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Vitamin D Status in Admitted Acute Ischemic Stroke Patients of a Tertiary Care Hospital in Bangladesh

Dr. Anil Kumar Shah[1*], Dr. Shahjada Selim[2], Prof. Md. Moniruzzaman Bhuiyan[3], Dr. Mostafa Hasan Rajib[4], Dr. Anil Yadav[5], Dr. Md. Shahed Morshed[6], Prof. M A Hasanat[7], Prof. Md. Farid Uddin[8]

[1]Lecturer, Department of Medicine (Endocrinology Unit), National Medical College, Birgunj, Nepal
[2]Associate professor, Department of Endocrinology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh
[3]Professor, Department of Neurology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh
[4]Medical Officer, Department of Endocrinology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh
[5]Lecturer, Department of Medicine (Endocrinology Unit), Birat Medical College Teaching Hospital, Morang, Nepal
[6]Emergency Medical Officer, Emergency, Kurmitola General Hospital, Dhaka, Bangladesh
[7]Professor, Department of Endocrinology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh
[8]Professor, Department of Endocrinology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

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***Corresponding author:** Dr. Anil Kumar Shah **Email:** anilshah2072@gmail.com

Abstract **Original Research Article**

***Background*:** Vitamin D deficiency (VDD) is found recently common across all age groups and may contribute to Ischemic stroke. V causing isch¬emic stroke has been documented in recent reports. Objectives: To determine the correlation between serum level of Vitamin D with acute ischemic stroke patients admitted to hospital. Materials and methods: A case-control study including 60 acute ischemic patients admitted in the department of neurology as case and 60 apparently healthy genders and age group matched as control were recruited. All the participants were interviewed for medical history. Physical examination was done and imaging reports confirmed the diagnosis of stroke by the neurologist. Data were collected by a pre-tested semi-structured questionnaire, clinical examination [body mass index (BMI), waist circumference (WC), blood pressure] For Laboratory investigation of serum 25-Hydroxy Vitamin D[25(OH)D], venous blood was collected and assayed by High-Performance Liquid Chromatography (HPLC) method. Results: About 94.74% of study populations were found to have low Vitamin D levels of which insufficiency and deficiency were 38.35% and 56.16% respectively. The mean (±SD) Vitamin D level of acute ischemic stroke patients was 18.68± 8.29 whereas 18.07±8.05 in healthy control. Conclusions: There is a high prevalence of biochemical hypovitaminosis D in Bangladeshi of all age and sex groups despite adequate sunshine. There is no significant difference in Vitamin D levels between stroke and healthy participants.

Keywords: 25(OH)D level, Acute Ischemic Stroke, Vitamin D deficiency.

INTRODUCTION

Vitamin D is the most common form in humans are Vitamin D3 and Vitamin D2 [1]. It is derived from sun exposure i.e. synthesized in the skin, and also from the diet including fish, milk, yogurt, orange juice, and cereals [2]. Although 1,25(OH)2D is the active form of Vitamin D, its serum level does not correlate with overall Vitamin D status and thus is generally not clinically useful [2]. Serum 25(OH)D is the circulating form of Vitamin D with a half-life of 2 to 3 weeks and is converted to the active form 1,25-(OH)2D3 in the kidneys. 25(OH)D is a marker of Vitamin D status in the human body [2]. Vitamin D is converted in the liver to 25(OH)D, which is the major circulating metabolite of Vitamin D. In the kidney, 25(OH)D is converted by 1α-hydroxylase to its active form, 1,25-dihydroxy Vitamin D [1,25(OH)2D], which plays a vital role in maintaining bone and muscle health by regulating calcium metabolism. It is widely considered to be the best indicator of Vitamin D status because of its stability, better co-relation with clinical features, an accurate reflection of body storage [3]. There are a number of techniques used to measure 25(OH)D, each with strengths and weaknesses. Different assays result in variation of up to 20% above or below those obtained using the 'gold standard' technique of ultra-performance liquid chromatography and tandem mass spectrometry (LCMS/MS) [4]. HPLC can provide nearly similar results for 25 (OH)D in comparison to LC-MS/MS. LC-MS/MS, however,

