Tohoku J. Exp. Med., 2015, **236**, 247-253

High Serum Osteoprotegerin Is Associated with Arterial Stiffness in Kidney Transplant Patients

Bang-Gee Hsu,[1,2] Ming-Hui Shih,[3] Yen-Cheng Chen,[4] Guan-Jin Ho,[4] Teng-Yi Lin[5] and Ming-Che Lee[2,4]

[1]Division of Nephrology, Buddhist Tzu Chi General Hospital, Hualien, Taiwan
[2]School of Medicine, Tzu Chi University, Hualien, Taiwan
[3]Department of Nursing, Buddhist Tzu Chi General Hospital, Hualien, Taiwan
[4]Department of Surgery, Buddhist Tzu Chi General Hospital, Hualien, Taiwan
[5]Department of Laboratory Medicine, Buddhist Tzu Chi General Hospital, Hualien, Taiwan

Osteoprotegerin (OPG) is a cytokine that regulates bone resorption by inhibiting osteoclastogenesis, and OPG has been implicated in the process that causes vascular stiffness. An increase in serum OPG level has been associated with the development of arterial stiffness. Kidney transplant (KT) patients are susceptible to aortic stiffness, which is considered to be a predictor of cardiovascular events in this patient population. Carotid-femoral pulse wave velocity (cfPWV) has emerged as a gold standard for non-invasive evaluation of aortic stiffness. The aim of this study was to evaluate the relationship between serum OPG concentration and cfPWV among KT patients. Fasting blood samples were obtained from 57 KT patients and their cfPWV was measured using applanation tonometry. The serum OPG levels were measured using an enzyme-linked immunosorbent assay. Univariable linear regression analysis showed that the cfPWV in KT patients was significantly and positively correlated with age, body weight, waist circumference, body mass index, log-creatinine, systolic blood pressure, diastolic blood pressure, pulse pressure, and the log-OPG concentration. KT patients with metabolic syndrome had higher cfPWV values than those without metabolic syndrome (P = 0.036), which indicates a higher incidence of aortic stiffness in this patient population. Multivariable forward stepwise linear regression analysis of the significant variables showed that the log-OPG (P = 0.001), the log-creatinine (P = 0.004), and the SBP (P = 0.005) remained as independent and positive predictors of cfPWV values. These findings indicate that serum OPG levels are positively associated with cfPWV in KT patients.

Keywords: arterial stiffness; carotid-femoral pulse wave velocity; kidney transplantation; osteoprotegerin; pulse pressure

Tohoku J. Exp. Med., 2015 August, **236** (4), 247-253.

Introduction

Cardiovascular (CV) disease is a leading cause of morbidity and mortality in kidney transplant (KT) patients (Lentine et al. 2012). Arterial stiffness is an established CV risk marker and an independent predictor of CV events and mortality among KT patients (Khoshdel and Carney 2008). Carotid-femoral pulse wave velocity (cfPWV), a measure of the intrinsic stiffness of the aortic wall, is a direct measurement of aortic stiffness and has been recommended as the gold-standard measurement for arterial stiffness (Tomlinson 2012). Recent studies have noted that cfPWV is potent means of predicting future CV events in KT patients and an increase in cfPWV by 1 m/s might increase the frequency of CV events by 35% to 45% (Verbeke et al. 2011; Claes et al. 2013).

Arterial stiffness is defined by a reduction in arterial distensibility and decreased arterial compliance is one of the earliest detectable manifestations of adverse structural and functional changes within the vessel wall (Tomiyama and Yamashina 2010). The pathogenesis of arterial stiffness in terms of vascular calcification is important (Karwowski et al. 2012). Vascular calcification is an active and cell-regulated process that involves mineralization and its net result is a trans-differentiation process changing vascular smooth muscle cells into chondrocyte-like or osteoblast-like cells. These changes seem to lead to increased arterial stiffness. Osteoprotegerin (OPG) is a glycoprotein that is involved in the regulation of the vascular calcification process and is a vascular calcification inhibitor (Venuraju et al. 2010). In recent studies, OPG has been associated with increased pulse wave velocity, progression of arterial calcification and

Received January 27, 2015; revised and accepted June 16, 2015. Published online July 3, 2015; doi: 10.1620/tjem.236.247.
Correspondence: Ming-Che Lee, Department of Surgery, Buddhist Tzu Chi General Hospital, No. 707, Section 3, Chung-Yang Road, Hualien 970,Taiwan.
e-mail: mingche1229@gmail.com

with mortality in both end-stage renal failure patients as well as the general population (Kiechl et al. 2004; Speer et al. 2008). Aortic calcification is a frequent finding in KT patients. A high level of OPG has been found to be significantly associated with the progression of abdominal aortic calcifications in KT patients (Meneghini et al. 2013). Thus OPG might be considered as a possible reliable marker for the progression of abdominal aortic calcifications in KT patients. Elevated serum OPG is an independent predictor of death from any cause or of CV death among KT patients (Hjelmesaeth et al. 2006). In the ALERT (Assessment of Lescol in Renal Transplantation) study, elevated serum OPG was also found to be independently associated with renal events, CV events and mortality among KT patients (Svensson et al. 2012). The aim of the current study was to determine the relationship between fasting serum OPG levels and aortic stiffness, as measuring by cfPWV, among KT patients.

