Original Research Article

Study of association of Ischaemic Stroke and serum 25-hydroxyvitamin-D Levels

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Abstract

Background and Objectives: The association of low vitamin D and cardiovascular diseases and risk factors has been explored in both animal and human studies. This study was undertaken to examine the association of serum 25-hydroxyvitamin D deficiency with ischemic stroke. **Material and Methods:** The present study comprised 41 consecutive ischemic stroke patients and 45 age and sex matched controls attending the medicine ward of MRA Medical College, Ambedkarnagar, Uttar Pradesh, India, from December 2016 to October 2018. All ischemic stroke patients underwent stroke subtyping. This study was approved by the Institutional Ethics Committee. Data were entered and analysed using the Microsoft excel. **Results:** Out of forty one stroke patients, 57.7% were men and mean age was 61.0±10.0 years. 25-hydroxy Vitamin D deficiency was observed in 65% stroke patients and 31% controls (P=0.0026). As NIHSS score increases, mean vitamin D levels decreases. Trend shows that negative association between levels of vitamin D severity of stroke. Statistically significant association was found in type of Infract and vitamin D deficiency status. **Conclusion**: This study found that 25-hydroxyvitamin D deficiency had association with ischemic stroke. Severity of ischemic stroke is inversely related to 25-hydroxyvitamin D levels. Vitamin D deficiency is associated with increased risk of having large stroke as compared to lacunar stroke.

Keywords: Ischemic stroke; Lacunar; 25-hyderoxy vitamin D; NIHSS

Introduction

Stroke is an unexpected onset of focal neurological shortage, a major cause of morbidity and mortality and the second leading cause of death all over the world. [1]. Stroke has a heterogeneous etiology including unmodifiable risk factors such as genetic, age and sex, and modifiable risk factors including diabetes mellitus, hypertension, sedentary lifestyle, dyslipid-emia, and smoking. Most of the studies have reported an association of deficiency of 25-hydroxy-vitamin D with ischemic stroke [2,3] and cardiovascular disease[4,5]. Vitamin D is a 9, 10 -seco steroid and is found usually in humans are vitamin D3 (chole-calciferol) and Vitamin D2 (ergocalciferol) [2].Vitamin D is important for the human body to sustain a balance between calcium and phosphorus Insufficient vitamin D can cause faintness, reduced bone mineralization, increased bone loss and hip fracture and its prevalence is high in both hemispheric populations[6,7]. Serum 25-hydroxyvitamin D is the circulating form of vitamin D with a half life of 2 to 3 weeks and is changed to the active form -1,25-dihydroxy vitamin D3 in the kidneys[8]. 25-hydroxy vitamin D is a marker of vitamin D status in the human body[9]. Vitamin D deficiency is associated with ischemic stroke risk and contributing factors being hypertension, hyperlipidemia, diabetes mellitus and ischemic heart disease[10]. Stroke is the second leading cause of death worldwide, accounting for 9% of deaths. There are an estimated 5.7 million stroke deaths which are expected to increase to 7.8 million by 2030 .[11,12] Low serum vitamin D levels have been found in acute stroke patients compared with normal controls.[12,13] Vitamin D is essential for regulation of brain growth, function, and cerebrovascular physiology [14].Moreover, deficiency of vitamin D influences vascular remodelling through modulation of smooth muscle cell proliferation, swelling, and thrombosis. These vascular changes can eventually cause stroke [15]. Beside this rising percentage of stroke in Asians countries, very few data are existing on the relationship between Vitamin D and stroke. Since Vitamin D levels are straight measurable and its deficiency can be treated, many trials are being done to assess its association with stroke and to prevent stroke if possible. This study aimed to investigate the association between serum 25-hydroxyvitamin D deficiency and ischemic stroke and its subtypes in Indian patients.

