Original Article

Serum Osteoprotegerin is Associated with Arterial Stiffness Assessed According to the Cardio-ankle Vascular Index in Hypertensive Patients

Chung-Jen Lee¹, Ji-Hung Wang^{2,3}, Mei-Ling Chen², Chiu-Fen Yang², Yu-Chih Chen² and Bang-Gee Hsu^{3,4}

¹Department of Nursing, Tzu Chi College of Technology, Hualien, Taiwan

²Division of Cardiology, Buddhist Tzu Chi General Hospital, Hualien, Taiwan

³School of Medicine, Tzu Chi University, Hualien, Taiwan

⁴Division of Nephrology, Buddhist Tzu Chi General Hospital, Hualien, Taiwan

Aim: Arterial stiffness is recognized to be an independent risk factor for cardiovascular morbidity and mortality. Recent studies have found that osteoprotegerin (OPG) is associated with increased pulse wave velocity and may reflect endothelial dysfunction. The aim of this study was to evaluate the relationship between the serum OPG level and arterial stiffness in hypertensive patients using the cardio-ankle vascular index (CAVI).

Methods: Fasting blood samples were obtained from 115 hypertensive patients and 52 healthy participants. The CAVI value was derived using the waveform device (CAVI-VaSera VS-1000). The serum OPG levels were measured using a commercially available enzyme-linked immunosorbent assay. A CAVI value of ≥ 9 defined the high arterial stiffness group.

Results: Sixty-five hypertensive patients (56.5%) were included in the high arterial stiffness group. Diabetes ($p=0.032$), smoking ($p=0.044$), age ($p<0.001$), systolic blood pressure ($p=0.001$), diastolic blood pressure ($p=0.024$), pulse pressure ($p=0.046$) and the creatinine ($p=0.013$) and serum OPG ($p<0.001$) levels were higher in the high arterial stiffness group than in the low arterial stiffness group, while the glomerular filtration rate ($p=0.003$) was lower in the high arterial stiffness group than in the low arterial stiffness group among the hypertensive patients. The results of the Spearman's rank correlation coefficient test also indicated a strong positive correlation between the OPG and CAVI values ($r=0.484$, $p<0.001$) in the hypertensive patients. In addition, a multivariate logistic regression analysis showed that age (odds ratio: 1.162, 95% confidence interval (CI): 1.070-1.263, $p<0.001$), diastolic blood pressure (odds ratio: 1.109, 95% CI: 1.033-1.190, $p=0.004$), and serum OPG level (odds ratio: 1.275, 95% CI: 1.030-1.580, $p=0.026$) were independent predictors of arterial stiffness in hypertensive patients.

Conclusions: The serum OPG level is positively associated with arterial stiffness in hypertensive patients.

See editorial vol. 22: 233-234

J Atheroscler Thromb, 2015; 22: 304-312.

Key words: Osteoprotegerin, Arterial stiffness, Cardio-ankle vascular index, Hypertension

Introduction

Osteoprotegerin (OPG) is a vascular calcification inhibitor whose level may reflect endothelial dysfunction¹⁾. The vascular role of OPG is multifaceted and depends on the interplay with its ligands, receptor activator of NF- κ B ligand (RANKL) and tumor necro-

Address for correspondence: Bang-Gee Hsu, Division of Nephrology, Buddhist Tzu Chi General Hospital, No. 707, Section 3, Chung-Yang Road, Hualien, Taiwan
E-mail: gee.lily@msa.hinet.net

Received: May 6, 2014

Accepted for publication: September 15, 2014

sis factor (TNF)-related apoptosis-inducing ligand (TRAIL), as well as bidirectional modulation involving osteogenic, inflammatory and apoptotic responses². Clinical studies suggest that the serum OPG level increases in association with vascular calcification, coronary artery disease, stroke and future cardiovascular events².

Arterial stiffness is recognized to be an independent risk factor for cardiovascular morbidity and mortality³. The pulse wave velocity (PWV) is currently the most popular index and is known to be a predictor of cardiovascular events. However, this parameter depends on the patient's blood pressure at the time of measurement⁴. The cardio-ankle vascular index (CAVI) is a new index of the overall stiffness of the arteries from the origin of the aorta to the ankle and is theoretically independent of changes in blood pressure^{4, 5}. Clinically, CAVI values are widely applied to assess arterial stiffness in subjects with known cardiovascular disease⁴. One study noted that the OPG/fetuin-A ratio is independently associated with the CAVI values in hemodialysis patients⁶. Hypertension is a significant public health concern of worldwide distribution and the most common risk factor for cardiovascular disease and mortality⁷. Arterial stiffness is an established cardiovascular risk factor that may prove to be a better risk index for target organ damage and cardiovascular events in the hypertension population⁸. Our previous study found the serum OPG level to be positively associated with the carotid-femoral pulse wave velocity in hypertensive patients⁹. However, to the best of our knowledge, there have been no prior studies regarding the serum OPG level and arterial stiffness measured according to the CAVI in hypertensive patients.

