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ORIGINAL ARTICLE

Vitamin D status, hypertension and ischemic stroke: a clinical perspective

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The relationship between vitamin D deficiency and stroke was cross-sectionally evaluated in the high-risk Asian Indian population. Age- and gender-matched, 239 ischemic stroke patients and 241 control subjects were recruited. Vitamin D status was estimated by measuring serum 25-hydroxyvitamin D (25(OH)D) levels. After multivariate adjustment for a range of potential covariates in a logistic regression model, an inverse association was found between serum 25(OH)D concentration and risk of ischemic stroke: subjects with severely low 25(OH)D levels ($\leqslant$9.33 ng ml^{-1}) were found to be at 3.13-fold (95% confidence interval (CI), (1.22–8.07)) increased risk of ischemic stroke as compared with those with high levels. Adjustment for systolic blood pressure levels was found to abrogate this association (odds ratio (OR) = 2.00, 95% CI = 0.61–6.50). On stratification, a pronounced association was found between low 25(OH)D and risk of ischemic stroke in hypertensives, OR = 13.54, 95% CI = 1.94–94.43 as compared with no association in non-hypertensives, ($P_{\text{interaction}} = 0.04$). We conclude that high blood pressure partly explains the association between 25(OH)D levels and ischemic stroke. Presence of hypertension amply aggravates the risk of ischemic stroke associated with low vitamin D levels. Meticulous management of hypertension, regular monitoring of serum 25(OH)D levels and treatment of severe vitamin D deficiency, particularly in hypertensive subjects, could help in effective prevention of stroke.

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INTRODUCTION

Owing to their skin pigmentation, sociocultural and dietary habits as well as genetic makeup, Asians, particularly Indians, are reported to be highly vitamin D deficient with lowest serum 25-hydroxyvitamin D (25(OH)D) levels amongst other ethnicities.[1–3] Indian estimates of the prevalence of vitamin D deficiency are very alarming and range as high as 70–100% in apparently healthy individuals.[1] The classical effects of vitamin D include bone and mineral metabolism. However, in the past few years, vitamin D has emerged as a potential risk factor for cardiovascular diseases, which has been linked to its vasoprotective potential.[4] The reported vasoprotective effects of vitamin D include slowing down of atherosclerosis, promotion of endothelial function, suppression of renin–angiotensin–aldosterone system and thereby reduction of the risk of hypertension.[4] In addition, various neuroprotective aspects of vitamin D action have also been suggested, that is, antioxidation, neuronal calcium regulation, enhanced nerve conduction and detoxification.[5] All these proposed biological attributes of vitamin D make it an appropriate candidate for cerebrovascular diseases like stroke. Stroke is a major public health problem in developing countries like India. Of particular concern is the steadfast increase in the incidence rates of stroke over the past few decades with current estimated prevalence rate of the disease being 545.10 per 100 000.[6] This could be explained by the fast pace of urbanization and the overall increase in elderly population due to increased longevity.[7] Owing to rising health care cost, prevention of stroke is of utmost importance. Identification of controllable and treatable risk factors is essential for effective prevention.[6,8] India, with a population size of 1.2 billion and co-occurring epidemics of vitamin D deficiency and stroke, provides a strong platform for assessment of the interrelationship between vitamin D status and risk of stroke. Reported clinical studies indicate an association of vitamin D status and stroke; however, most of these results are based on populations of Caucasian descent.[9–16] Generalization or extrapolation of results of association studies across various populations may not be judicious.[17] To date, only one study from India has reported an association between vitamin D deficiency and ischemic stroke.[16] India is a vast country with a lot of genetic and cultural diversity that is evident even among geographically proximal regional communities of India.[18] Hence, independent assessments of impact of vitamin D status on risk of stroke across different subpopulations become necessary. In view of these discrepancies, we undertook the present study to assess the association between vitamin D status and risk of ischemic stroke based on a population from Karnataka, a southern state of India.

