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A Cross-Sectional Study of the Association between Circulating 25-Hydroxyvitamin D Levels and Predicted Operative Mortality of Patients with Cardiovascular Disease

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Summary Recent studies have suggested that low levels of 25-hydroxyvitamin D (25OHD) are associated with cardiovascular risks in medical patients. However, these associations have not been well documented in high risk surgical patients. We hypothesized that serum 25OHD, 1,25-dihydroxyvitamin D (1,25OHD) would be associated with the cardiac operative risk stratification score. The study was conducted with a cross-sectional design at a single academic medical center in Japan. Two hundred five adult patients scheduled for major cardiovascular surgery were included consecutively. Cardiac operative risk was evaluated with the European System for Cardiac Operative Risk Evaluation (EuroSCORE) scoring system. Correlations between 25OHD and 1,25OHD, and EuroSCORE were assessed using simple and multiple linear regression models. Mean 25OHD and 1,25OHD were 20.1±7.1 ng/mL and 51.2±19.2 pg/mL, respectively. Half and 88% of the study population showed deficient (<20 ng/mL) and insufficient (<30 ng/mL) 25OHD levels, respectively. In contrast, only 3% showed 1,25OHD levels lower than normal (<20 pg/mL). Circulating 25OHD levels, but not 1,25OHD levels, were negatively correlated with EuroSCORE (p=0.005) even after adjusted for body mass index, albumin, hypertension, dyslipidemia, diabetes mellitus, creatinine, use of statin, high sensitive C-reactive protein, and intact parathyroid hormone. These results suggest that serum 25OHD levels are inversely associated with operative risk severity of patients undergoing major cardiovascular surgery.

Key Words vitamin D, 25-hydroxyvitamin D, European System for Cardiac Operative Risk Evaluation, cross-sectional study

Vitamin D is mainly synthesized through exposure to sunlight and ingested from the diet and/or through supplements, which are metabolized to the major circulating form of vitamin D, 25-hydroxyvitamin D (25OHD) in the liver. 25OHD is metabolized in the kidneys by 25-hydroxyvitamin D-1α-hydroxylase (1αOHase) to its active form, 1,25-dihyroxyvitamin D (1,25OHD); serum levels of 1,25OHD are tightly regulated in the range of 20 to 60 pg/mL by parathyroid hormone (PTH) as well as calcium (Ca) and phosphate levels (*1*). Secretion of 1,25OHD into the serum facilitates not only intestinal Ca and phosphate absorption, but also bone calcification. Deficiency of 1,25OHD is known to cause rickets, osteopenia, osteoporosis, and osteomalacia, and to simultaneously increase the risk of fracture.

In addition to these bone diseases, recent epidemiologic evidence suggests that 25OHD deficiency, but not 1,25OHD deficiency, is associated with an increased risk of many chronic diseases including cardiovascular disease (CVD), various cancers, and infectious diseases (*2*). In the Framingham Offspring Study, 25OHD deficiency was associated with incident CVD (*3*). The risk for CVD may be associated with ubiquitous expression of both 1αOHase and vitamin D receptor (VDR) in a variety of cell types including vascular smooth muscle cells, endothelial cells, and cardiomyocytes (*4*). Various experimental studies also suggest that vitamin D supplements provide cardiovascular protection including anti-atherosclerotic, anti-inflammatory, and direct cardio-protective actions, as well as beneficial effects on classic cardiovascular risk factors and suppression of PTH levels (*5*). Observational studies and meta-analyses of randomized controlled trials indicate that vitamin D supplements may reduce CVD-related disease and all-cause mortality (*6*), although a cause and effect relationship is still controversial (*7*). Of critically ill patients, similarly, Lee et al. identified the possibility of a relationship between low levels of 25OHD, and high morbidity and mortality

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although the sample size was small and adjustment for well-known risk factors was not performed (*8*).

Given the epidemiological information about 25OHD and 1,25OHD above, we hypothesized that serum 25OHD and 1,25OHD would be associated with the cardiac operative risk stratification score. In this cross-sectional study, we tested this hypothesis with use of a larger number of patients than previous studies along with multivariate adjustment to determine associations between circulating levels of 25OHD as well as 1,25OHD, and predicted operative mortality among cardiovascular patients undergoing cardiovascular surgery.