Aim

The aim of the present study was to determine the relationship between the serum OPG level and arterial stiffness in hypertensive patients using the CAVI values.

Patients and Methods

Patients

Between January and December 2012, 167 participants from a medical center located in Hualien, Taiwan were enrolled in this study (115 patients with hypertension and 52 healthy participants). Trained staff measured blood pressure (BP) in the morning in all participants using a standard mercury sphygmomanometer with an appropriate cuff size after the partici-

pant remained in a sitting position for at least 10 minutes. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) values were obtained at the points of appearance and disappearance, respectively, of the Korotkoff sounds. The SBP and DBP values were obtained three times at five-minute intervals and averaged for the analysis. In the prevalence survey, hypertension was defined as a SBP of ≥ 140 mmHg and/or DBP of ≥ 90 mmHg or use of antihypertensive medication within the past two weeks. A patient was regarded as being diabetic if their fasting plasma glucose level was 126 mg/dL or greater or if he/she was using diabetes medications (oral drugs or insulin)¹⁰. Cardiovascular disease was defined as a history of coronary artery disease or stroke. Coronary artery disease defined as $> 50\%$ stenosis in any segment on coronary angiography. The pulse pressure was calculated by subtracting the DBP from the SBP. Patients were excluded if they had acute infection, acute myocardial infarction or pulmonary edema at the time of blood sampling, were receiving oral calcium preparations, bisphosphonates, teriparatide, estrogen medications or agents for osteoporosis and/or they refused to sign the informed consent form for study participation. The Protection of Human Subjects Institutional Review Board of Tzu-Chi University and Hospital approved the study protocol.

Anthropometric Analysis

Body weight was measured to the nearest half-kilogram with the patient in light clothing and without shoes. Height was measured to the nearest half-centimeter, and waist circumference was measured to the nearest half-centimeter at the shortest point below the lower rib margin and iliac crest. Body mass index (BMI) was calculated as the weight (kilograms) divided by the height squared (meters)^{9, 11}.

Biochemical Measurements

The fasting blood samples (approximately 5 mL) were immediately centrifuged at 3,000 g for 10 minutes. The serum levels of blood urea nitrogen (BUN), creatinine (Cre), fasting glucose, total cholesterol (TCH), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total calcium, phosphorus and C-reactive protein (CRP) were measured using an autoanalyzer (COBAS Integra 800, Roche Diagnostics, Basel, Switzerland)^{9, 11}. The serum OPG levels (eBioscience Inc., San Diego, CA, USA) were determined using a commercially available enzyme-linked immunosorbent assay (ELISA)⁹. The serum intact parathyroid hormone levels (iPTH) (Diagnostic Systems Labora-

Table 1. Clinical and analytical characteristics of the 167 participants with and without hypertension

Items	Healthy participants (<i>n</i> = 52)	Hypertension (<i>n</i> = 115)	<i>p</i> value
Age (years) ^a	66.27 ± 9.14	64.76 ± 10.13	0.359
Height (cm) ^a	160.54 ± 6.12	161.40 ± 8.68	0.517
Body weight (kg) ^a	66.13 ± 11.84	70.34 ± 12.90	0.047*
Waist circumference (cm) ^a	91.87 ± 11.81	93.50 ± 10.73	0.377
Body mass index (kg/m ²) ^a	25.60 ± 3.95	26.88 ± 3.70	0.044*
Cardio-ankle vascular index ^a	9.29 ± 1.62	9.39 ± 2.35	0.778
Systolic blood pressure (mmHg) ^a	118.38 ± 9.35	136.24 ± 17.04	<0.001*
Diastolic blood pressure (mmHg) ^a	69.27 ± 7.84	74.48 ± 10.30	0.001*
Pulse pressure (mmHg) ^a	49.11 ± 10.88	61.76 ± 15.80	<0.001*
Total cholesterol (mg/dL) ^a	171.81 ± 37.14	172.14 ± 36.95	0.957
Triglyceride (mg/dL) ^b	130.98 ± 72.79	160.58 ± 127.24	0.182
High density lipoprotein (mg/dL) ^a	48.13 ± 14.42	44.80 ± 12.69	0.134
Low density lipoprotein (mg/dL) ^a	100.69 ± 27.75	101.69 ± 29.39	0.837
Fasting glucose (mg/dL) ^b	120.77 ± 42.62	134.37 ± 57.76	0.216
Blood urea nitrogen (mg/dL) ^b	16.77 ± 5.32	17.35 ± 6.12	0.734
Creatinine (mg/dL) ^b	0.99 ± 0.18	1.12 ± 0.33	0.024*
Glomerular filtration rate (mL/min) ^a	74.40 ± 13.95	69.70 ± 21.45	0.150
Total calcium (mg/dL) ^a	9.09 ± 0.33	9.15 ± 0.36	0.352
Phosphorus (mg/dL) ^a	3.56 ± 0.53	3.50 ± 0.52	0.480
Calcium-phosphorous product ^a	32.43 ± 5.33	32.04 ± 5.10	0.657
Intact parathyroid hormone (pg/mL) ^b	58.21 ± 33.29	52.87 ± 28.70	0.271
C-reactive protein (mg/dL) ^b	0.28 ± 0.37	0.34 ± 0.40	0.242
Osteoprotegerin (pg/L) ^b	4.22 ± 5.48	4.17 ± 4.65	0.692
Log-osteoprotegerin (pg/L) ^a	0.34 ± 0.51	0.37 ± 0.49	0.745

The data are expressed as the mean ± SD.