MATERIALS AND METHODS

Study population

This study was approved by the ethics committee of our Institute and written informed consent was obtained from all the participants. We recruited 239 stroke patients, who presented at the Neurological Services of the National Institute of Mental Health and Neuro Sciences (NIMHANS), a tertiary care center for neurological disorders located in Bangalore, India, from January 2012 to January 2013. Only patients with a clinical diagnosis of ischemic stroke confirmed by cranial computed tomography scan and/or magnetic resonance imaging were considered for the study. Patients with hemorrhagic stroke or stroke secondary to neuroinfections, trauma and malignancy were excluded. Our control group comprised of 241 healthy volunteers with no prior history of cerebrovascular diseases.

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Controls were matched with patients for age, gender and ethnicity. All the subjects were of Karnataka origin, speaking Kannada, a Dravidian language of this southern state of India. Blood samples were collected in the morning after 12-h fasting, immediately centrifuged and stored at − 70 °C.

Demographic and anthropometric analyses included the following variables: age, sex, smoking habits, alcoholism, height, weight and blood pressure. Clinical laboratory measurements included serum levels of 25 (OH)D, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglycerides. Serum triglycerides, total cholesterol and high-density lipoprotein cholesterol concentrations were determined enzymatically using commercially available kits (Beckman Coulter, Brea, CA, USA) and auto analyzer (Olympus AU680, Beckman Coulter) and plasma low-density lipoprotein cholesterol concentration was calculated using Friedewald's formula.[19] Serum 25(OH)D was measured using enzyme immunoassay kits (Immunodiagnostic Systems Ltd., Bolden, UK) and quality-control materials provided by the manufacturer. The intra- and interassay coefficients of variation were 2.70 and 3.59%, respectively.

Statistical analysis

Statistical analysis was performed using SPSS 22.0 software (Chicago, IL, USA). Mean ± s.d. were calculated for continuous variables. The paired '*t*' test and Mann–Whitney *U*-tests were applied to test the differences in continuous variables with normal and skewed distributions, respectively. All *P*-values were two sided.

On the basis of their distribution in controls, 25(OH)D concentrations were grouped into four quartiles followed by an additional separate inclusion of the lowest level, 1–9th percentile and thereby generation of five distribution levels of 25(OH)D levels, 1–9th, 10–24th, 25–49th, 50–74th and 75–100th percentiles. The substratification of the lowest quartile was done to analyze the effects of severely low 25(OH)D concentrations, that is, the 1–9th percentile, which corresponded to 25(OH)D values $\leqslant 9.33$ ng ml^{-1}. Further splitting of the lower percentiles was not considered owing to limited sample size. Similar stratifications have been reported in another study.[14] The five distribution levels of 25(OH)D values were $\leqslant 9.33$ ng ml^{-1} (23.32 nmol l^{-1}), 9.34–12.55 ng ml^{-1} (23.35–31.37 nmol l^{-1}), 12.56–17.86 ng ml^{-1} (31.4–44.65 nmol l^{-1}), 17.87–23.37 ng ml^{-1} (44.67–58.42 nmol l^{-1}) and $\geqslant 23.38$ ng ml^{-1} (58.45 nmol l^{-1}). The association between 25(OH)D status and ischemic stroke risk was evaluated using logistic regression analysis. Confidence intervals (95% CI) were calculated for odds ratios (ORs). In the multivariable analysis, we controlled for covariates that may confound the association of interest: in minimally adjusted analyses, we adjusted for age and gender as these were the matching variables. In addition, we adjusted for the potential confounders in separate models. Linear trends in ORs were performed by assigning the median value of 25(OH)D for each quartile as a continuous covariate in the logistic regression model. We also evaluated the relationship between 25(OH)D and anthropometric parameters like age, gender, body mass index (BMI) and systolic blood pressure amongst controls. Modification of the effect of 25(OH)D by potential risk factors like age, gender, hypertension, smoking, BMI multivitamin supplementation was evaluated by testing for multiplicative interaction by including interaction terms. A cross-product interaction term was computed in the logistic regression model to assess multiplicative interaction between vitamin D levels and covariates.