MATERIALS AND METHODS

Study design. This cross-sectional study was performed at the intensive care unit of Jichi Medical University Saitama Medical Center. The data monitoring center was at the Division of Molecular Epidemiology, Jikei University School of Medicine. Planned analyses are in full compliance with the principles of the Declaration of Helsinki. The study protocol was reviewed and approved by the ethics committee of Jichi Institutional Review Board, Jichi Medical University School of Medicine, and Jikei Institutional Review Board, Jikei University School of Medicine. The study design and protocol, data monitoring, and analyses were performed only by academic authors; there was no industry support or involvement in the study.

Study population, eligibility, and consent. Two hundred five patients aged 26 to 86 y old and scheduled for major cardiovascular surgery at Jichi Medical University Saitama Medical Center were included consecutively. Eligible patients were suffering from either coronary artery disease (CAD), valvular heart disease (VHD) or aortic disease (AD), and diagnosed by cardiac surgeons. Patients who were taking oral vitamin D supplements or active vitamin D therapy, or who had kidney dysfunction (defined as creatinine [Cr] levels of $\geq$2.26 mg/dL), were excluded. All patients were asked to participate in this study on the day before surgery by the anesthesiologists in charge and provided written informed consent. The accrual period was from January 28, 2010, to October 29, 2010.

Outcome measure. We used the additive European System for Cardiac Operative Risk Evaluation scoring system (EuroSCORE) to evaluate predicted operative mortality for patients undergoing cardiovascular surgery (*9–11*). The additive EuroSCORE has been validated by databases in Europe, North America (*12*), and Japan (*10*), and is easy to use at the bedside. Relevant factors that contribute to the EuroSCORE include patient-related factors (age, gender, renal dysfunction [divided by serum Cr $<$2.26 mg/dL or $\geq$2.26 mg/dL], peripheral artery disease, and chronic pulmonary disease), cardiac-related factors (recent cardiac status and left ventricular ejection fraction (LVEF)), and operation-related factors (surgical procedure and urgency of treatment) (*9*). Scoring was performed by one author (Y.T.), who was blinded to serum 25OHD and 1,25OHD levels.

Clinical evaluation. Clinical information such as baseline characteristics, comorbidity factors and medication history was abstracted from medical records and preanesthetic interview forms. Body mass index (BMI) was obtained by weight (kg)/[height (m)]^2. LVEF was measured by transthoracic echocardiography with the Method of Disks within 1 mo prior to the date of surgery. Laboratory data for peripheral blood Cr, albumin, high-sensitivity C reactive protein (hsCRP), ionized calcium (iCa) (normal range: 1.21 to 1.36 mmol/L) and intact PTH (iPTH) (normal range: 10 to 65 pg/mL) were collected at the study entry. Hypertension was defined as systolic blood pressure $\geq$140 mmHg, diastolic blood pressure $\geq$90 mmHg, or use of antihypertensive medications (*13*). Dyslipidemia was defined as fasting LDL-cholesterol $\geq$140 mg/dL, triglycerides $\geq$220 mg/dL, or use of lipid-altering agents (*14*). Diabetes mellitus was defined as a fasting plasma glucose $\geq$126 mg/dL, hemoglobin A1C $\geq$6.5%, or use of insulin or hypoglycemic medications.

Samples and 25OHD/1,25OHD measures. Serum levels of 25OHD (ng/mL) and 1,25OHD (pg/mL) were measured at SRL Inc. (Hachioji, Tokyo, Japan) as described previously (*15, 16*). In each case, blood samples were collected in the operating room prior to anesthesia induction to avoid the hemodilution effect of intravenous fluid infusion (*17*) and stored at -80°C until measurements. All data were measured twice by radioimmunoassay. When duplicated data differed by 5 ng/mL or more, measures were repeated.