**p* < 0.05 was considered to be statistically significant according to Student's *t*-test or the Mann-Whitney *U* test.

^aData were tested using Student's *t*-test.

^bData were tested using the Mann-Whitney *U* test.

tories, Webster, Texas, USA) were measured using a commercially available ELISA¹¹⁾. The estimated glomerular filtration rate (GFR) was calculated using the Modification of Diet in Renal Disease (MDRD) equation.

Cardio-ankle Vascular Index Measurements

The measurements were obtained in a quiet, temperature-controlled room with the patient in the supine position after 10 minutes of rest in accordance with recommendations. The CAVI was measured according to methods described previously (VaSera VS-1000, Fukuda Denshi Co. Ltd., Tokyo, Japan)^{4, 11)}. Briefly, a cuff was applied to the bilateral upper arms and ankles with the subject supine and the head held in the midline position. We then placed the phonocardiography microphone and electrocardiography electrodes. After the subject had rested for 10 minutes, the VaSera VS-1000 was used to measure the blood

pressure and pulse wave velocity, and the CAVI was calculated automatically. In this study, the high arterial stiffness group included patients with a CAVI value of ≥ 9, whereas a CAVI value of < 9 was used to define the low arterial stiffness group¹¹⁾.

Statistical Analysis

The data are expressed as the mean ± standard deviation (SD) and were tested for a normal distribution using the Kolmogorov-Smirnov test. Comparisons between patients were made using the Student's independent *t*-test (two-tailed) for normally distributed data or the Mann-Whitney *U* test for parameters that presented with a non-normal distribution (triglycerides, fasting glucose, BUN, iPTH, CRP and OPG). Variables expressed as the number of patients were analyzed using the χ^2 test. Because the OPG distribution was not normal, these data were transformed logarithmically (base 10), and the log-OPG values

Table 2. Clinical variables of the 115 hypertensive patients with and without arterial stiffness

Items	Low arterial stiffness group (<i>n</i> = 50)	High arterial stiffness group (<i>n</i> = 65)	<i>p</i> value
Age (years) ^a	60.42 ± 9.58	68.09 ± 9.29	< 0.001*
Height (cm) ^a	162.54 ± 8.92	160.53 ± 8.45	0.220
Body weight (kg) ^a	72.47 ± 12.64	68.69 ± 12.96	0.120
Waist circumference (cm) ^a	93.64 ± 10.16	93.40 ± 11.22	0.906
Body mass index (kg/m ²) ^a	27.37 ± 3.80	26.50 ± 3.60	0.215
Cardio-ankle vascular index ^a	7.36 ± 1.45	10.96 ± 1.59	< 0.001*
Systolic blood pressure (mmHg) ^a	130.44 ± 11.86	140.71 ± 19.05	0.001*
Diastolic blood pressure (mmHg) ^a	72.02 ± 8.82	76.37 ± 10.94	0.024*
Pulse pressure (mmHg) ^a	58.42 ± 10.71	64.34 ± 18.47	0.046*
Total cholesterol (mg/dL) ^a	172.54 ± 35.23	171.83 ± 38.49	0.919
Triglyceride (mg/dL) ^b	166.42 ± 150.74	156.09 ± 106.72	0.821
High density lipoprotein (mg/dL) ^a	44.34 ± 12.36	45.15 ± 13.03	0.735
Low density lipoprotein (mg/dL) ^a	102.70 ± 27.77	100.91 ± 30.77	0.747
Fasting glucose (mg/dL) ^b	132.38 ± 62.21	135.91 ± 54.55	0.588
Blood urea nitrogen (mg/dL) ^b	16.26 ± 6.34	18.18 ± 5.86	0.095
Creatinine (mg/dL) ^a	1.03 ± 0.23	1.19 ± 0.38	0.013*
Glomerular filtration rate (mL/min) ^a	76.30 ± 18.79	64.63 ± 22.11	0.003*
Total calcium (mg/dL) ^a	9.12 ± 0.38	9.17 ± 0.35	0.454
Phosphorus (mg/dL) ^a	3.48 ± 0.51	3.52 ± 0.52	0.686
Calcium-phosphorous product ^a	31.71 ± 4.87	32.30 ± 5.28	0.541
Intact parathyroid hormone (pg/mL) ^b	47.85 ± 20.97	56.78 ± 33.16	0.182
C-reactive protein (mg/dL) ^b	0.35 ± 0.43	0.34 ± 0.39	0.403
Osteoprotegerin (pg/L) ^b	2.48 ± 3.51	5.47 ± 5.01	< 0.001*
Log-osteoprotegerin (pg/L) ^a	0.16 ± 0.45	0.53 ± 0.46	< 0.001*

The data are expressed as the mean ± SD.