Materials and Methods

Patients

Between May and August 2013, 57 KT patients from a medical center located in Hualien, Taiwan, were enrolled in this study. The Human Subjects Institutional Review Board of Tzu-Chi University and General Hospital approved this study. Blood pressure was measured in the morning using standard mercury sphygmomanometers with appropriate cuff sizes, after the participants had been sitting for at least 10 minutes. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were taken at the points of appearance and disappearance, respectively, of the Korotkoff sounds. SBP and DBP were taken three times at five-minute intervals and were averaged for analysis. Pulse pressure was calculated by subtracting DBP from SBP. Patients were excluded if they had any acute infection, malignancy, acute rejection, acute myocardial infarction or pulmonary edema at the time of blood sampling as well as if they had an arterial-venous shunt or had received a graft in the hands. Patients using medications related to calcium, active vitamin D metabolites, bisphosphonates, teriparatide, or estrogen, as well as if they refused to provide informed consent, were also excluded.

Anthropometric analysis

The participants' weights were measured in light clothing and without shoes to the nearest 0.5 kilograms, and their height was measured to the nearest 0.5 cm. Waist circumference was measured using a tape around patient's waist from the point between patient's lowest ribs and patient's hip bones after the patient's hands had been placed on patient's hips. Body mass index (BMI) was calculated as the weight in kilograms divided by the height in meters squared (Chen et al. 2013; Wang et al. 2014).

Biochemical investigations

Fasting blood samples (approximately 5 mL) were immediately centrifuged at 3,000 *g* for 10 min. The serum levels of blood urea nitrogen (BUN), creatinine (Cre), fasting glucose, total cholesterol (TCH), triglycerides (TG), high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, albumin, globulin, alkaline phosphatase, total calcium, and phosphorus were measured using an autoanalyzer (COBAS Integra 800, Roche Diagnostics, Basel, Switzerland) (Chen et al. 2013; Wang et al. 2014). The serum OPG levels (eBioscience Inc., San Diego, CA, USA) were measured using a commercial available enzyme-linked immunosorbent assay (ELISA) (Wang et al. 2014). The serum intact parathyroid hormone (iPTH) (Diagnostic Systems Laboratories, Texas, USA) levels were measured using a commercially available enzyme-linked immunosorbent assays (ELISA) (Chen et al. 2013). The estimated glomerular filtration rate (GFR) calculation in this study used the equation from the Modification of Diet in Renal Disease (MDRD). The blood samples were taken on the same day as the cfPWV value was measured.

Carotid-femoral pulse wave velocity measurements

The cfPWV was measured in KT patients using applanation tonometry (SphygmoCor system, AtCor Medical, Australia), which allowed transcutaneous recording of the pressure pulse waveform in the underlying artery as previously described (Norton et al. 2012; Wang et al. 2014). All measurements were performed in the morning in the supine position after at least 10-min rest in a quiet, temperature-controlled room. The recordings were made simultaneously with an ECG signal, which provided an *R*-timing reference. Pulse wave recordings were performed consecutively at two superficial artery sites (carotid-femoral segment). Integrated software was used to process each set of pulse wave and the ECG data to calculate the mean time difference between *R*-wave and pulse wave on a beat-to-beat basis for an average of 10 consecutive cardiac cycles. The cfPWV was calculated using the distance and mean time difference between the two recorded points. Quality indices, included in the software, were used to ensure uniformity of the data obtained.

Metabolic syndrome and its components

The prevalence of metabolic syndrome (MetS) was defined using the International Diabetes Federation definition (Alberti et al. 2006). Chinese people were classified as having MetS if they had central (abdominal) obesity with a waist circumference ≥ 90 cm (men) or ≥ 80 cm (women) and matched two or more of the following criteria: fasting serum glucose ≥ 110 mg/dL, triglycerides ≥ 150 mg/dL, HDL-C level ≤ 40 mg/dL in men or ≤ 50 mg/dL in women, or blood pressure of ≥ 130/85 mmHg. The use of antihypertensive medication was considered as high blood pressure in this analysis. Type 2 diabetes was determined according to World Health Organization criteria (Alberti and Zimmet 1998). A person was regarded as diabetic if the fasting plasma glucose was ≥ 126 mg/dL, or if the 2-h glucose during an oral glucose tolerance test was ≥ 200 mg/dL, or if he/she was using diabetes medications (oral or insulin).

