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Parathyroid Hormone and Vitamin D Levels are Independently Associated With Calcific Aortic Stenosis

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Background In calcific aortic valve disease, the early lesion is similar to atherosclerotic plaque, but later calcification prevails. Parathyroid hormone (PTH) and vitamin D are the principal calcium pool regulators, so the present study was designed to assess their association with aortic stenosis (AS) in patients with significant coronary artery disease (CAD), and preserved renal function.

Methods and Results The 122 consecutive patients with AS (mean gradient ≥30 mmHg) plus CAD, and 101 patients with nonobstructive aortic sclerosis (mean gradient ≤10 mmHg) plus CAD, as controls, were prospectively enrolled. The AS patients were older (71±7 vs 66±7 years; p<0.001), had higher serum intact (i)PTH (51.4 [39–70] vs 37.4 [27–50] pg/ml; p<0.001), and lower plasma vitamin D (32.0 [25–40] vs 35.8 [27–55] nmol/L; p=0.003) levels than those with aortic sclerosis. The groups did not differ significantly in creatinine level (93 [82–105] vs 96 [85–107] μmol/L, p=0.19), calcium – phosphate product, occurrence of hypertension, smoking, diabetes, dyslipidemia, or body mass index. The iPTH (odds ratio (OR) 1.04, 95% confidence interval (CI) 1.02–1.05; p<0.001) and vitamin D levels (OR 0.97, 95% CI 0.95–0.99; p=0.003) were independently associated with AS.

Conclusion Higher serum iPTH with lower vitamin D levels were independently associated with calcific AS in CAD patients. (*Circ J* 2008; **72:** 245 – 250)

Key Words: Aortic stenosis; Calcium; Coronary artery disease; Valvular disease

The pathogenesis of aortic stenosis (AS) is an active process with 2 distinguishable stages: nonobstructive aortic valve thickening, or sclerosis, and progressive calcification, which causes valve obstruction. Aortic sclerosis is a common finding, present in 25–28% of the elderly[1] and the underlying inflammatory cusp lesion is similar to atherosclerotic plaque[2] In only approximately 2.5% of these patients, progressive calcification leads to severe AS[3,4] This process shares features of skeletal bone formation: cells with osteoblast phenotype have been identified in the stenotic aortic valve[5] and mature lamellar bone commonly occurs in end-stage AS[6]

Parathyroid hormone (PTH) and vitamin D – endocrine system are the principal regulators of the calcium balance and bone mineral turnover. We hypothesized that their dysregulation may be associated with advanced acquired calcific AS with preserved renal function, and we aimed to assess the association of vitamin D and PTH plasma or serum levels with AS on the background of advanced atherosclerosis, in a prospectively collected sample of patients with significant coronary artery disease (CAD), and moderate to severe AS or nonobstructive aortic valve sclerosis.

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Methods

Patient Population

The study was conducted at 2 cardiovascular centers in the Czech Republic. The study protocol complied with the Declaration of Helsinki and was approved by the local scientific and ethical committees of both centers. The study has been registered in the system of the National Institutes of Health and FDA ClinicalTrials.gov as No. NCT 00375336. All patients gave written informed consent to participate in the study.

Between January 2005 and June 2006 we prospectively enrolled consecutive patients admitted to hospital for evaluation for common causes such as dyspnea, chest pain, fatigue or syncope, and who fulfilled the 2 inclusion criteria: (1) angiographically significant CAD, and (2) AS (mean transvalvular aortic gradient ≥30 mmHg) or nonobstructive aortic sclerosis (mean gradient ≤10 mmHg) diagnosed by echocardiography. The patient enrollment was regularly dispersed over the whole 18-month period.

We excluded patients with acute coronary syndrome within the last 3 months before admission, rheumatic heart disease (defined by fusion of commissures between the

valve cusps plus rheumatic mitral valve disease), prosthetic valves, congenital heart disease except for bicuspid aortic valve, moderate to severe aortic regurgitation (>2/4), Marfan syndrome, infectious endocarditis, hypertrophic obstructive cardiomyopathy, known primary hyperparathyroidism, severe heart failure New York Heart Association class IV, end-stage renal disease requiring dialysis, and those with other systemic disease or malignity severely limiting prognosis. We also excluded from the final data analysis any patients treated with vitamin D and calcium supplements.

