25-Hydroxy-vitamin D level may predict presence of coronary collaterals in patients with chronic coronary total occlusion

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Abstract

Introduction: Sufficient coronary collateral circulation (CCC) protects myocardial tissue against ischemia in patients with coronary chronic total occlusion (CTO). Vitamin D is a steroid hormone which has been related to increased prevalence of hypertension, left ventricular hypertrophy, heart failure, peripheral artery disease, coronary artery disease, myocardial infarction and cardiovascular mortality.

Aim: To investigate whether there is an association between serum 25-hydroxy-vitamin D levels and development of CCC in patients with coronary CTO.

Material and methods: A total of 188 patients with CTO at coronary angiography were included in this study. Vitamin D and parathyroid hormone (PTH) levels were measured on the day of coronary angiography. Development of collateral circulation was graded according to the Rentrop classification after coronary angiography. Then, patients were divided into two groups on the basis of CCC grades: group 1 included 68 (36%) patients with poorly developed CCC, and group 2 included 120 (64%) patients with well-developed CCC.

Results: Patients with poorly developed CCC had significantly lower serum 25-hydroxy-vitamin D levels compared to those with well-developed CCC (20 ±3 vs. 30 ±6 ng/ml, p < 0.0001). Multivariate logistic regression analysis indicated serum 25-hydroxyvitamin D (25(OH)D) (OR = 1.794, 95% confidence interval (CI): 1.453–2.216; p < 0.001) as an independent predictor of poor collateral flow in patients with CTO.

Conclusions: Low vitamin D level is an independent predictor of poor CCC in patients with CTO.

Key words: chronic total occlusion, vitamin D, coronary collateral circulation.

Introduction

Coronary collaterals are anastomotic channels between portions of the same coronary artery or between different coronary arteries, and have long been considered as an alternative source of blood supply to the jeopardized ischemic myocardium. Presence of coronary collateral circulation (CCC) is of great importance particularly in patients with a chronic total occlusion (CTO, Figure 1). Previous studies assessing prevalence have differed with regard to the reference population. A previous study showed that the overall prevalence of CTO in patients with coronary artery disease (CAD) referred for elective coronary angiography is 18.4% [1, 2]. Collateral vessels develop from congenital intracoronary anastomosis following chronic ischemia or hypoxia and growth factors and inflammatory cells play a significant role in development of CCC [3, 4]. It has been shown that well-grown CCC has beneficial effects on infarct size, aneurysm formation and ventricular function.

Vitamin D is a kind of steroid hormone produced via sun exposure in the skin. Once consumed or made in the skin, vitamin D undergoes two hydroxylation steps to generate the final hormonal form. It is hydroxylated first in the liver to produce 25-hydroxyvitamin D₃ (25(OH)D₃) and then second in the kidney to create the hormonal form, 1α,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃). Vitamin D deficiency is associated with several chronic disease states including one affecting the cardiovascular system. Many studies have demonstrated that low serum 25-hy-
The serum level of vitamin D is associated with increased prevalence of hypertension, left ventricular hypertrophy, heart failure, peripheral artery disease, coronary artery disease, myocardial infarction and cardiovascular mortality [5–10]. However, there are limited data regarding the role of vitamin D level in development of CCC in patients with CTO.

**Aim**

In this study, we aimed to evaluate whether there is an association between serum vitamin D level and CCC in patients with CTO.

**Material and methods**

**Study population**

A total of 173 patients with a CTO in at least one coronary artery, who were referred to our hospital for coronary angiography between March 2013 and January 2014, were included in this observational study.

Patients with a history of acute coronary syndromes within the last 3 months, chronic inflammatory disease, coronary bypass surgery, severe cardiac valvular diseases, chronic kidney disease (creatinine > 1.4 mg/dl), chronic pulmonary disease, known malignity and those with active infection were excluded.

Risk factors for CAD including hypertension, diabetes mellitus, hyperlipidemia and smoking were recorded in all patients. Left ventricular ejection fraction (LVEF) was calculated from conventional apical 2- and 4-chamber images using biplane Simpson’s technique. Following coronary angiography, patients were divided into 2 groups based on the degree of CCC development as follows: group 1, patients with poorly developed CCC (Rentrop 0, 1); and group 2, those with well-developed CCC (Rentrop 2, 3).