**p* < 0.05 was considered to be statistically significant according to Student's *t*-test or the Mann-Whitney *U* test.

^aData were tested using Student's *t*-test.

^bData were tested using the Mann-Whitney *U* test.

found to be normally distributed. The associations between the OPG concentration and age, CAVI, SBP, DBP, pulse pressure, Cre and GFR were evaluated using the nonparametric Spearman's rank correlation coefficient. Variables found to be significantly associated with arterial stiffness in the hypertensive patients were tested for independence according to a multivariate logistic regression analysis (adopted factors: diabetes, smoking, gender, age, SBP, DBP, pulse pressure, BUN, Cre, GFR and OPG). The data were analyzed using the SPSS software program for Windows (version 19.0; SPSS, Inc., Chicago, IL, USA). A *p*-value of less than 0.05 was considered to be statistically significant.

Results

The clinical and laboratory characteristics of the 52 healthy participants and 115 hypertensive patients

are presented in **Table 1**. The hypertensive patients had higher body weight (*p* = 0.047), BMI (*p* = 0.044), SBP (*p* < 0.001), DBP (*p* = 0.001), pulse pressure (*p* < 0.001) and Cre (*p* = 0.024) values than the healthy participants.

The demographic, biochemical and clinical characteristics of the 115 hypertensive patients and the hypertensive patients in the high arterial stiffness group versus the low arterial stiffness group are presented in **Table 2**. Sixty-five patients (56.5%) were assigned to the high arterial stiffness group. Age (*p* < 0.001), SBP (*p* = 0.001), DBP (*p* = 0.024), pulse pressure (*p* = 0.046), Cre (*p* = 0.013), serum OPG (*p* < 0.001) and log-OPG (*p* < 0.001) were higher in the high arterial stiffness group than in the low arterial stiffness group, while the GFR values (*p* = 0.003) were lower in the high arterial stiffness group than in the low arterial stiffness group. The log-OPG levels did not differ statistically with high arterial stiffness group

Table 3. Baseline characteristics of the 115 hypertensive patients with and without arterial stiffness

Characteristics	Low arterial stiffness group (%)	High arterial stiffness group (%)	<i>p</i> value
Gender			
Male	36 (72.0)	38 (58.5)	0.133
Female	14 (28.0)	27 (41.5)	
Diabetes			
No	36 (72.0)	34 (52.3)	0.032*
Yes	14 (28.0)	31 (47.7)	
Dyslipidemia			
No	29 (58.0)	31 (47.7)	0.273
Yes	21 (42.0)	34 (52.3)	
Cardiovascular disease			
No	17 (34.0)	23 (35.4)	0.877
Yes	33 (66.0)	42 (64.6)	
Smoking			
No	45 (90.0)	49 (75.4)	0.044*
Yes	5 (10.0)	16 (24.6)	
ACE inhibitor			
No	32 (64.0)	43 (66.2)	0.810
Yes	18 (36.0)	22 (33.8)	
ARB			
No	22 (44.0)	32 (49.2)	0.577
Yes	28 (56.0)	33 (50.8)	
β -blocker			
No	20 (40.0)	26 (40.0)	1.000
Yes	30 (60.0)	39 (60.0)	
CCB			
No	29 (58.0)	29 (44.6)	0.155
Yes	21 (42.0)	36 (55.4)	
Statin			
No	21 (42.0)	33 (50.8)	0.350
Yes	29 (58.0)	32 (49.2)	
Fibrate			
No	42 (84.0)	58 (89.2)	0.409
Yes	8 (16.0)	7 (10.8)	
Aspirin			
No	20 (40.0)	30 (46.2)	0.509
Yes	30 (60.0)	35 (53.8)	
Clopidogrel			
No	37 (74.0)	51 (78.5)	0.576
Yes	13 (26.0)	14 (21.5)	

* $p < 0.05$ was considered to be statistically significant.

The data are expressed as the number of patients, and the analysis was performed using the χ^2 test.

($n = 31$, 0.42 ± 0.57 pg/L) compared with low arterial stiffness group ($n = 21$, 0.23 ± 0.39 pg/L) in healthy participants ($p = 0.179$, data not shown).

