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Relation of Serum Vitamin D to Risk of Mitral Annular and Aortic Valve Calcium (from the Multi-Ethnic Study of Atherosclerosis [MESA]).

Brief Title: Vitamin D and Valvular Calcium.

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ABSTRACT

Serum 25(OH)D concentration has been identified as a possible modifiable risk factor for cardiovascular disease (CVD). We hypothesized that serum 25(OH)D concentration would be associated with calcifications of the left-sided heart valves, which are markers of CVD risk. Aortic Valve Calcium (AVC) and Mitral Annular Calcium (MAC) were quantified from cardiac CT scans performed on 5,530 MESA participants at the baseline exam (2000-2002) and at a follow-up visit at either Exam 2 (2002-2004) or Exam 3 (2004-2005). 25(OH)D was measured from serum samples collected at the baseline exam. Using relative risk regression, we evaluated the multivariable-adjusted risk of prevalent and incident AVC and MAC in this ethnically diverse population free of clinical CVD at baseline. The mean age of participants was 62±10 years; 53% were women, 40% white, 26% black, 21% Hispanic and 12% Chinese. Prevalent AVC and MAC were observed in 12% and 9%, respectively. There were no significant associations between 25(OH)D and prevalent AVC or MAC. Over a mean follow-up of 2.5 years, 4.1% developed incident AVC and 4.6% developed incident MAC. After adjusting for demographic variables, each 10 ng/ml higher serum 25(OH)D was associated with a 15% [RR=0.85 (95% CI 0.74, 0.98)] lower risk of incident MAC but not AVC. However, this association was no longer significant after adjusting for lifestyle and CVD risk factors. Results suggest a possible link between serum 25(OH)D and the risk for incident MAC, but future studies with longer follow-up are needed to further test this association.

Key words: Vitamin D, Valvular Calcium, AVC, MAC, Epidemiology, Prevention

INTRODUCTION:

Mineral metabolism has been recognized as a major risk factor for vascular calcification through trans-differentiation of smooth muscle cells to an osteogenic phenotype.[1] Specifically, higher serum concentrations of phosphate have been shown to be associated with aortic valve calcium (AVC) and mitral annular calcium (MAC) in the setting of kidney disease[2,3] and in a general population of older adults without known kidney disease or hyperparathyroidism.[4,5] In addition to its central role in mineral metabolism, activated vitamin D modulates inflammation, which is a key component of coronary artery and valvular calcification as well as atherosclerosis.[6] An independent association of serum 25-hydroxyvitamin D [25(OH)D] with coronary artery calcium (CAC) has been reported,[7] but whether there is a relation of 25(OH)D to valvular calcium is unknown. This study examines whether serum concentrations of 25(OH)D are associated with the prevalence and incidence of AVC and MAC using data from a large multi-ethnic community-based cohort. We hypothesized that both deficient and excess 25(OH)D concentrations would be associated with increased valvular calcium.

METHODS:

Detailed description of the Multi-Ethnic Study of Atherosclerosis (MESA) has been published elsewhere.[8] In brief, 6,814 Caucasian, African-American, Hispanic and Chinese participants aged 45-84 years and free of clinical cardiovascular disease (CVD) at time of enrollment were recruited from six U.S cities (Baltimore, Maryland; Chicago, Illinois; Forsyth County, North Carolina; Los Angeles, California; New York, New York

and St. Paul, Minnesota). MESA was designed to investigate the significance of subclinical CVD. The first examination (baseline) took place between 2000 and 2002. Follow-up visits took place between 2002-2004 for Exam 2 and 2004-2005 for Exam 3. By study design, all participants underwent a cardiac computed tomography (CT) scan at baseline; a random half had a follow-up cardiac CT at Exam 2 and the other half at Exam 3 (thus varying the time between the baseline and follow-up CTs).

Of the 6,814 participants enrolled in MESA, after accounting for the exclusions noted in **Figure 1**, a total of 5,530 participants were included in our analytic sample. Institutional Review Boards of all participating sites approved the study, and all participants signed informed consent for the MESA Study.

From serum samples that were collected at the baseline MESA exam and stored at -70^{o}C, 25(OH)D concentrations were measured using high-performance HPLC–tandem mass spectrometry at the University of Washington,[9] and calibrated to National Institute of Standards and Technology standards.[10] The interassay coefficient of variation was 4.4% at 10.4 ng/mL with a lower limit of detection of 2.0 ng/mL for 25(OH)D_3 and a coefficient of variation of 4.4% at 9.4 ng/mL with a lower limit of detection of 0.5 ng/mL for 25(OH)D_2.[11] 25(OH)D_3 and 25(OH)D_2 were combined for total 25(OH)D concentrations. Seasonal variation in serum levels of 25(OH)D was accounted for by using a cosinor model to derive annualized 25(OH)D concentrations as previously described.[11,12] To convert 25(OH)D levels to nmol/L from ng/ml, multiply by 2.496.