Covariates

Covariates were identified based on the prior knowledge of possible confounding effect of the variables on the association between serum 25 (OH)D levels and ischemic stroke. Covariates included were age, gender, BMI, month of 25(OH)D assessment, habit of smoking or drinking, presence of diabetes mellitus or hypertension and intake of calcium supplements. Hypertension was defined as diastolic blood pressure $\geqslant 90$ mm Hg and/or systolic blood pressure $\geqslant 140$ mm Hg[20] and/or use of antihypertensive medication. Diabetes was diagnosed based on fasting plasma glucose values > 126 mg dl^{-1} or the subjects' self-reported history of diabetes or use of antidiabetic medication.[21] BMI was calculated as weight in kg divided by height in m^2 and coded as low (< 23 kg m^{-2}) or elevated ($\geqslant 23$ kg m^{-2}) according to appropriate Indian cutoffs of BMI.[22] Dyslipidemia was defined by the presence of one or more than one of the abnormal serum lipid concentrations: total cholesterol $\geqslant 200$ mg dl^{-1}, low-density lipoprotein cholesterol $\geqslant 130$ mg dl^{-1}, high-density lipoprotein cholesterol < 40 mg dl^{-1} for men and < 50 mg dl^{-1} for women, very low-density lipoprotein cholesterol $\geqslant 130$ mg dl^{-1} and triglycerides $\geqslant 150$ mg dl^{-1}.[23] Months of 25(OH)D assessment were included to account for the seasonal variations in vitamin D levels. Data for serum 25(OH)D were grouped into two seasonal categories, that is, summer (March to September) and winter (October to February).

RESULTS

Distribution of the covariates among patients and controls is presented in Table 1. As the samples were age and gender matched, there was no significant difference in the distribution of these two parameters across the subjects. Prevalence of hypertension was high in ischemic stroke patients as compared with controls ($P < 0.05$). Stroke patients were more frequently cigarette smokers and alcohol drinkers as compared with their control counterparts ($P < 0.05$). Prevalence of dyslipidemia (81.25 vs 78.42%) and elevated BMI (83.64 vs 77.42%) were found to be

Table 1. Distribution of the covariates between ischemic stroke cases and controls

Variables	*Ischemic stroke (n = 239)*	*Controls (n = 241)*	P-*value*
Age (years)	47.82 ± 15.28	46.43 ± 10.12	0.273
Gender (%)			
Male	75.31	70.83	0.303
Female	24.69	29.47	
BMI, kg m^{-2} (%)			
Normal, < 23	16.36	22.58	0.303
Elevated, $\geqslant 23$	83.64	77.42	
Smoking (%)			
Current/former smokers	31.38	18.26	0.003
Never	68.62	81.74	
Alcohol drinking (%)			
Yes	31.80	16.60	0.001
No	68.20	83.40	
Hypertension (%)			
Yes	47.70	31.80	0.001
No	52.30	68.20	
Diabetes (%)			
Yes	16.32	12.45	0.289
No	83.68	87.55	0.312
Dyslipidemia (%)			
Yes	81.25	78.42	0.507
No	18.75	21.58	
Season of sample collection (%)			
October–February (winter)	49.79	46.06	0.522
March–September (summer)	50.21	53.94	
Usage of multivitamins and calcium (%)			
Yes	20.92	16.60	0.243
No	79.08	83.40	
Vitamin D, ng ml^{-1}	17.99 ± 9.05	18.49 ± 7.95	0.380
Vitamin D deficiency (%)	61.92	58.10	0.456

Abbreviation: BMI, body mass index. For continuous variables, values were expressed as mean ± s.d.; for categorical variables, % was used. *P*-values were based on Student's *t*-test for continuous variables or Pearson χ^2-test for categorical variables.