Statistical analysis

The collected data are expressed as the mean ± standard deviation (SD) and were tested for normal distribution using Kolmogorov-Smirnov statistics. Comparisons between subjects were performed using the Student's independent *t*-test (two-tailed) for normally distributed data, or the Mann-Whitney U test for parameters that presented a non-normal distribution. The glucose, BUN, Cre, TG, and OPG datasets showed skewed non-normal distributions and therefore these were recalculated by transformation to the logarithm to the base 10; after this transformation the log-glucose, log-BUN, log-Cre, log-TG, and log-OPG then became normally distribution. Clinical variables that correlated with cfPWV values in KT patients were evaluated by univariable linear regression analysis first. Variables that were significantly associated with cfPWV in the KT patients were

tested for independence by multivariable forward stepwise regression analysis (the factors adopted consisted of age, body weight, waist circumference, BMI, log-Cre, SBP, DBP, pulse pressure, MetS and log-OPG). All data were analyzed using SPSS for Windows (version 19.0; SPSS Inc., Chicago, IL, USA). A P-value of < 0.05 was considered statistically significant.

Results

The clinical and laboratory characteristics of the anthropometric and biochemical data of the 57 KT patients are presented in Table 1. Table 2 shows that 34 patients had diabetes (59.6%), and 15 had hypertension (26.3%). The immunological medications prescribed to the KT patients included tacrolimus (n = 34, 59.6%), mycophenolate mofetil or mycophenolic acid (n = 45, 78.9%), steroids (n = 46, 80.7%), rapamycin (n = 11, 19.3%), and cyclosporine (n = 12, 21.1%). There is no statistically difference in cfPWV values based on gender, transplantation model, diabetes, hypertension, or use of the immunological medications listed. However, 18 KT patients with MetS had significantly higher cfPWV values than the 39 KT patients without MetS (P = 0.036, Table 2).

The univariable linear analysis of the cfPWV values of the 57 KT patients is presented in Table 3. Among the nine anthropometric factors and sixteen biochemical factors studied, the following nine factors showed a positive correlation with cfPWV value: age (r = 0.262; P = 0.049), body weight (r = 0.360; P = 0.006), waist circumference (r = 0.350; P = 0.008), BMI (r = 0.332; P = 0.012), log-Cre (r = 0.441; P = 0.001), SBP (r = 0.406; P = 0.002), DBP (r = 0.290; P = 0.029), pulse pressure (r = 0.306; P = 0.021), and the log-OPG concentration (r = 0.479; P < 0.001).

A multivariable forward stepwise linear regression analysis was carried out that including all the variables that were significantly associated with cfPWV values; these were age, body weight, waist circumference, BMI, log-Cre, SBP, DBP, pulse pressure, MetS and log-OPG. Table 4 showed that three of the ten factors remained significant and have independent predictive value with respect to cfPWV values in our patients; these are log-OPG (β = 0.364, R square = 0.229, P = 0.001), log-Cre (β = 0.322, R square = 0.120, P = 0.004), and SBP (β = 0.309, R square = 0.092, P = 0.005).

Discussion

The results of our study show that ten factors (age, body weight, waist circumference, BMI, SBP, DBP, pulse pressure, metabolic syndrome, log-Cre and log-OPG concentrations) are positively correlated with cfPWV values in KT patients by univariable analysis. After adjusting these significant variables using multivariable forward stepwise linear regression analysis, we found that SBP, log-Cre, and log-OPG concentration were the three independent predictors of the cfPWV values among our KT patients.

Aortic stiffness, as expressed by cfPWV, is a strong predictor of future CV events and all-cause mortality in humans (Vlachopoulos et al. 2010). In addition, cfPWV is a strong predictor of future CV events in KT patients (Verbeke et al. 2011; Claes et al. 2013). Aortic stiffness leads to an increase in SBP because hearts are ejecting into a stiffer arterial bed that is less able to accommodate the volume of blood ejected by the left ventricle. The greater pressure increment during systole exposes the myocardium to higher SBP, which results in left ventricular hypertrophy and fibrosis (Stehouwer et al. 2008). Reduced aortic elastic recoil and reservoir capacity leads to a fall in DBP, which

Table 1. Clinical and laboratory characteristics of the 57 kidney transplantation patients.