At the baseline exam and at either Exam 2 or 3 (randomly assigned), participants underwent ECG-gated cardiac CT scanning by electron-beam CT at 3 centers and a four-slice multi-detector row helical CT at the other 3 centers. Participants underwent

two consecutive scans at the same visit and results were averaged to enhance the accuracy of calcium assessments. From these cardiac CTs, AVC and MAC were quantified by the Agatston scoring method.[13] All studies were interpreted at one central reading center (Harbor-UCLA Research and Education Institute, Los Angeles, CA). Any detectable calcium was defined as a score >0 Agatston units (AU). Details of the image acquisition and interpretation protocols, quality control measures and interobserver reliability characteristics have been reported.[14]

Demographics, behavioral risk factors, medical history and medication history were obtained using standardized questionnaires at Exam 1. The following covariates were included in our analyses: age, sex, race/ethnicity (White, Chinese, Black, or Hispanic), education (<high school; high school or vocational school; college, graduate or professional school), cigarette smoking status (current; former; never) and physical activity level (MET-hours/week of moderate or vigorous activity). We also included baseline CVD risk factors: systolic blood pressure, high-sensitivity C-reactive protein (CRP), diabetes (defined as fasting blood glucose ≥126 mg/dl, or nonfasting glucose ≥200 mg/dl or medication use), body mass index (BMI, as a continuous variable), estimated glomerular filtration rate (eGFR) derived from the CKD-EPI equation,[15] total cholesterol, HDL-cholesterol, and medication usage (lipid-lowering therapy and antihypertensive).

Prevalent AVC or MAC was defined as Agatston score >0 at baseline and incident AVC or MAC was defined as detectable AVC or MAC at a follow-up examination in a participant free of AVC or MAC at baseline.

We stratified baseline characteristics by serum 25(OH)D categories defined using the Institute of Medicine guidelines, deficient 25(OH)D as levels <20 ng/ml and adequate as levels ≥20 ng/ml.[16] Frequencies and proportions were reported for categorical variables, and means with standard deviations or medians with interquartile ranges for continuous variables. Variables that were highly skewed (CRP and physical activity) were natural log transformed to approximate a normal distribution. After confirming a linear assumption was not violated, relative risk regression models with robust variance estimation were used to estimate the adjusted prevalence risk ratio (adjPRR) and relative risk (adjRR) of incident AVC or MAC [with 95% confidence intervals (CI)] for each 10 ng/ml higher in serum 25(OH)D. All analyses were progressively adjusted as follows: Model 1 adjusted for demographics (age, sex, race/ethnicity, education, site and CT scan type) and time between CT for incidence analysis, Model 2 additionally adjusted for behavioral risk factors (physical activity, BMI and smoking), and Model 3 additionally adjusted for potential mediators of the association between 25(OH)D and calcification (diabetes, systolic blood pressure, eGFR, total cholesterol, HDL cholesterol, CRP, use of lipid lowering medication and use of antihypertensive medications). To determine if there was a U-shaped distribution of risk as hypothesized[17], we modeled the association between 25(OH)D with AVC and MAC using a restricted cubic spline model, adjusted for demographic variables (Model 1). In sensitivity analyses, we also evaluated the multivariable-adjusted risk of prevalent and incident AVC or MAC by quintiles of 25(OH)D with median quintile as reference. Multiplicative interaction terms were created to evaluate for effect modification by

variables that may affect vitamin D metabolism. These included: race/ethnicity, age, sex, and eGFR.

All statistical analyses were performed using STATA 13 (StataCorp LP, College Station, TX) and significance was considered at *P* value of 0.05 or less.

RESULTS:

Of the 5,530 participants included in our analysis, the mean age was 62 ±10 years and 47% were men. Deficient serum 25(OH)D (<20ng/ml) was associated with younger age, black race, presence of diabetes, current smoking status, higher BMI, higher CRP level and lower HDL cholesterol **(Table 1)**. At baseline, 12.3% of participants had prevalent AVC, leaving 87.7% at risk for incident AVC **(Figure 1)**. Also, 9.0% had prevalent MAC, leaving 91.0% at risk for incident MAC **(Figure 1)**.

Compared to persons with 25(OH)D<20ng/ml, persons with 25(OH)D≥20ng/ml were more likely to have prevalent MAC at baseline **(Table 1)**. However, there was no statistically significant association between 25(OH)D levels and prevalent MAC nor AVC in multivariable-adjusted analysis **(Table 2)**.

Over a mean follow-up of 2.5 years, 4.1% of participants free of AVC at baseline developed incident AVC and 4.6% free of MAC developed incident MAC **(Figure 1)**. After adjusting for demographic variables, each 10 ng/ml higher serum 25(OH)D was associated with a 15% lower risk of incident MAC but not AVC **(Table 3,** Model 1**)**. However, this association was no longer significant after adjusting for lifestyle and CVD risk factors. In adjusted restricted cubic spline models, the associations of 25(OH)D levels with incident MAC **(Figure 2)** and incident AVC **(Figure 3)** were generally monotonic.

In sensitivity analyses, results for prevalent and incident AVC and MAC were similar in models using 25(OH)D categories instead of continuous 25(OH)D levels **(Supplemental Tables S1 and S2).** We did not find any association of 25(OH)D concentrations with the severity of MAC and AVC scores at baseline or follow-up for those with score>0. Also, there were no significant interactions by age, sex, race/ethnicity, or eGFR for the associations tested.

