Received: 20 April 2022 | Revised: 7 September 2022 | Accepted: 8 September 2022

DOI: 10.1002/ajhb.23810

ORIGINAL ARTICLE

American Journal of Human Biology WILEY

The association of 25 (OH) D3 serum level with ischemic cerebrovascular accident risk, severity and outcome in Iranian population

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Abstract

Objectives: The role of combined presence of vitamin D deficiency and other risk factors of stroke in ischemic cerebrovascular accident (CVA) development in Iranian adults has been unclear, so far. The association of vitamin D status at admission with ischemic CVA severity and outcome in this community is not yet well elucidated. This study aimed to clarify these ambiguities.

Methods: In a cross-sectional study 104 hospitalized ischemic CVA patients and 104 healthy controls participated. The serum level of 25 (OH) D3 and baseline biochemical parameters were measured in ischemic patients within the first 24 h of admission, as well as healthy controls. The severity of CVA and clinical outcome were assessed using National Institutes Health Stroke Scale and Modified Rankin Scale, respectively. Data were analyzed using the Chi-square test, independent *t*-test, and multiple logistic regression.

Results: There was a significant difference between patients and controls regarding the presence of vitamin D3 deficiency, hypertension, smoking, and baseline level of LDL and FBS. Vitamin D3 deficiency boosted the risk of ischemic in males and those having family history of CVA. A low serum level of 25 (OH) D3 was associated with more severity and poor outcome of CVA. The CVA severity, vitamin D3 deficiency, and hypertension were predictors of poor outcome.

Conclusions: The study highlights the increased risk of ischemia in Iranians by cooccurrence of vitamin D3 deficiency and other risk factors of CVA. Clinical significance of vitamin D3 deficiency control may be suggested in those at risk of CVA and functional poor outcomes.

1 | INTRODUCTION

Cerebrovascular accident (CVA) is characterized by the acute onset of local neurological dysfunctions with a vascular origin, lasting for a minimum of 24 h (Simon et al., 2009). CVA is the fourth main cause of death. It is also the most common debilitating neurological disease (Sohrabji et al., 2013). The severity of CVA-induced neurological impairment may be high at first, which is common in CVA due to embolism. Alternatively, it may

gradually progress within a few seconds to a couple of hours, which is commonly the case in stroke due to progressive arterial thrombosis or recurrent embolisms (Aminoff et al., 2015). The risk of CVA increases with age, and it is a common health dilemma in elderly communities (Ingall, 2004), imposing a high financial burden on healthcare costs (Ingall, 2004). According to the literature, ischemia and hemorrhage account for about 90% and 10% of the CVA cases, respectively (Simon et al., 2009).

Evidently, genetics also plays a role in CVA (Ohira et al., 2006; Sug Yoon et al., 2001). However, CVA is often multifactorial, and occurs as the result of interactions of environmental and genetic factors (Ohira et al., 2006). Some risk factors for CVA such as aging, male gender, low birth weight, African American race, and positive family history cannot be altered. However, some other risk factors such as hypertension, smoking, peripheral vascular disease, asymptomatic carotid stenosis, congestive heart failure, coronary artery disease, atrial fibrillation, diabetes mellitus, intake of contraceptives, obesity, high total cholesterol level, low HDL (<40 mg/dL), or sedentary lifestyle can be fixed or treated to lower the risk of CVA (Ingall, 2004; Simon et al., 2009; Sug Yoon et al., 2001).

Vitamin D deficiency is highly prevalent worldwide. Recently, vitamin D deficiency has been suggested as a contributing factor of atherosclerosis (Targher et al., 2012; Wang et al., 2008). It has been reported that vitamin D deficiency can cause hypercoagulation and subsequently increase the risk of CVA (Wang et al., 2008). A previous cohort study showed that low dietary vitamin D was an independent risk factor for the incidence of all types of strokes in Japanese-American males (Kojima et al., 2012). Another study showed that a low level of 25 (OH) D3 was associated with increased carotid intima-media thickness (Lu et al., 2020). A prior study reported low 25 (OH) D3 serum levels in the majority of patients with acute stroke (Poole et al., 2006; Zhang et al., 2022). A meta-analysis also showed an association between vitamin D deficiency and a slightly increased risk of CVA (Sun et al., 2012). It seems that vitamin D3 deficiency plays a role in the pathogenesis of cardiovascular diseases.

