

Serum 25-Hydroxyvitamin D and the risk of stroke in Hong Kong Chinese

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ABSTRACT

Introduction: Low vitamin D levels have been associated with various cardiovascular diseases, however, whether it is associated with stroke remains inconclusive. We aimed to evaluate the association between serum 25-hydroxyvitamin D and risk of stroke.

Materials and methods: A cohort study consisting of 3,458 participants from the Hong Kong Osteoporosis Study aged ≥ 45 at baseline, examined between 1995 and 2010 and followed up using electronic medical records. Ischemic and hemorrhagic stroke were defined using the ICD-9 code.

Results: In multivariable Cox-proportional hazard regression, quintiles 1 and 4 were significantly associated with increased risk of stroke when compared to the highest quintile (Quintile 1: HR, 1.78; 95% CI, 1.16-2.74 and quintile 4: HR, 1.61; 95% CI, 1.07-2.43). A similar association was observed in both men and women. In subgroup analysis, the association was specifically observed for ischemic stroke, but not hemorrhagic stroke. Using a penalized regression spline, the association between vitamin D and risk of stroke was in a reverse J-shape, with the lowest risk of stroke being observed at 25(OH)D levels between 70 and 80nmol/L.

Conclusion: In conclusion, a low vitamin D level is associated with increased risk of ischemic stroke, however whether high vitamin D level is also associated with increased risk of stroke requires further study.

INTRODUCTION

Vitamin D deficiency is prevalent and affects over 1 billion people worldwide. Vitamin D is a fat-soluble steroid hormone synthesized in the skin mainly when exposed to ultraviolet (UV) light (1), although it can also be acquired from food and supplements. The inactive vitamin D is hydroxylated in the liver to form 25-hydroxyvitamin D (25[OH]D), which is the major circulating metabolite (2). The most well established function of vitamin D is on bone and mineral metabolism. Recently, a role of vitamin D on cardio- and cerebrovascular diseases has been suggested based on experimental (3-5) and epidemiological studies (6-11), which may be contributed by the vasoprotective effects of vitamin D on the promotion of endothelial function, modulation of the renin-angiotensin-aldosterone system (RAAS), inhibition of angiogenesis and vascular calcification (3-5). The effects of vitamin D on RAAS have been extensively demonstrated in animal studies; for example, vitamin D receptor knockout mice developed high renin expression, arterial hypertension, and cardiac hypertrophy (12, 13). In addition, several neuroprotective functions of vitamin D have also been reported, such as anti-oxidation, immunomodulation, neuronal calcium regulation, enhanced nerve conduction and detoxification mechanisms (14). Taken together, low vitamin D status is considered a risk factor of cerebrovascular diseases.

Although previous studies have shown that a low level of 25(OH)D is associated with stroke, inconsistent findings were observed in human studies (4, 15-18). Some, but not all (19, 20), prospective follow-up studies and meta-analyses have shown that low

vitamin D levels were associated with higher risk of stroke in normal subjects (16, 21) or hemodialysis patients (22). Given that there may be racial differences in the association of 25(OH)D with stroke (23), whether serum vitamin D is associated with incident stroke in Chinese has remained largely unstudied.

In this study, we aimed to evaluate the relationship of total 25(OH)D with stroke risk in Hong Kong Chinese.

METHOD

Subjects

The study participants were from the Hong Kong Osteoporosis Study (HKOS) (24-26). HKOS is a prospective follow-up study in Hong Kong Chinese population aiming to investigate the genetic and environmental risk factors of osteoporosis and related traits. Briefly, the cohort participants were Southern Chinese descendants residing in the local Hong Kong community and were recruited in baseline examinations from 1995-2010. A total of 9,453 participants were recruited. After providing written informed consent, the participants were interviewed, underwent examination and their blood was sampled, together with height, weight, and bone density measurement performed. Demographic data such as anthropometric measurements, socioeconomic status, education level, medical, and reproductive history were collected by trained interviewers using a structured questionnaire. Lifestyle information including smoking status, drinking

habits and physical activities were also obtained. Among these, 3,458 who were aged ≥ 45 and had total 25(OH)D measured were included.

Clinical data collection

The electronic medical record (EMR), the Clinical Data Analysis and Reporting System (CDARS), of the Hong Kong Hospital Authority was used to retrieve the stroke records of the participants retrospectively using a unique reference key. Stroke events were defined by International Classification of Diseases, 9th Revision (ICD-9) codes (ICD9 CODES: 430-438), whereas ischemic stroke (ICD-9 codes 433–438) and hemorrhagic stroke (ICD-9 codes 430–432) were also identified separately. History of lipid lowering medications and anti-hypertensive drugs (angiotensin-converting-enzyme inhibitor, angiotensin II receptor blockers, beta blocker, calcium channel blocker, and diuretics) were retrieved from the CDARS database.