Statistical analysis. All statistical analyses were performed using STATA 12.0 (STATA Corp., College Station, TX). For continuous variables with a normal distribution, the mean$\pm$SD is reported, and for non-normally distributed variables, medians and interquartile ranges are reported. To compare baseline characteristics, patients were divided according to underlying cardiac disease (i.e., CAD, VHD, or AD). Analysis of variance was used for the continuous variables including 25OHD and 1,25OHD that were distributed normally. Non-parametric continuous variables such as age, LVEF, Cr, hsCRP, iCa, iPTH levels were analyzed by the Kruskal-Wallis equality-of-populations rank test, because assumptions of normality of the distribution or homogeneity of variances were not verified. For categorical data, the χ^2 test was performed, except when expected cells were found to be less than five, in which case we used Fisher's exact test. Pearson's coefficient (r) was used to study the relationship between 25OHD and 1,25OHD levels. Multiple linear regression analysis was performed to evaluate the independent risk factor of EuroSCORE. In this analysis, clinical factors, and possible CVD risk factors were included to which EuroSCORE was significantly correlated in simple linear regression analysis. Associations between EuroSCORE and 25OHD levels as well as 1,25OHD levels were assessed using multiple linear regression models in multivariate analysis. Coefficiency with 95% confidence intervals was computed. We did not use seasonal variation in the multivariate analysis because we could not determine any seasonal variations in this study.

Table 1. Baseline characteristics according to underlying disease.

	Total (n=205)	CAD[1] (n=56)	VHD[2] (n=100)	AD[3] (n=49)	p-value[4]
Age (y)	69 (62–75)	70 (62–75)	68 (61–75)	70 (62–76)	0.61[5]
Gender, n (%)					0.001[6]
Male	137 (66.8)	47 (83.9)	55 (55.0)	35 (71.4)	
Female	68 (33.2)	9 (16.1)	45 (45.0)	14 (28.6)	
BMI (kg/m^2)	23.0±3.5	24.1±3.9	22.0±3.0	23.8±3.6	0.0004[7]
LVEF (%)	59 (50–67)	57 (38–67)	59 (49–68)	62 (55–68)	0.056[5]
Cr (mg/dL)	0.86 (0.72–1.04)	0.92 (0.78–1.13)	0.82 (0.67–0.99)	0.86 (0.76–1.03)	0.012[5]
Albumin (g/dL)	3.9±0.5	3.8±0.5	3.9±0.4	3.9±0.5	0.35[7]
hsCRP (ng/mL)	761 (301–2,240)	1,100 (383–4,385)	549 (208–1,470)	947 (384–2,070)	0.009[5]
iCa (mmol/L)	1.33 (1.26–1.37)	1.33 (1.24–1.38)	1.32 (1.25–1.36)	1.33 (1.27–1.40)	0.102[5]
iPTH (pg/mL)	40 (29–64)	38 (30–51)	38 (28–58)	51 (30–92)	0.029[5]
25OHD (ng/mL)	20.1±7.1	19.1±6.6	20.6±7.2	20.3±7.2	0.44[7]
1,25OHD (pg/mL)	51.2±19.2	45.5±18.6	53.5±20.9	53.1±15.0	0.033[7]
Comorbidity (%)					
Hypertension	79.0	83.9	69.0	93.9	0.001[6]
Dyslipidemia	53.7	76.8	43.0	49.0	<0.001[6]
Diabetes mellitus	28.8	50.0	27.0	8.2	<0.001[6]
Medication (%)					
Statin	44.9	64.3	38.0	36.7	0.003[6]
ACE/ARB[8]	62.9	67.9	62.0	59.2	0.633[6]

[1] Coronary artery disease (CAD) included unstable angina (51) and acute myocardial infarction (5).
[2] Valvular heart disease (VHD) included aortic stenosis (38), aortic regurgitation (21), mitral stenosis (8), mitral regurgitation (32) and tricuspid regurgitation (1).
[3] Aortic disease (AD) included thoracic aortic aneurysm (41) and aortic dissection (8).
[4] $p<0.05$ was considered statistically significant. Continuous data are presented as mean±SD or median (interquartile range).
[5] Data were evaluated with Kruskal-Wallis equality-of-populations rank test.
[6] Data were evaluated with chi-square test.
[7] Data were evaluated with analysis of variance.
[8] ACE/ARB: angiotensin converting enzyme inhibitor or angiotensin II receptor blocker.