The comorbid conditions of the 115 hypertensive patients included diabetes ($n = 45$; 39.1%), dyslipidemia ($n = 55$; 47.8%) and cardiovascular disease

($n = 75$; 65.2%). The medications prescribed in the hypertensive patients included angiotensin receptor blockers (ARBs; $n = 61$; 52.2%), angiotensin-converting enzyme inhibitors (ACEIs; $n = 40$; 34.8%), calcium channel blockers (CCBs; $n = 62$; 49.6%), β -blockers ($n = 69$; 60.0%), statins ($n = 61$; 53.0%), fibrates ($n =$

Table 4. Spearman correlation coefficients between the osteoprotegerin levels and clinical variables in the hypertensive patients

Variables	Spearman coefficient of correlation	<i>p</i> value
Age (years)	0.317	0.001*
Cardio-ankle vascular index	0.484	<0.001*
Systolic blood pressure (mmHg)	0.250	0.007*
Diastolic blood pressure (mmHg)	0.021	0.820
Pulse pressure (mmHg)	0.286	0.002*
Creatinine (mg/dL)	0.118	0.210
Glomerular filtration rate (mL/min)	-0.144	0.124

**p* < 0.05 was considered statistically significant by using the nonparametric Spearman's rank correlation coefficient.

15; 13.0%), aspirin (*n*=65; 56.5%) and clopidogrel (*n*=27; 23.5%). Diabetes (*p*=0.032) and smoking (*p*=0.044) were more frequent in the high arterial stiffness group than in the low arterial stiffness group. The groups did not differ statistically in terms of gender, co-existing dyslipidemia, cardiovascular disease and use of ARBs, ACEIs, CCBs, β -blockers, statins, fibrates, aspirin or clopidogrel (**Table 3**). The log-OPG levels did not differ statistically between hypertensive patients with (*n*=75, 0.36 ± 0.48 pg/L) and without cardiovascular disease (*n*=40, 0.39 ± 0.52 pg/L) (*p*=0.702, data not shown).

The correlations between OPG and age, cardio-ankle vascular index, systolic blood pressure, diastolic blood pressure, pulse pressure, creatinine and the glomerular filtration rate in the 115 hypertensive patients are presented in **Table 4**. The results of the Spearman test indicated strong positive correlations between OPG and age (*r*=0.317, *p*=0.001), CAVI (*r*=0.484, *p*<0.001), SBP (*r*=0.250, *p*=0.007) and pulse pressure (*r*=0.286, *p*=0.002).

The multivariable logistic regression analysis of the variables that were significantly associated with arterial stiffness (diabetes, smoking, gender, age, SBP, DBP, pulse pressure, BUN, Cre, GFR, and OPG) among hypertensive patients showed that age (odds ratio: 1.162, 95% confidence interval (CI): 1.070-1.263, *p*<0.001), DBP (odds ratio: 1.109, 95% CI: 1.033-1.190, *p*=0.004), and serum OPG level (odds ratio: 1.275, 95% CI: 1.030-1.580, *p*=0.026) were independent predictors of arterial stiffness in these patients (**Table 5**).

Discussion

Our results showed that age, DBP and OPG were positively correlated with arterial stiffness in the hypertensive patients based on the CAVI values.

Arterial stiffness is a well-recognized predictor of cardiovascular morbidity and mortality¹². Hyperten-

sion itself is strongly associated with cardiovascular disease¹³. Many papers have reported that the CAVI values are high in patients with hypertension^{4, 14}. In this study, 56.5% of the hypertensive patients were assigned to the high arterial stiffness group based on the CAVI.

The CAVI values are reportedly higher in patients with diabetes mellitus¹⁵. A high glucose level may modulate the arterial wall, thus increasing stiffness within a relatively short time and resulting in an increased CAVI⁴. The association between the CAVI and the progression of diabetes mellitus, another well-known condition that contributes to vascular stiffness, has also been extensively studied^{4, 5}, and diabetes is an independent determinant of the CAVI values in hypertensive patients¹⁶. Smoking has been found to be a risk factor for increasing arterial stiffness¹⁷. One study noted that smoking causes a significant increase in arterial stiffness as measured by the CAVI and that the CAVI correlates with the Brinkman index, which suggests that the CAVI is a useful index of the degree of arterial stiffness caused by smoking¹⁸. Another study found that complete smoking cessation improves the CAVI value¹⁹. Similarly, we found that, among the hypertensive patients included in our study, diabetes and smoking were more prevalent in the high arterial stiffness group than in the low arterial stiffness group.

Aging of the arterial system is accompanied by progressive structural changes, consisting of fragmentation and degeneration of elastin, increases in collagen, thickening of the arterial wall, endothelium damage and progressive dilation of the arteries²⁰. In healthy, normotensive individuals, the CAVI values nonetheless increase with age²¹. In the current study, among the hypertensive patients, the subjects in the high arterial stiffness group tended to be older than those in the low arterial stiffness group. Arterial stiffness leads to an increase in SBP because the heart is

Table 5. Multivariate logistic regression analysis of factors correlated with arterial stiffness in the 115 hypertensive patients

Variables	Odds ratio	95% confidence interval	<i>p</i> value
Age (per 1 year)	1.162	1.070-1.263	<0.001*
Diastolic blood pressure (per 1 mmHg)	1.109	1.033-1.190	0.004*
Osteoprotegerin (per 1 pg/L)	1.275	1.030-1.580	0.026*

* $p < 0.05$ was considered to be statistically significant in the multivariate logistic regression analysis (adopted factors: diabetes, smoking, gender, age, systolic blood pressure, diastolic blood pressure, pulse pressure, blood urea nitrogen, creatinine, glomerular filtration rate and OPG).