Table 2. Odds ratio (95% CI) of ischemic stroke for five levels of serum 25(OH)D

25(OH)D percentile divisions (ng ml^{-1})	*Patients (n = 239), n*	*Controls (n = 241), n*	*Model 1*	*Model 2*	*Model 3*	*Model 4*	*Model 5*	*Model 6*
0–10 (⩽9.33)	38	25	1.55 (0.82–2.93)	1.58 (0.80–3.13)	2.49 (1.10–5.60)	3.13 (1.22–8.07)	2.00 (0.61–6.50)	2.03 (0.62–6.68)
11–24 (9.34–12.55)	30	36	0.78 (0.49-1.25)	0.68 (0.41–1.13)	0.61 (0.34–1.10)	0.92 (0.46–1.84)	0.79(0.38–1.66)	0.79 (0.38–1.66)
25–49 (12.56–17.86)	55	60	0.79 (0.47–1.33)	0.60 (0.33–1.09)	0.59 (0.30–1.17)	1.03 (0.46–2.30)	0.92 (0.38–2.22)	0.92 (0.38–2.22)
50–74(17.87–23.37)	52	60	0.77 (0.45–1.33)	0.57 (0.30–1.09)	0.68 (0.34–1.37)	0.93 (0.41–2.13)	0.53 (0.20–1.38)	0.56 (0.20–1.50)
75–100 (>23.38)	64	60	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
P_{trend}			0.65	0.72	0.55	0.55	0.55	0.55

Abbreviations: CI, confidence interval; 25(OH)D, 25-hydroxyvitamin D. Model 1, adjusted for age (years) and gender (male/female); Model 2, additionally adjusted for seasonal variations (summer/winter); Model 3, additionally adjusted for body mass index levels (kg m^{-2}); Model 4, additionally adjusted for smoking status(yes/no), alcohol drinking (yes/no), diabetes (yes/no) and dyslipidemia (yes/no); Model 5, additionally adjusted for systolic blood pressure levels (mmHg), Model 6, additionally adjusted for usage of multivitamin and calcium supplements.

Table 3. Odds ratio (95% confidence interval) of ischemic stroke for Vitamin D-deficient status (comparison groups, <20 ng ml^{-1} vs $\geqslant 20$ ng ml^{-1})

	Vitamin D deficiency
Patients/controls nos	148/140
Model 1	1.14 (0.79–1.64)
Model 2	1.03 (0.69–1.53)
Model 3	1.00 (0.64–1.57)
Model 4	1.33 (0.79–2.26)
Model 5	1.23 (0.69–2.19)
Model 6	1.23 (0.69–2.19)

Model 1, adjusted for age and gender; Model 2, additionally adjusted for seasonal variations; Model 3, additionally adjusted for body mass index; Model 4, additionally adjusted for smoking, drinking, diabetes and dyslipidemia; Model 5, additionally adjusted for systolic blood pressure levels; Model 6, additionally adjusted for usage of multivitamin and calcium supplements.

very high in both patients as well as controls and their differential distributions in the two study groups were not significantly different ($P>0.05$). There was a very slight but nonsignificant difference in the mean 25(OH)D levels between stroke patients and controls ($P=0.380$). Prevalence of vitamin D deficiency defined as 25(OH)D levels <20 ng ml^{-1},[10,13,16] was higher in patients (61.92%) as compared with controls (58.10%): however, the difference was not statistically significant.