Items	Parameter		Parameter	
Anthropometric data	Height (cm)	162.11 ± 8.53	Waist circumference (cm)	84.82 ± 11.88
	Body weight (kg)	62.32 ± 12.70	Age (years)	50.82 ± 8.95
	Body mass index (kg/m^2)	23.65 ± 4.37	KT duration (months)	67.77 ± 39.67
	SBP (mmHg)	138.44 ± 16.22	DBP (mmHg)	87.33 ± 10.71
	Pulse pressure (mmHg)	51.44 ± 11.40	cfPWV (m/s)	9.19 ± 3.31
Biochemical data	Triglyceride (mg/dL)	154.84 ± 116.18	Total cholesterol (mg/dL)	193.53 ± 45.68
	HDL-cholesterol (mg/dL)	51.72 ± 15.92	LDL-cholesterol (mg/dL)	114.37 ± 39.39
	Albumin (g/dL)	4.14 ± 0.50	Globulin (g/dL)	2.80 ± 0.61
	ALP (U/L)	111.46 ± 53.19	Creatinine (mg/dL)	2.15 ± 1.58
	Fasting glucose (mg/dL)	124.05 ± 38.06	GFR (ml/min)	44.14 ± 21.17
	BUN (mg/dL)	25.77 ± 13.63	Phosphorus (mg/dL)	3.29 ± 0.83
	Total Calcium (mg/dL)	9.27 ± 1.12	iPTH (pg/mL)	115.74 ± 107.02
	Ca × P product (mg^2/dL^2)	30.14 ± 6.63	Osteoprotegerin (pg/L)	5.21 ± 5.05

Data are expressed as means ± standard deviations.
KT, kidney transplantation; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-cholesterol, high-density lipoprotein cholesterol; LDL-cholesterol, low-density lipoprotein cholesterol; ALP, alkaline phosphatase; BUN, blood urea nitrogen; GFR, glomerular filtration rate; Ca × P product, calcium-phosphorus product; iPTH, intact parathyroid hormone; cfPWV, carotid-femoral pulse wave velocity.

Table 2. Clinical characteristics and carotid-femoral pulse wave velocity levels of the 57 kidney transplantation patients.

Characteristic		Number (%)	cfPWV (m/s)	*P* value
Gender	Male	28 (49.1)	9.67 ± 3.54	0.289
	Female	29 (50.9)	8.73 ± 3.07	
Diabetes	No	23 (40.4)	9.20 ± 1.76	0.981
	Yes	34 (59.6)	9.18 ± 4.07	
Hypertension	No	42 (73.7)	8.78 ± 3.54	0.118
	Yes	15 (26.3)	10.34 ± 2.28	
Transplantation model	Cadaveric	49 (86.0)	9.02 ± 3.44	0.328
	Living	8 (14.0)	10.26 ± 2.27	
Metabolic syndrome	No	39 (68.4)	8.57 ± 2.45	0.036*
	Yes	18 (31.6)	10.54 ± 4.45	
Tacrolimus use	No	23 (40.4)	8.29 ± 3.29	0.092
	Yes	34 (59.6)	9.80 ± 3.23	
Mycophenolate mofetil or mycophenolic acid use	No	12 (21.1)	10.12 ± 2.63	0.280
	Yes	45 (78.9)	8.94 ± 3.45	
Steroid use	No	11 (19.3)	9.89 ± 5.90	0.440
	Yes	46 (80.7)	9.02 ± 2.39	
Rapamycin use	No	46 (80.7)	9.38 ± 3.56	0.393
	Yes	11 (19.3)	8.42 ± 1.90	
Cyclosporine use	No	45 (78.9)	9.43 ± 3.01	0.289
	Yes	12 (21.1)	8.28 ± 4.30	

Data are expressed as means ± standard deviations.
*$P < 0.05$ was considered statistically significant by the Student *t*-test.
cfPWV, carotid-femoral pulse wave velocity.

results in the widened pulse pressure (Cavalcante et al. 2011). Lower DBP further reduces coronary artery perfusion and promotes subendocardial ischemia, which is exacerbated by left ventricular hypertrophy (Laurent and Boutouyrie 2007). 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 arteries (Lakatta 2003). Recently, reference and normal values for cfPWV from a large multicenter European cohort (n = 11,092) were published and it was noted that there was a linear and age related increase in cfPWV values (Reference Values for Arterial Stiffness' Collaboration 2010). Age-related widening of the pulse pressure of individuals is the major cause of the age-related increase in the prevalence of hypertension and has been attributed to aortic stiffening. Our study shows that age, SBP, DBP, and pulse pressure are positively correlated with cfPWV values among our KT patients**.** SBP was also an independent clinical predictor of cfPWV values among KT patients in our study after multivariable analysis.

