

## Research Article

# Ethnic Differences in Association of Vitamin D Levels with Incident Stroke Cases between Ethnic Chinese and South Asians

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## Abstract

**Introduction:** Published data suggests that vitamin D deficiency is associated with risk of coronary events and stroke mortality in whites but not in blacks. We aimed to investigate ethnicity interaction effects in the vitamin D-ischemic stroke relationship among ethnic Chinese and South Asians.

**Materials & Methods:** We recruited 271 ischemic stroke patients (87% Chinese, 13% South Asian, similar to the national ethnic distribution) with blood samples taken within 2 weeks of stroke onset to reflect pre-stroke levels, and 271 stroke-free controls matched for age, sex and ethnicity from population-based studies. Serum 25-hydroxyvitamin D levels [25(OH)D] were measured by competitive electroluminescence immunoassay blinded to clinical data.

**Results:** Mean 25(OH)D levels were lower in South Asians than Chinese among stroke cases (19.1±8.4 vs. 24.6±9.3 ng/mL;  $P=0.001$ ) as well as among controls (16.7±6.6 vs. 29.3±9.7 ng/mL;  $P<0.001$ ). Among Chinese, 25(OH)D levels were significantly lower in stroke cases than in controls ( $P<0.001$ ), however this was not the case among South Asians ( $P=0.188$ ). There was an interaction between ethnicity with the association of 25(OH)D levels and stroke ( $P<0.001$ ), which remained even after adjusting for covariates of diabetes, hypertension, hyperlipidemia, previous myocardial infarction and smoking status as well as calcium, phosphate and parathyroid hormone levels ( $P=0.003$ ).

**Conclusion:** These data add to the evidence that the deleterious effects of vitamin D deficiency varies between ethnicities with differing skin pigmentation, with an association being found in ethnic Chinese, but not in ethnic South Asians.

**Keywords:** Vitamin D; Stroke; Asians; Ethnicity

## Abbreviations

25(OH)D: 25-hydroxyvitamin-D; ANOVA: Analysis of Variance; CI: Confidence Interval; IQR: Interquartile Range; LSM: Least Squares Mean; PTH: Parathyroid Hormone; SAS: Statistical Analysis Systems; SD: Standard Deviation; TOAST: Trial of Org 10172 in Acute Stroke Treatment

## Introduction

Ethnic differences have been reported in the association of vitamin D deficiency with its postulated deleterious effects including vascular risk [1-3]. Low vitamin D levels are associated with coronary heart events in whites but not blacks [1]. There are limited data on ethnic differences for stroke risk. One study showed a significant increased risk of fatal stroke with vitamin D deficiency among whites but not blacks [2]. Another used ethnic-specific tertile of vitamin D levels and found no influence of white versus black ethnicity on strength of association between vitamin D deficiency and incident stroke [4]. In Singapore, there are ethnic Chinese and South Asian populations who have lighter and darker skin pigmentation respectively as evidence by different melanin skin content [5]. We matched ischemic stroke patients with population-based controls among ethnic Chinese and

ethnic South Asians (who have darker skin pigmentation and lower vitamin D levels [6]). Our aim was to investigate the effect of Chinese versus South Asian ethnicity on 25-hydroxyvitamin-D [25(OH)D] levels (ng/mL) in a comparison of stroke cases and controls, to explore the ischemic stroke-vitamin D relationship between these two ethnic groups.

## Materials and Methods

We recruited 271 acute ischemic stroke patients each of Chinese and South Asian ethnicity admitted to two large tertiary hospitals in Singapore from July 2000 to September 2012. The study was approved by the institution review committees of both hospitals, and all subjects gave written informed consent. Stroke cases were matched on sex, race and age (within 5 years) to population-based controls with no previous self-reported history of stroke from two community-based epidemiological studies, the Singapore Chinese Eye Study (SCES) and the Singapore Indian Eye Study (SINDI) [7].

Ethnicity was ascertained during the patient interview or from the identification card issued to all Singapore citizens and residents. Ethnic South Asians are defined as people whose origin is from South Asian countries, namely India, Pakistan, Bangladesh, Nepal, Bhutan,

**Table 1:** Mean 25-hydroxyvitamin-D levels (ng/mL) showing ethnicity×group interaction.

Ethnicity	Study Group		Difference (95% CI)	Difference P Value	Ethnicity×Group Interaction P Value <sup>c</sup>
	Stroke (n=271)	Control (n=271)			
Sample means <sup>a</sup> ± SD					
Chinese (n=474)	24.6 ± 9.3 (n=237)	29.3 ± 9.7 (n=237)	-4.63 (-6.23, -3.04)	<0.0001	0.002
South Asian (n=68)	19.1 ± 8.4 (n=34)	16.7 ± 6.6 (n=34)	2.44 (-1.77, 6.66)	0.255	
Least squares means (95% CI) adjusted for covariables <sup>b</sup>					
Chinese (n=474)	24.5 (23.2, 25.8)	28.8 (27.4, 30.2)	-4.32 (-6.00, -2.65)	<0.0001	0.003
South Asian (n=68)	19.4 (16.3, 22.4)	16.8 (13.6, 19.9)	2.61 (-1.59, 6.81)	0.222	

**Abbreviations:** CI: Confidence Interval; SD: Standard Deviation.

<sup>a</sup>Tests the null hypothesis that the differences between Stroke and Control groups are the same for both Chinese and South Asians.

<sup>b</sup>Same as ANOVA least squares means unadjusted for confounders.

<sup>c</sup>P values: Group  $P=0.462$ , Ethnicity  $P<0.0001$ , age  $P<0.0001$ , diabetes  $P=0.222$ , hypertension  $P=0.523$ , hyperlipidemia  $P=0.184$ , previous myocardial infarction  $P=0.813$ , smoking status  $P=0.004$ , calcium  $P=0.120$ , phosphate  $P=0.483$ , and parathyroid hormone  $P=0.024$ .