Particularly, a piece of evidence has indicated that vitamin D deficiency in turn might be associated with some risk factors of CVA such as hypertension, gender and smoking. During last decade, it has been revealed that vitamin D deficiency might be considered as a new risk factor of hypertension (Mehta & Agarwal, 2017). Geographically, vitamin D deficiency might be related to gender in some areas of Iran (Heshmat et al., 2008). Furthermore, smoking appears to be associated with vitamin D deficiency (Yang et al., 2021). Besides, the high prevalence of vitamin D deficiency (Tabrizi et al., 2018) and hypertension were reported in the Iranian population (Haghdoost et al., 2008). Likewise, previous studies have supported the sex and race differences in the association between the incidence of ischemic CVA and risk factors (Howard et al., 2019). There is also a body of evidence regarding the substantial differences in the vitamin D levels of inhabitants of Middle Eastern countries and European countries, signifying the dependency of vitamin D deficiency on geography and race (Siddiqee et al., 2021). However, it is unclear whether the cooccurrence of vitamin D deficiency and each one of the risk factors of CVA including hypertension, gender, smoking, and family history may increase the risk of ischemic CVA in the Iranian community. Moreover, the role of vitamin D deficiency in CVA severity and outcome is not yet well elucidated

Hence, we aimed to investigate the role of the combined presence of vitamin D deficiency, measured by 25-hydroxyvitamin D3 (25 (OH) D3), the best marker of vitamin D status (Chen et al., 2019), and individual factors such as gender, hypertension, smoking, and CVA in first-degree relatives in Iranian people in comparison with control. In addition, we studied the association between vitamin D3 deficiency and ischemic CVA severity. Here, we also determined the effective factors of ischemic CVA development in people with vitamin D3 deficiency and short-time poor clinical outcomes in ischemic patients in Iranian population.

2 | MATERIALS AND METHODS

2.1 | Participants

This cross-sectional study was conducted on 104 Iranian ischemic CVA patients admitted to the neurology unit of Poursina Hospital in Rasht in less than 24 h following the onset of their symptoms and 104 gender- and age-matched healthy controls. The study was approved by the ethics committee of Guilan University of Medical Sciences (Ethics code: IR.GUMS.REC.B94.621) and was conducted according to the Declaration of Helsinki.

The diagnosis of CVA was made by a neurologist according to the clinical signs and symptoms of patients following neurological examination. Computed tomography was also performed to confirm the diagnosis. All patients and controls signed informed consent forms before participation in the study.

Those with an experience of some diseases and conditions such as calcinosis tumor, primary hyperparathyroidism, sarcoidosis, tuberculosis, idiopathic

hypercalciuria, cardiac failure, ischemic heart disease or other cardiovascular diseases, menopause-induced osteoporosis, chronic renal disease, hypoparathyroidism, tumor-induced osteomalacia, rickets, disability, multiple sclerosis, and history of vitamin D3 supplementation in the past year were excluded.