Waist circumference has been found to be positively correlated with cfPWV values and was independent of age, sex, blood pressure, glucose and lipids, while BMI has been found not to be associated with cfPWV values in a study of Chinese community-dwelling adults (Fu et al. 2013). In another study, an increase in waist circumference has also been found to be independently associated with higher cfPWV values among youth with type 1 diabetes (Dabelea et al. 2013). However, in contrast, yet another study has noted that cfPWV values are correlated positively with BMI, but are not correlated with waist circumference in a Brazilian population (Rodrigues et al. 2012). Increased aortic stiffness, a major mechanical factor predicting cardiovascular risk, has been clearly identified as playing a role in MetS (Safar et al. 2013). Our study showed that waist circumference, BMI and MetS were positively correlated with cfPWV values among our KT patients. Furthermore, it has been found that a decrease in GFR exhibits a significant reverse association with cfPWV values in women with normal to mildly impaired renal function (Bian et al. 2012). Another report has also noted that a decreased GFR is a major determinant of aortic stiffness in hypertensive patients with normal renal function (Schillaci et al. 2006). Reduced GFR has been associated with central arterial stiffness in terms of a higher augmentation index (Andrade et al. 2008). Coronary artery disease (CAD) patients with impaired renal function have greater cfPWV values compared to those with CAD and normal renal function (Rossi et al. 2013). Our study showed that log-Cre is positively correlated with cfPWV values among our KT patients. At the univariable analysis level, a reduced GFR shows a trend toward a positive correlation with cfPWV values among our KT patients, but it just missed statistical

Table 3. Correlation of carotid-femoral pulse wave velocity and clinical-laboratory variables using univariable linear regression analysis among the 57 kidney transplantation patients.

Variable	*R* value	*P* value
Age (years)	0.262	0.049*
Kidney transplantation duration (months)	−0.073	0.589
Height (cm)	0.071	0.599
Body weight (kg)	0.360	0.006*
Waist circumference (cm)	0.350	0.008*
Body mass index (BMI; kg/m^2)	0.332	0.012*
Total cholesterol (mg/dL)	−0.161	0.231
Log-Triglyceride (mg/dL)	0.100	0.461
HDL-cholesterol (mg/dL)	−0.066	0.623
LDL-cholesterol (mg/dL)	0.054	0.689
Albumin (g/dL)	0.150	0.267
Globulin (g/dL)	0.012	0.928
Alkaline phosphatase (U/L)	−0.059	0.661
Log-Glucose (mg/dL)	0.010	0.940
Log-BUN (mg/dL)	0.040	0.765
Log-Creatinine (mg/dL)	0.441	0.001*
Glomerular filtration rate (ml/min)	−0.241	0.071
Systolic blood pressure (mmHg)	0.406	0.002*
Diastolic blood pressure (mmHg)	0.290	0.029*
Pulse pressure (mmHg)	0.306	0.021*
Total calcium (mg/dL)	−0.067	0.621
Phosphorus (mg/dL)	0.008	0.953
Calcium-phosphorus product (mg^2/dL^2)	−0.032	0.811
iPTH (pg/mL)	0.106	0.434
Log-OPG (pg/L)	0.479	< 0.001*

*$P < 0.05$ is considered statistically significant in the univariable linear analyses.
Data for BUN, creatinine, triglyceride, fasting glucose, iPTH, and OPG levels showed skewed distributions and therefore were log-transformed before analysis.
BUN, blood urea nitrogen; HDL-cholesterol, high-density lipoprotein cholesterol; LDL-cholesterol, low-density lipoprotein cholesterol; iPTH, intact parathyroid hormone; OPG, osteoprotegerin.

Table 4. Multivariable stepwise linear regression analysis of carotid-femoral pulse wave velocity among the 57 kidney transplantation patients.

Items	Beta	R square	R square change	*P* value
Log-OPG (pg/L)	0.364	0.229	0.229	0.001*
Log-Creatinine (mg/dL)	0.322	0.349	0.120	0.004*
Systolic blood pressure (mmHg)	0.309	0.441	0.092	0.005*

Data for creatinine and OPG levels showed skewed distribution and therefore were log-transformed before analysis.
*$P < 0.05$ was considered statistically significant in the multivariable stepwise linear regression analysis (adopted factors: age, body weight, waist circumference, body mass index, log-creatinine, systolic blood pressure, diastolic blood pressure, pulse pressure, metabolic syndrome and log-OPG).
OPG, osteoprotegerin.

significance ($P = 0.071$).