Serum 25(OH)D levels were found to be significantly and inversely correlated with age (Pearson correlation coefficient, (r) $=-0.137$, $P=0.034$), BMI ($r=-0.130$, $P=0.044$) and systolic blood pressure ($r=-0.183$, $P=0.009$). However, significant correlations could not be established between 25(OH)D levels and other anthropometric parameters, that is, serum triglyceride ($r=0.090$, $P=0.172$) and cholesterol levels ($r=-0.001$, $P=0.130$). As expected, 25(OH)D levels were higher during summer season (mean ± s.d. = 20.59 ± 8.68 ng ml^{-1}) as compared with winter (16.65 ± 6.81 ng ml^{-1}), $P<0.05$.

We carried out logistic regression analyses to assess the association between the levels of 25(OH)D and risk of ischemic stroke (Table 2). The risk of ischemic stroke did not show a linear trend across the increasing levels of 25(OH)D (Table 2). After accounting for matching variables, none of the lower levels of 25 (OH)D were found to be significantly associated with the risk of ischemic stroke as compared with the highest levels of 25(OH)D (75th–100th percentile; Table 2). However, when further adjusted for BMI values, the association became significant for the extremely low values of 25(OH)D (1st–9th percentile; OR = 2.49, 95% CI; 1.10–5.60; Table 2). Additional adjustment for other covariates like seasonal variation, presence of diabetes, smoking and drinking status and dyslipidemia strengthened this association (OR = 3.13, 95% CI = 1.22–8.02; Table 2). However, further adjustment for systolic blood pressure (SBP) levels completely attenuated the association (OR = 2.00, 95% CI = 0.61–6.50; Table 2). Additional adjustment for the usage of dietary calcium and multivitamin supplements did not alter the OR effectively; OR = 2.03, 95% CI = 0.62–6.68 (Table 2). We also tested the association between vitamin D status and ischemic stroke using the commonly applied clinical cutoff points of <20 ng ml^{-1} and $\geqslant 20$ ng ml^{-1} (Table 3). However, we failed to establish any association; when adjusted for age and sex, the subjects with low vitamin D levels (<20 ng ml^{-1}) were found to have nonsignificantly higher 1.14-fold risk of ischemic stroke as compared with those with high vitamin D levels ($\geqslant 20$ ng ml^{-1}; Table 3). After further adjustments for other potential covariates, the association remained nonsignificant (Table 3).

We performed secondary analyses to explore the association between 25(OH)D levels and risk of ischemic stroke in various subgroups of covariates (Table 4). We analyzed the interaction between these covariates and 25(OH)D levels. We found a significant effect modification of ischemic stroke risk by 25(OH)D status and hypertension interaction ($P=0.04$); hypertensives with low 25(OH)D were found to be at very high risk of ischemic stroke as compared with those with high 25(OH)D levels. The ORs (95% CI) were 13.54 (1.94–94.43) and 0.22 (0.01–4.08) in hypertensives and non-hypertensives, respectively. Other covariates were not found to significantly modify the effects of low 25(OH)D levels (Table 4).

DISCUSSION

The plethora of recent scientific reports highlight a wide sphere of physiological effects mediated by vitamin D. This multifunctionality has been attributed to the elicitation of physiological responses mediated by Vitamin D in $\geqslant 36$ cell types that express its specific receptor, Vitamin D receptor.[24] There have been many new advances in the knowledge of the biology of vitamin D that include its effects on diverse and major health outcomes like cancer, autoimmune and cardiovascular diseases. However, despite the mounting awareness of the beneficial aspects of vitamin D, there is a pandemic of vitamin D deficiency.[25] The present study targeted the very high-risk Indian population facing multiple epidemics of various cardiovascular diseases and risk factors.[1,6,26] On the basis of the defined cutoff of 20 ng ml^{-1},[10,13,16] we found 58.10% of the control population to be vitamin D deficient. In addition, we found high prevalence rates of cardiovascular risk factors, that is, hypertension, elevated BMI and dyslipidemia in our control population which demands health awareness.