Laboratory analyses

Coagulated blood samples were collected and processed under standard laboratory procedure. Serum samples were stored at -70°C until analysis. Serum total 25(OH)D were measured by direct enzymatic immunoassay EIA, (IDS Ltd, UK). The minimum detectable concentration of 25(OH)D was 4.8ng/mL and the coefficient of variation was less than 10%.

Bone mineral density (BMD) measurements

BMD at the femoral neck was measured using Hologic QDR 2000 plus and Hologic QDR 4500 plus in the visit before and after mid-2002, respectively. Presence of osteoporosis is defined as BMD T-score at the femoral neck lower or equal to -2.5.

Statistical Analysis

Quintiles were described and compared with respect to baseline characteristics and concentrations of vitamin D. Descriptive statistics of baseline characteristics were expressed as mean \pm standard deviation (SD) for continuous variables or frequencies for categorical variables. Differences according to quintiles of vitamin D biomarkers were determined with chi-square tests for categorical variables and t tests for continuous measurements.

The association between 25(OH)D status and risk of stroke was evaluated using multivariable Cox-proportional hazard regression analysis. The overall proportional hazards assumption was evaluated for the variables in the fully adjusted model by including time dependent covariates in the Cox model. Quintile of parathyroid hormone was included as a stratum in the Cox-regression models to avoid violation of the proportional hazards assumption. Variables that were not normally distributed were log-transformed. Factors that may affect serum 25(OH)D or the risk of stroke were included in the multivariable Cox-regression models. The simple model was adjusted for demographic factors (age, gender, BMI); the full model was further adjusted for the behavioral and socioeconomic variables (education, smoking, drinking, sport habit),

and biomarkers related to vitamin D metabolism (season, eGFR, serum calcium, serum phosphate, serum alkaline phosphatase, and serum parathyroid hormone). Using the highest quintile as the reference, the hazard ratio (HR) and 95% CI of vitamin D status and incident stroke for each quintile were calculated. Statistical significance was defined at the P value <0.05 level using two-sided tests for all analyses. The P-spline method was used to study the relationship between the hazard ratio of stroke with 25(OH)D as the continuous variable. P-spline is penalized B-spline to avoid data overfitting by fitting data to a set of spline basis functions with a reduced set of knots, combined with the roughness penalty of smoothing splines (27). Analyses were performed using SPSS 21.0 software (SPSS Inc., Chicago, USA) or R software (V3.1.2).

RESULTS

Baseline characteristics of the study population according to quintiles of serum total 25(OH)D levels are summarized in Table 1. In the 3,458 participants, there were 2,157 women (62.3%), with a mean age of 63.2yrs±10.2 and mean serum 25(OH)D of 56.5nmol/L±16.9. Subjects with lower 25(OH)D level were more likely to be older, female, smokers, more educated, less physically active, having had a longer history of anti-hypertensive medication, and usually had the study visit in autumn/winter. They tended to have higher BMI, eGFR, higher serum parathyroid hormone and lower serum corrected calcium. The prevalence of incident stroke observed was 7.0% (244 events in 3,458 subjects).

During a median of 10.3 years (range 0.1 to 17.9 years) and 31,526.9 person-years, 244 participants had incident stroke, with an estimated incidence rate of 7.7 per 1,000 person-years. The relationship between HR of incident stroke and 25(OH)D quintiles is shown in Table 2. In the simple adjusted model, participants in quintiles 1 and 4 had increased risk of stroke when compared to the highest quintile (Quintile 1: HR, 1.74; 95% CI, 1.14-2.64 and quintile 4: HR, 1.54; 95% CI, 1.03-2.32). After further adjustment for confounding factors, the association remained statistically significant (Quintile 1: HR, 1.78; 95% CI, 1.16-2.74 and quintile 4: HR, 1.61; 95% CI, 1.07-2.43). Although no vitamin D by sex interaction was observed (data not shown), the association was significant in men ($P=0.019$) but marginally significant in women ($P=0.09$, Supplementary table 1). Figure 1 shows the relationship between continuous vitamin D levels and risk of stroke.

The association of vitamin D with subtypes of stroke is shown in Table 3. The lowest quintile of vitamin D was significantly associated with increased risk of ischemic stroke (HR, 1.81; 95% CI, 1.16-2.83) when compared with the highest quintile in the simple adjusted model. A similar result (HR, 1.84; 95% CI, 1.16-2.91) was observed in the fully adjusted model. On the other hand, null association was observed between vitamin D and hemorrhagic stroke.

We also evaluated if other bone and mineral biomarkers were associated with incident stroke (Table 4). In general, no significant association was observed between serum calcium, phosphate, alkaline phosphate, and risk of stroke.