Parathyroid hormone (PTH) plays an active role in the regulation of vascular calcification (Karwowski et al. 2012). PTH level is a significant predictor of aortic stiffness, irrespective of cardiovascular risk factors and of factors involved in bone formation in postmenopausal women (Pirro et al. 2012). Hyperparathyroidism has been also associated with the coronary artery calcification progression among KT patients (Mazzaferro et al. 2009). However, in community-dwelling elderly individuals, no statistically significant association between iPTH values and cfPWV values has been found after adjustment for several con-

founders. Our study, it should be noted, also found that iPTH levels are not correlated with cfPWV values in KT patients.

OPG strongly inhibits bone resorption and may also serve as a vascular calcification inhibitor (Venuraju et al. 2010). Arterial stiffness is associated with vascular calcification (Persy and D'Haese 2009). OPG is expressed in vivo by endothelial cells, vascular smooth muscle cells, and osteoblasts (Venuraju et al. 2010). Production of OPG is enhanced by inflammatory cytokines and may reflect endothelial dysfunction. Additionally, a failing myocardium, plaque rupture and the presence of other inflamed tissues, could contribute to an elevation in circulating OPG concentration (Van Campenhout and Golledge 2009; Venuraju et al. 2010). Clinical studies have suggested that an increase in serum OPG levels is associated with renal events, CV events and mortality among KT patients (Hjelmesaeth et al. 2006; Svensson et al. 2012). A high level of serum OPG is significantly associated with the progression of abdominal aortic calcifications in KT patients who were followed up for 2 years (Meneghini et al. 2013). However, another study noted that serum OPG levels were independently associated with coronary artery calcification at baseline, are dramatically reduced after transplantation and do not show a significant association with OPG and coronary artery calcification at 1-year after KT (Bargnoux et al. 2009). Our study shows that serum log-OPG concentrations are positively correlated with cfPWV values among our KT patients. This relationship remained significant even after adjustment for several confounders affecting the KT patients.

Our study has some limitations. Firstly, this study had a cross-sectional design and therefore, our findings need to be investigated using long-term prospective studies before a causal relationship between serum OPG levels and cfPWV in KT patients can be established. Secondly, this study was restricted to a limited number of KT patients and there were no age-matched control participants; thus the possibility of selection bias cannot be excluded. Moreover, the observational design of this study does not allow us to reach conclusions on the mechanism underlying the observed statistical association between OPG and cfPWV.

In conclusion, the present study showed a positive association between cfPWV and log-OPG, SBP, and log-Cre among KT patients. We feel further studies and investigations are needed to understand the effects of these factors on cfPWV in KT patients.

Acknowledgments

This study was supported by a grant from Buddhist Tzu Chi General Hospital, Hualien, Taiwan (TCRD102-26). We also thank Professor Yeu-Tsu Margaret Lee (Department of Surgery, School of Medicine, University of Hawaii, USA) for corrected grammatical and typographical errors in this manuscript.

Conflict of Interest

The authors declare no conflict of interest.

References

Alberti, K.G. & Zimmet, P.Z. (1998) Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet. Med.*, **15**, 539-553.

Alberti, K.G., Zimmet, P. & Shaw, J. (2006) Metabolic syndrome: a new world-wide definition. A consensus statement from the International Diabetes Federation. *Diabet. Med.*, **23**, 469-480.

Andrade, J., Er, L., Ignaszewski, A. & Levin, A. (2008) Exploration of association of 1,25-OH2D3 with augmentation index, a composite measure of arterial stiffness. *Clin. J. Am. Soc. Nephrol.*, **3**, 1800-1806.

Bargnoux, A.S., Dupuy, A.M., Garrigue, V., Jaussent, I., Gahide, G., Badiou, S., Szwarc, I., Deleuze, S., Vernhet, H., Cristol, J.P. & Mourad, G. (2009) Evolution of coronary artery calcifications following kidney transplantation: relationship with osteoprotegerin levels. *Am. J. Transplant.*, **9**, 2571-2579.

Bian, S.Y., Guo, H.Y., Ye, P., Luo, L.M., Wu, H.M., Xiao, W.K., Qi, L.P., Yu, H.P. & Duan, L.F. (2012) Association of glomerular filtration rate with arterial stiffness in Chinese women with normal to mildly impaired renal function. *J. Geriatr. Cardiol.*, **9**, 158-165.

Cavalcante, J.L., Lima, J.A., Redheuil, A. & Al-Mallah, M.H. (2011) Aortic stiffness: current understanding and future directions. *J. Am. Coll. Cardiol*., **57**, 1511-1522.

