

Association between Vitamin D Status and Risk of Peripheral Arterial Disease: The Dong-gu Study

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Low levels of vitamin D have been associated with increased cardiovascular disease risk. However, few studies have evaluated the association between vitamin D status and peripheral arterial disease (PAD). We therefore aimed to investigate whether low 25-hydroxyvitamin D (25(OH)D) levels were associated with increased risk of PAD in the Korean population. This cross-sectional study was conducted among 8,960 subjects aged 50 years or older without known myocardial infarction or stroke. PAD was defined by an ankle brachial blood pressure index < 0.9. Multivariate logistic regression was used to evaluate the association between serum 25(OH)D levels and risk of PAD. Of the 8,960 subjects, 3.0% had PAD and the age and sex adjusted prevalence of PAD decreased with the increasing 25(OH)D quartile. After adjusting for potential confounders and parathyroid hormones, serum 25(OH)D levels were associated with a significantly decreased risk of PAD (OR for one SD increase, 0.98; 95% CI, 0.95-1.0, P for trend=0.040). Compared with the first 25(OH)D quartile, the odds of PAD were 0.86 (95% CI, 0.62-1.21), 0.67 (95% CI, 0.46-0.97), and 0.71 (95% CI, 0.49-1.04) for the second, third, and fourth quartiles, respectively. In this cross-sectional study, we found that low serum 25(OH)D levels were associated with an increased risk of PAD, independent of traditional cardiovascular risk factors and parathyroid hormone. Our findings suggest that low vitamin D levels may contribute to PAD in the Korean population.

Key Words: 25-Hydroxyvitamin D; Peripheral Arterial Disease; Atherosclerosis

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INTRODUCTION

Vitamin D is a steroid hormone. It is mainly subdivided into vitamin D₂ (ergocalciferol) and vitamin D₃ (cholecalciferol).¹ It can be produced in the skin with adequate ultraviolet exposure or ingested through diet and supplementation.² However, vitamin D deficiency has become a universal public health issue. Vitamin D deficiency is very common, and between 30 and 50% of the world's population suffers from it.³ Similarly, the Korea National Health and Nutrition Examination Survey data show that 50-60% of men and 60-70% of women experience severe vitamin D

deficiency.^{4,5}

Vitamin D deficiency is related to hypertension, diabetes mellitus, dyslipidemia, metabolic syndrome, stroke, myocardial infarction, and atherosclerosis.⁶⁻¹⁰ Peripheral arterial disease (PAD) is a chronic occlusive arterial disease caused by atherosclerosis. The primary risk factors for PAD are increasing age, smoking, hypertension, diabetes mellitus, and dyslipidemia.¹¹ It has not yet been determined, however, whether vitamin D deficiency or insufficiency is a risk factor for PAD. Some prior studies have reported that low serum 25(OH)D concentrations are associated with a higher prevalence of PAD,¹²⁻¹⁶ whereas others

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have not.^{3,17,18}

Although several case-control studies have evaluated the association between vitamin D status and PAD¹⁹ in CVD patients, there have been few population-based studies, and those were performed in Western populations.²⁰ Although ethnic differences could influence the relationship between serum 25(OH)D concentrations and the risk of PAD, to date, no studies have been performed in Asians. We therefore aimed to investigate whether low 25-hydroxyvitamin D (25(OH)D) levels were associated with increased risk of PAD in a Korean population.

MATERIALS AND METHODS

1. Subjects

The Dong-gu Study is an ongoing prospective study designed to investigate the prevalence and incidence of the risk factors for chronic diseases targeting the elderly population who live in Dong-gu.²¹ In total, 9,260 subjects aged 50 years and older were enrolled (3,711 males and 5,549 females) from 2007 to 2010. This cross-sectional study was conducted among 8,960 participants and excluded due to missing information on: vitamin D measurement (n=58), peripheral arterial disease (n=76), medication intake (n=166). All of the participants provided signed consent forms, and the study was performed according to the guidelines of the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board (IRB) of Chonnam National University Hospital [No I-2008-05-056].