Clinical Evaluation

All patients underwent a detailed history of their cardiovascular risk factors, and their medication, including intake of vitamin D or calcium supplements, was recorded. At physical examination, blood pressure was measured under standard conditions, and waist circumference and body mass index (BMI) were assessed.

Laboratory Parameters

Blood samples were drawn between 06.00 and 07.00 h, after a 12-h fast. The serum or plasma were separated within 30–60 min of collection and the aliquots were frozen to –80°C and kept on site without freeze–thaw cycles.

After study completion, the following analytes were determined at 1 core laboratory: plasma level of vitamin D (25-hydroxyvitamin D, calcidiol) and the serum level of its active metabolite 1,25-dihydroxyvitamin D (calcitriol), both by radioimmunoassay (Immunodiagnostic Systems, Ltd, Tyne and Wear, UK). The analyses were done in duplicate with a mean interassay variability of 4.6%. Serum intact (i) PTH was assessed by chemiluminiscent immunoanalysis (Access PTH, Beckmann Coulter, Inc, Fullerton, CA, USA). The reproducibility of the test was in accordance with the values declared by the manufacturer, with a variation coefficient of 3–6%. High-sensitivity C-reactive protein (hs-CRP) was assessed by the immunoturbidimetric method (Orion Diagnostica OY, Espoo, Finland). The serum level of inorganic phosphate, total calcium, creatinine, urea and uric acid were measured by standard photometric methods with the kits from Olympus Diagnostica (Clare, Ireland) and an Olympus AU 2700 analyzer (Mishima, Japan). Homocysteine concentration was measured by photometric method with kits from Carolina Liquid Chemistries (Brea, CA, USA).

The ionized calcium level in the arterial blood was measured on site by a selective electrode using the ABL 715 analyzer (Radiometer, Copenhagen, Denmark). Total cholesterol was measured by enzymatic method using kits from Dialab (Vienna, Austria), high-density lipoprotein-cholesterol (HDL-C) by a direct method with kits from Human (Wiesbaden, Germany), and the concentration of low-density lipoprotein-cholesterol (LDL-C) was calculated according to Friedwald's formula. Twenty-four hours urinary phosphorus and calcium excretions were determined using standard methods. Creatinine clearance was calculated using the Cockroft – Gault formula.

Hyperparathyroidism was defined as serum intact parathyroid hormone (iPTH) >65 pg/ml. Vitamin D insufficiency was defined as calcidiol plasma level 25–50 nmol/L, vitamin D defficiency as calcidiol plasma level <25 nmol/L.[7,8]

Echocardiography

Standard echocardiographic examination was performed in all patients (System FiVE, Vivid Five, GE Vingmed, Horten, Norway, and iE33, Philips, Andover, MA, USA). The data were stored on videotapes and reviewed by 2 cardiologists who were unaware of the other results. Discrepances in evaluation were solved by consensus between them. Aortic sclerosis was defined as higher echogenicity, or thickening of the leaflets,[1,9] with peak aortic flow velocity ≤2.5 m/s or mean transaortic gradient <10 mmHg.

Mean and peak transaortic gradients were calculated by the modified Bernoulli equation at the time of examination. Aortic regurgitation was evaluated according to the American Society of Echocardiography (ASE) criteria.[10] The left ventricular dimensions were measured from the 2-dimensional long-axis parasternal view by the leading edge to leading edge method. The ejection fraction was assessed by the biplane modified Simpson's method.

Coronary Angiography

Coronary angiography was performed in all patients using the femoral approach with 4F or 5F instruments. The coronary artery tree was divided into 3 compartments (left anterior descending artery, circumflex artery and right coronary artery) to diagnose the 1–3-vessel or left main CAD. The reference diameter of the artery within the assessed lesion had to be greater than 1.5 mm to diagnose a "diseased artery". In the case of left-dominant or balanced-dominant circulation, the assessment was modified according to actual coronary morphology. Significant CAD was defined as >50% diameter stenosis. Radiation exposure per patient and examination was 10 mSv. All examinations were evaluated by an interventional cardiologist unaware of the other study results. Quantitative analysis was used only in borderline cases.