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Materials and Methods

Forty one patients with acute ischemic stroke enrolled in medicine ward of MRA Medical College, Ambedkarnagar, Uttar Pradesh. Forty five age and sex matched controls were

recruited from healthy volunteers with no prior history of stroke or transient ischemic attacks. This study consisted of patient attendees and people volunteering for blood donation. The study period was between December 2016 and October 2018. This study was approved by the Institutional Ethics Committee and informed consent was obtained from controls and patients and if patients were severely ill, consent was taken from their relatives.

Selection of cases: Stroke patients met the following criteria: first ischemic stroke, admitted within 10 days of stroke onset. Stroke was defined according to the World Health organization as "rapidly developing clinical signs of focal/global disturbance of cerebral function, with no apparent cause other than of vascular origin."[16] Cerebral infarction was diagnosed on the basis of the first Computer tomography (CT) or Magnetic Resonance Imaging (MRI) brain scan. If patients had a normal CT scan brain, then ischemic stroke was diagnosed based on diffusion weighted MRI.All stroke patients underwent CT scan of brain (to rule out hemorrhagic stroke) initially, followed by MRI of brain. Ischemic stroke subtypes were classified as large artery atherosclerosis, small vessel disease (lacunar stroke) and stroke of undetermined etiology[17].

Exclusion criteria: Patients on vitamin D and calcium supplements**,** Aged less than18 years**,** Patients with renal and hepatic impairment**,** Patients who underwent thrombolysis and Cardio- embolic stroke patients.

The National Institutes of Health Stroke Scale, or NIH Stroke Scale (NIHSS) is a tool used by healthcare providers to objectively quantify the impairment caused by a stroke. The NIHSS is composed of 11 items, each of which scores a specific ability between a 0 and 4. For each item, a score of 0 typically indicates normal function in that specific ability, while a higher score is indicative of some level of impairment[18]. The individual scores from each item are summed in order to calculate a patient's total NIHSS score. The maximum possible score is 42, with the minimum score being a zero[19,20].

Blood collection: Blood collection was done at the time of enrolment of cases and controls; 5 mL blood sample was used for estimation of 25-hydroxyvitamin D. We used chemiluminescent microparticle immunoassay (CMIA) with automated instruments for estimation of 25- hydroxyvitamin D.Values≤20 ng/mL were diagnosed as 25-hydroxyvitamin D deficiency. [21-23] Values from 11-20 ng/mL were considered mild and <10 ng/mL were diagnosed as severe vitamin D deficiency

Statistical analysis: Data were entered and analysed using the Microsoft excel. Quantitative variables were expressed as mean ± standard deviation (SD), whereas qualitative variables were expressed as frequencies and percentages. Comparison between patients and controls was performed using Student t test or chi-square test as deemed appropriate. P values ≤ 0.05 and ≤ 0.01 were considered significant and highly significant, respectively.

Results

Table 1: Distribution of mean vitamin D levels over different age groups and gender amongst cases and controls.

Variables	Mean Vitamin D levels	
	Cases(mean±SD)	Controls(mean±SD)
Age groups(years)		
45-55	27.27 ±09	22.18±11
56-65	16.55±12	27.56±10
66-75	27.55±13	23.93±12
76—90	20.17±11	27.91±13
Gender		
Male	21.48±15	24.58±22
Female	22.40±18	31.27±12

Table 1 shows that the mean and standard deviation of vitamin D levels over different age groups and gender. In cases mean vitamin D was lowest in the age group 56-65 years and highest in the age group 45-55 years. In control mean vitamin D was lowest in the age group 45-55 years and highest in the age group 76-90 years. Mean vitamin D levels in females were higher a compared to male in cases. (table 1)

Table 2: Distribution of vitamin D levels amongst cases and controls

Vitamin D Levels (ng/ml)	Cases	Controls
0-10	3(7.3%)	0(0.0%)
10.1-20	24(60.9%)	14(31.1%)
20.1-30	11(26.8%)	13(28.8%)
>30.1	3(7.3%)	18(40%)
Total	41(100.0%)	45(100.0%)