2. Anthropometric data and lifestyle factors

Demographic characteristics, medical history, and lifestyle were assessed by standardized questionnaires. Smoking status was divided into smokers (including ex-smokers) and non-smokers. Alcohol consumption was classified into current drinkers and non-drinkers. Education was categorized into middle school completion or less and high school completion or more. Exercise was classified as regular exercise or no regular exercise based on the weekly frequencies of recreational activities. Self-rated health status was classified as good (very good, good) or poor (average, poor, very poor). We examined whether participants had taken medications during the previous 2 weeks for hypertension, diabetes, or dyslipidemia. Waist circumference was measured at the midpoint between the ribs and pelvis, and body mass index (BMI) value was computed using height (m) and weight (kg) with the formula $BMI = \text{kg}/\text{m}^2$. Blood pressure was measured using a mercury sphygmomanometer (Baumanometer; WA Baum Co, Inc., Copiague, NY, USA) placed around the right upper arm after 5 minutes of quiet sitting. Three consecutive readings of systolic and diastolic blood pressure were taken once per minute, and the average value was used for the analysis.

3. Laboratory measurements

Blood samples were drawn in the morning after a 12-h overnight fast. Levels of total cholesterol, triglycerides,

high-density lipoprotein (HDL) cholesterol, and blood glucose were measured via enzymatic methods using an automatic analyzer (Model 7600; Hitachi, Ltd., Tokyo, Japan). High-sensitive C-reactive protein (CRP) levels were locally determined using a BN II nephelometer (Dade Behring, Marburg, Germany). Hemoglobin A1c (HbA1c) levels were measured using high-performance liquid chromatography (HPLC) using the Bio-Rad VARIANT II system (Bio-Rad, Hercules, CA, USA). Levels of 25(OH)D and parathyroid hormone (PTH) were determined by chemiluminescent microparticle immunoassay (CMIA) (ARCHTECT i2000, Abbott, Chicago, IL, USA).

TABLE 1. Characteristics of participants according to peripheral arterial disease

Variable	No PAD (N=8,688)	PAD (N=272)	p-value
Age, years	65.0±8.1	70.1±8.5	< 0.001
Male, %	3,438 (39.6)	142 (52.2)	< 0.001
Less than middle school education, %	5,427 (62.5)	185 (68.0)	0.035
Body mass index, kg/m ²	24.3±2.9	24.4±3.5	0.928
Waist circumference, cm	88.0±8.5	89.0±9.3	0.046
Ever smoking, %	2,711 (31.2)	131 (48.2)	< 0.001
Current alcohol consumption, %	4,022 (46.3)	110 (40.4)	0.238
Regular Exercise, %	2,817 (32.4)	69 (25.4)	< 0.001
Good self-rated health, %	1,852 (21.3)	48 (17.6)	0.081
Hypertension medication use, %	3,040 (35.0)	150 (55.1)	< 0.001
Diabetes medication use, %	1,087 (12.5)	66 (24.3)	< 0.001
Dyslipidemia medication use, %	739 (8.5)	25 (9.2)	0.377
Systolic blood pressure, mmHg	123.2±16.8	128.3±19.8	< 0.001
Diastolic blood pressure, mmHg	74.3±10.2	74.5±10.7	0.730
History of myocardial infarction, %	109 (1.3)	7 (2.6)	< 0.001
History of Stroke, %	343 (4.0)	29 (10.7)	< 0.001
C-Reactive Protein, mg/dL	0.2±0.4	0.3±0.6	< 0.001
HbA1c, %	5.8±0.9	6.1±1.1	< 0.001
Triglyceride, mg/dL	142.9±101.4	142.6±83.1	0.967
Total cholesterol, mg/dL	201.3±40.0	200.8±41.1	0.847
HDL cholesterol, mg/dL	51.6±12.0	50.3±11.4	0.065
Glucose, mg/dL	109.4±24.9	115.1±29.9	< 0.001
Parathyroid hormone, pg/mL	43.1±21.4	47.7±27.1	< 0.001
25-hydroxylated vitamin D, ng/mL	16.7±5.8	16.0±5.6	0.040

Data are expressed as mean±standard deviation or number and percentage in parentheses.