Statistical Analysis

The categorical variables are described by number and percentages. The continuous variables are shown as mean and standard deviation, or median and 25–75th percentile in the case of non-Gaussian distribution. The categorical variables were compared by the chi-quadrate test, the continuous variables by t-test or by the Mann-Whitney U-test as appropriate.

First, we compared the clinical, echocardiographic, angiographic and laboratory characteristics between the patients with AS and aortic sclerosis. Then we compared their calcium metabolism parameters, including fasting serum iPTH and plasma vitamin D levels adjusted for age, using multivariate logistic regression. Third, we evaluated the association of iPTH with known risk factors of secondary hyperparathyroidism[11] (ie, vitamin D level, age, creatinine clearance, and BMI) using univariate linear regression. Finally, we assessed the association of the serum iPTH and plasma vitamin D levels with AS in the multivariate logistic regression model. We used the Statgraphics Centurion Version XV.2 (Statpoint Inc, Herndon, VA, USA) for statistical evaluation. The $p<0.05$ was considered statistically significant.

Results

Study Sample

We enrolled 223 patients with significant CAD (mean age range 50–87 years), of whom 122 had AS, and 101 patients with aortic sclerosis as controls. The patients with AS and CAD were older (71±7 vs 66±7 years, $p=0.001$); the comparison of their clinical, echocardiographic, angio-

Table 1 Comparison of Basic Patient Characteristics

	Aortic stenosis (n=122)	Aortic sclerosis (n=101)	p value
Age (years)	71±7	66±7	0.001
Women	37 (30%)	25 (25%)	0.30
Hypertension	97 (80%)	85 (85%)	0.36
Diabetes mellitus	43 (35%)	41 (41%)	0.34
Metabolic syndrome	15 (12%)	22 (22%)	0.02
Current or past smokers	70 (57%)	64 (63%)	0.18
Dyslipidemia	104 (85%)	93 (92%)	0.08
BMI (kg/m^2)	29±4	29±3	0.94
Waist (cm)	101±17	103±9	0.19
Systolic BP (mmHg)	142±17	136±17	0.007
Diastolic BP (mmHg)	80±10	80±9	0.70
Laboratory profile			
Total cholesterol (mmol/L)	4.9±1.0	5.0±1.2	0.33
HDL-cholesterol (mmol/L)	1.21±0.35	1.24±0.39	0.49
LDL-cholesterol (mmol/L)	3.01±0.98	3.07±0.94	0.85
hs-CRP (mg/L)	1.94 (0.79–5.04)	2.93 (1.32–5.34)	0.25
Creatinine (μmol/L)	93 (82–105)	96 (85–107)	0.19
Urea (mmol/L)	7.4 (5.6–8.5)	5.5 (4.6–6.6)	<0.001
Uric acid (μmol/L)	365±99	316±93	<0.001
Homocysteine (μmol/L)	13.9±5	14.2±3.9	0.19
Echocardiography			
AVAI (cm^2/m^2)	0.43±0.1	–	–
Maximal gradient (mmHg)	73±22	–	–
Mean gradient (mmHg)	47±15	–	–
AR degree 0–1	115 (95%)	96 (95%)	1.00
LV end-diastolic diameter (mm)	49±7	50±8	0.21
LV mass index (g/m^2)	150±38	116±33	<0.001
LV ejection fraction (%)	57±12	55±11	0.35
E/Ea$_{lat}$	11.2±5	8.5±3	<0.001
Bicuspid aortic valve	9 (7%)	1 (1%)	0.02
Coronary angiography			
1 vessel	40 (33%)	28 (28%)	0.34
≥2 vessels	61 (50%)	67 (66%)	0.013
Left main coronary artery	21 (17%)	6 (6%)	0.017

Continuous data are shown as mean±standard deviation, categorical variables as number and percentage, variables with non-Gaussian distribution as median (25–75th percentile).
BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; AVAI, aortic valve area index; AR, aortic regurgitation; LV, left ventricular; Ea$_{lat}$, early lateral annulus velocity by tissue doppler imaging.

