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Original Article

Low serum-free oxygen radicals defense level is associated with peripheral arterial stiffness in kidney transplantation patients

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Abstract: Oxidative stress is a causative mechanism of vascular alterations resulting in arterial stiffness. The aim of this study was to evaluate the relationship between oxidative stress and arterial stiffness among kidney transplantation (KT) patients. Fasting blood samples were obtained from 70 KT patients. Arterial stiffness was measured by brachial-ankle pulse wave velocity. Oxidative stress was measured by free oxygen radicals testing (FORT) and free oxygen radicals defense (FORD). We found that diabetes ($P = 0.005$), hypertension ($P = 0.001$), metabolic syndrome ($P = 0.029$), age ($P = 0.007$), KT duration ($P = 0.007$), waist circumference ($P = 0.039$), systolic blood pressure ($P < 0.001$), diastolic blood pressure ($P = 0.004$), pulse pressure ($P = 0.001$), triglycerides ($P = 0.049$), fasting glucose ($P = 0.003$), insulin ($P = 0.011$), homeostasis model assessment of insulin resistance (HOMA-IR, $P = 0.002$), and FORT ($P = 0.014$) were all higher in the high arterial stiffness group, while HDL-C ($P = 0.004$) and FORD ($P = 0.009$) were lower. Multivariate logistic regression analysis of all significant variables showed that FORD level (OR: 0.849, 95% CI: 0.740-0.973, $P = 0.019$) is inversely associated with arterial stiffness. Logarithmically transformed HOMA-IR ($\beta = -0.280$, $P = 0.019$) was independently associated with FORD levels in KT patients. Our study showed that KT patients with higher serum FORT level and lower FORD level had high arterial stiffness. Low serum FORD level is an independent predictor of peripheral arterial stiffness in KT patients. Among these KT patients, logarithmically transformed HOMA-IR is negatively associated with serum FORD level.

Keywords: Kidney transplantation, free oxygen radicals testing, free oxygen radicals defense, arterial stiffness, brachial-ankle pulse wave velocity

Introduction

Arterial stiffness represents vascular damage and is an independent predictor of cardiovascular disease [1]. Cardiovascular disease is also the leading cause of death in kidney transplant (KT) patients [2, 3]. Pulse wave velocity (PWV) reflects segmental arterial elasticity and is one of the noninvasive methods currently used to assess arterial stiffness [4]. Peripheral arterial stiffness is defined by measured PWV from brachial to ankle regions (baPWV) [4]. Evaluation of arterial stiffness with baPWV can be used as one of the screening tests for predicting cardiovascular disease in KT patients [5, 6].

Endothelial cells regulate several arterial properties, including arterial vascular tone, permeability, angiogenesis, and vascular inflammatory response [7]. Arterial stiffness is associated with the increased activity of angiotensin II, which results in increased NADPH oxidase activity, reduced nitric oxide bioavailability, and increased production of reactive oxygen species [8]. Excessive production of reactive oxygen species and reactive nitrogen species leads to oxidative the modification of proteins, DNA, and lipids, which accumulate in cells leading to impaired cellular and vascular function [9, 10]. Thus, oxidative stress can be associated with endothelial dysfunction and may be a cause of arterial stiffness [11]. The aim of the present

study was to determine the relationship between serum oxidative stress and peripheral arterial stiffness among KT patients.

Materials and methods

Patients

This was a prospective, cross-section study conducted at a medical center in Hualien, Taiwan from May to August 2013 where 70 KT patients were enrolled. The study was approved by the Protection of Human Subjects Institutional Review Board of Tzu-Chi University and Hospital and is consistent with the Declaration of Helsinki. Patients were excluded if they had any acute infection, malignancy, acute rejection, acute myocardial infarction, or pulmonary edema at the time of blood sampling or if they had an arterial-venous shunt or graft in either hand, used antioxidants such as vitamin supplements or fish oil capsules, or if they refused to give informed consent for the study.

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 the iliac crest. Body mass index was calculated as weight (kilograms) divided by height squared (meters) [12-15]. Bioimpedance measurements of fat mass were performed at the bedside according to the standard tetrapolar whole body (hand-foot) technique, using a single-frequency (50-kHz) analyzer (Biodynamic-450, Biodynamics Corporation, Seattle, USA). Measurements were carried out by the same operator [16].