ejecting into a stiffer arterial bed that is less able to accommodate the volume of blood ejected by the left ventricle, resulting in a greater increase in systolic pressure²²). Furthermore, reduced aortic elastic recoil and a decreased reservoir capacity lead to a fall in DBP, resulting in a widened pulse pressure²³). In the current analysis, we found that the SBP, DBP and pulse pressure values were higher in the high arterial stiffness group than in the low arterial stiffness group. The CAVI correlates independently with the estimated GFR in the general Japanese population²⁴), whereas, in renal insufficiency patients, the CAVI values are closely associated with the cystatin C levels²⁵). Another report noted a significant correlation between the serum cystatin C level and the CAVI only in women in a non-chronic kidney disease population²⁶). A decreased GFR is a key determinant of arterial stiffness in hypertensive patients with a normal renal function²⁷). In our study population, among the hypertensive patients, the BUN and Cre values were higher, while the GFR values were lower in the high arterial stiffness group than in the low arterial stiffness group. In addition, the multivariable analysis showed age and DBP to be independent predictors of arterial stiffness in the hypertensive patients.

The pathogenesis of arterial stiffness induced by vascular calcification is very important²⁸). Vascular calcification is an active and complex process that involves numerous mechanisms responsible for calcium deposition in the arterial wall, leading to an increase in arterial stiffness²⁹). Studies conducted both *in vitro* and in animal models suggest that OPG is a vascular calcification inhibitor as well as a signaling molecule involved in bone remodeling and has been implicated in the regulation of vascular calcification and atherogenesis²). The mechanisms underlying the inhibitory effects of OPG on vascular calcification in animal models may be passive or cellular^{2, 30}). However, clinical studies suggest that the serum OPG level increases in association with vascular calcification, and the potential of OPG as a biomarker of vascular disease has been reported³⁰). This phenomenon may involve vascular calcification that induces the further

production of OPG to compensate for the process of vascular calcification. However, the exact significance and mechanisms by which this bone regulatory protein influences cardiovascular pathophysiology remains unclear^{2, 30}). Clinical observational studies have shown a positive association between the serum OPG levels and cardiovascular disease²), and the OPG/fetuin-A ratio is independently associated with the CAVI values in hemodialysis patients⁶). Azelnidipine, but not indapamide, when combined with olmesartan treatment for 12 months has been demonstrated to improve arterial stiffness, as measured by the CAVI, and is associated with a significant decrease in the OPG concentrations in hypertensive patients³¹). In the current study, the serum OPG concentrations were higher in the hypertensive patients in the high arterial stiffness group than in those in the low arterial stiffness group. This association remained significant even after adjusting for various confounders common in hypertensive patients. The results of the Spearman's rank correlation coefficient test also indicated a strong positive correlation between OPG and CAVI. Compared to our previous study using the carotid-femoral pulse wave velocity⁹), the present results also showed that the serum OPG level is positively associated arterial stiffness, measured according to the CAVI, in hypertensive patients.

Pharmacologic interventions have been shown to influence the CAVI values in humans. For example, in type 2 diabetes patients with hypertension, olmesartan, an angiotensin II receptor blocker, has been observed to significantly decrease the CAVI scores after 12 months of treatment, in contrast to amlodipine, which did not have this effect³²). In addition, treatment with efonidipine significantly decreases the CAVI values after 12 months in type 2 diabetes patients with hypertension and nephropathy receiving angiotensin receptor II blockers³³), while therapy with azelnidipine combined with olmesartan for 12 months decreases the CAVI values in hypertensive patients³¹). Telmisartan-based therapy also has beneficial effects on arterial stiffness, as assessed using the CAVI, compared with calcium channel blocker-based therapy³⁴).

Furthermore, treatment with pitavastatin for 12 months significantly decreases the CAVI scores in type 2 diabetes patients³⁵⁾. However, our results did not show a relationship between arterial stiffness, as measured according to the CAVI, and treatment with statins, fibrates or other medications (ARBs, ACEIs, CCBs, β -blockers, aspirin or clopidogrel) in the hypertensive patients. Further studies are therefore required to elucidate the relationship between medications and the CAVI levels in hypertensive patients.

There are some limitations associated with the current study. First, the number of patients enrolled was small, thereby weakening the statistical power of the results. Second, the study had a cross-sectional design; therefore, the findings should be investigated in long-term prospective studies before the causal relationship between the serum OPG level and arterial stiffness in hypertensive patients can be established. Third, since the CAVI reflects both organic and functional stiffness, it is inappropriate to consider it a pure indicator of organic changes in the arterial wall, as occur with arteriosclerosis and aging³⁶⁾. Hence, further studies are needed to assess the effects of OPG on arterial stiffness in hypertensive patients.

In conclusion, the present findings indicate that the serum OPG level is positively associated with arterial stiffness as assessed by the CAVI in hypertensive patients.

Conflicts of Interest/Disclosures

The authors declare no conflicts of interest or disclosures.

