

Association of Vitamin D Status with Ischemic Stroke and Its risk Factors in Bangladeshi Patients: A case-control study

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INTRODUCTION

Stroke is a common medical emergency caused by either a sudden reduction in the blood to the brain or rupture of intra cerebral vessel result in hemorrhage, causes focal neurological deficit. Most strokes are arterial in origin (>99%), among them 85% are ischemic and rest 15% are haemorrhagic .

It is the 2nd leading cause of death and 3rd most common cause of disability in the world. In Bangladesh, stroke is the third leading cause of death. However, in recent years, accumulating evidence has supported clearly that the most common etiology of ischemic stroke (atherosclerosis) and common modifiable risk factors of stroke are linked with vitamin-D status by different mechanism.

Vitamin-D is an essential fat-soluble vitamin that acts and regulate physiology of body as a steroid hormone. Vitamin D₃ is produced, mostly (80%) from skin on exposure to sunlight or rest from animal and plant sources. vitamin D₂ or D₃, whatever it is, needs two hydroxylations to become biologically active form of vitamin D. Though 25(OH)D is inactive form, it is the best biomarker to quantify vitamin-D status.

After identification of VDR several studies have revealed that, vitamin-D related extra skeletal effects are mainly due to regulating the transcription of multiple genes result in immunomodulatory, anti-inflammatory, anti proliferative and regulation of cell differentiation, apoptosis, angiogenesis, oxidative stress, membrane transport, cell adhesion, renin angiotensin aldosteron system and neurotropic factor expression function.

So, it is now proved by several studies that, extra skeletal effect of vitamin-D deficiency can cause hypertension, type-2 diabetes mellitus, neurological diseases including stroke, other cardiovascular diseases, obesity, dyslipidaemia, autoimmune diseases , cancers, some infectious diseases, depression, muscle weakness and fall .

In this study we intended to find out whether there is any association of vitamin-D with ischemic stroke in our community/setting. This will help us to develop necessary therapeutic strategies in stroke prevention and better post stroke outcome.

General objective:

**To find out the association of vitamin D status
in patients with ischemic stroke and its risk
factors.**

Specific Objectives:

- **To document the socio-demographic characteristics of the stroke patients in our setting.**
- **To describe the distribution of risk factors of ischemic stroke among stroke patients.**
- **To assess the risk factors of vitamin-D deficiency in Ischemic stroke patients.**
- **To determine the prevalence of vitamin D deficiency among ischemic stroke patients and healthy control.**
- **To identify the association of vitamin-D level with risk factors of Ischemic stroke among stroke patients.**

Materials and method

Type of study: This was a case control study

Study population: A total of 44 patients diagnosed as ischemic stroke within 7 days of onset fulfilling both inclusion and exclusion criteria were selected as case in this study. Equal number of age and sex matched healthy people enrolled as control.

Sampling technique: Consecutive sampling

- **List of variables**
 - **Socio demographic variables.**
 - **Predictor variables for vitamin D status.**
 - **Risk factor of ischemic stroke.**
- **Other clinical and laboratory variables :**
Height, weight, SBP,DBP, Fasting lipid profile,
Serum Calcium, Serum 25(OH)D .

Selection criteria for case:

(a) Inclusion criteria:

- Clinically and Radiologically (C.T scan of Head/MRI of brain) documented new case of Ischemic stroke.
- Presented within 7 days of onset of stroke
- Age upto 70 years.

- **(b) Exclusion criteria**

- Patient rejecting to participate in this study.
- Patient with hemorrhagic stroke and stroke secondary to neuro-infection, trauma, malignancy etc. and other stroke-mimics, recurrent stroke.
- Patient with chronic liver disease, chronic hepatitis, chronic kidney disease or ESRD, severe sepsis, malabsorption syndrome, IBD, RA, SLE, systemic sclerosis, COPD, bronchial asthma and Nephrotic syndrome.
- Patient with atrial fibrillation and valvular heart disease.
- Patient with H/O MI (within 6 weeks), H/O fracture

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- Known case of anti phospholipid syndrome, protein C & S deficiency, H/O DVT
- Drug history like steroid, oral contraceptive Pill, Bisphosphonate antiepileptic, antiretroviral.
- H/O Taking calcium & vitamin-D supplements (last 1 month after stroke).
- Pregnancy & Lactation.
- Hyper viscosity syndrome (Known or new case).

Study procedure:

All CT/MRI confirmed acute ischemic patients (case) and controls (age and sex-matched) to their legal guardians were thoroughly informed about the objectives and procedure of the study. After getting informed written consent, clinical history, relevant physical examination findings and base line investigations (ECG,CBC,FBS/RBS,S Creatinine, urine R/E etc) were recorded in the enrollment form to select as case or control as per inclusion and exclusion criteria of each. Then socio-demographic characteristics, anthropometric, clinical data, relevant examination findings and laboratory findings were recorded step by step in predesigned case recorded (CRF).