2.2 | Procedures

Demographic characteristics of patients and controls including age, gender, and medical history of their first-degree relatives were recorded by asking the patients or their companions. About 3 mL of venous blood was collected from all patients within the first 24 h after admission as well as control subjects. Blood sampling was done at 8 a.m. for all subjects. Serum 25 (OH) D3 levels were measured by enzyme-linked immunosorbent assay using the available kit [25OH-VIT.D3-RIA-CT (KIP1961), IBL-America, USA] according to the manufacturer's instructions. Routine chemical parameters were quantified using typical kits and techniques in the hospital's lab. Blood pressure measurements were carried out by mercury sphygmomanometer and hypertension was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg, or the use of antihypertensive therapy (Chobanian et al., 2003). Criteria for diabetes mellitus were an FPG ≥126 mg% (7 mmol/L) or HbA1c ≥6.5% or use of insulin or oral hypoglycemic agents (DeSisto et al., 2014). Likewise, 25 (OH) D3 level lower than 20 ng/mL was considered deficient. All subjects were classified into two groups according to this cutoff point of 25 (OH) D3 serum level. In order to determine the CVA severity at admission, we applied the National Institutes of Health Stroke Scale which is a tool to objectively quantify impairment intensity caused by stroke. This scale consists of 11 items that assess specific abilities including consciousness levels, horizontal eye movement, visual field test, facial palsy, motor, and speech and language functions. A score ranging from 0 (normal function) to 2 or 3 or 4 (complete impairment) can be considered for each specific ability according to clinical examination by a physician. The total score of the scale is between 0 and 42. Stroke severity can be categorized based on total score as no stroke symptoms (0), minor stroke (1–4), moderate score (5–15), moderate to severe (16–20), and severe stroke (21–42) (Directorate General of Health Services, 2010). CVA short-time outcome during discharge was evaluated using modified Rankine scale (mRS) as a 7-level scale ranging from 0 to 6 for measuring the degree of disability in the daily activities of stroke patients. According to mRS scoring system, the clinical outcomes of stroke patients can be graded as no symptoms at all (0), no significant disability despite symptoms (1), slight disability (2), moderate disability (3), moderately severe disability (4), severe disability (5), and death (6). We considered the short-time clinical outcome of CVA based on the mRS score as poor >2 and good ≤2 (Bruno et al., 2010).

2.3 | Statistical analysis

All data were analyzed using SPSS version 22. The normal distribution of the serum 25 (OH) D3 levels was determined by the Kolmogorov–Smirnov test. Thus, we applied the parametric independent *t*-test to compare the serum level of vitamin D3, other chemical measures and age between the CVA and control groups. We also stratified all subjects according to gender, hypertension, smoking and family history of CVA and compared the frequency of vitamin D3 deficiency between CVA and control subjects in males, females and those with and without a family history of CVA, smoking and hypertension, separately. The Chi-square test was applied to compare the frequency of qualitative variables among independent groups. Multiple logistic regression was done to scrutinize the effective factors on the development and outcome of CVA. Quantitative data was shown as mean ± SD, median and interquartile ranges. Qualitative data were presented as the frequency and percentage N (%). All tests were considered two-tailed and significance was reported at $p < .05$.

3 | RESULTS

As shown in Table 1, the mean age of CVA patients and control was 69.40 ± 12.78 years and 70.04 ± 11.42 years, respectively. Among CVA patients, 53 (50.96%) were males and 51 (49.04%) were females. In control group, about 56 (53.85%) and 48 (46.15%) were male and female, respectively. There was no significant difference in gender frequency and mean age between the control and CVA groups ($p > .05$). The percentage of hypertension in CVA patients (74.03%) was significantly higher than in controls (39.42%). There was no observed significant difference between patients (44%) and controls (48%) regarding the presence of diabetes mellitus. There was a substantial difference in the frequency of smokers between the patient (54.8%) and control (30.76%) groups. We did not find a statistically significant difference in BMI mean between patients (27.41 ± 2.08) and control (26.93 ± 1.75) groups ($p = .204$). Interestingly, the difference in the mean serum level of 25 (OH) D3 between the patient (18.85 ± 10.39) and control (21.61 ± 11.55) groups

TABLE 1 Demographic, past medical history, baseline chemical parameters and CVA severity and outcome at discharge in patients and controls