requires expensive instrumentation and considerable technical expertise, whereas the proposed HPLC method is less technically demanding and the costs are lower [5]. 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 [6, 7]. Stroke has a heterogeneous etiology, caused by modifiable and unmodifiable risk factors. Recent studies have strongly suggested an association of Vitamin D deficiency (VDD) with ischemic stroke [8]. Lower 25(OH)D concentration was shown to be an independent risk factor for atherosclerosis, coronary calcification, and cardiovascular death [9, 10]. In addition, smooth muscle cells, and lymphocytes express receptors for Vitamin D and convert circulating 25(OH)D to 1,25(OH)2D. 1,25(OH)2D, in turn, reduces the proliferation of lymphocytes and the production of cytokines. As there is increasing evidence that systemic inflammation leads to atherosclerosis [11-13] observed a significantly higher proportion of stroke patients with elevated levels of alkaline phosphatase and decreased phosphate levels compared with control participants, this anti-inflammatory effect may have a protective role. This is advocated by decreased serum phosphate and increased alkaline phosphatase are caused by VDD [14, 15]. Alkaline phosphatases may contribute to atherosclerosis by promoting vascular calcification [16]. Elevated alkaline phosphatases have also been shown to be an independent predictor of mortality after ischemic stroke [17]. Recent studies have advocated that CRP levels are elevated in VDD [18]. This furthers emphasizes the role of vitamin D in reducing inflammation and thus reducing atherogenesis. Several studies have shown a strong association of VDD with cardiovascular disease [19-21]. In a study it was demonstrated low levels of 25(OH)D as a high-risk factor for myocardial infarction [18]. VDD in stroke patients preceded stroke and prevalence of VDD was more in stroke patients than general medical patients [22]. Vitamin D supplementation in post-stroke patients is helpful in the prevention of recurrent stroke and is suggested to reduce neurological, psychological, and musculoskeletal disorders [23]. It also reduces morbidity, mortality and improves functional outcomes after stroke [24]. Stroke patients with low Vitamin D levels have large ischemic infarcts, suffer severe strokes and have poor health outcomes after stroke than those with normal Vitamin D levels found in American Stroke Association International Stroke Conference 2015 [25]. There is a paucity of data on the prevalence of VDD in Bangladesh. In a small-scale study hypovitaminosis, D (≤25 nmol/L) was observed in premenopausal Bangladeshi women of both urban and rural areas [26]. VDD was also observed in Bangladeshi women of reproductive age 27 yrs [28]. There is also a lack of data on the prevalence of VDD among ischemic stroke peoples in Bangladesh. Hence, this study was undertaken to investigate the prevalence of VDD in ischemic stroke patients among the Bangladeshi population.

METHODS AND MATERIALS

This was a case-control study conducted in the In-patient Department of Neurology, Bangabandhu Sheikh Mujib Medical University, Dhaka. Total 60 adult patients with acute ischemic stroke within 14 days of the first occurrence of clinical signs and symptoms admitted in the Department of Neurology, BSMMU and an equal number of 60 apparently healthy genders and age group matched control were recruited as the study population. Written informed consent was obtained from all and the study was undertaken after due approval of the hospital ethics committee. Patients with a history of past or recurrent stroke, currently on calcium alone or calcium and Vitamin D together or history of calcium and Vitamin D supplement within past 4 months, endocrine disorders other than DM, receiving any medications that may alter Vitamin D level, any malignancies, liver, renal disease, and severe heart failure, and connective tissue diseases like gout, rheumatoid arthritis, malabsorption syndrome, gastric or small bowel surgery, inflammatory bowel disease, Pregnancy and lactation were excluded from the study. The reference values of 25(OH)D measured by HPLC assay in the laboratory have been taken from the recommendation of Endocrine Society, Vitamin D sufficiency- ≥30ng/ml, vitamin D insufficiency 21-29 ng/ml, Vitamin D deficiency ≤20 ng/ml. Vitamin D was measured using an automated analyzer: ARCHITECT Plus ci4100 25(OH)D assay, which is a SIL 20 series prominence HPL for the quantitative determination of 25(OH)D in human serum. Data were collected in a pretested semi-structured questionnaire after completion of detailed history and physical examination. Venous blood was collected by venipuncture. Blood samples were centrifuged for 10-15 minutes at 3000 rpm to obtain serum after 10-15 minutes of collection. Samples were preserved appropriately and were analyzed for serum 25(OH)D within a week. The data were analyzed by the SPSS 22.0 for windows software. For comparison of baseline variables between the groups, the Chi-Square ($\chi 2$) or Fisher's exact test was performed for categorical data, the Student's t-test, and one-way ANOVA for normally distributed continuous data. Multivariate logistic regression analyses were performed to identify factors associated with Vitamin D deficiency and adjust for potential confounding factors. Odds ratios (OR) with a 95 % confidence interval (CI) will be provided.