HDL: High density lipoprotein cholesterol, 25(OH)D: 25-hydroxylated vitamin D.

4. Definition of peripheral arterial disease

PAD was defined as a ankle brachial blood pressure <0.9. ABI was provided automatically using an oscillometric method²² with the VP-1000 system (Colin Co., Komaki, Japan). After 5 minutes of relaxation in the supine position, BP cuffs were placed around the subjects' arms and ankles. ABI was measured by the ratio of the ankle systolic blood pressure to the arm systolic blood pressure. If the ABI value was lower than 0.9, the patient was defined as having PAD.

5. Statistical analysis

Baseline characteristics were analyzed for PAD values and compared using the t-test and chi-squared test, as appropriate. Multivariate, logistic regression was used to evaluate the relationship between serum 25(OH)D levels and the risk of PAD, adjusting for age, sex, smoking, alcohol intake, medication use (hypertension, diabetes, and dyslipidemia), systolic and diastolic blood pressure, total cholesterol, HDL cholesterol, triglyceride, fasting glucose, and hs-CRP. We further adjusted for PTH to evaluate the mediating effect of PTH. Odds ratios (ORs) are expressed per standard deviation (SD) increase in 25(OH)D. All statistical tests were two-tailed, and the statistical significance level was set at $p < 0.05$. Statistical analyses were performed using SPSS version 22.0 (SPSS, Inc., IBM, Chicago, IL, USA).

RESULTS

Among the 8,960 subjects, 272 (3.0%) had PAD. The mean ages of those with and without PAD were 70.1 ± 8.5 and 65.0 ± 8.1 years, respectively ($p < 0.001$). Participants with PAD were more likely to be male, to have had smoked, to have increased systolic blood pressure, HbA1c, CRP, glucose, parathyroid hormone, to use anti-hypertensive medication and anti-diabetic medication, and to have a history of myocardial infarction and stroke, and they were less likely to perform regular exercise (Table 1).

The prevalence of PAD according to the 25(OH)D quartile is presented in Fig. 1. The age and sex adjusted prevalence of PAD decreased with increasing 25(OH)D quartile.

The prevalence of PAD according to the 25(OH)D quartile was 4.0%, 3.4%, 2.5%, and 2.4% for the first through fourth quartiles, respectively.

The association between serum 25(OH)D and the prevalence of PAD is presented in Table 2. When adjusted for age and sex, increased serum 25(OH)D levels were significantly associated with decreased risk of PAD (OR for one SD increase, 0.96; 95% CI, 0.94-0.99). Further adjustments for potential confounders attenuated this association (OR for one SD increase, 0.97; 95% CI, 0.95-1.0). When we further adjusted for parathyroid hormone to evaluate its mediating effect, the association was slightly attenuated (OR for one SD increase, 0.98; 95% CI, 0.95-1.0). Compared with the first quartile of 25(OH)D, the odds of PAD were 0.86 (95% CI, 0.62-1.21), 0.67 (95% CI, 0.46-0.97), and 0.71 (95% CI, 0.49-1.04) for the second, third, and fourth quartiles, respectively.