Table 2 Comparison of Calcium Metabolism Parameters

	Aortic stenosis (n=122)	Aortic sclerosis (n=101)	OR (95%CI)	p value
S-total calcium (mmol/L)	2.40±0.11	2.38±0.10	14 (0.83–242)	0.06
S-inorganic phosphate (mmol/L)	1.17±0.18	1.14±0.23	2.12 (0.53–8.58)	0.29
Calcium–phosphate product	2.85±0.50	2.74±0.58	1.53 (0.88–2.68)	0.13
Ionized calcium (mmol/L)	1.18±0.16	1.17±0.08	0.78 (0.00–42)	0.90
dU-Ca (mmol/24 h)	2.18 (1.19–3.6)	2.25 (1.3–3.26)	1.02 (0.88–1.19)	0.75
dU-P (mmol/24 h)	19.9 (12.8–24.4)	13.7 (9.2–23.2)	1.03 (1.00–1.06)	0.035
S-iPTH (pg/ml)	51.4 (39–70)	37.4 (27–50)	1.03 (1.02–1.05)	<0.001
P-vitamin D (nmol/L)	32.0 (25–40)	35.8 (27–55)	0.97 (0.95–0.99)	0.003

P values adjusted for age. Continuous data are shown as mean±standard deviation, variables with non Gaussian distribution as median (25–75th percentile).
OR, odds ratio; CI, confidence interval; dU-Ca, daily calcium urinary excretion; dU-P, daily phosphorus urinary excretion; S-iPTH, serum intact parathormone.

graphic and selected laboratory characteristics is shown in Table 1. Two or more major cardiovascular risk factors were present in 87% and 95% of patients with AS and aortic sclerosis, respectively. Hemoglobin A$_{1c}$ was above the recommended level of 4.5% in two-thirds of the diabetic patients in both groups. The creatinine clearance was lower in patients with AS (67±24 ml/min vs 75±18 ml/min, p=0.044), but this difference was no more significant after adjustment for age (odds ratio (OR) 0.99, 95% confidence interval (CI) 0.45–2.21, p=0.9).

Calcium–Phosphate Metabolism

The comparison of the calcium–phosphate metabolism parameters between patients with AS and aortic sclerosis is shown in Table 2. In the whole sample, the level of iPTH ranged from 10 to 135 pg/ml. Hyperparathyroidism (iPTH >65 pg/ml) was more frequent in patients with AS than in those with aortic sclerosis (27% vs 7%, OR 5.0, 95%CI

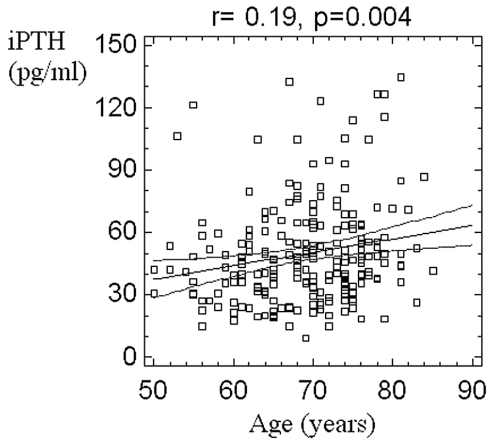

Fig 1. Association of serum intact parathyroid hormone (iPTH) level with age in 223 patients with coronary artery disease and aortic stenosis or sclerosis. Regression line, and the 95% confidence limit lines are shown.

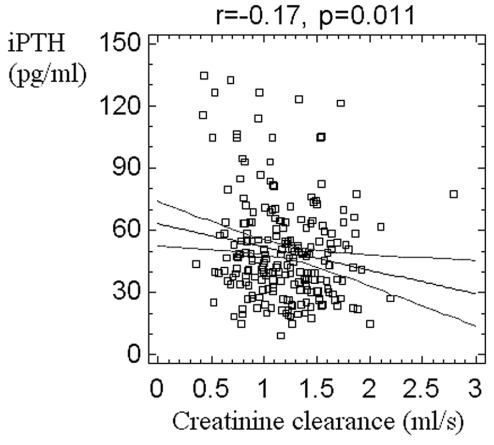

Fig 2. Association of serum intact parathyroid hormone (iPTH) level with creatinine clearance in 223 patients with coronary artery disease and aortic stenosis or sclerosis. Regression line, and the 95% confidence limit lines are shown.