- **After keeping fasting for 8-12 hours blood sample for fasting lipid profile and serum calcium, serum 25 (OH) Vitamin D were collected and measured.**
- **For controls, same precaution, procedure and technology were followed/applied as for case (Stroke patient). Then results were recorded.**

Statistical and analytical methods:

- Categorical variables were expressed as frequency and percentage. Continuous data were expressed either in mean (\pm SD) or median (Interquartile range) as appropriate according to their distribution.**
- logistic regression model was used to estimate adjusted effect of vitamin D and to determine the independent predictors of ischemic stroke and also the odds ratios (ORs). P <0.05 was considered statistically significant and all P-values were two sided.**

- **Fisher's exact test/Pearson's chi-square test was used which one required.**
- **Independent sample t test was used to assess the differences of means of continuous variables .**
- **The normality of data distribution was checked by the Shapiro-Wilk test.**
- **One-way ANOVA (F test) used for continuous variables .**
- **Bivariate logistic regression analysis was used to define the significant predictor, with Odds Ratio.**

Observations and Results:

Table I: Comparison of Demographic characteristics between two Groups

Variables		Stroke patients (n=44)	Control subjects (n=44)	P value
Age, in years	Mean \pm SD	54.57 \pm 11.02	54.55 \pm 11.10	0.992*
	Range	42-70	42-70	
Sex	Male	27 (61.4%)	27 (61.4%)	1.0*
	Female	17 (38.6%)	17 (38.6%)	
Living area	Rural	37 (84.1%)	29 (65.9%)	0.049*
	Urban	7 (15.9%)	15 (34.1%)	
Religion	Islam	34 (77.3%)	37 (84.1%)	0.354*
	Hinduism	7 (15.9%)	5 (11.4%)	
	Buddhism	3 (6.8%)	2 (4.5%)	
Ethnicity	Bengali	41 (93.2%)	44 (100%)	0.241†
	Tribal	3 (6.8%)	0 (0%)	

Table III: Comparison of factors related to Vitamin D between two groups

Variables		Stroke patients (n=44)	Control subjects (n=44)	P value
Daily activity	Mainly outdoor	10 (22.7%)	16 (36.4%)	0.163*
	Mainly indoor	34 (77.3%)	28 (63.6%)	
Dietary habit^a	Adequate	4 (9.1%)	3 (6.8%)	1.0 [†]
	Inadequate	40 (90.9%)	41 (93.2%)	
Sun exposure^b	Adequate	13 (29.5%)	25 (56.8%)	0.01*
	Inadequate	31 (70.5%)	19 (43.2%)	
Alcohol habit	No	42 (95.5%)	44 (100%)	0.494 [†]
	Yes	2 (4.5%)	0 (0%)	
Smoking habit	Never/Ex-smoker	35 (79.5%)	27 (61.4%)	0.062*
	Current smoker	9 (20.5%)	17 (38.6%)	

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Tobacco leaf	No	34 (77.3%)	44 (100%)	0.001*
	Yes	10 (22.7%)	0 (0%)	
Skin color	Fair	16 (36.4%)	24 (54.5%)	0.087*
	Dark	28 (63.6%)	20 (45.5%)	
Clothing type	Wide coverage	22 (50.0%)	18 (40.9%)	0.392*
	Others	22 (50.0%)	26 (59.1%)	

Table IV: Distribution of the risk factors of ischemic stroke by Groups

Variables		Strokepatients (n=44)	Control subjects (n=44)	P value
Hypertension	Absent	14 (31.8%)	27 (61.4%)	.005*
	Present	30 (68.2%)	17 (38.6%)	
Diabetes mellitus	Absent	30 (68.2%)	37 (84.1%)	0.08*
	Present	14 (31.8%)	7 (15.9%)	
Obesity ^a	Absent	21 (47.7%)	21 (47.7%)	1.0*
	Present	23 (52.3%)	23 (52.3%)	
Dyslipidaemia ^b	Absent	9 (20.5%)	5 (11.4%)	.244 [†]
	Present	35 (79.5%)	39 (88.6%)	
BMI	Mean ±SD	26.08±4.74	25.57±4.38	.604 [#]

Except serum triglyceride and 25(OH)D all other laboratory parameters (total cholesterol, HDL, LDL, FBS and serum calcium) are similar in both groups. Serum 25(OH)D and triglyceride level are significantly less among stroke patients than healthy control.