DISCUSSION

We found a significant, inverse relationship between serum 25(OH)D status and the risk of PAD in these Korean

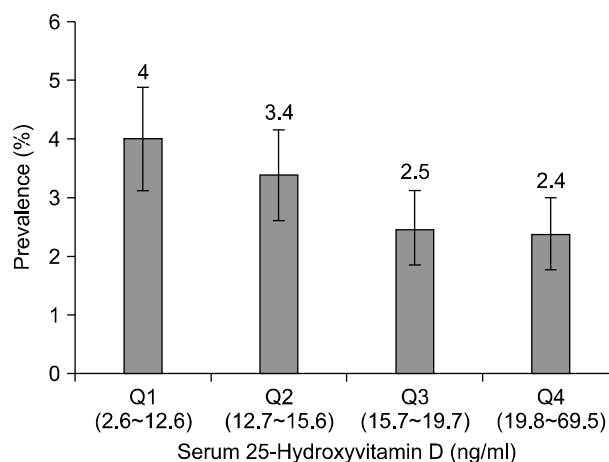


FIG. 1. Age and sex adjusted prevalence of peripheral arterial disease according to the quartile of serum 25-hydroxyvitamin D.

TABLE 2. Association between serum 25-hydroxyvitamin D (ng/mL) and peripheral arterial disease

	Affected/unaffected	Model 1	Model 2	Model 3
25(OH)D, per 1 ng/ml increment		0.96 (0.94-0.99)	0.97 (0.95-1.0)	0.98 (0.95-1.0)
1 st quartile (2.6-12.5)	84/2,149	1.00 (reference)	1.00 (reference)	1.00 (reference)
2 nd quartile (12.6-15.6)	70/2,155	0.83 (0.60-1.16)	0.84 (0.60-1.18)	0.86 (0.62-1.21)
3 rd quartile (15.7-19.7)	56/2,196	0.59 (0.41-0.85)	0.64 (0.44-0.92)	0.67 (0.46-0.97)
4 th quartile (19.8-69.5)	62/2,188	0.59 (0.41-0.84)	0.67 (0.46-0.97)	0.71 (0.49-1.04)
P for trend		0.001	0.014	0.040

Data are odds ratio (95% confidence intervals).

Model 1 was adjusted for age and sex. Model 2 was further adjusted for education, body mass index, waist circumference, smoking, alcohol, exercise, self-rated health, hypertension medication, diabetes mellitus medication, dyslipidemia medication, systolic blood pressure, HbA1c, cholesterol, high density lipoprotein cholesterol, glucose, log C-Reactive Protein, log triglyceride. Model 3 was further adjusted for log parathyroid hormone.

subjects aged 50 and older.

Three studies^{13,15,18} have examined the relationship between vitamin D and PAD in the general population. Of those, two^{13,15} were conducted using the US National Health and Nutrition Examination Survey (NHANES) 2001-2004 data. Melamed et al.¹³ reported that the prevalence rate ratio for the lowest quartile was 1.8 (95% CI, 1.19-2.74) compared with the highest quartile, which is in accordance with the present study. Reis et al.¹⁵ investigated whether there were racial differences in the association between vitamin D levels and the prevalence of PAD. They found that the prevalence of PAD was significantly lower in white than in black adults (5.3% and 8.5%, respectively); after adjusting for racial differences in 25(OH)D, the OR for PAD decreased by 31%, suggesting that racial differences in vitamin D status may explain racial difference in PAD prevalence. However, a prospective study performed in an older population of 1,568 Italian community-dwelling adults \geq 65 years old with a mean follow-up duration of 4.4 years found no association between baseline serum 25(OH)D and an incident of PAD.¹⁸ This study had a small sample size and relatively short follow-up duration of 4.4 years. In addition, half of the study participants had sufficient levels of 25(OH)D (\geq 30 ng/mL), whereas only 7% had deficient levels of 25(OH)D ($<$ 10 ng/mL), which may have hindered the evaluation of the association between vitamin D status and PAD incidence.