Table 4. Odds ratio (95% CI) of ischemic stroke for 1th–9th percentile vs 75th–100th percentile of 25(OH)D levels by various risk factors

	Patients/ controls (%)	*Odds ratio (95% CI)*	$P_{interaction}$
Age (years)			
< 49	37.62/57.14	7.71 (1.39–42.71)	0.64 (NS)
$\geqslant 49$	62.38/42.86	0.04 (0.00–8.44)	
Gender			
Males	71.29/64.28	0.64 (0.12–3.32)	0.43 (NS)
Females	28.71/35.72	1.00 (0.53–1.27)	
BMI, kg m^{-2}			
< 23	40.59/20.24	0.26 (0.003–25.77)	0.17 (NS)
$\geqslant 23$	59.41/79.76	1.84 (0.56–6.10)	
Cigarette smoking			
Yes	24.75/17.86	NA	0.26 (NS)
No	75.25/82.14	1.19 (0.30–4.73)	
Alcohol drinking			
Yes	29.70/25.18	0.50 (0.65–1.42)	0.16 (NS)
No	70.30/74.82	1.63 (0.42–6.34)	
Hypertension			
Yes	49.50/29.76	13.54 (1.94–94.43)	0.04
No	50.10/70.24	0.22 (0.01–4.08)	
Diabetes			
Yes	14.85/14.29	NA	0.80 (NS)
No	85.15/85.71	1.67 (0.48–5.75)	
Dyslipidemia			
Yes	42.57, 80.95	1.00 (0.24–4.21)	0.90 (NS)
No	57.43, 19.05	4.70 (0.08–282.32)	
Season of blood collection			
March– October	41.43/55.95	12.57 (1.08–146.29)	0.64 (NS)
November–February	58.57/44.05	1.33 (0.22–8.22)	
Usage of multivitamins and calcium supplements			
Yes	21.78/11.90	NA	0.29 (NS)
No	78.22/88.10	1.19 (0.33–4.31)	

Abbreviations: BMI, body mass index; CI, confidence interval; NA, not applicable; NS, not significant; 25(OH)D, 25-hydroxyvitamin D. Odds ratios were maximally adjusted for the full set of covariates as in Model 5, Table 2, but with the exclusion of the stratifying covariate.