**Biochemical analysis**

Blood samples were drawn to evaluate serum 25(OH)D levels. Serum 25(OH) D levels were measured with a HPLC device using the chromatographic method (Shimadzu LC 20AD/T, Kyoto, Japan). Serum creatinine, calcium, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride, total cholesterol, glucose, high-sensitivity C reactive protein (hs-CRP), and parathroid hormone (PTH) levels were also measured in the blood samples drawn.

**Coronary angiography**

Coronary angiography was performed via femoral catheterization by the Judkins method. Patients with at least a CTO in one coronary artery were included in this study. Collateral circulation was graded according to the Rentrop classification as follows: 0, no marked collateral circulation; 1) collateral circulation at lateral branches but not reaching the epicardial coronary artery; 2) partial filling of the epicardial segment through collateral channels; 3) presence of complete filling in the epicardial coronary artery [11].

**Statistical analysis**

All analyses were carried out using SPSS 15.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Continuous variables were given as mean ± standard deviation; categorical variables were defined as percentages. The variables were investigated using the Kolmogorov-Smirnov test to determine whether they are normally distributed. Independent samples t test was used to compare continuous variables between the two groups. Non-parametric values were compared with the Mann-Whitney U test. The χ² test was used to compare categorical data. Pearson and Spearman’s correlation coefficient was used to examine the association between serum 25(OH)D levels and Rentrop score. The effects of different variables on CCC were calculated using univariate analysis. The variables for which the unadjusted p was < 0.10 in logistic regression analysis were identified as potential risk markers and included in the full model. We reduced the model using backward elimination multivariate logistic regression analyses, and we eliminated potential risk markers using likelihood ratio tests. A two-tailed p value < 0.05 was considered as significant.

**Results**

A total of 188 patients (mean age 65 ±11 years, 120 men) were included in this study. Group 1 included 68 patients with poorly developed CCC (Rentrop 0, 1) and group 2 included 120 patients with well-developed CCC (Rentrop 2, 3). Comparison of baseline characteristics of patients with poorly developed CCC and well-developed CCC are shown in Table I. Mean age, sex, body...
mass index, smoking history, ejection fraction, presence of diabetes mellitus and hypertension were similar in the two groups. The levels of fasting glucose, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride, creatinine, calcium and white blood cell count were also similar between the two groups (Table II). However, patients with poorly developed CCC had significantly lower serum 25 (OH)D levels compared to those with well-developed CCC (20 ±3 vs. 30 ±6 ng/ml, p < 0.0001, Figure 2).

Correlation analysis showed a significant correlation between vitamin D levels and the Rentrop score.

### Table I. Demographic characteristics of the study population

| Parameter               | Poorly developed CCC (n = 68) | Well-developed CCC (n = 120) | Value of p |
|-------------------------|------------------------------|------------------------------|------------|
| Age [years]             | 65 ±12                       | 65 ±11                       | 0.890      |
| Gender, male            | 41 (60%)                     | 79 (66%)                     | 0.192      |
| Diabetes, n             | 26 (38%)                     | 42 (35%)                     | 0.757      |
| Hypertension, n         | 41 (60%)                     | 76 (63%)                     | 0.686      |
| Smoking, n              | 30 (44%)                     | 61 (51%)                     | 0.178      |
| BMI [kg/m²]             | 28 ±5                        | 27 ±5                        | 0.136      |
| Ejection fraction (%)   | 41 ±8                        | 43 ±9                        | 0.473      |
| Ocluded artery, n (%)   |                              |                              |            |
| LAD                     | 11                           | 41                           | 0.011      |
| LCX                     | 25                           | 31                           | 0.073      |
| RCA                     | 36                           | 55                           | 0.340      |
| Rentrop score, n:       |                              |                              |            |
| 0                       | 9                            |                              |            |
| 1                       |                              | 59                           |            |
| 2                       | 68                           |                              |            |
| 3                       | 52                           |                              |            |

Notes: BMI – Body mass index, CCC – coronary collateral circulation, LAD – left anterior descending artery, LCX – left circumflex artery.

