamin D levels were inversely correlated with age (r=-0.307, p=0.003) and lesion volume (r=-0.494, p<0.001) in patients with ischemic stroke and significantly higher in men than women in both groups (Table 4).

Table 4. Vitamin D serum levels in terms of gender in both groups.

Group	Gender	Vit D (Mean± SD)	P value
Patient	Male	31.48± 8.8	0.001
	Female	24.7± 9.1	
Control	Male	33.54± 7.6	0.001
	Female	25.45± 8.1	

In the patient group, serum vitamin D levels were not significantly associated with ischemic stroke risk factors including diabetes, hypercholesterolemia, coronary artery disease, smoking background, and hypertension (Table 5).

Table 5. Vitamin D serum levels in terms of every risk factor in the patient group

Risk factor		Number	Percent	Vit D(Mean ± SD)		P value
Hypertension	+	71	76.3	28.08 ±	10.0	0.138
	-	22	25.7	31.05 ±	7.3	
Diabetes Mellitus	+	29	31.1	27.59 ±	10.3	0.416
	-	64	68.9	29.33 ±	9.1	
Hyperlipidemia	+	11	11.8	28.91 ±	10.3	0.966
	-	82	88.2	28.77 ±	9.4	
Ischemic heart disease	+	21	22.5	27.05 ±	9.9	0.344
	-	72	77.5	29.29 ±	9.3	
Congestive Heart Failure	+	9	9.6	25.11 ±	8.9	0.227
	-	84	90.4	29.18 ±	9.5	
Smoking	+	10	10.8	32.40 ±	8.1	0.171

There was a significant direct relationship between the admission and discharge MRS scores in the patient group (r= 0.891, p<0.001).Gender had no significant relationship with the admission MRS scores (r= 0.099, p=0.334) and discharge (MRS) scores (r= 0.140, p=0.181). However, age had a significant direct relationship with the admission MRS scores (r=0.269, p=0.009) and the discharge (MRS) scores (r=0.315, p=0.002). Vitamin D levels were inversely correlated

with the admission MRS scores (r=-0.503, p<0.001), and the discharge (MRS) scores (r=-0.642, p<0.001).

DISCUSSION

This research is one of the first studies conducted in Iran to investigate the relationship between vitamin D serum levels, ischemic stroke severity, and stroke-induced lesion volume. The results suggested that the serum vitamin Dlevelswere28.78±9.5 $ng.ml^{-1}$ (mean ±SD) ranging between10-49 $ng.ml^{-1}$in patients with ischemic stroke, and 29.11±8. $ng.ml^{-1}$(mean ±SD) ranging between 12-51 in the control group; no significant difference was observed between them. However, serum vitamin D levels were lower than normal levels [20]. The mean volume of stroke-induced lesions in the present study was 12.41±10.51 mm^3 (mean ±SD), and serum levels of vitamin D had a significant negative relationship with the lesion volumes. In the present study, stroke severity in patients was evaluated based on MRS; i.e., MRS was calculated and recorded at the time of admission and discharge. MRS score of higher than 5 (indicating high stroke severity) was found in 32.3% of the patients at the time of admission and in 23.6% of them at the time of discharge. In addition, the mean vitamin D level in patients with high (MRS) scores (23.78 $ng.ml^{-1}$) was significantly lower than this value in patients with lower (MRS) scores. Based on MRS scores, serum vitamin D levels had a significant negative relationship with the stroke severity at the time of admission (r=-0.503, p<0.001).This vitamin had also a significant negative relationship with the stroke severity at the time of discharge (r=-0.642, p<0.001). Therefore, lower serum levels of vitamin D are associated with more severe strokes. In a study conducted in the UK, serum vitamin D levels were measured in 44 patients with a first-time stroke and hemiplegic during the first 30 days after stroke. The results of this study showed that vitamin D deficiency was more prevalent in these patients than in the healthy population[22]. These results are consistent with the findings of our study.

Trotskyet employed MRS and NIHSS to assess the stroke severity at baseline and 90 days later, and showed that the effects of stroke on brain were doubled with every 10 $ng.ml^{-1}$ decrease in vitamin D [20]. Studies performed in China and France in 2012 and several other studies the use of MRS and serum vitamin D levels in stroke patients showed that vitamin D levels were significantly higher in patients with less severe stroke and better outcome [19, 21, 23 and 25]. These results were consistent with the findings of our study. In a study conducted in Australia to determine the relationship between malnutrition-related vitamin D deficiency and the consequences of ischemic stroke in mice evaluating the volume of stroke-induced lesion in the vitamin D-deprived mice group; the results suggested that vitamin D deficiency had no relationship with the infarct size or with the neurological consequences of stroke, or at least, it had no effect on its outcome in the acute phase of the stroke [32]. The results of this study are in conflict with those of the present research.