Variable	CVA patients (n = 104)	Controls (n = 104)	*p*-value	OR (95%CI)
Age, years (mean ± SD)	69.40 ± 12.78	70.04 ± 11.42	.706	
Gender N (%)			.677	0.89 (0.52–1.54)
Male	53 (50.96%)	56 (53.85%)		
Female	51 (49.04%)	48 (46.15%)		
Hypertension N (%)			*.01*	*4.38 (2.43–7.90)*
Yes	77 (74.03)	41 (39.42)		
Diabetes mellitus N (%)			.753	0.86 (0.50–1.48)
Yes	46 (44.23)	50 (48.07)		
Smokers N (%)	57 (54.8)	32 (30.76)	*.011*	*2.73 (1.55–4.82)*
Familial history of CVA N (%)				
Yes	73 (70.19)	66 (63.46)	.603	1.36 (0.76–2.42)
Vitamin D serum level (mean ± SD)	18.85 ± 10.39	21.61 ± 11.55	.072	
Presence of vitamin D deficiency N (%)			*.036*	*1.80 (1.04–3.13)*
Deficient vitamin D [≤ 20 ng/mL]	65 (62.50)	50 (48.08)		
Sufficient vitamin D [>20 ng/mL]	39 (37.50)	54 (51.92)		
Total Cholesterol [mg/dL] (mean ± SD)	83.96 ± 46.3	173 ± 33.81	.16	
LDL-C [mg/dL] (mean ± SD)	117.73 ± 43.5	90.6 ± 33.21	*.02*	
HDL-C [mg/dL] (mean ± SD)	38.14 ± 8.82	42.11 ± 22.62	.18	
TG [mg/dL] (mean ± SD)	144.78 ± 59.21	148.47 ± 71.16	.76	
FBS [mg/dL] (mean ± SD)			*.001*	
NIHSS score at admission, median (IQR)	235 ± 89.03	124.41 ± 40.26		
CVA severity at admission N (%)	5 (2–13)	–		
NIHSS score <5 (Minor)				
5 ≤ NIHSS score <16 (Moderate)	52 (50)	–		
NIHSS ≥16 (Moderate to severe & Severe)	29 (27.88)	–		
mRS score at discharge, median (IQR)	23 (22.11)	–		
Clinical outcome at discharge N (%)	2 (0–3.2)	–		
Poor (mRS >2)	40 (38.46)	–		

Note: Italic values are indicating significant differences between groups.
Abbreviations: IQR, interquartile; SD, standard deviant.

was not significant ($p > .05$). However, the percentage of vitamin D3 deficiency was considerably higher in CVA patients (62.5%) than in healthy controls (48.08%). About 37.5% of CVA patients and 51.92% of healthy controls demonstrated sufficient vitamin D3 ($p = .036$). There was no significant difference between patients and controls in terms of total cholesterol, HDL-C, and TG levels ($p > .05$). Nevertheless, a remarkable difference in FBS ($p < .001$) and LDL-C ($p = .02$) levels were seen between CVA patients and healthy controls. Likewise, it was revealed that about 50% and 27.88% of patients experienced minor and moderate CVA, respectively. Moreover, the percentage of moderate to severe and severe CVA was approximately 22.11% in patients. The poor short-time outcome was seen in 38.46% of patients. Gender (OR = 0.89), diabetes mellitus (OR = 0.86), and family history of CVA (OR = 1.36) alone did not increase the risk of ischemic CVA in Iranian population. However, hypertension (OR = 4.38), smoking (OR = 2.37), and vitamin D3 deficiency (OR = 1.8) each one alone substantially increased the risk of ischemic CVA in the Iranian. More details are presented in Table 1.

According to Table 2, a significant difference was shown in the frequency of vitamin D3 deficiency between CVA patients and controls with a positive history of CVA in their first-degree relatives ($p = .016$).

TABLE 2 Association of vitamin D status with risk of CVA in groups stratified according gender, familiar history of CVA and presence of hypertension

Variables		Group	With vitamin D deficiency (n = 115)	Without vitamin D deficiency (n = 93)	*p*-value*	OR (95%CI)
Gender	Male (n = 109)	CVA	35 (68.16)	16 (31.37)	.022	2.59 (1.14–5.87)
		Control	22 (45.83)	26 (54.17)		
	Female (n = 99)	CVA	30 (56.60)	23 (43.40)	.490	1.30 (0.61–2.77)
		Control	28 (50.0)	28 (50.0)		
Family history of CVA	Positive (n = 139)	CVA	49 (67.12)	24 (32.88)	*.016*	*2.31 (1.16–4.58)*
		Control	31 (46.97)	35 (53.03)		
	Negative (n = 69)	CVA	16 (51.61)	15 (48.39)		
		Control	19 (50.0)	19 (50.0)		
Hypertension	Yes (n = 118)	CVA	64 (83.11)	13 (16.99)	*.01*	*3.15 (1.33–7.49)*
		Control	25 (60.97)	16 (39.03)		
	No (n = 90)	CVA	17 (62.96)	10 (37.04)		
		Control	9 (14.28)	54 (85.72)		
Smoking	Yes (n = 89)	CVA	43 (75.43)	14 (24.57)	*.017*	*2.75 (1.08–6.80)*
		Control	17 (53.12)	15 (46.87)		
	No (n = 119)	CVA	22 (46.8)	25 (53.19)		
		Control	33 (45.83)	39 (54.17)		

Note: Italic values are indicating significant differences between groups.
Abbreviations: CI, confidence interval; OR, odds ratio.
*Chi-square test.