### Table II. Comparison of laboratory features of patients with poorly developed and well-developed coronary collateral circulation

| Parameter                  | Poorly developed CCC (n = 68) | Well-developed CCC (n = 120) | Value of p |
|----------------------------|------------------------------|------------------------------|------------|
| Fasting glucose [mg/dl]    | 128 ±36                      | 118 ±30                      | 0.145      |
| Creatinine [mg/dl]         | 1.1 ±0.9                     | 1 ±0.9                       | 0.693      |
| LDL cholesterol [mg/dl]    | 123 ±49                      | 128 ±46                      | 0.484      |
| HDL cholesterol [mg/dl]    | 40 ±8                        | 41 ±8                        | 0.859      |
| Triglyceride [mg/dl]       | 201 ±98                      | 196 ±121                     | 0.826      |
| 25(OH)D₃ [ng/ml]           | 20 ±3                        | 30 ±6                        | < 0.001    |
| Ca                         | 8.9 ±0.5                     | 9.2 ±0.5                     | 0.393      |
| Leucocyte count [× 10⁶/µl] | 8.8 ±0.3                     | 9.0 ±0.3                     | 0.684      |
| Hemoglobin [g/dl]          | 14.1 ±3                      | 14.6 ±3                      | 0.328      |
| Platelet count [10³/mm³]   | 244 ±75                      | 252 ±76                      | 0.519      |
| Hs-CRP [mg/l]              | 4.3 ±0.3                     | 3.4 ±0.4                     | 0.035      |

Notes: Ca – Calcium, HDL – high density lipoprotein cholesterol, LDL – low density lipoprotein cholesterol, Hs-CRP – high sensitivity C-reactive protein.
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\[ r = 0.714 \text{ and } p < 0.001, \text{ Figure 3}. \] Serum 25(OH)D (OR = 1.794, 95% confidence interval: 1.453–2.161; \( p < 0.001 \)) and hs-CRP levels (OR = 0.910, 95% CI: 0.830–0.996; \( p = 0.041 \)) were independent predictors of poor collateral flow in multivariate logistic regression analysis (Table III).

**Discussion**

The present study shows that patients with CTO and poorly developed CCC have lower serum 25(OH)D levels compared to patients with well-developed CCC. This study also shows that lower serum 25(OH)D levels may independently predict poorly developed CCC in patients with coronary CTO.

Development of CCC occurs through either angiogenesis of novel capillaries arising from available blood vessels or proliferation and maturation (arteriogenesis) of congenital intracoronary anastomoses following chronic ischemia or hypoxia. The main factor affecting development of collateral coronary vessels is the pressure gradient between segments localized at proximal and distal ends of the occlusion [12]. Growth factors released from endothelial cells and inflammatory cells recruited to ischemic tissue such as platelets and monocytes play a sig-

**Figure 2.** Comparison of 25(OH)D levels in patients with poorly developed coronary collaterals and well developed coronary collaterals

**Figure 3.** Relation between Rentrop Score and serum 25(OH)D levels in patients with a chronic coronary total occlusion

**Table III.** Predictors of well developed coronary collaterals in univariate and multivariate logistic regression analyses

| Variables           | Unadjusted OR | 95% CI      | Value of \( p \) | Adjusted OR | 95% CI      | Value of \( p \) |
|---------------------|---------------|-------------|------------------|-------------|-------------|------------------|
| Male gender         | 1.335         | 0.689–2.587 | 0.392            |             |             |                  |
| Age                 | 1.002         | 0.975–1.029 | 0.889            |             |             |                  |
| Diabetes            | 2.197         | 1.161–4.158 | 0.016            | 1.590       | 0.553–4.575 | 0.389            |
| Hypertension        | 0.903         | 0.469–1.738 | 0.760            |             |             |                  |
| Smoking             | 0.619         | 0.334–1.150 | 0.129            |             |             |                  |
| SBP                 | 1.000         | 0.987–1.013 | 0.975            |             |             |                  |
| BMI                 | 0.953         | 0.894–1.016 | 0.139            |             |             |                  |
| LAD involvement     | 0.362         | 0.162–0.810 | 0.013            | 0.553       | 0.150–2.034 | 0.373            |
| LCx involvement     | 1.830         | 0.942–3.555 | 0.074            | 2.086       | 0.608–7.154 | 0.242            |
| Vit. D level        | 1.817         | 1.491–2.215 | < 0.001          | 1.794       | 1.453–2.216 | < 0.001          |
| Creatinine          | 0.936         | 0.674–1.300 | 0.693            |             |             |                  |
| Hs-CRP              | 0.914         | 0.867–0.963 | 0.001            | 0.910       | 0.830–0.996 | 0.041            |
| Leucocyte count     | 1.022         | 0.922–1.132 | 0.682            |             |             |                  |