Table 2 shows that the vitamin D levels in frequency and percentage for cases and controls. Majority of the subjects in case group had vitamin D levels between 10.1 – 20 ng/dl. Maximum number of subjects in control group had vitamin D levels 30 ng/ml and above. Minimum number of subjects were found in both cases and controls. (table 2)

Table 3: Distribution of cases and controls according to Vitamin D deficiency status (Cut off 20 ng/ml)

Vitamin D status	Cases	Controls	P value
Vitamin D deficiency	27(65%)	14(31%)	0.000
Normal Vitamin D	14(35%)	31(69%)	
Total	41(100.0%)	45(100.0%)	

Contingency table 3 shows that the distribution of cases and controls according to Vitamin D deficiency status (Cut off 20 ng/ml). Sixty five percent of cases had vitamin D deficiency and 31% controls had vitamin D deficiency. While 35% cases had normal vitamin, D. Applying Chi square test gives p value of less than 0.05 which was statistically significant. (table 3)

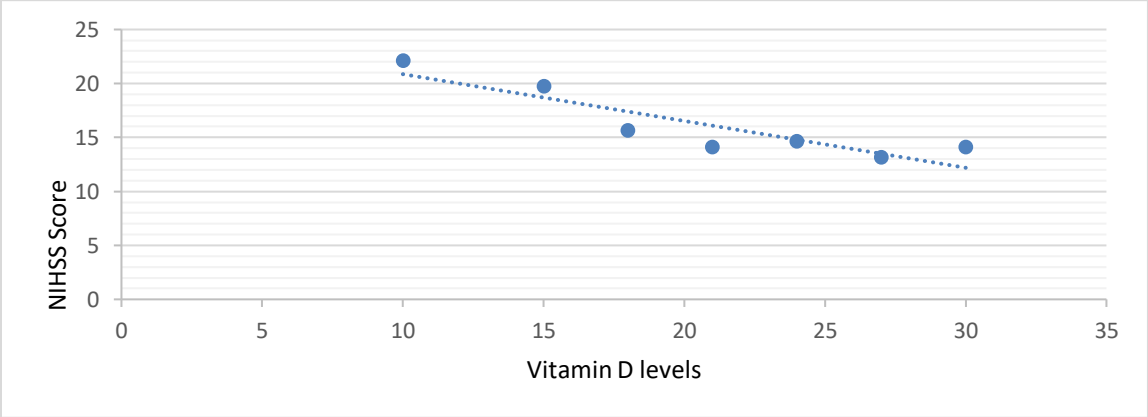

Fig 1:Showing increasing NIHSS Score of case against mean vitamin D Levels

Figure 1 depicts that the relationship between NIHSS Score and mean vitamin D levels. As NIHSS score increases mean vitamin D levels decreases. Trend shows that negative association between levels of vitamin D severity of stroke.

Table 4: Distribution of outcome (at day 7) in cases with their vitamin D deficiency status (Cut off value 20 ng/ml)

Vitamin D status	Expired by day 7	Survived by day 7	Total	P value
Vitamin D deficiency	14(87%)	11(44%)	25(61%)	0.014
Normal Vitamin D	2(13%)	14(56%)	16(39%)	
Total	16(39%)	25(61%)	41(100%)	

Table 4 shows that the distribution of outcome in cases with their vitamin D deficiency status. Fourteen subjects with vitamin D deficiency expired by day seven as compared to only 2 subjects with normal vitamin D. Applying chi square test to above contingency table, we get a p value 0.014 which was statistically significant.(Table 4)

Table 5: Distribution of type of Infract according to vitamin D deficiency status (Cut off value 20 ng/ml)

Vitamin D status	Subjects with Large Artery Infarct	Subjects with Lacunar Infarct	Total	P value
Vitamin D deficiency	21(91%)	2(9%)	23(100%)	0.011
Normal Vitamin D	10(58%)	7(42%)	17(100%)	
Total	31	9	40	