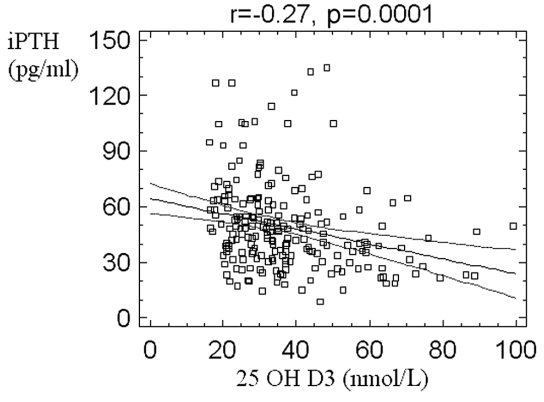

Fig 3. Association of serum intact parathyroid hormone (iPTH) with plasma vitamin D level in 223 patients with coronary artery disease and aortic stenosis or sclerosis. Regression line, and the 95% confidence limit lines are shown. 25 OH D3, 25-hydroxycholecalciferol, calcidiol.

2–12, p=0.0001). No case of primary hyperparathyroidism (elevated iPTH and ionized calcium level plus elevated 24-h urinary calcium excretion) was found. The elevated iPTH level did not correlate with the active metabolite calcitriol and ionized calcium levels (data not shown), which also suggested secondary etiology.

Vitamin D insufficiency (plasma level 25–50 nmol/L) was more frequent in patients with AS than in those with aortic sclerosis (59% vs 46%, OR 1.7, 95%CI 1.0–2.9, p=0.044); however, the frequency of overt vitamin D deffi-ciency (plasma level <25 nmol/L) was 30% in both groups.

Furthermore, in the whole study sample, we evaluated the association of the serum iPTH with the following known risk factors for secondary hyperparathyroidism;[11] the iPTH level correlated with age and negatively with vitamin D level and creatinine clearance (Figs 1–3), but not with BMI, hs-CRP, smoking or lipid levels (data not shown).

As the iPTH modestly correlated with the vitamin D level, their association with AS was evaluated separately in 2 multivariate models (Table 3). The iPTH and vitamin D levels, respectively, were independently associated with AS after adjustment for age, male gender, hs-CRP, and BMI.

Discussion

This is the first study to assess the serum iPTH and plasma vitamin D levels in nondialyzed patients with calcific AS.

We found increased iPTH with lower vitamin D level was independently associated with AS in a prospectively collected sample of patients with significant CAD, and moderate to severe AS or nonobstructive aortic valve sclerosis, with preserved renal function. Our results suggest dysregulation of calcium–phoshate metabolism, with elevated iPTH and lack of vitamin D, as a possible pathogenetic factor in AS in CAD patients.

In patients with end-stage renal disease, secondary hyperparathyroidism is common and higher iPTH has been found to be associated with more rapid AS progression[12] and cardiovascular calcification![13] In primary hyperparathyroidism, Stefenelli et al observed higher prevalence of aortic valve calcification than in controls (50% vs 12.5%);[14] the mostly mild to moderate degree of these changes may have indicated short duration of disease.

The association between serum calcium level and AS in patients with normal renal function has been assessed in 2 studies: the calcium–phosphate product correlated with AS severity![15] and calcium levels were negatively associated with valve calcification in males with severe AS in another study![16] However, it may be difficult to associate parameters that may rapidly change within hours or even minutes with processes that progress over years, and only higher phosphate urinary excretion suggested higher bone mineral turnover in our patients with AS.