Table V: Distribution of the laboratory parameters by Groups

Variables	Stroke patients (n=44)	Control subjects (n=44)	P value
Total cholesterol, mg/dl	204.39±48.99	204.68±40.82	0.976 [†]
LDL, mg/dl	128.59±37.54	123.73±29.46	0.501 [†]
HDL, mg/dl	42.57±6.29	41.89±5.66	0.594 [†]
Triglyceride, mg/dl	118 (97-187)	155 (110-212)	<0.001**
FBS, mmol/L	7.13±2.79	6.66±2.05	0.42 [†]
25(OH)D, ng/ml	19.00±7.79	31.05±8.46	<0.001 [†]
Calcium, mg/dl	8.77±0.59	8.64±0.52	0.604 [†]

Unadjusted effect of vitamin D level on acute ischemic stroke is described in Table VI. Majority of the stroke patients (56.8%) are vitamin D deficient whereas majority of the control (61.4%) have sufficient vitamin D.

Table VI: Comparison of vitamin D status between two Groups

Vitamin D status	Stroke patients (n=44)	Control subjects (n=44)	Odds ratio (95% CI)	P value*
Sufficient (≥30ng/ml)	6 (13.6%)	27 (61.4%)	Reference category	-----
Insufficient (20-29.99 mg/dl)	9 (20.5%)	12 (27.3%)	3.37 (0.98-11.62)	0.054
Deficient (10-19.99 mg/dl)	25 (56.8%)	5 (11.4%)	28.13 (7.09-111.47)	<0.001
Severely deficient (<10 mg/dl)	4 (9.1%)	0 (0%)	18.0 (1.69-191.23)	0.017

Table VII: Demographic, epidemiological, and clinical characteristics of acute ischemic stroke patients, according to their vitamin D status

Variables		Ischemic Stroke Patients (n=44)			P value
		Vitamin D Deficient (n=29)	Vitamin D Insufficient (n=9)	Vitamin D Sufficient (n=6)	
Vitamin D (ng/ml)					
	Mean ±SD	14.43±3.6	23.74±2.3	34.01±2.7	<0.001 [¥]
Age (years)					
	Mean ±SD	54.79±10.6	56.29±12.1	50.0±11.8	0.497 [¥]
Sex					
	Male	17 (58.6%)	6 (66.7%)	4 (66.7%)	1.0 [†]
	Female	12 (41.4%)	3 (33.3%)	2 (33.3%)	
Ethnicity					
	Bengali	29 (100%)	8 (88.9%)	4 (66.7%)	0.018 [†]
	Tribal	0 (0%)	1 (11.1%)	2 (33.3%)	
BMI					
	Mean ±SD	26.7±4.8	24.2±2.2	25.5±5.6	0.383 [¥]
Diabetes mellitus					
	Present	10 (34.5%)	3 (33.3%)	1 (16.7%)	0.796 [†]
Hypertension					
	Present	27 (57.4%)	10 (21.3%)	10 (21.3%)	<0.001 [†]
Dyslipidemia					
	Present	25 (86.2%)	6 (66.7%)	4 (66.6%)	0.281 [†]

Table VIII: Odds ratio (95% confidence interval) of acute ischemic stroke for vitamin D status

Vitamin D status	Model 1	Model 2	Model 3
Normal (≥ 30 ng/ml)	1 (Reference)	1 (Reference)	1 (Reference)
Insufficient (VDI) (20-29.9 ng/ml)	3.37 (0.98-11.62) P=0.054	3.25 (0.91-11.74) P=0.072	2.28 (0.67-12.31) P=0.153
Deficient (VDD) (< 20 ng/ml)	26.1 (7.1-95.5) P<0.001	27.6 (7.06-107.83) P<0.001	22.49 (4.9-103.15) P<0.001

Model 1: unadjusted

Model 2: adjusted for age (years), sex, residence, BMI

Model 3: additionally adjusted for smoking, hypertension, diabetes mellitus, dyslipidemia

Table IX: Odds ratio (95%) CI for the predicting effect of the contributing factors of ischemic stroke

Variables	P value	Odds ratio (OR)	95% CI for OR	
			Lower	Upper
Vitamin D (≥ 30 ng/ml vs. < 30)	0.003	10.713	2.212	51.887
Smoking (Never/Ex vs. current)	0.034	5.430	1.135	25.989
Activities (outdoor vs. indoor)	0.466	2.112	0.283	15.780
Sun exposure (Adequate vs. inadequate)	0.949	0.931	0.105	8.256
Skin color (Black vs. fair)	0.370	1.766	0.509	6.134
Hypertension (Absent vs. present)	0.143	3.212	0.673	15.323
DM (Absent vs. present)	0.440	1.789	0.408	7.831
Dyslipidaemia (Absent vs. present)	0.408	0.465	0.076	2.850

Table X: Association of the risk factors of ischemic stroke with vitamin D status (n=88)