However, it was exhibited that difference in the frequency of vitamin D3 deficiency between CVA patients and controls with a negative history of CVA in their first-degree relatives was not significant ($p = .894$). It was clear that the frequency of vitamin D3 deficiency in males having CVA was significantly more than in male controls ($p = .022$). This difference was not significant between females of the CVA and control groups ($p = .490$). The risk of CVA was boosted by the cooccurrence of vitamin D3 deficiency and family history of CVA (OR = 2.59); and vitamin D3 deficiency and male gender (OR = 2.31). In hypertensive subjects, more vitamin D3 deficiency was manifested in CVA patients than in controls ($p = .01$). In the population without a hypertension history, There was a higher percentage of vitamin D3 deficiency in the CVA patients than in the control ($p = .012$). Furthermore, smokers with vitamin D3 deficiency were markedly more frequent in CVA patients as compared with controls ($p = .017$). However, no significant difference in vitamin D3 deficiency frequency between nonsmokers with CVA and healthy control was observed. The risk of CVA was increased by 3.15-fold and 2.71-fold when the presence of vitamin D3 deficiency accompanied by a history of hypertension and smoking, respectively (Table 2).

Table 3 displays a significant difference between several CVA severity categories as well as poor and good clinical outcomes regarding the presence of vitamin D3 deficiency ($p = .001$). It was clarified that the majority of patients with moderate to severe and severe CVA (73.91%) conspicuously possessed vitamin D3 deficiency. Notably, the percentage of patients with moderate CVA having vitamin D3 deficiency (72.41%) was higher than those with moderate CVA and sufficient vitamin D3 (27.58%). Furthermore, poor outcome was substantially beholden in the patients with vitamin D3 deficiency (72.5%) as compared with those with sufficient vitamin D3 (27.5%).

Based on Table 4, it was disclosed that hypertension ($p = .001$, OR = 2.80), history of smoking ($p = .029$, OR = 2.27), male gender ($p = .037$, OR = 1.77), and history of CVA in first-degree relatives ($p = .045$, OR = 1.36) were four strong predictors of CVA development in people with vitamin D3 deficiency. More information was depicted in Table 4. It was also found that CVA severity at admission ($p = .001$, OR = 4.44), serum 25 (OH) D3 level ≤20 ng/mL ($p = .019$, OR = 3.16) and hypertension ($p = .027$, OR = 2.53) were main effective factors for the prediction of CVA short-time outcome. More details are shown in Table 5.

TABLE 3 Association of vitamin D status with CVA severity at admission and clinical outcome at discharge in CVA patients

Variables	Serum 25 (OH) D ≤20 ng/mL (n = 65)	Serum 25 (OH) D >20 ng/mL (n = 39)	*p*-value*
CVA severity N (%)			
Mild[a] (n = 52)	27 (51.92)	25 (48.08)	*001*
Moderate[b] (n = 29)	21 (72.41)	8 (27.58)	
Moderate to severe & Severe[c] (n = 23)	17 (73.91)	6 (26.09)	
Clinical outecome N (%)			
Poor[d] (n = 40)	29 (72.5)	11 (27.5)	*.001*
Good[e] (n = 64)	36 (56.25)	28 (43.75)	

Note: Italic values are indicating significant differences between groups.
[a]NIHSS <5; [b]5 ≤ NIHSS <16; [c]NIHSS ≥16; [d]mRS <2; [e]mRS >2.
*Chi-square test.