DISCUSSION

In this study, we found that lower circulating concentrations of total 25(OH)D is a predictor of ischemic stroke in Hong Kong Chinese. However, there was no evidence of a role in hemorrhagic stroke.

When 25(OH)D levels were modeled as a continuous variable, a reverse J-shape association was observed between 25(OH)D and risk of stroke, as shown in the regression spline. The lowest risk of stroke was observed in the range of 25(OH)D levels between 70 and 80 nmol/L, whereas the risk of stroke increased below and above this range. Although we did not observe such reverse J-shape association in Tables 2 and 3, this was observed if we used 25(OH)D of 69.05 to 80 nmol/L as the reference group (Supplementary Table 2). This finding is indeed in agreement with a recent study showing that there was a reverse J-shape association between serum 25(OH)D and stroke mortality, with the lowest risk of stroke death observed at 70nmol/L (28). Our result was also in line with a previous study that demonstrated a U-shape association of 25(OH)D with major cardiac and cerebrovascular events, with an optimal level of 70-100nmol/L (29). There is growing evidence that vitamin D has its detrimental effect to health with a level of less than approximately 75nmol/L and a level of 120nmol/L or

greater, resulting in U-shape or reverse J-shape association of 25(OH) with all-cause mortality (11, 30, 31). Vitamin D intoxication caused by overdosed vitamin D supplements is rare and related to 25(OH)D levels higher than 375nmol/L (32). However, the underlying physiological basis of how mildly elevated serum vitamin D may affect general or cardiovascular health is still unknown and inconclusive; more studies are required to elucidate the relationship.

Our findings are consistent with a meta-analysis showing that low 25(OH)D levels were associated with higher risk of ischemic stroke, but not hemorrhagic stroke (2). Similarly, a prospective study in the Nurses' Health Study and meta-analysis also provided evidence that low vitamin D levels were modestly associated with risk of stroke (33). The mechanism as to how vitamin D is related to ischemic stroke is unclear. Mineral metabolism has been suggested to play a role in cardiovascular and stroke risk (34-36). Given that vitamin D is correlated with other biomarkers of mineral metabolism, such as calcium and phosphate, we performed a fully adjusted model after adjusting for mineral biomarkers and found that the association between vitamin D and risk of stroke remained robust. Moreover, among all mineral biomarkers studied, only vitamin D showed significant association with a risk of stroke (Table 4). Similarly, osteoporosis and low BMD have been shown to be associated with cardiovascular disease or stroke (37-39). Given that osteoporosis may confound the association, we first tested if there was an interaction between osteoporosis and vitamin D by introducing an interaction term in the model and found that there was no significant interaction (data not shown).

Second, we performed the association analysis with further adjustment for osteoporosis or BMD Z-score at the femoral neck (Supplementary Table 3), and the findings were essentially unchanged. As previously noted (40), men were shown to have a higher prevalence of vitamin D deficiency and insufficiency than in women. This discrepancy does not appear to be related to increased osteoporosis among men and we are currently investigating these findings. Although observational studies showed that lower serum vitamin D levels are associated with a higher risk of stroke, randomized clinical trials have not obtained similar results (19, 20). In a recent meta-analysis of randomized controlled trials (RCTs) involving 11,841 subjects, vitamin D supplementation showed no effect on risk of stroke (risk ratio of 1.09; 95% CI: 0.92-1.3) (41). However, it should be noted that the intervention varied greatly between studies, for example, intervention was given with or without calcium and various doses of vitamin D were used. Moreover, most of the trials had a short one-year follow-up. Thus, whether supplementation of vitamin D reduces the risk of stroke remains to be proven in clinical trials.

Our study has strengths and limitations. To our knowledge, this was the first study evaluating the association between risk of stroke and total 25(OH)D in Chinese. Stroke events were precisely classified according to ICD-codes and stratified into hemorrhagic and ischemic strokes for analysis. The outcome was validated in our previous study showing a positive predictive value of 0.9 (42). There are several limitations in this study. First, a causal relationship between either low or high 25(OH)D levels and

increased risk of stroke could not be inferred from this observational study. Second, 25(OH)D levels were measured by immunoassay rather than obtained by direct measurement. However, the method we used is known to be highly correlated with direct measurement ($R>0.93$) (43). Third, a single measurement of the levels of vitamin D was used. Fourth, vitamin D intake and the use of supplements of vitamin D was not assessed, whereas the most important source of vitamin D, from sunlight, could not be quantified.

In conclusion, a significant association was observed between low levels of 25(OH)D and a higher risk of ischemic stroke in middle-age to older adults in Hong Kong Chinese population. Further studies, such as the ongoing VITAL study (44, 45), are needed to assess a causal effect of vitamin D on the risk of stroke.

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