Variables	Vitamin D status		Odds ratio (95%CI)	P value	
	Sufficient	Deficient			
	(≥30ng/ml) (n=33)	(<30ng/ml) (n=55)			
Hypertension	Absent	23 (69.7%)	18 (32.7%)	4.73 (1.86- 12.01)	0.003*
	Present	10 (30.3%)	37 (67.3%)		
Diabetes mellitus	Absent	28 (84.8%)	39 (70.9%)	2.29 (0.75- 7.1)	0.137*
	Present	5 (15.2%)	16 (29.1%)		
Obesity*	Absent	19 (57.6%)	23 (41.8%)	1.88 (0.18- 2.17)	0.432*
	Present	14 (42.4%)	32 (58.2%)		
Dyslipidaemia*	Absent	4 (12.1%)	10 (18.2%)	0.62 (0.18- 2.17)	0.432*
	Present	29 (87.9%)	45 (81.8%)		

Table X: Association of the risk factors of ischemic stroke with vitamin D status (n=88)

Variables		Vitamin D status		Odds ratio (95% CI)	P value
		Sufficient (≥30ng/ml) (n=33)	Deficient (<30ng/ml) (n=55)		
Hypertension	Absent	23 (69.7%)	18 (32.7%)	4.73 (1.86-12.01)	0.003*
	Present	10 (30.3%)	37 (67.3%)		
Diabetes mellitus	Absent	28 (84.8%)	39 (70.9%)	2.29 (0.75-7.1)	0.137*
	Present	5 (15.2%)	16 (29.1%)		
Obesity ^a	Absent	19 (57.6%)	23 (41.8%)	1.88 (0.18-2.17)	0.452*
	Present	14 (42.4%)	32 (58.2%)		
Dyslipidaemia ^b	Absent	4 (12.1%)	10 (18.2%)	0.62 (0.18-2.17)	0.452*
	Present	29 (87.9%)	45 (81.8%)		

Discussion

In this present study, Vitamin D deficiency (cut off value <20ng/ml) were found in 65.9% in ischemic stroke patient and 11.4% in control. Our results are generally consistent with two earlier case control studies. Daniela FA, et al, 2016 reported that vitamin-D deficiency in case & control were 43.45% and 5.08% respectively. Laleh A. et al, 2015 reported that severe vitamin D deficiency in Stroke patient were 30% and in control were 11%. Reports from Indian studies also consistent.

In our study mean serum 25 (OH) D level in stroke patient (19.00 + 7.79 ng/ml) is less than control subjects (31.05+8.46 ng/ml), found Statistically significance (P <0.001), Odds ratio for Vitamin-D deficient group is 28.13 (7.09-111.47), P<0.001, Which is highly significant, When cut off value taken (≥ 30 verses < 30 ng/ml) OR is 10.71 (95%CI, 2.21-51.88). This result also consistent with two recent case control study. Alfieri et al in Barzil found OR 16.64 (95%CI, 3.66-42.92) and Tan et al is china found OR 12.92 (95%CI, 6.23-26.82). A meta analysis in 2018 by Ren Zhou et al, included 19 Studies (cohort, RCT, case control study), also consistent with our findings, the pooled RR was 1.62 (95%.CI, 1.34-1.96)

Even after controlling for the classical variable having confounding effect, patient with vitamin D deficiency were 22.49 more likely to have acute ischemic stroke compare with vitamin D sufficient status. Danhiela FA et al. 2016, found VDD were 16.64 more likely to have ischemic stroke than VDS.

Deficiency of vitamin D has been associated with hypertension, diabetes mellitus, and dyslipidaemia. Now study proved that Vitamin D status linked inversely with renin angiotensin system.

We found hypertension is VDD, VDI, VDS are 57.4%, 21.3% & 21.3% respectively and statistically significant, OR for stroke by hypertension is 3.21.

V Majumder et al reported, OR for stroke by hypertension was 13.54 (95% CI; 1.94-94.43). As hypertension is modifiable risk factor of stroke, maintaining sufficient Vitamin D level is crucial.

Though Several studies claimed that vitamin D deficiency related with DM, dyslipidemia, obesity, but we have not gotten statistically significant result as these risk factors of stroke might be related with another mechanism.

We found, Sun exposure significantly inadequate in stroke patient and found statistically significant. In this study other factors related with Vitamin D status were statistically insignificant.

In conclusion, Our study showed that ischemic stroke patients have a higher prevalence of Vitamin D deficiency. It is clear that, Vitamin D deficiency has an independent association with ischemic stroke and also with hypertension.

Recommendation:-

- 1. Further studies with large sample size are warranted to better understanding.**
- 2. RCT are needed to verify this association and explore the prevention effectiveness of Vitamin D supplement.**
- 3. Public awareness is very important.**
- 4. Health policy should be adopted by government.**

THANK YOU ALL