TABLE 4 The results of multiple logistic regression to determine the predictors of CVA occurrence in subjects with vitamin D deficiency

Predictors	*b*	*p*-value	OR	95% CI
Hypertension	1.03	*.001*	2.80	1.18–7.04
Smoking history	0.82	*.029*	2.27	1.34–4.94
Men	0.57	*.037*	1.77	1.02–3.18
Positive familial history of CVA	0.3	*.045*	1.36	1.07–1.56

Note: Italic values are indicating significant differences between groups.
Abbreviations: CI, confidence interval; OD, odds ratio.

TABLE 5 The results of multiple logistic regression to determine the predictors of CVA poor outcome

Predictors	*b*	*p*-value	OR	95% CI
CVA severity at admission	1.49	*.001*	4.44	3.73–5.15
Serum 25 (OH) D ≤20 ng/mL	1.15	*.019*	3.16	2.21–4.11
Hypertension	0.93	*.027*	2.53	1.43–3.63

Note: Italic values are indicating significant differences between groups.
Abbreviations: CI, confidence interval; OD, odds ratio.

4 | DISCUSSION

In this study, we showed a significant association of vitamin D3 deficiency, smoking and hypertension with CVA risk. We also found that male gender and family history of CVA alone was not the risk factor for CVA in Iranians while these factors could be significantly accounted for the risk factor of CVA if they were accompanied by vitamin D3 deficiency. Likewise, the study indicated that hypertension, smoking, and vitamin D3 deficiency each one alone increased the risk of CVA. It seems that vitamin D3 deficiency as an independent risk factor when added to each one of the other two factors potentiated the risk rate of CVA than when there was vitamin D3 deficiency alone. We have realized vitamin D3 deficiency in CVA patients may lead to poor clinical outcomes and intensifying CVA. Likewise, the present study highlighted that hypertension, smoking, male gender and CVA family history were the main risk factors for CVA in people with vitamin D3 deficiency. The more CVA severity, the less 25 (OH) D3 serum levels, the higher blood pressure, the more possibility of poor clinical outcomes in CVA patients would be.

In accordance with our results, a prior study (Bhougal et al., 2016) compared the 25 (OH) D3 level between CVA patients and healthy controls in an Indian population and reported a significant difference between the two groups, but in another study conducted in north Indian (Gupta et al., 2014), vitamin 25 (OH) D level was not significantly different between ischemic CVA patients and control.

Consistent with the result of our study, a prior study exhibited vitamin D3 deficiency was associated

with CVA risk in the Iranian population (Talebi et al., 2020).

Our finding was also substantiated by another piece of evidence (Chaudhuri et al., 2014) signifying that vitamin D deficiency was more common in Indian CVA patients than in healthy controls. According to the literature, the frequency of CVA in those with severe vitamin D deficiency (<10 ng/mL) was significantly higher than that in individuals with mild (10.1–20 ng/mL) vitamin D deficiency. It seems that the level of 25-hydroxyvitamin D less than 20 ng/mL is a common finding in CVA patients, which may be due to decreased exposure to sunlight, as well as malnutrition (Pilz et al., 2008).

Another main finding of our study was that males not female with vitamin D3 deficiency were more susceptible to ischemic CVA experience. This result might arise from a higher incidence of CVA in men and can be partly explained by a higher frequency of several traditional CVA risk factors such as smoking, alcohol intake, hypertension in men (Wang et al., 2019), that seems in turn increase CVA risk if accompanied by the presence of vitamin D3 deficiency.

An outstanding finding of this project was that the risk enhancement of ischemic in subjects with vitamin D3 deficiency and hypertension or smoking or family history of CVA was more than those who experienced solely vitamin D3 deficiency. This study uncovered that hypertension and smoking were the paramount first and second effective factors of CVA development in individuals with vitamin D3 deficiency. Particularly, this project also highlighted an interaction between hypertension and vitamin D3 deficiency for the CVA appearance. Mechanistically, vitamin D acts as an endogenous inhibitor of the Renin–Angiotensin System and attenuates blood pressure and endothelial dysfunction. There is a piece of evidence that expressed the presence of hypertension plentifully aggravates the risk of ischemic stroke associated with low vitamin D levels (Majumdar et al., 2015). Another study also supported an inverse association between 25 (OH) D3 serum levels with blood pressure (Valle & Giannini, 2020). Given that both hypertension and vitamin D deficiency are controllable parameters, precise monitoring and management of vitamin D deficiency and hypertension are suggested to decrease the risk of stroke.