RESULTS

Table-1: Characteristics of the study participants (N=120)

Parameter	Case (n=60)	Control (n=60)	p-Value
Age (In Years)			0.620
<40 yrs.			
40-49 yrs.			
≥ 60 yrs.			
Mean (± SD)	52.05±13.28	50.88±12.64	
Gender			
Male	42(70.0)	39(65.0)	0.560
Female	18(30.0)	21(35.0)	
Residence			
Urban	27(45.0)	40(66.7)	0.020
Rural	33(55.0)	20(33.3)	
Family history of stoke			
Yes	6(10.0)	2(3.3)	0.150
No	54(90.0)	58(96.7)	
Adequate sun exposure			
Yes	9(15.0)	2(3.3)	0.030
No	51(85.0)	58(96.7)	
BMI (kg/m2)	24.93±4.89	24.78±4.85	0.87
SBP (mmHg)	122.30±8.33	120.65±7.27	0.25
DBP (mmHg)	76.95±5.91	76.75±5.49	0.85

The characteristics of study participants were shown in Table 1. Mean age of the acute ischemic stroke and apparently healthy controls was significantly different (57.63±12.90 vs. 50.30±13.01 yr, p=0.02); but when compared among age groups it was not significantly different (p=0.39). There were more male participants than female in both groups. Among cases, 27 were from urban and 33 were from rural area whereas 40 were from urban and 20 were from rural area in control group (p=0.020), family history of stroke was found to be significantly different between both groups (p=0.150). Most of the study population from both case and control had inadequate sun exposure 85.0% and 96.7% respectively (p=0.030). Mean BMI of stroke patients was 24.93±4.89 kg/m^2 and of healthy population control was 24.78±4.85 kg/m^2 (p=0.87). Meansystolic blood pressure among stroke patients was 122.30±8.33 mmHg whereas in healthy population was 120.65±7.27 mmHg (p=0.25). Mean diastolic blood pressure of stroke patients was 76.95±5.91 mm/Hg whereas in healthy population was 76.75±5.49 mm/Hg (p=0.85).

Table-2: Distribution of the study participants by status of vitamin D (N=120)

Vitamin D status	Case (n=60)	Control (n=60)	Total (N=120)	p-Value
Sufficiency (≥30 ng/ml)	4 (6.7)	3 (5.0)	7 (5.8)	0.650
Insufficiency (20-29.99 ng/ml)	21 (35.0)	20 (33.3)	41 (34.2)	
Deficiency (<20 ng/ml)	35 (58.3)	37 (61.7)	72 (60.0)	

Table 2 shows distribution of the study participants by status of Vitamin D, among stroke patients 4(6.7%) were Vitamin D sufficient, 21(35.0%) were insufficient and 35(58.3%) were deficient. Whereas in healthy population 3(5.0%) were Vitamin D sufficient, 20(33.3%) were insufficient and 37(61.7%) were deficient (p=0.650).

Table 3: Mean Serum Vitamin D level between two groups of the participants (N=120)

Parameter	Case (n=60) Mean ± SD	Control (n=60) Mean ± SD	p-Value
Serum 25(OH)D (ng/ml)	18.68±3.61	18.07 ± 3.41	0.340

Table 3 shows the mean Vitamin D level in the study population, among the stroke patients it was 18.68± 3.61 ng/ml and in healthy population 18.07 ± 3.41 ng/ml (p=0.340).