Acknowledgement

This study was supported by a grant from Buddhist Tzu Chi General Hospital, Hualien, Taiwan (TCRD-100-02).

References

- 1) Venuraju SM, Yerramasu A, Corder R, Lahiri A, Venuraju SM: Osteoprotegerin as a predictor of coronary artery disease and cardiovascular mortality and morbidity. *J Am Coll Cardiol*, 2010; 55: 2049-2061
- 2) Van Campenhout A, Golledge J: Osteoprotegerin, vascular calcification and atherosclerosis. *Atherosclerosis*, 2009; 204: 321-329
- 3) Vlachopoulos C, Aznaouridis K, Stefanadis C: Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *J Am Coll Cardiol*, 2010; 55: 1318-1327
- 4) Shirai K, Hiruta N, Song M, Kurosu T, Suzuki J, Tomaru T, Miyashita Y, Saiki A, Takahashi M, Suzuki K, Takata M: Cardio-ankle vascular index (CAVI) as a novel indicator of arterial stiffness: theory, evidence and perspectives. *J Atheroscler Thromb*, 2011; 18: 924-938
- 5) Sun CK: Cardio-ankle vascular index (CAVI) as an indicator of arterial stiffness. *Integr Blood Press Control*, 2013; 6: 27-38
- 6) Kim HR, Kim SH, Han MJ, Yoon YS, Oh DJ: The ratio of osteoprotegerin to fetuin-a is independently associated with vascular stiffness in hemodialysis patients. *Nephron Clin Pract*, 2013; 123: 165-172
- 7) Damasceno A, Azevedo A, Silva-Matos C, Prista A, Diogo D, Lunet N: Hypertension prevalence, awareness, treatment, and control in mozambique: Urban/rural gap during epidemiological transition. *Hypertension*, 2009; 54: 77-83
- 8) Trudeau L: Central blood pressure as an index of antihypertensive control: determinants and potential value. *Can J Cardiol*, 2014; 30 (5 Suppl): S23-S28
- 9) Wang JH, Lee CJ, Chen ML, Yang CF, Chen YC, Hsu BG: Association of serum osteoprotegerin levels with carotid-femoral pulse wave velocity in hypertensive patients. *J Clin Hypertens (Greenwich)*, 2014; 16: 301-308
- 10) Alberti KG, Zimmet PZ: Definition, diagnosis and classification of diabetes mellitus and its complications. I. Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med*, 1998; 15: 539-553
- 11) Chen YC, Lee MC, Lee CJ, Ho GJ, Yin WY, Chang YJ, Hsu BG: N-terminal pro-B-type natriuretic peptide associated with arterial stiffness by cardio-ankle vascular index in renal transplant recipients. *J Atheroscler Thromb*, 2013; 20: 647-654
- 12) Antonini-Canterin F, Carerj S, Di Bello V, Di Salvo G, La Carrubba S, Vriz O, Pavan D, Balbarini A, Nicolosi GL; Research Group of the Italian Society of Cardiovascular Echography (SIEC): Arterial stiffness and ventricular stiffness: a couple of diseases or a coupling disease? A review from the cardiologist's point of view. *Eur J Echocardiogr*, 2009; 10: 36-43
- 13) Ohira T, Iso H: Cardiovascular disease epidemiology in Asia: an overview. *Circ J*, 2013; 77: 1646-1652
- 14) Shirai K, Utino J, Saiki A, Endo K, Ohira M, Nagayama D, Tatsuno I, Shimizu K, Takahashi M, Takahara A: Evaluation of blood pressure control using a new arterial stiffness parameter, cardio-ankle vascular index (CAVI). *Curr Hypertens Rev*, 2013; 9: 66-75
- 15) Iбата J, Sasaki H, Kakimoto T, Matsuno S, Nakatani M, Kobayashi M, Tatsumi K, Nakano Y, Wakasaki H, Furuta H, Nishi M, Nanjo K: Cardio-ankle vascular index measures arterial wall stiffness independent of blood pressure. *Diabetes Res Clin Pract*, 2008; 80: 265-270
- 16) Masugata H, Senda S, Inukai M, Murao K, Himoto T, Hosomi N, Murakami K, Noma T, Kohno M, Okada H, Goda F: Association of cardio-ankle vascular index with brain natriuretic peptide levels in hypertension. *J Atheroscler Thromb*, 2012; 19: 255-262
- 17) Doonan RJ, Hausvater A, Scallan C, Mikhailidis DP, Pilote L, Daskalopoulou SS: The effect of smoking on arterial stiffness. *Hypertens Res*, 2010; 33: 398-410