In addition, smoking appears to exacerbate the risk of CVA in people having vitamin D deficiency. Based on a growing number of studies, serum level of 25 (OH) D in smokers was substantially less than in nonsmokers (Jääskeläinen et al., 2013; Thuesen et al., 2012). The exact mechanism(s) of smoke effect on the vitamin D metabolism is still not clear. A possible explanation may be that smokers often had a less healthy lifestyle such as bad dietary habits and less physical activity, leading to reduced sun exposure and thus the synthesis of vitamin D (Kassi et al., 2015; Shinkov et al., 2015). Recently, smoke exposure has been identified as an independent predictor of vitamin D deficiency in pediatrics (Nwosu & Kum-Nji, 2018). Therefore, it is conceivable that smoking might bring about vitamin D deficiency and subsequently beget vascular perturbation and CVA development. Functionally, it remains to be well elucidated which mediators implicate smoking-induced vitamin D deficiency. However, it has been believed that smoking presumably might result in hypoparathyroidism due to nicotine receptors activation in the parathyroid gland (Díaz-Gómez et al., 2007) and adversely impact the mineral metabolism through downregulation and dysfunction of parathyroid hormone as a primary factor that activates the enzyme, 1α-hydroxylase, which converts 25 (OH) D to the biologically active form, 1,25-dihydroxy vitamin D (Khundmiri et al., 2011).

Moreover, it is thought that inflammatory pathways could link smoking and vitamin D deficiency in stroke. As known, acute stroke could activate inflammatory responses by triggering proinflammatory cytokines (Jin et al., 2010). Likewise, it has been discovered that vitamin D plays an important antiinflammatory role by inhibiting of production of proinflammatory cells (Yin & Agrawal, 2014). Besides, smoking may intermeddle with the antiinflammatory action of vitamin D (Lange et al., 2012). It has been well documented that endothelial dysfunction as a hallmark of atherosclerosis may be generated by oxidative stress and inflammation processes (Carnevale et al., 2018). Some studies also suggested vitamin D deficiency and smoking were associated with oxidative stress (Isik et al., 2007; Sziva et al., 2020). Thus, as another plausible mechanism, smoking might exert the detrimental effects on the antiinflammatory and antioxidative cascades triggered by vitamin D. This event as an addendum to already vitamin D deficiency might drastically potentiate the risk of CVA.

Furthermore, CVA mostly occurred in those with positive family history and vitamin D deficiency. It is obvious that vitamin D deficiency is responsible for the aggravation of atherosclerosis (Kassi et al., 2013; Lu et al., 2020). Moreover, patients with a positive family history of CVA have a higher frequency of risk factors for cardiovascular diseases such as atherosclerosis (Scheuner et al., 2008), which may explain the role of vitamin D deficiency in this group.

This study indicated that CVA severity and low serum level of 25 (OH) D3 at admission and hypertension can predict the short-time clinical outcome of CVA. The current study was also portrayed that more severity and poor clinical outcome of CVA was plentifully observed in the

patients with 25(OH) D3 serum level ≤20 ng/mL as compared with those with 25 (OH) D3 serum level >20 ng/mL.

It seems that the proinflammatory reactions induced by vitamin D deficiency (Yin & Agrawal, 2014) might be associated with deleterious biological mechanisms that negatively affect severity and clinical outcome in CVA patients. Further, the neuroprotective function of vitamin D (Makariou et al., 2014) might be abolished in CVA patients with deficient 25 (OH) D, leading to the debilitation and delay of spontaneous neural restoration responses and consequently severe CVA and poor clinical outcomes.