The vitamin D level decreases with age, and gets into the range of insufficiency (25–50 nmol/L) in more than 50% of the elderly European population![17,18] Serum PTH has been shown to progressively rise with vitamin D levels below 27.5–37.5 nmol/L![19]

The calcidiol (25-hydroxyvitamin D, vitamin D) level is generally considered the most reliable measure of the patient's overall vitamin D status![8] Calcitriol (1, 25-hydroxyvitamin D) is produced by renal hydroxylation of calcidiol stimulated by PTH. It is the active metabolite that tightly regulates the ionized calcium level within narrow limits. The

Table 3 Association of iPTH and Vitamin D Levels With Aortic Stenosis

	OR (95%CI)	*p value*		*OR (95%CI)*	*p value*
iPTH	*1.04 (1.02–1.05)*	*<0.001*	*Vitamin D*	*0.97 (0.95–0.99)*	*0.003*
Age	*1.09 (1.04–1.15)*	*<0.001*		*1.10 (1.05–1.16)*	*<0.001*
BMI	*1.05 (0.96–1.15)*	*0.29*		*1.03 (0.94–1.17)*	*0.55*
Male sex	*1.46 (0.70–3.01)*	*0.31*		*1.22 (0.60–2.48)*	*0.57*
hs-CRP	*1.04 (0. 94–1.14)*	*0.45*		*1.02 (0.93–1.11)*	*0.74*
Creatinine clearance	*1.02 (0.38–2.74)*	*0.97*		*1.03 (0.40–2.63)*	*0.95*

Fully adjusted models for possible confounding variables.
iPTH, intact parathormone. Other abbreviations see in Tables 1,2.

stable ionized calcium and calcitriol levels are the crucial results of a homeostatic control system, and are attained at the expense of PTH increase. Therefore, secondary hyperparathyroidism caused by vitamin D insufficiency involves elevated PTH, which modestly correlates with decreased calcidiol, but not with the calcitriol level[7,8] These relationships were also found in the present study.

Vitamin D insufficiency or overt defficiency was present in the majority of these patients, and the serum iPTH correlated significantly and negatively with plasma vitamin D levels in both groups. However, it is important to note that we found an independent association of iPTH with AS against the background of advanced atherosclerosis present in all patients. Moreover, both groups did not differ in the extremely adverse, inadequately controlled cardiovascular risk profile, nor in the level of systemic inflammation assessed by the serum hs-CRP level.

Cardiovascular calcification is an active process driven by osteoblast-like cells in situ[5,20,21] and thus is conceivably linked with regulators of the processes in skeletal bone. PTH acts directly on bone osteoblasts; however, direct effect of PTH on vascular cells has not been established in an experimental model[22] Direct effect of vitamin D on vascular tissue has been observed, and a biphasic curve of a dose-related response to vitamin D is suggested by animal and human studies[23] Vitamin D excess achieved by high, toxic doses of vitamin D causes mediocalcinosis, whereas physiologic levels may exert direct antiinflammatory and calcification blocking effects,[24] which diminish again with vitamin D defficiency.[25] In the aortic valve, these effects remain as yet to be studied.

Overall, the vitamin D status in patients without severe renal or liver disease is the result of a combination of sunlight exposure, dietary vitamin D intake, and intestinal absorption.[7] We speculate that the interplay of these factors would also explain the differences in the present patients. However, detailed analysis of these factors to assess the causes of lower vitamin D levels in patients with AS deserves further extensive study.

We are aware that the cause–effect relationship could not be assessed in this cross-sectional design. As PTH levels may change throughout the day and season of the year, we collected the blood samples at the same time of the day, and the patient enrollment in both study groups was regularly dispersed over the whole 18-month period. Further, the iPTH assay is not able to discriminate the inactive PTH N-terminal fragments; however, this has not been found to cause significant error in the range of values between 10 and 99 pg/ml,[26] which was the case for the majority of the study patients.

In conclusion, we found increased serum iPTH with lower vitamin D levels was independently associated with calcific AS in patients with significant CAD without severe renal disease. Our results suggest that dysregulation of calcium metabolism may be involved in AS pathogenesis in CAD patients with preserved renal function. In patients with AS, vitamin D status should be evaluated and treatment considered. We speculate that treatment of the calcium metabolism dysregulation may have a beneficial effect by slowing or reversing AS progression, but a prospective study is needed to test this hypothesis.

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