Table 4: Serum Vitamin D as risk factor of Stroke (N=120)

Variable of interest	Stroke Vs. Healthy Control	p-Value
	OR (95% CI)	0.330
Vitamin D (<20 ng/ml)	1.15 (0.63- 2.08)	

Table 4 shows whether Vitamin D deficiency is a risk factor of stroke. With Vitamin D level <20ng/ml, OR(95%CI) 1.15(0.63- 2.08), p=0.330.

DISCUSSION

In this case-control study, most of the participants were male (66.17%). The mean age in years of the case and controls were (52.05±13.28 vs 50.88±12.64) with 0.620, this significantly different value was due to the high number of cases above 60 years of age and unavailability of control in equal number above 60 years of age, but age group was matched. Other confounding variables, area of residence, occupation, education, socioeconomic, smoking, adequacy of sun exposure, and physical activity level were significantly different between the groups. Despite abundant sunlight, this condition is quite prevalent in Bangladesh. This is in agreement with the reported high frequency (100%) of hypovitaminosis D among the Bangladeshi apparently healthy population [29] and 77.5% of stroke patients [30]. Some other studies conducted in Bangladesh have also shown a high prevalence of Vitamin D deficiency especially among premenopausal women where they found 58.90% of the study population had hypovitaminosis D, using cutoff for insufficiency and deficiency as 15ng/ml and 10ng/ml respectively and in female garment workers they found about 99% of the study population had either deficiency or insufficiency according to endocrine society criteria [26, 31]. In the current study mean Vitamin D level was found significantly different (18.68±3.61vs. 18.07 ± 3.41, p=0.330). This is in agreement with north Indian study [32] where they found, median value (IQR) of serum Vitamin D level was 7.94 ng/mL (4.59-14.00) in the cases and it was 8.82 ng/mL (5.59-14.70) in the controls [32]. The difference between the serum 25(OH)D levels of the two groups was not found to be statistically significant. Another similar study also conducted on north Indian patients[33] found no significant difference in the prevalence of Vitamin D deficiency/insufficiency (P = 0.25), mean 25(OH) D levels (P=0.75) between cases and controls. The mean Vitamin D levels in cases and controls were 24.8±16.2 ng/ml and 26.8 ±14.6 ng/ml, respectively [12]. Similar study conducted in Bangladesh [30] to establish the association between low serum Vitamin D and stroke, found that low serum Vitamin D were independently significant predictors for having a stroke [32]. Lower 25(OH)D levels were associated with an elevated risk of ischemic stroke in the Nurse's Health Study: the OR (95% CI) comparing women in the lowest versus highest tertiles was 1.49 (1.01–2.18; P=0.04).34 Most of the patients (61.6%) had hypovitaminosis D out of which Vitamin D insufficiency (43.3%) and Vitamin D deficiency (18.3%) and Vitamin D level was significantly associated with the previous history of stroke [35]. 960 participants developed stroke, where the incidence of stroke was significantly higher in the lowest dietary Vitamin D quartile compared with the highest (all stroke: 6.38 vs. 5.14 per 1000 person-years follow-up, P=0.03), in a 34 years follow-up study conducted on Japanese-American men found [36]. These variations in the results of different studies might be explained by differences in dietary intake, sun avoidance behaviors, and geographical variations of environment, skin color, ethnicity, or genetic predisposition. In addition, the area of study and socioeconomic status of the population, different cut-off Vitamin D levels, may influence the rate of Vitamin D deficiency. There is a high frequency of biochemical hypovitaminosis D in apparently healthy Bangladeshi of all age and sex groups despite adequate sunshine in light of the present consensus of measurement of Vitamin D by the laboratory method. The present study did not find any significant difference in mean Vitamin D level between stroke and healthy population; thus, infers that deficiency of Vitamin D may not be of much concern for the contribution of stroke.

CONCLUSION

There is a high frequency of biochemical hypovitaminosis D in apparently healthy Bangladeshi of all age and sex groups despite adequate sunshine. There is no significant difference in mean Vitamin D level between stroke and healthy population.

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