There is a growing body of evidence about the lower serum levels of 25 (OH) D3 and ischemic stroke, higher mortality, and poor outcome (Daumas et al., 2016; Qiu et al., 2017). A previous study also pointed to the potential role of vitamin D3 in CVA and cognitive impairment (Judd et al., 2012) and a vitamin-D-free regimen may intensify learning deficit in Alzheimer's disease (Taghizadeh et al., 2011). Literature has shown that low levels of 25 (OH) D3, and 1, 25-dihydroxy vitamin D3 were independent predictors of fatal stroke. It has been suggested that vitamin D3 supplementation as a promising approach for the prevention of CVA (Pilz et al., 2008; Yarlagadda et al., 2020) might be as effective as statins (Wilding, 2012). Hypertension as the third predictor of CVA outcome might deteriorate vascular dysfunction in stroke patients, leading to weak clinical outcomes.

It should be noted that patients with factors decreasing the serum level of 25 (OH) D3 such as immobility and some certain diseases were not included in this study; thus, the effect of such confounding factors on the results was minimized. This was the superiority of this study and was not performed in many previous studies.

Some lifestyle properties such as nutrition status, physical activity, exposure to sunlight, as well as factors related to vitamin D metabolism including serum calcium and phosphate, inflammatory markers, serum parathyroid hormone, and alkaline phosphatase were not considered in our project. It is seriously recommended these variables be taken into account in the next investigations.

Furthermore, 25 (OH) D3 is used to serve as a marker for vitamin D deficiency in our study and a large number of previous studies and we compare our results with studies in which 25 (OH) D3 was measured. Although there are concerns about using 25 (OH) D3 and the overestimation of vitamin D deficiency, in contrast, there are doubts about using a total 25 (OH) D to assess vitamin D deficiency. Some investigators reported that measurement of a total 25 (OH) D may have some significant disadvantages (Acar & Özkan, 2021). In this method, both 25 (OH) D2 and 25 (OH) D3 are measured as a total of 25 (OH) D and this may lead to misinterpretation in countries that use ergocalciferol in treatment. The potency of 25 (OH) D2 is considered to be less than one-third of the potency of 25 (OH) D3, and when we measure the total 25 (OH) D, due to their inability to differentiate between these compounds, may overestimate the in vivo vitamin D status.

However, further multicenter studies on a large scale in other regions of Iran are suggested urgently to support our findings. A cross-sectional design was another limitation of this study, which does not allow for finding a causal relationship. Future longitudinal studies are required to further elucidate this matter.

5 | CONCLUSION

Taken together, this study provides evidence on the augmentation of ischemic risk in individuals with vitamin D3 deficiency having a male gender or a history of hypertension or smoking or CVA in first degree-relatives. According to the findings of this investigation, precise monitoring of serum levels of 25 (OH) D3 especially in males and those with a history of CVA in first-degree relatives is obligatory. Here, it is found that the presence of vitamin D3 deficiency might intensify CVA and weaken clinical outcomes in patients. Furthermore, CVA severity, vitamin D3 decline, and elevated blood pressure are the strongest predictors of CVA clinical outcome denoting the clinical significance of controlling these factors in the management of CVA patients to reach a better outcome.

AUTHOR CONTRIBUTIONS

Babak Bakhshayesh Eghbali: conception and design of the study. **Sara Ramezani:** drafting the manuscript and tables. **Cyrus Emir Alavi:** editing final original draft. **Amir Reza Ghayeghran:** sampling. **Sina Sedaghat Herfeh:** investigation, project administration, and editing revised draft. **Amirhomayoun Atefi:** revising the manuscript. **Sepideh Rahimi Limouei:** data collection and Statistical analysis. **Malek Moein Ansar:** supervising and drafting of introduction.

ACKNOWLEDGMENTS

The authors would like to thank all staff of Neuroscience Research Center and Clinical Research Development Unit of Poursina Hospital at Guilan University of Medical Sciences, Rasht, who helped us in performing the data collection and study processes, and Mr. Mostafa Haghiri for English editing services.

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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How to cite this article: Eghbali, B. B., Ramezani, S., Alavi, C. E., Ghayeghran, A. R., Herfeh, S. S., Atefi, A., Limouei, S. R., & Ansar, M. M. (2022). The association of 25 (OH) D3 serum level with ischemic cerebrovascular accident risk, severity and outcome in Iranian population. *American Journal of Human Biology*, *34*(12), e23810. https://doi.org/10.1002/ajhb.23810