DISCUSSION:

We evaluated the association between serum 25(OH)D levels and cardiac valvular calcium (AVC and MAC) by means of quantitative serial CT scan measurements. Over a mean follow-up of 2.5 years, higher serum 25(OH)D was significantly associated with a lower risk of incident MAC but not AVC, however, this association was no longer significant after further adjustment for behavioral and traditional CVD risk factors. Contrary to some suggestions of a U-shaped distribution of risk reported with vitamin D and CVD in the literature,[17] we found that the association between serum 25(OH)D and valvular calcium was generally monotonic.

To our knowledge, this is the first study to report a prospective association between serum 25(OH)D and valvular calcium in a large racially/ethnically diverse population free of preexisting clinical CVD. Dishmon et al reported an association between vitamin D deficiency and echocardiographic evidence of valvular calcium in a small sample of patients with dilated cardiomyopathy.[18] Similar to our findings, prior work in MESA revealed an inverse association between serum 25(OH)D and the risk of incident CAC by cardiac CT.[7] Interestingly, MAC and CAC have been shown to share the same traditional CVD risk factors,[19,20] and given the strong association between

MAC and CAC,[21] the same biological mechanisms may underlie both diseases.

However, the association of higher serum 25(OH)D with lower risk of incident MAC was no longer significant after further adjustment for lifestyle and CVD risk factors. One possible explanation is that our analyses may be have been underpowered given the small proportion of incident MAC (4%) after an average follow-up period of only 2.5 years. Alternatively, the relationship between 25(OH)D and MAC may be confounded by lifestyle and CVD risk factors, as deficient 25(OH)D may be a marker of a poorer health state. Studies show that increasing BMI and low physical activity level, which are well-established risk factors for CVD, are also associated with lower serum 25(OH)D levels.[22-24]

It is unclear why serum 25(OH)D was not associated with incident AVC. One possible explanation may be because of the differences in anatomy and hydrodynamics between the aortic and mitral valves. The mitral valve annulus is a C-shaped segment of the fibrous skeleton at the base of the left ventricle and may present a larger surface area for progressive calcium deposition compared to the aortic valve (which has a smaller annulus). In addition, the aortic valve is a high-flow area compared to the mitral valve and may take longer for progressive calcium deposition to build up. Additionally, previous analysis in Cardiovascular Health Study (CHS) revealed that mineral metabolism markers were mainly associated with MAC, whereas measures of lipid metabolism were associated with AVC.[25] This could therefore explain the lack of significant association between 25(OH)D and AVC.

The findings from this study are partly consistent with the large body of observational evidence that serum 25(OH)D deficiency is a risk marker for future

CVD.[7,26,27] However whether vitamin D supplementation, a widely available and low cost remedy, can reduce CVD risk remains uncertain at this time.[27] The recent Vitamin D Assessment Study failed to show that high-dose vitamin D supplementation prevented CVD events.[28] However, confirmatory results from other large randomized clinical trials [such as the ongoing Vitamin D and Omega-3 Trial (VITAL) study[29]] that include more diverse populations and include individuals with 25(OH)D deficiency are needed to definitively determine the benefit of vitamin D supplementation on cardiovascular outcomes.

This analysis was conducted in a racially/ethnically diverse community-based population. Also, participants were free of clinical CVD at the time of enrollment, reducing the likelihood of confounding by poorer health status. AVC and MAC were assessed with serial CT scan measurements and potential confounders were also well characterized in MESA. Nonetheless, there are some limitations to this study that must be acknowledged. First, 25(OH)D was measured only once at baseline. Although serum 25(OH)D is reflective of the composite production of vitamin D from endogenous and exogenous sources, a single measurement of 25(OH)D does not represent cumulative vitamin D levels. However, we did account for seasonal variation. Second, follow-up time of almost 2.5 years was short, leading to a very low incidence of AVC and MAC in this population. Finally, the temporal association observed between 25(OH)D and MAC does not necessarily demonstrate causality.

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institutions can be found at http://www.mesa-nhlbi.org.

Conflicts of interest:

Dr. Michos has the following disclosures:

- Consultant: Siemens Healthcare Diagnostics (unrelated to topic)

Dr. Budoff has received research funds from GE Healthcare.

No other authors declare a conflict of interest.

FIGURE LEGENDS:

Figure 1. A flow diagram illustrating the prevalence of AVC and MAC at baseline and the percentage of participants at risk who developed incident AVC/MAC on follow-up CT.

Figure 2. Adjusted restricted cubic spline model of the association between serum 25(OH)D level and incident MAC. Solid line represents the point estimate and dashed lines represent the 95% confidence intervals. Adjusted per model 1 (see footnote to Table 3).

Figure 3. Adjusted restricted cubic spline model of the association between serum 25(OH)D level and incident AVC. Solid line represents the point estimate and dashed lines represent the 95% confidence intervals. Adjusted per model 1 (see footnote to Table 3).

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