Maldives and Sri Lanka [8]. Patients of mixed ethnicity were excluded. Stroke etiology was categorized by the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification [9]. Diabetes was defined as a self-reported history, use of diabetic medications or by the World Health Organization diagnostic guidelines [10], hypertension as a self-reported history, use of antihypertensive medication or by the Joint National Committee hypertension guidelines [11], hyperlipidemia as a self-reported history, use of lipid-lowering medication or by the National Cholesterol Education Program Adult Treatment Panel guidelines [12], previous myocardial infarction (MI) as a self-reported history and smoking as any history of smoking in the past or current smoking. For stroke cases, blood samples were taken within two weeks of stroke onset (median, 3 days; interquartile range [IQR], 2-5 days). Serum 25(OH)D was measured using competitive electrochemiluminescence protein binding immunoassay, employing a vitamin D binding protein as a capture protein with a Coefficient of Variance of 18.5% (Roche Diagnostics, Mannheim, Germany) [13] and was masked to clinical data. Serum calcium, phosphate and albumin were measured using colorimetric methods with ADVIA 2400 (Siemens Healthcare Diagnostics, NY, USA) and parathyroid hormone (PTH) measure using chemiluminometric two site sandwich assay (Siemens Healthcare Diagnostics, NY, USA).

Mixed model Analysis of Variance (ANOVA) was performed on 25(OH)D concentrations using the GLIMMIX procedure of the Statistical Analysis Systems (SAS) software package, version 9.3 (SAS Institute, Inc., Cary, NC). A two-factor unadjusted analysis model included main effects for ethnicity and group (case/control), and an ethnicity×group interaction. The same two-factor model augmented with available co variables was used in an adjusted analysis. In both analyses, matched pairs were incorporated as random subject effects. Subject effects and residual errors were assumed to be normally distributed. Statistical significance was declared for  $P$  values  $\leq 0.05$ .

## Results

The ethnic distribution for both stroke cases and controls was 87.5% Chinese (n=474, stroke=237, non-stroke=237) and 12.5% South Asian (n=68, stroke=34, non-stroke=34), similar to the national ethnic distribution in Singapore. The median age was 60 years (IQR 15) and 80% were male. The TOAST subtype distribution did not differ between ethnic Chinese patients (large vessel 14%, cardioembolic

10%, small vessel 46%, other etiology 1%, undetermined 29%) and ethnic South Asian (large vessel 21%, cardioembolic 6%, small vessel 41%, other etiology 3%, undetermined 29%) patients ( $P=0.389$ ). Mean 25(OH)D levels were lower in South Asians than in Chinese for both stroke cases (19.1±8.4 vs. 24.6±9.3 ng/mL;  $P=0.001$ ) and controls (16.7±6.6 vs. 29.3±9.7 ng/mL;  $P<0.001$ ). In Chinese patients, the least squares mean (LSM) adjusted 25(OH)D concentration was lower in stroke cases than in controls (24.5 vs. 28.8 ng/mL, LSM difference -4.32;  $P<0.001$ ); however, this was not the case in the South Asians (LSM difference 2.61;  $P=0.222$ ) (Table 1). The ethnicity×group interaction was statistically significant in the unadjusted analysis ( $P=0.002$ ) and persisted after adjustment for covariates of diabetes, hypertension, hyperlipidemia, previous myocardial infarction, smoking status, calcium, phosphate, and PTH levels ( $P=0.003$ ).

## Discussion

This study found a significant ethnic interaction between low vitamin D levels and stroke between ethnic Chinese and ethnic South Asians. The vitamin D-stroke association in lighter-skinned Chinese but not in darker-skinned South Asians, is consistent with prior data for coronary disease and bone consequences [2,3]. The finding of lower vitamin D levels in ethnic Indians compared to Chinese is consistent with published literature [6] and may be contributed by darker skin pigmentation which reduces cutaneous vitamin D production [14].

The lack of vitamin D-stroke association in ethnic South Asians, which concurs with prior findings [15], may be due to adaptive mechanisms in vitamin D metabolism in darker-pigmented races [1]. Lower levels of vitamin D-binding proteins were observed in blacks versus whites which is postulated to provide protection against conditions associated with low levels of 25(OH)D [16]. Target organ effects of vitamin D deficiency may vary between races of differing skin pigmentation. It has been shown that there is skeletal resistance to PTH-stimulated bone resorption in blacks compare to whites [17].

Ethnicity should be an important consideration in studies investigating vascular risks of vitamin D deficiency. Emerging data show that low vitamin D is associated with poorer prognosis, including larger lesion volume [18], worse functional outcome [19,20] and poorer cognitive impairment [20] after stroke. Trials studying

the benefits of vitamin D supplementation for vascular risk reduction and improving outcome after stroke should consider ethnicity as a confounding factor. Asians of differing skin pigmentations, such as Chinese and South Asians, should not be categorized together in such studies.

Strengths of this study include early blood sampling in stroke patients to reflect pre-stroke levels and obviate post-stroke confounders. There is likely no seasonal or latitude variations in vitamin D level in Singapore which is small and has uniform temperatures and hours of sunlight year-round. This is a case-control study and the demonstrated associations do not prove causality. Some potential 25(OH)D level confounders such as adiposity, dietary intake and vitamin supplementation were not considered. The proportion of cases by ethnicity (87% Chinese, 13% South Asian) is uneven, and is consistent with the general population in Singapore (74% Chinese, 9% South Asian) [21].

## Conclusion

In conclusion, our study showed there was a vitamin D-stroke association in ethnic Chinese, but not in ethnic South Asians.

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