**BMI** – Body mass index, **LAD** – left anterior descending artery, **LCx** – left circumflex artery, **SBP** – systolic blood pressure, **Hs-CRP** – high sensitivity C-reactive protein.
significant role both atherosclerosis and angiogenesis. Although the precise mechanism underlying the association between vitamin D deficiency and coronary collateral development is not fully understood, several potential mechanisms have been proposed.

Vitamin D is involved in the pathogenesis of vascular diseases. In angiogenesis, vitamin D plays a key role in the development and maturation of the collateral circulation, which is crucial for restoring coronary blood flow in patients with chronic ischemia.

Collateral development is a complex process involving the growth and stabilization of new blood vessels. Several factors, including genetic predispositions, lifestyle, and environmental factors, influence the development of collateral vessels.

One potential mechanism to explain the association between vitamin D levels and collateral development could be mediated through the modulation of the interplay between apoptosis and autophagy. This effect is achieved through inhibition of nitric oxide production and upregulation of superoxide anion generation.

In a recent study by Amer et al., it was observed that serum 25(OH)D levels are related to coronary collateral development. A lower vitamin D level is associated with a higher risk of poor collateral development, which may result in impaired collateral development.

In addition, vitamin D deficiency is associated with an increase in the incidence and severity of immune-inflammatory disorders. High-sensitivity CRP, an indicator of inflammatory activity, was observed to be associated with lower vitamin D levels and better collateral development.

Furthermore, it is well known that an elevated level of CRP is associated with increased cardiovascular risk. Moreover, vitamin D deficiency is associated with increased CRP levels, which may result in impaired collateral development and worse outcomes in patients with cardiovascular disease.

A recent systematic review and meta-analysis conducted long-term cross-sectional prospective studies. It was found that vitamin D deficiency predicts coronary collateral development by increasing activity of endothelial cells and smooth muscle cells. Moreover, low vitamin D levels were associated with impaired development of endothelial and smooth muscle cells.

Collateral formation was assessed by coronary angiography in patients with acute myocardial infarction. Nitric oxide production, an indicator of endothelial function, plays a major role in the collateral function of the coronary circulation.

In our study, we established the association of serum 25(OH)D levels and vitamin D levels with coronary collateral development. Vitamin D levels were found to be inversely related to coronary collateral development. It was observed that the highest rate of mortality occurred in patients with serum 25(OH)D levels in the lowest quartile. However, in that study, only 48% of subjects and in the control group 52% of subjects had a totally occluded coronary artery. Moreover, in a European study that enrolled patients who underwent coronary angiography, it was found that 25(OH)D levels were associated with impaired development of macrovascular smooth muscle cell proliferation and expression of endothelial adhesion molecules. In addition, it was noted that visible coronary collateral vessels may even be associated with poor outcomes in patients with ACS.

Recently, Molinari et al. reported that vitamin D deficiency is associated with poor collateral development and increased mortality. They described beneficial effects of vitamin D on vascular remodeling, which may result in impaired collateral development.

Moreover, it is also known that vitamin D has anti-inflammatory properties, which may contribute to improved collateral development. It was also observed that vitamin D can prevent vascular calcification by inhibiting the expression of endothelial adhesion molecules and suppressing inflammation that triggers the migration of macrophages to foam cells. This effect is achieved through inhibition of nitric oxide production and upregulation of superoxide anion generation.

Another potential mechanism to explain the association between vitamin D levels and coronary collateral development could be mediated through the modulation of the interplay between apoptosis and autophagy. This effect is achieved through inhibition of nitric oxide production and upregulation of superoxide anion generation.
Conclusions

The present study shows that patients with a CTO and poor CCC have lower serum 25(OH)D levels compared to those with well-developed CCC. Our study also shows that, in patients with a CTO, low serum 25(OH)D level is an independent predictor of poor CCC. According to our results, we speculate that blunted collaterals might be a cause of poor cardiovascular outcomes in patients with CAD and vitamin D deficiency.

Conflict of interest

The authors declare no conflict of interest.

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