- 18) Kubozono T, Miyata M, Ueyama K, Hamasaki S, Kusano K, Kubozono O, Tei C: Acute and chronic effects of smoking on arterial stiffness. *Circ J*, 2011; 75: 698-702
- 19) Noike H, Nakamura K, Sugiyama Y, Iizuka T, Shimizu K, Takahashi M, Hirano K, Suzuki M, Mikamo H, Nakagami T, Shirai K: Changes in cardio-ankle vascular index in smoking cessation. *J Atheroscler Thromb*, 2010; 17: 517-525
- 20) Lakatta EG: Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part III: cellular and molecular clues to heart and arterial aging. *Circulation*, 2003; 107: 490-497
- 21) Choi SY, Oh BH, Bae Park J, Choi DJ, Rhee MY, Park S: Age-associated increase in arterial stiffness measured according to the cardio-ankle vascular index without blood pressure changes in healthy adults. *J Atheroscler Thromb*, 2013; 20: 911-923
- 22) Stehouwer CD, Henry RM, Ferreira I: Arterial stiffness in diabetes and the metabolic syndrome: a pathway to cardiovascular disease. *Diabetologia*, 2008; 51: 527-539
- 23) Cavalcante JL, Lima JA, Redheuil A, Al-Mallah MH: Aortic stiffness: current understanding and future directions. *J Am Coll Cardiol*, 2011; 57: 1511-1522
- 24) Kubozono T, Miyata M, Ueyama K, Nagaki A, Hamasaki S, Kusano K, Kubozono O, Tei C: Association between arterial stiffness and estimated glomerular filtration rate in the Japanese general population. *J Atheroscler Thromb*, 2009; 16: 840-845
- 25) Nakamura K, Iizuka T, Takahashi M, Shimizu K, Mikamo H, Nakagami T, Suzuki M, Hirano K, Sugiyama Y, Tomaru T, Miyashita Y, Shirai K, Noike H: Association between cardio-ankle vascular index and serum cystatin C levels in patients with cardiovascular risk factor. *J Atheroscler Thromb*, 2009; 16: 371-379
- 26) Yamashita H, Nishino T, Obata Y, Nakazato M, Inoue K, Furusu A, Takamura N, Maeda T, Ozono Y, Kohno S: Association between cystatin C and arteriosclerosis in the absence of chronic kidney disease. *J Atheroscler Thromb*, 2013; 20: 548-556
- 27) Schillaci G, Pirro M, Mannarino MR, Pucci G, Savarese G, Franklin SS, Mannarino E: Relation between renal function within the normal range and central and peripheral arterial stiffness in hypertension. *Hypertension*, 2006; 48: 616-621
- 28) Karwowski W, Naumnik B, Szczepanski M, Mysliwiec M: The mechanism of vascular calcification - a systematic review. *Med Sci Monit*, 2012; 18: RA1-11
- 29) Persy V, D'Haese P: Vascular calcification and bone disease: the calcification paradox. *Trends Mol Med*, 2009; 15: 405-416
- 30) Baud'huin M, Duplomb L, Teletchea S, Lamoureux F, Ruiz-Velasco C, Maillason M, Redini F, Heymann MF, Heymann D: Osteoprotegerin: multiple partners for multiple functions. *Cytokine Growth Factor Rev*, 2013; 24: 401-409
- 31) Uzui H, Morishita T, Nakano A, Amaya N, Fukuoka Y, Ishida K, Arakawa K, Lee JD, Tada H: Effects of combination therapy with olmesartan and azelnidipine on serum osteoprotegerin in patients with hypertension. *J Cardiovasc Pharmacol Ther*, 2014; 19: 304-309
- 32) Miyashita Y, Saiki A, Endo K, Ban N, Yamaguchi T, Kawana H, Nagayama D, Ohira M, Oyama T, Shirai K: Effects of olmesartan, an angiotensin II receptor blocker, and amlodipine, a calcium channel blocker, on Cardio-Ankle Vascular Index (CAVI) in type 2 diabetic patients with hypertension. *J Atheroscler Thromb*, 2009; 16: 621-626
- 33) Sasaki H, Saiki A, Endo K, Ban N, Yamaguchi T, Kawana H, Nagayama D, Ohhira M, Oyama T, Miyashita Y, Shirai K: Protective effects of efonidipine, a T- and L-type calcium channel blocker, on renal function and arterial stiffness in type 2 diabetic patients with hypertension and nephropathy. *J Atheroscler Thromb*, 2009; 16: 568-575
- 34) Kinouchi K, Ichihara A, Sakoda M, Kurauchi-Mito A, Murohashi-Bokuda K, Itoh H: Effects of telmisartan on arterial stiffness assessed by the cardio-ankle vascular index in hypertensive patients. *Kidney Blood Press Res*, 2010; 33: 304-312
- 35) Miyashita Y, Endo K, Saiki A, Ban N, Yamaguchi T, Kawana H, Nagayama D, Ohira M, Oyama T, Shirai K: Effects of pitavastatin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, on cardio-ankle vascular index in type 2 diabetic patients. *J Atheroscler Thromb*, 2009; 16: 539-545
- 36) Shirai K, Song M, Suzuki J, Kurosu T, Oyama T, Nagayama D, Miyashita Y, Yamamura S, Takahashi M: Contradictory effects of β 1- and α 1-adrenergic receptor blockers on cardio-ankle vascular stiffness index (CAVI)-CAVI independent of blood pressure. *J Atheroscler Thromb*, 2011; 18: 49-55