The mechanism underlying the association between low serum 25(OH)D and risk of PAD can be explained as follows. Increased blood levels of 25(OH)D have been associated with decreased blood pressure, which could influence the risk of PAD. In the Health Professionals' Follow-up Study and the Nurses' Health Study, subjects whose 25(OH)D concentrations were less than 15 ng/mL had an 8.1 times and 2.7 times increased risk of hypertension, respectively.²³ The mechanism linking vitamin D with hypertension may be explained by its role as a negative regulator of the renin-angiotensin system (RAS) and its influence on vascular endothelial function or vascular smooth muscle intracellular concentrations of calcium.²⁴ In addition, vitamin D inhibits the progression of inflammation and atherosclerosis by adjusting T helper cells, which in turn inhibit the development and progression of coronary artery disease and PAD.²⁵

Although the present study had the advantage of being a large population-based study, it has several limitations. First, because the design was cross sectional, the established associations do not necessarily indicate a causal relationship. Second, levels of sunlight exposure and intakes for vitamin D were not measured, and both likely had effects on serum 25(OH)D levels. Third, a low ABI ($<$ 0.9) which was used in the present study may underestimate the prevalence of PAD.²⁶ However, the ABI is a reliable screening test for PAD and a low ABI (\leq 0.9) independently predicts future cardiovascular events.²⁷

In conclusion, low serum 25(OH)D levels were associated with the risk of PAD, independent of the traditional

cardiovascular risk factors and parathyroid hormone. Our findings suggest that low vitamin D levels contribute to PAD in the Korean population.

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CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. Chua GT, Chan YC, Cheng SW. Vitamin D status and peripheral arterial disease: evidence so far. *Vasc Health Risk Manag* 2011;7:671-5.
2. Kim J. Association between serum vitamin D, parathyroid hormone and metabolic syndrome in middle-aged and older Korean adults. *Eur J Clin Nutr* 2015;69:425-30.
3. Liew JY, Sasha SR, Ngu PJ, Warren JL, Wark J, Dart AM, et al. Circulating vitamin D levels are associated with the presence and severity of coronary artery disease but not peripheral arterial disease in patients undergoing coronary angiography. *Nutr Metab Cardiovasc Dis* 2015;25:274-9.
4. Joo NS, Dawson-Hughes B, Yeum KJ. 25-Hydroxyvitamin D, calcium intake, and bone mineral content in adolescents and young adults: analysis of the fourth and fifth Korea National Health and Nutrition Examination Survey (KNHANES IV-2, 3, 2008-2009 and V-1, 2010). *J Clin Endocrinol Metab* 2013;98:3627-36.
5. Joo NS, Dawson-Hughes B, Kim YS, Oh K, Yeum KJ. Impact of calcium and vitamin D insufficiencies on serum parathyroid hormone and bone mineral density: analysis of the fourth and fifth Korea National Health and Nutrition Examination Survey (KNHANES IV-3, 2009 and KNHANES V-1, 2010). *J Bone Miner Res* 2013;28:764-70.
6. May HT, Bair TL, Lappé DL, Anderson JL, Horne BD, Carlquist JF, et al. Association of vitamin D levels with incident depression among a general cardiovascular population. *Am Heart J* 2010;159:1037-43.
7. Barnard K, Colón-Emeric C. Extraskeletal effects of vitamin D in older adults: cardiovascular disease, mortality, mood, and cognition. *Am J Geriatr Pharmacother* 2010;8:4-33.
8. Makariou S, Liberopoulos EN, Elisaf M, Challa A. Novel roles of vitamin D in disease: what is new in 2011? *Eur J Intern Med* 2011;22:355-62.
9. Rosen CJ. Clinical practice. Vitamin D insufficiency. *N Engl J Med* 2011;364:248-54.
10. Reis JP, von Mühlen D, Michos ED, Miller ER 3rd, Appel LJ, Araneta MR, et al. Serum vitamin D, parathyroid hormone levels, and carotid atherosclerosis. *Atherosclerosis* 2009;207:585-90.
11. Aronow WS. Peripheral arterial disease in the elderly. *Clin Interv Aging* 2007;2:645-54.
12. Kim DH, Sabour S, Sagar UN, Adams S, Whellan DJ. Prevalence of hypovitaminosis D in cardiovascular diseases (from the