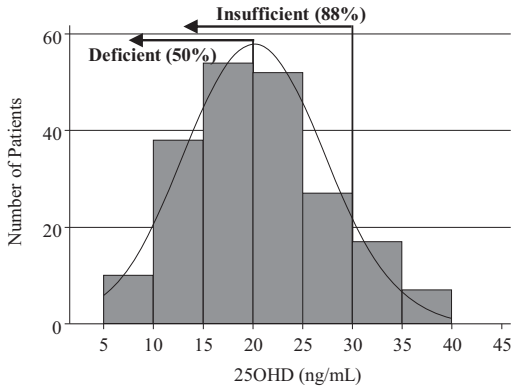

Fig. 1. Histogram of circulating 25OHD levels. Serum 25OHD levels were measured by radioimmunoassay.

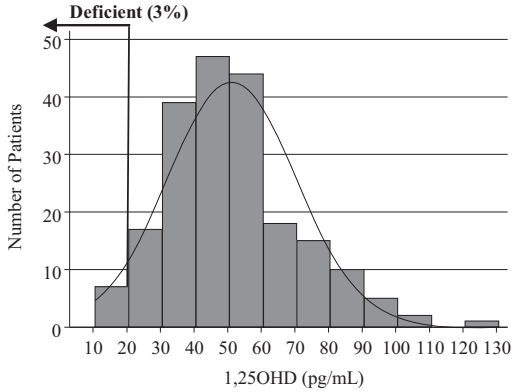

Fig. 2. Histogram of circulating 1,25OHD levels. Serum 1,25OHD levels were measured by radioimmunoassay.

RESULTS

Patients' characteristics

A total of 205 patients participated in the study. Baseline characteristics of the study population divided by underlying CVDs are shown in Table 1. Among three types of CVDs, there were significantly higher numbers of males in the CAD and AD groups than in the VHD group. Mean BMI and median Cr and hsCRP in the CAD group were higher than in the VHD group. Levels of iPTH in the AD group were higher compared with the CAD and VHD groups and 1,25OHD in the CAD group was lower compared with the other groups. The AD group had the highest proportion of patients suffering from hypertension, whereas the CAD had the highest proportion of patients with dyslipidemia, diabetes mellitus, and on statin therapy among the three underlying CVDs. No other baseline characteristic differed signifi-

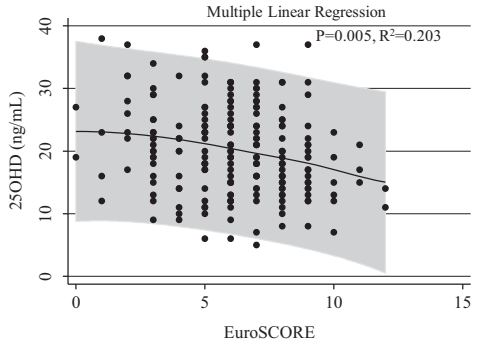

Fig. 3. Associations between EuroSCORE and serum 25OHD levels for patients with cardiovascular disease. For patients with cardiovascular disease, we used linear regression models to assess the association between EuroSCORE and 25OHD levels in multivariable analyses using BMI, albumin, hypertension, dyslipidemia, diabetes mellitus, Cr, use of statin, hsCRP and iPTH.

cantly among the three groups.

Distribution of 25OHD and 1,25OHD

Histograms of circulating 25OHD (mean±SD, 20.1±7.1 ng/mL; median, 20 ng/mL) and 1,25OHD levels (mean±SD, 51.2±19.2 pg/mL; median, 49.1 pg/mL) are shown in Figs. 1 and 2. Half and 88% of the study population showed deficient (<20 ng/mL) and insufficient (<30 ng/mL) 25OHD levels, respectively (Fig. 1). In contrast, only 3% showed 1,25OHD levels lower than normal (<20 pg/mL) (Fig. 2). Serum 25OHD levels had a positive association with 1,25OHD levels (r=0.34, p<0.0001).