Table 5 shows that the distribution of type of Infract according to vitamin D deficiency status. In subjects with vitamin D deficiency 21(91%) subjects were having large artery infarct as compared to 2(9%) subjects with lacunar infarct. Applying chi square test to above contingency table, we get a p value 0.011 which was statistically significant. (Table 5)

Discussion

In the present total of 86 subjects were evaluated. Out of 86 subjects enrolled, 41 subjects were ischemic stroke cases and 45 subjects were of age and sex matched controls selected from healthy ambulatory adults living in and around Ambedkarnagar. Age may impact vitamin D levels. Vitamin D levels decline with increasing age. Studies done by MacLaughlin et al and Gillor et al. support this fact. Skin is source of 90% of vitamin D in body[24,25]. In addition Harinarayan et al did not find any significant difference among various age groups[26].We did not find any significant association between Vitamin D levels and gender distribution. Mean vitamin D levels in females were higher compared to male in cases. This is contrary to the study done by Johnson et al which showed significant Vitamin D

deficiency among men than women[27]. In present study, significant association was found between 25-hydroxyvitamin-D deficiency and ischemic stroke. Similar findings showed by Chaudhuri et al.,[28]Majumdar et al.,[29] Poole et al.[30]. In present study, NIHSS score increases, mean vitamin D levels decreases. Trend shows that negative association between levels of vitamin D severity of stroke. In addition, Kasukurthy et al reported that the serum25-hydroxyvitamin-D deficiency inmoderately severe stroke patients and severe stroke patients based on NIHSS10 at admission were 51.2% and 54.5% and severity of stroke is higher in patients with serum 25-hydroxy vitamin-D deficiency when compared to patients with normal serum 25-hydroxyvitamin-D levels[31]. Wang et al., found that the serum 25-hydroxyvitamin-D[32]. Levels at admission had inversely correlated with infarct volume and admission neurological deficit assessed by NIHSS[33]. Daubail et al. reported low serum 25-hydroxyvitamin-D level is a predictor of severity of stroke[34]. It has been suggested that serum 25-hydroxy vitamin-D has neuroprotective properties and supplementation could contribute to reduce volume of cerebral infarct in animal models of stroke[35,36]. In present study, reported thatout of vitamin D deficiencysubjects, 91% were having large artery infarct as compared to only 9% subjects with lacunar infarct. Statistically significant association was found in type of Infract and vitamin D deficiency status. Moreover, Study conducted in China by TuWJ et al.[37] suggested low serum 25-hydroxy vitamin-D as an independent predictor of functional outcome at 90 days in acute stroke. The possible pathophysiological mechanisms underlying the association between low serum 25-hydroxyvitamin-D and outcome could be the larger infarct volume in low serum 25-hydroxyvitamin-D levels. Vitamin D may exert anti-inflammatory effects, and post-stroke inflammatory response might be augmented in patients with vitamin D deficiency[38]. In present study, fourteen subjects with vitamin D deficiency expired by day seven as compared to only two subjects with normal vitamin D. Statistically significant association found between result (at day 7) in cases with their vitamin D deficiency status. In addition, Vitamin D deficiency in stroke patients preceded stroke and prevalence of vitamin D deficiency was more in stroke patients than general medical patients[39]. Matthews LR administ-ered 50,000-100,000 IU vitamin D immed-iately after admission and continued to supplement with vitamin D for up to five days and suggested significant development in functional reult in three months, fewer deaths and fewer disabilities[40]. Park et al., found that giving 50,000 IU/day, for first few days after stroke is safe and improves the outcome[41].

Conclusion

This study shows that 25-hydroxyvitamin D deficiency had association with ischemic stroke. Severity of ischemic stroke is inversely related to 25-hydroxy-vitamin D levels. Vitamin D deficiency is associated with increased risk of having large stroke as compared to lacunar stroke.

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