Chen, Y.C., Lee, M.C., Lee, C.J., Ho, G.J., Yin, W.Y., Chang, Y.J. & Hsu, B.G. (2013) N-terminal pro-B-type natriuretic peptide is associated with arterial stiffness measured using the cardio-ankle vascular index in renal transplant recipients. *J. Atheroscler. Thromb.*, **20**, 646-653.

Claes, K.J., Heye, S., Bammens, B., Kuypers, D.R., Meijers, B., Naesens, M., Vanrenterghem, Y. & Evenepoel, P. (2013) Aortic calcifications and arterial stiffness as predictors of cardiovascular events in incident renal transplant recipients. *Transpl. Int.*, **26**, 973-981.

Dabelea, D., Talton, J.W., D'Agostino, R. Jr., Wadwa, R.P., Urbina, E.M., Dolan, L.M., Daniels, S.R., Marcovina, S.M. & Hamman, R.F. (2013) Cardiovascular risk factors are associated with increased arterial stiffness in youth with type 1 diabetes: the SEARCH CVD study. *Diabetes Care*, **36**, 3938-3943.

Fu, S., Luo, L., Ye, P., Liu, Y., Zhu, B., Zheng, J., Bai, Y. & Bai, J. (2013) Overall and abdominal obesity indicators had different association with central arterial stiffness and hemodynamics independent of age, sex, blood pressure, glucose, and lipids in Chinese community-dwelling adults. *Clin. Interv. Aging*, **8**, 1579-1584.

Hjelmesaeth, J., Ueland, T., Flyvbjerg, A., Bollerslev, J., Leivestad, T., Jenssen, T., Hansen, T.K., Thiel, S., Sagedal, S., Røislien, J. & Hartmann, A. (2006) Early posttransplant serum osteoprotegerin levels predict long-term (8-year) patient survival and cardiovascular death in renal transplant patients. *J. Am. Soc. Nephrol.*, **17**, 1746-1754.

Karwowski, W., Naumnik, B., Szczepański, M. & Myśliwiec, M. (2012) The mechanism of vascular calcification: a systematic review. *Med. Sci. Monit.*, **18**, RA1-11.

Khoshdel, A.R. & Carney, S.L. (2008) Arterial stiffness in kidney transplant recipients: an overview of methodology and applications. *Urol. J.*, **5**, 3-14.

Kiechl, S., Schett, G., Wenning, G., Redlich, K., Oberhollenzer, M., Mayr, A., Santer, P., Smolen, J., Poewe, W. & Willeit, J. (2004) Osteoprotegerin is a risk factor for progressive atherosclerosis and cardiovascular disease. *Circulation*, **109**, 2175-