- National Health and Nutrition Examination Survey 2001 to 2004). *Am J Cardiol* 2008;102:1540-4.
13. Melamed ML, Muntner P, Michos ED, Uribarri J, Weber C, Sharma J, et al. Serum 25-hydroxyvitamin D levels and the prevalence of peripheral arterial disease: results from NHANES 2001 to 2004. *Arterioscler Thromb Vasc Biol* 2008;28:1179-85.
 14. van de Luijngaarden KM, Voûte MT, Hoeks SE, Bakker EJ, Chonchol M, Stolker RJ, et al. Vitamin D deficiency may be an independent risk factor for arterial disease. *Eur J Vasc Endovasc Surg* 2012;44:301-6.
 15. Reis JP, Michos ED, von Mühlen D, Miller ER 3rd. Differences in vitamin D status as a possible contributor to the racial disparity in peripheral arterial disease. *Am J Clin Nutr* 2008;88:1469-77.
 16. Amer M, Narotsky DL, Qayyum R. 25-Hydroxyvitamin D and ankle-brachial blood pressure index in adults without peripheral artery disease. *Clin Transl Sci* 2014;7:391-5.
 17. McDermott MM, Liu K, Ferrucci L, Tian L, Guralnik J, Kopp P, et al. Vitamin D status, functional decline, and mortality in peripheral artery disease. *Vasc Med* 2014;19:18-26.
 18. Veronese N, De Rui M, Bolzetta F, Toffanello ED, Coin A, Zambon S, et al. Serum 25-hydroxyvitamin D and the incidence of peripheral artery disease in the elderly: the Pro.V.A study. *J Atheroscler Thromb* 2015;22:726-34.
 19. Nsengiyumva V, Fernando ME, Moxon JV, Krishna SM, Pinchbeck J, Omer SM, et al. The association of circulating 25-hydroxyvitamin D concentration with peripheral arterial disease: a meta-analysis of observational studies. *Atherosclerosis* 2015;243:645-51.
 20. Choi EY. 25(OH)D status and demographic and lifestyle determinants of 25(OH)D among Korean adults. *Asia Pac J Clin Nutr* 2012;21:526-35.
 21. Kweon SS, Shin MH, Jeong SK, Nam HS, Lee YH, Park KS, et al. Cohort Profile: The Namwon Study and the Dong-gu Study. *Int J Epidemiol* 2014;43:558-67.
 22. Tomiyama H, Yamashina A, Arai T, Hirose K, Koji Y, Chikamori T, et al. Influences of age and gender on results of noninvasive brachial-ankle pulse wave velocity measurement—a survey of 12517 subjects. *Atherosclerosis* 2003;166:303-9.
 23. Forman JP, Giovannucci E, Holmes MD, Bischoff-Ferrari HA, Tworoger SS, Willett WC, et al. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. *Hypertension* 2007;49:1063-9.
 24. Vaidya A, Williams JS. The relationship between vitamin D and the renin-angiotensin system in the pathophysiology of hypertension, kidney disease, and diabetes. *Metabolism* 2012;61:450-8.
 25. Zagura M, Serg M, Kampus P, Zilmer M, Eha J, Unt E, et al. Aortic stiffness and vitamin D are independent markers of aortic calcification in patients with peripheral arterial disease and in healthy subjects. *Eur J Vasc Endovasc Surg* 2011;42:689-95.
 26. Wikström J, Hansen T, Johansson L, Lind L, Ahlström H. Ankle brachial index < 0.9 underestimates the prevalence of peripheral artery occlusive disease assessed with whole-body magnetic resonance angiography in the elderly. *Acta Radiol* 2008;49:143-9.
 27. Moyer VA; U.S. Preventive Services Task Force. Screening for peripheral artery disease and cardiovascular disease risk assessment with the ankle-brachial index in adults: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med* 2013;159:342-8.