In view of the paucity of studies done for the assessment of relationship between vitamin D levels and stroke in Asian populations, we undertook the present cross-sectional study. We hypothesized that an interrelationship between vitamin D deficiency and ischemic stroke might explain the observed temporal trends in their increased coincidence rates in India over recent decades. Most of the prior studies were based on populations of Caucasian descent and indicated an inconsistent inverse relationship between vitamin D levels and risk of stroke.[9–16] Recent two meta-analyses studies summarized the results of previous studies and presented statistically significant pooled estimates of relative risks of stroke comparing low vs high vitamin D status.[13,14] After pooling results from seven prospective studies that included a total of 1214 stroke cases, Sun *et al*.[13] reported a pooled relative risk of 1.52 with 95% CI (1.20, 1.85). Similarly, in a meta-analysis comparing lowest vs highest quartile of 25(OH)D concentrations, the multivariate adjusted OR of ischemic stroke was found to be 1.54 (1.43–1.65) by Brøndum-Jacobsen *et al*.[14] These summarized estimates indicate a potentially significant contribution of low vitamin D status to stroke risk. In line with these studies, we found a strong and significant association between 25(OH)D status and risk of ischemic stroke in Asian Indian population. The lowest level of 25(OH)D was found to be associated with increased risk of ischemic stroke when compared with the highest level in the a model adjusted for age, sex, seasonal variation, BMI, cigarette smoking, alcohol drinking, presence of diabetes mellitus and dyslipidemia, OR = 3.13, 95% CI, (1.22–8.07). However, adjustment for SBP levels was found to abrogate this association (OR = 2.00, 95% CI = 0.61–6.50), which implies that the increased risk of ischemic stroke by low 25(OH)D levels was mediated by its effect on arterial hypertension. This is in line with the observed significant interaction between 25(OH)D levels and hypertension, $P_{interaction} = 0.04$ and strong correlation found between SBP and 25 (OH)D levels found in the present study ($r = -0.183$, $P = 0.009$). However, in our previous study, we could not relate vitamin D with hypertension.[27] This difference could be due to the inclusion of only older subjects (above 40 years) in the present study; advancing age made the effects of vitamin D on blood pressure levels more pronounced. On the contrary, the prior study constituted of a wide range of age of the study subjects including substantial number of young adults of age 18–35 years (~20%). We speculate that young subjects are more resistant and able to combat the adverse effects of low vitamin D levels on SBP and hence the overall effect of low vitamin D on SBP could not be detected in the prior study. Hypertension is an established risk factor for stroke.[4] Basic science research indicates a role of vitamin D in arterial hypertension via the suppression of the renin–angiotensin–aldosterone system. Vitamin D receptor knockout mice have been demonstrated to exhibit increased renin expression and arterial hypertension.[4] However, in transgenic mice overexpressing the human Vitamin D receptor in the juxtaglomerular cells, renal renin mRNA levels and plasma renin activity have been reported to be significantly suppressed.[28] RAS system is the key regulator of blood pressure, electrolyte and volume homeostasis[28] and its overactivation leads to hypertension. It appears that vitamin D has a key role in the homeostasis of cardiovascular system via suppression of the renin–angiotensin–aldosterone system. This clinical finding supports the experimentally proven role of Vitamin D in arterial hypertension.[4] Importantly, we found a strong effect modification of the 25(OH)D levels on the risk of ischemic stroke in hypertensive subjects. As our results indicate that hypertension mediates the effect of low vitamin D levels on the risk of stroke, in hypertensive subjects, the biological effects of low vitamin D levels are aggravated leading to a pronounced increase in the risk of stroke. Fortunately, both hypertension and vitamin D deficiency are controllable and treatable parameters, hence we suggest that effective healthcare programs should be organized countrywide for monitoring and management of the serum levels of vitamin D and hypertension. Random clinical trials should also focus on supplementation of vitamin D in subjects with severe vitamin D deficiency and particularly those with a background of hypertension.

The subjects in the present study were relatively younger when compared with stroke patients in similar European and American studies.[9–15] This could be due to the different population age structure resulting from greater mortality rates in India when compared with more developed countries. It has been reported that the average age of patients with stroke in low-income countries is 15 years below that in high-income countries.[29]

BMI was found to be a negative confounder in this study. There was a high prevalence of elevated BMI in our apparently healthy control subjects. As confirmed in the present study, BMI levels are well known to inversely correlate with vitamin D levels (Pearson correlation coefficient, $(r) = -0.130$, $P = 0.044$); this could be explained by the solubility and storage of vitamin D in the body fat and subsequent reduced serum vitamin D levels in individuals

with high BMI.[30] An overall elevated BMI in the studied population would have contributed to equivalently lower physiological vitamin D concentrations in controls as compared with the patients and masked the genuine association of low vitamin D levels with risk of stroke. On a different note, our results also highlight the future possibility for the risk of ischemic stroke in apparently healthy but obese Indian population with low vitamin D levels. This could explain the rapid and parallel epidemiological co-transitions in the incidences of obesity, vitamin D deficiency and stroke in India.[1,7,26]

We could not validate the association between 25(OH)D levels and ischemic stroke risk using the widely used deficiency cutoff for vitamin D levels of 20 ng ml^{-1}. This emphasizes the need for thorough reevaluation of population-based vitamin D cutoffs in the context of diseases and therapeutics. In view of this, we consider referencing the new serum 25(OH)D thresholds set by National Osteoporosis Society for the UK population, which suggests >20 ng ml^{-1} levels of vitamin D as sufficient and only levels of 12 ng ml^{-1} or less as deficient.[31] As these thresholds have been set in respect to bone health, evaluation of these cutoff levels is necessary for cardiovascular diseases. This also explains the failures observed in previous random clinical trials conducted in general population wherein no significant beneficial effect of vitamin D supplementation was observed for incidence of stroke.[32] Hence, the beneficial effects of vitamin D treatment against stroke incidence could be more effective when targeted to the group of individuals with very low vitamin D levels instead of the general population.[32]