2180.
Lakatta, E.G. (2003) Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part III: cellular and molecular clues to heart and arterial aging. *Circulation*, **107**, 490-497.
Laurent, S. & Boutouyrie, P. (2007) Arterial stiffness: a new surrogate end point for cardiovascular disease? *J. Nephrol.*, **20** Suppl 12, S45-50.
Lentine, K.L., Costa, S.P., Weir, M.R., Robb, J.F., Fleisher, L.A., Kasiske, B.L., Carithers, R.L., Ragosta, M., Bolton, K., Auerbach, A.D. & Eagle, K.A.; American Heart Association Council on the Kidney in Cardiovascular Disease and Council on Peripheral Vascular Disease; American Heart Association; American College of Cardiology Foundation (2012) Cardiac disease evaluation and management among kidney and liver transplantation candidates: a scientific statement from the American Heart Association and the American College of Cardiology Foundation: endorsed by the American Society of Transplant Surgeons, American Society of Transplantation, and National Kidney Foundation. *Circulation*, **126**, 617-663.
Mazzaferro, S., Pasquali, M., Taggi, F., Baldinelli, M., Conte, C., Muci, M.L., Pirozzi, N., Carbone, I., Francone, M. & Pugliese, F. (2009) Progression of coronary artery calcification in renal transplantation and the role of secondary hyperparathyroidism and inflammation. *Clin. J. Am. Soc. Nephrol.*, **4**, 685-690.
Meneghini, M., Regalia, A., Alfieri, C., Barretta, F., Croci, D., Gandolfo, M.T., Vettoretti, S., Rastaldi, M.P. & Messa, P. (2013) Calcium and osteoprotegerin levels predict the progression of the abdominal aortic calcifications after kidney transplantation. *Transplantation*, **96**, 42-48.
Norton, G.R., Majane, O.H., Maseko, M.J., Libhaber, C., Redelinghuys, M., Kruger, D., Veller, M., Sareli, P. & Woodiwiss, A.J. (2012) Brachial blood pressure-independent relations between radial late systolic shoulder-derived aortic pressures and target organ changes. *Hypertension*, **59**, 885-892.
Persy, V. & D'Haese, P. (2009) Vascular calcification and bone disease: the calcification paradox. *Trends Mol. Med.*, **15**, 405-416.
Pirro, M., Manfredelli, M.R., Helou, R.S., Scarponi, A.M., Schillaci, G., Bagaglia, F., Melis, F. & Mannarino, E. (2012) Association of parathyroid hormone and 25-OH-vitamin D levels with arterial stiffness in postmenopausal women with vitamin D insufficiency. *J. Atheroscler. Thromb.*, **19**, 924-931.
Reference Values for Arterial Stiffness' Collaboration (2010) Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values'. *Eur. Heart J.*, **31**, 2338-2350.
Rodrigues, S.L., Baldo, M.P., Lani, L., Nogueira, L., Mill, J.G. & Sa Cunha, Rd. (2012) Body mass index is not independently associated with increased aortic stiffness in a Brazilian population. *Am. J. Hypertens.*, **25**, 1064-1069.
Rossi, S.H., McQuarrie, E.P., Miller, W.H., Mackenzie, R.M., Dymott, J.A., Moreno, M.U., Taurino, C., Miller, A.M., Neisius, U., Berg, G.A., Valuckiene, Z., Hannay, J.A., Dominiczak, A.F. & Delles, C. (2013) Impaired renal function impacts negatively on vascular stiffness in patients with coronary artery disease. *BMC Nephrol.*, **14**, 173.
Safar, M.E., Balkau, B., Lange, C., Protogerou, A.D., Czernichow, S., Blacher, J., Levy, B.I. & Smulyan, H. (2013) Hypertension and vascular dynamics in men and women with metabolic syndrome. *J. Am. Coll. Cardiol.*, **61**, 12-19.
Schillaci, G., Pirro, M., Mannarino, M.R., Pucci, G., Savarese, G., Franklin, S.S. & Mannarino, E. (2006) Relation between renal function within the normal range and central and peripheral arterial stiffness in hypertension. *Hypertension*, **48**, 616-621.
Speer, G., Fekete, B.C., El Hadj Othmane, T., Szabó, T., Egresits, J., Fodor, E., Kiss, I., Logan, A.G., Nemcsik, J., Szabó, A., Németh, Z.K., Szathmári, M. & Tislér, A. (2008) Serum osteoprotegerin level, carotid-femoral pulse wave velocity and cardiovascular survival in haemodialysis patients. *Nephrol. Dial. Transplant.*, **23**, 3256-3262.
Stehouwer, C.D., Henry, R.M. & Ferreira, I. (2008) Arterial stiffness in diabetes and the metabolic syndrome: a pathway to cardiovascular disease. *Diabetologia*, **51**, 527-539.
Svensson, M., Dahle, D.O., Mjøen, G., Weihrauch, G., Scharnagl, H., Dobnig, H., März, W., Jardine, A., Fellström, B. & Holdaas, H. (2012) Osteoprotegerin as a predictor of renal and cardiovascular outcomes in renal transplant recipients: follow-up data from the ALERT study. *Nephrol. Dial. Transplant.*, **27**, 2571-2575.
Tomiyama, H. & Yamashina, A. (2010) Non-invasive vascular function tests: their pathophysiological background and clinical application. *Circ. J.*, **74**, 24-33.
Tomlinson, L.A. (2012) Methods for assessing arterial stiffness: technical considerations. *Curr. Opin. Nephrol. Hypertens.*, **21**, 655-660.
Van Campenhout, A. & Golledge, J. (2009) Osteoprotegerin, vascular calcification and atherosclerosis. *Atherosclerosis*, **204**, 321-329.
Venuraju, S.M., Yerramasu, A., Corder, R. & Lahiri, A. (2010) Osteoprotegerin as a predictor of coronary artery disease and cardiovascular mortality and morbidity. *J. Am. Coll. Cardiol.*, **55**, 2049-2061.
Verbeke, F., Maréchal, C., Van Laecke, S., Van Biesen, W., Devuyst, O., Van Bortel, L.M., Jadoul, M. & Vanholder, R. (2011) Aortic stiffness and central wave reflections predict outcome in renal transplant recipients. *Hypertension*, **58**, 833-838.
Vlachopoulos, C., Aznaouridis, K. & Stefanadis, C. (2010) Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *J. Am. Coll. Cardiol.*, **55**, 1318-1327.
Wang, J.H., Lee, C.J., Chen, M.L., Yang, C.F., Chen, Y.C. & Hsu, B.G. (2014) Association of serum osteoprotegerin levels with carotid-femoral pulse wave velocity in hypertensive patients. *J. Clin. Hypertens.* (*Greenwich*), **16**, 301-308.