In an interventional study in India investigating the stroke patients admitted to the neurology ward from April 2014 until March 2016, first serum vitamin D levels were measured, and then, patients with normal vitamin D were excluded from the research. The remaining 60 patients who were included in the study were and only divided into the two groups of (A) and (B). The stroke severity was measured at the time of admission using the long term Siriraj stroke score (SSS). Group (A) received 6 units of intramuscular cholecalciferol, but group (B) did not. Both groups received the routine stroke treatment and the necessary physiotherapy. The SSS was re-measured after three months in both groups to determine the stroke outcome. Comparison of the differences in the baseline scores and those calculated after three months three months in the two groups showed that the differences in patients with vitamin D deficiency were significantly higher in group (A) compared to group (B). However, the differences were not significant in patients with vitamin D deficiency, and it indicates improvement in ischemic stroke outcomes after vitamin D supplementation [33].

In this study, the patients' average age was 72.27±12 years, and vitamin D levels in patients with ischemic stroke had a significant negative correlation with their age. In addition, vitamin D levels were significantly higher in male patients with ischemic stroke than female patients. Other factors examined in this study included

the relationship between serum vitamin D levels and the presence or absence of ischemic stroke risk factors including diabetes, hyperlipidemia, ischemic heart disease, heart failure, smoking and hypertension. Out of the 93studiedpatients, seventy-one (76.3%) patients were hypertensive, eleven (11.8%) had hyperlipidemia, twenty-one (22.6%) had ischemic heart disease, nine (9.7%) had a heart failure record, and ten (10.8%) were smokers. There was no significant relationship between serum vitamin D levels and the presence or absence of these risk factors. These results are inconsistent with the findings of Kojima who showed that vitamin D levels had a significant relationship with BMI, hypertension, and smoking [23].

In a study performed by Wang in China, vitamin D levels decreased with age growth and there was a significant negative relationship between serum vitamin D levels and the stroke severity. These results are consistent with our findings. However, they found no relationship between patients' gender and vitamin D levels; this finding is contrary to our results[19].In a study in performed in Dijon, France, patients with low vitamin D levels were older and there was a higher diabetes rate among them. In this study, no correlation was found between ischemic stroke risk factors and the patients' serum vitamin Dlevels[25]; this result is consistent with the findings of the present research. The wide variation in the results of different studies in this area can be attributed to climatic differences, different prevalence rates of diseases, stroke risk factors, and different causes and factors involved in this risk among different communities.

Besides its strengths, the present research had some constraints as other studies. The most important constraints included the measurement tool and the small number of patients. Regarding the large number of the subject and the numerous known and unknown factors affecting serum vitamin D levels (including the types of clothing, skin color, types of diets, durations of exposure to sunlight, and underlying diseases), these constraints make it impossible to determine a certain relationship between serum vitamin D levels, stroke severity, the resulting lesion volume. Therefore, it is suggested to perform more extensive studies all over Iran in the future moving the mentioned constraints.

CONCLUSIONS

In general, the results of this study showed a significant negative relationship between vitamin D serum levels and the ischemic stroke severity. In addition, serum vitamin D levels had a significant negative relationship with stroke-induced lesions volume. However, further national studies with larger sample sizes are suggested to be done. Moreover, future interventional studies are also suggested to clarify this issue. Nevertheless, it seems that vitamin D deficiency can exacerbate ischemic stroke and cause larger infarct lesions through unknown mechanisms. Discovering the mechanisms of these effects requires performing further studies on genetic variants of this vitamin receptor and on the factors involved in regulating serum vitamin D levels.

Constraints

Although this study revealed some differences in serum Vitamin D levels, stroke severity, and lesion volume in patients with ischemic stroke, because of the sample size and orientation of the study, generalization of results should be done with caution. Also, lack of follow-up due to financial limitation is another constraint of this study.

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Conflict of interest

The author(s) declare no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

Ethical considerations

The code of ethics was obtained from the relevant University. The information was collected confidentially and kept by the researchers. All participants entered the study with the written informed consent and full

knowledge of the study process, and they had the right to withdraw from the study at any time. No cost or harm was imposed on the participants.

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