25OHD, 1,25OHD, and EuroSCORE

Simple linear regression was used to clarify the relationship between EuroSCORE and possible CVD risk factors. There was a significant association between EuroSCORE and 25OHD (p=0.001), hsCRP (p=0.003) and iPTH (p=0.006). However, no significant trend was observed for 1,25OHD levels and iCa levels (Table 2, Simple). Multiple linear regression analysis was performed to evaluate independent risk factors of EuroSCORE. In this analysis, in addition to clinical factors such as BMI, albumin, hypertension, dyslipidemia, diabetes mellitus, Cr, and use of statin, possible CVD risk factors such as hsCRP and iPTH were included to which EuroSCORE was significantly correlated in simple linear regression analysis. In multiple linear regression, higher 25OHD levels, but not 1,25OHD levels, were significantly associated with lower EuroSCORE (p=0.005, R^2=0.203) (Table 2, Multiple 1, 2, Fig. 3). After stratification for underlying diseases, a significant association between EuroSCORE and 25OHD remained in the VHD group, not in the CAD and AD groups (p=0.047).

DISCUSSION

In this study, we found that circulating 25OHD levels were deficient in half of patients who underwent cardiovascular surgery, and higher levels of 25OHD were significantly associated with a lower EuroSCORE even after multivariate adjustment for possible confounders. In contrast, circulating 1,25OHD levels were not reduced in most patients undergoing surgery and were not associated with EuroSCORE even after multivariate adjustment for possible confounders (Tables 1 and 2). Lee et al. first noted that lower levels of 25OHD were correlated with predicted hospital mortality evaluated by the Simplified Acute Physiology Score II in 42 patients admitted to the intensive care unit and referred to the Department of Endocrinology (*8*), which is consistent with our results. Levels of hsCRP and iCa were reported to be associated with severity and prognosis of CVD (*18*), and Anderson et al. identified the possibility of a relationship between elevated iPTH levels, and incidence of cardiovascular risk factors and mortality (*19*). Therefore, we used them as possible confounders for EuroSCORE in our multivariate analyses. As a result, however, we did not include iCa as a confounder in multiple linear regression analyses, because EuroSCORE was not significantly correlated with iCa in simple linear regression analysis. Additionally, we included statin therapy as a covariate, because statin therapy has been shown to increase circulating vitamin D levels (*20*) and may be a confounder behind the negative association between 25OHD as well as 1,25OHD levels, and EuroSCORE (*21*). In contrast, we did not use underlying diseases (i.e., CAD, VHD and AD) as confounders because EuroSCORE already accounts for these operation-related factors. In our study, 25OHD levels remained a significant risk factor of EuroSCORE in all patients even after adjustment for cardiovascular risk factors and other standard risk factors. Furthermore, in stratified analyses of underlying diseases, a significant association between EuroSCORE and 25OHD still remained in the VHD group, not in the CAD and AD groups. One of the most feasible reasons for these results may be that the number of patients in each group is too small for the differences to be significant in stratified analyses compared with the number of independent variables.

It is unclear why 25OHD levels were so low in our patients. Patients with severe CVD may not be able to go out because of the restricted body activity and may have less sun exposure, which could explain why patients with severe CVD had lower 25OHD levels (*22*). On the other hand, lower 25OHD levels may be a risk factor for CVD. It is also unclear why predicted operative mortality was associated with 25OHD levels but not 1,25OHD levels. We assumed that 1) like macrophages (*23*), cardiac cells take up circulating 25OHD rather than 1,25OHD, because circulating levels of 25OHD are 1,000 times higher than that of 1,25OHD (*1*); 2) cardiac cells activate 25OHD into 1,25OHD by 1αOHase in the cells (*1*); 3) the 1,25OHD binds to VDR in the cell; 4) the complex of 1,25OHD and VDR moves into the nucleus and binds to vitamin D response elements around promoter regions (*24*); 5) this reaction regulates a variety of gene transcriptions (*25*, *26*); 6) as a result, some gene transcriptions protect against progression of atherosclerosis, inflammation, and fibrotic scarring (5). In contrast to 25OHD, most of the circulating 1,25OHD levels were within normal range and 1,25OHD levels were not asso-

Table 2. Simple and multiple linear regression models for EuroSCORE of all patients with cardiovascular disease.