Our study has exclusively targeted ischemic stroke as a disease outcome which is definitely an advantage over most of the previously reported association studies on vitamin D status and risk of stroke. Except for a few, most of the studies have presented combined estimates of risk associated with low vitamin D levels for hemorrhagic and ischemic strokes.[9–16] Only four major studies have focused on ischemic stroke alone or separately for the stroke subtypes.[10,13,14,16] Diagnostically, the two stroke subtypes are different and have distinct underlying etiologies.[33] The former is caused by arterial occlusion or stenosis and the latter by leakage or rupture of an artery.[33] Therefore, it is important to analyze their etiological associations with vitamin D status, separately. This notion is supported by the separate analysis done for ischemic stroke by two different group of authors, Kilkkinen *et al.*[10] and Brøndum-Jacobsen *et al.*[14], wherein they found a significant inverse association between baseline 25(OH)D levels and risk of ischemic stroke but no association with hemorrhagic stroke.

The present study is limited by its cross-sectional design and small sample size. The cross-sectional design challenges the causal interpretation of the observed association between 25(OH)D status and disease risk. Moreover, the causal effects of vitamin D in disease pathologies have been challenged by the notion that low 25(OH)D levels in patients could be a mere consequence of their poor health status, morbidity, and decreased outdoor and physical activity.[4] On a contrasting note, the observed impact of interaction between 25(OH)D and hypertension on the risk of ischemic stroke enhances the possibility of a causal rather than consequential relationship. Another shortcoming of the present study is the lack of information on some of the key parameters like physical activity, levels of inflammatory markers, 1,25-dihydroxvvitamin D levels, parathyroid hormone, phosphate and fibroblast growth factor 23. Hence, we could not control for the confounding or effect modification due to these variables on the observed association between 25(OH)D status and risk of ischemic stroke. The present study is also limited by the immunoassay-based estimation of vitamin D. Isotope dilution liquid chromatography-tandem mass spectrometry has been recognized as a reference method by the Joint Committee for Traceability in Laboratory Medicine.[34]

This study is among one of the very few attempts to explore the relationship between vitamin D and a cerebrovascular outcome in an Asian Indian population. In line with a recent report from a different state of South India (Andhra Pradesh), we could replicate an association between vitamin D status and risk of ischemic stroke.[16] However, we could not validate the association using the cutoff 25(OH)D levels of 20 ng ml^{-1}, as was reported in this study.[16] This difference demonstrates the influence of dietary habits, socioeconomic culture and genetic variations on the optimal physiological range and related metabolic actions of vitamin D. Hence, large-scale validation of association studies across different subpopulations of the country is warranted.

In conclusion, as most of the potential links between vitamin D deficiency and cardiovascular diseases, including stroke, are based on observational studies, we emphasize the need for large-scale, well-designed random clinical trials to confirm the role of vitamin D deficiency in these diseases.

What is known about topic
- Low serum 25 (OH)D levels inconsistently associated with increased risk of stroke
- Relationship between 25(OH)D levels and ischemic stroke not clear in Asians

What this study adds
- Severely low 25(OH)D levels associated with ischemic stroke in Asian Indians
- Hypertension clearly aggravates the risk of ischemic stroke associated with low 25(OH)D levels

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

VM contributed to the design of the study, biochemical analysis, acquisition and analysis of data and manuscript writing. PP assisted VM in biochemical analysis and data acquisition. GBK provided the study materials and contributed to the interpretation of the data. RC contributed to the conception and design of the study and interpretation of data and carried out the overall supervision of the work.

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