	EuroSCORE[1]								
	Simple			Multiple 1[2]			Multiple 2[2]		
	β[3]	95% CI[4]	*p*-value[5]	β[6]	95% CI[4]	*p*-value[5]	β[6]	95% CI[4]	*p*-value[5]
25OHD (ng/mL)	−0.08	−0.120 to −0.030	0.001	−0.070	−0.11 to −0.02	0.005			
1,25OHD (pg/mL)	−0.012	−0.029 to 0.005	0.178				−0.014	−0.033 to 0.004	0.148
hsCRP (ng/mL)	0.00009	0.00003 to 0.00014	0.003	0.00007	0.00002 to 0.0001	0.013	0.00008	0.00002 to 0.0001	0.008
iCa (mmol/L)	−1.002	−2.40 to 0.40	0.159						
iPTH (pg/mL)	0.015	0.004 to 0.026	0.006	0.007	−0.004 to 0.018	0.202	0.011	−0.0005 to 0.023	0.060
BMI				−0.141	−0.234 to −0.047	0.003	−0.149	−0.244 to −0.054	0.002
Albumin				−0.198	−0.951 to 0.554	0.603	−0.334	−1.112 to 0.445	0.399
Hypertension				0.946	0.167 to 1.724	0.018	1.031	0.243 to 1.821	0.011
Dyslipidemia				0.042	−0.733 to 0.817	0.915	0.097	−0.692 to 0.885	0.809
Diabetes mellitus				−0.527	−1.242 to 0.188	0.148	−0.394	−1.112 to 0.324	0.280
Creatinine				1.686	0.454 to 2.919	0.008	0.993	−0.307 to 2.293	0.133
Use of statin				0.116	−0.686 to 0.917	0.776	0.138	−0.676 to 0.952	0.739

[1] European System for Cardiac Operative Risk Evaluation.
[2] Multiple linear regression adjusted for BMI, albumin, hypertension, dyslipidemia, diabetes mellitus, Cr, use of statin, hsCRP, and iPTH.
[3] β Coefficient of simple linear regression.
[4] Confidence interval.
[5] $p<0.05$ was considered statistically significant.
[6] β Coefficient of multiple linear regression.

ciated with predicted operative mortality in this study. These results indicates that circulating 1,25OHD may not be a predictor for operative mortality as the assay for 1,25OHD is less reliable than that for 25OHD because of a short half-life of 1,25OHD (*27*) and it may be tissue rather than circulating 1,25OHD sufficiency which is more important in cardiac operative risks. Additionally, as circulating 1,25OHD levels are tightly regulated by PTH, Ca and phosphate levels, circulating 1,25OHD levels stay within the normal range until severe deficiency of 1,25OHD levels.

There are several limitations to this study. First, the study design was cross-sectional; thus, no cause and effect relationship can be established. Second, we did not collect data on sun exposure or diet except for vitamin D supplements, so we could not determine the association of these factors with 25OHD levels and the severity of patients' conditions. Third, preoperative heart diseases were heterogeneous among our patients. On the other hand, the study population ($n=205$) was large enough to compute multivariate analyses for all patients using possible and known CVD risk factors, including BMI (*28*, *29*), albumin (*30*), hypertension (*31*), dyslipidemia (*28*), diabetes mellitus (*28*), renal dysfunction (*32*), use of statin (*20*), hsCRP (*33*), iCa (*18*) and iPTH (*19*). This is a strength of this study.

CONCLUSIONS

These results suggest that higher 25OHD levels may be associated with a milder EuroSCORE in patients undergoing major cardiovascular surgery.

Competing interests

The authors declare that they have no competing interests.

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