Biochemical determinations

Fasting blood samples of approximately 5 ml were immediately centrifuged at 3,000 *g* for 10 min after collection. Serum samples were stored at 4°C and used for biochemical analyses within 1 h of collection. Serum levels of blood urea nitrogen (BUN), creatinine (Cre), fasting glucose, total cholesterol (TCH), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), total calcium, and phosphorus were measured using an autoanalyzer (COBAS Integra 800, Roche Diagnostics, Basel, Switzerland). Serum insulin (Labor Diagnostika Nord immunoassays, Nordhorn, Germany) and intact parathyroid hormone (iPTH) (Diagnostic Systems Laboratories, Texas, USA) concentrations were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) [12-15]. Insulin resistance was evaluated using a calculated homeostasis model assessment of insulin resistance (HOMA-IR) as follows: HOMA-IR = fasting plasma glucose (mg/dl) × fasting serum insulin (μU/ml)/405 [16]. The estimate glomerular filtration rate (GFR) calculation in this study was used Modification of Diet in Renal Disease (MDRD) equation.

FORT and FORD determination

FORM plus apparatus (Callegari, Parma, Italy) was used for measuring the total antioxidant capacity and the amount of free radicals. Free oxygen radicals testing (FORT) and free oxygen radicals defense (FORD) tests were used for determination of oxidative stress in human blood. The FORT test principle is based on the fact that transition metals such as iron can catalyze the formation of free radicals in the presence of hydroperoxides according to the Fenton reaction. These free radicals are then trapped by an amine derivative that changes color, detectable at 505 nm. The intensity of the color correlates directly to the amount of radicals in the solution. The principle of the FORD test is the use of free radicals that are formed from reagents before adding of the blood sample and the change of the absorbance of light passing through the sample according to the Lambert-Beer law. This absorbance is proportional to the concentration of antioxidants in the added blood sample. In the presence of an acid buffer (pH 5.2) and an oxidant ($FeCl_3$), the chromogen (amine derivative) forms a stable colored compound (cation), which is detected at a 505 nm wavelength. Antioxidant compounds reduce the cation causing discoloration of the solution. The FORD test results of antioxidant concentration in the sample are given in the equivalent concentration of trolox, which is a water-soluble analog of vitamin E [17].

Metabolic syndrome and its components

The prevalence of metabolic syndrome was defined using the International Diabetes Federation definition [18]. Patients enrolled into

Table 1. Clinical variables of the 70 kidney transplantation patients with or without arterial stiffness

Items	All patients (*n* = 70)	Low arterial stiffness group (*n* = 36)	High arterial stiffness group (*n* = 34)	*P* value
Age (years)[a]	52.00 ± 9.53	49.08 ± 9.13	55.09 ± 9.07	0.007*
KT duration (months)[a]	70.99 ± 43.30	57.58 ± 28.36	85.18 ± 51.60	0.007*
Height (cm)[a]	162.57 ± 8.30	162.75 ± 8.84	162.38 ± 7.82	0.855
Body weight (kg)[a]	63.27 ± 12.41	61.86 ± 10.52	64.76 ± 14.15	0.331
Waist circumference (cm)[a]	85.46 ± 11.50	82.86 ± 9.98	88.50 ± 12.42	0.039*
Body mass index (kg/m^2)[a]	23.89 ± 4.21	23.41 ± 4.02	24.40 ± 4.40	0.330
Body fat mass (%)[a]	30.00 ± 4.35	29.66 ± 4.29	30.35 ± 4.45	0.510
Left-baPWV (m/s)[a]	13.96 ± 2.58	12.18 ± 1.35	15.79 ± 2.26	< 0.001*
Right-baPWV (m/s)[a]	14.17 ± 2.74	12.49 ± 1.59	15.95 ± 2.58	< 0.001*
Systolic blood pressure (mmHg)[a]	138.47 ± 16.79	129.86 ± 12.09	147.59 ± 16.36	< 0.001*
Diastolic blood pressure (mmHg)[a]	85.87 ± 10.97	82.25 ± 8.90	89.71 ± 11.74	0.004*
Pulse pressure (mmHg)[a]	52.60 ± 13.06	47.61 ± 8.94	57.88 ± 14.69	0.001*
Total cholesterol (mg/dl)[a]	197.76 ± 46.72	187.70 ± 40.25	208.41 ± 51.17	0.063
Triglyceride (mg/dl)[b]	151.06 ± 114.11	123.69 ± 77.91	180.03 ± 138.23	0.049*
HDL-C (mg/dl)[b]	51.41 ± 16.17	55.22 ± 14.93	47.38 ± 16.66	0.004*
LDL-C (mg/dl)[a]	110.66 ± 37.71	105.33 ± 27.56	116.29 ± 45.86	0.227
Fasting glucose (mg/dl)[b]	111.13 ± 46.48	93.50 ± 16.11	129.79 ± 59.533	0.003*
Blood urea nitrogen (mg/dl)[b]	26.14 ± 14.41	25.33 ± 17.20	27.00 ± 10.92	0.115
Creatinine (mg/dl)[b]	1.74 ± 0.78	1.68 ± 0.85	1.79 ± 0.70	0.147
Glomerular filtration rate (ml/min)[a]	47.10 ± 19.39	49.08 ± 21.81	44.18 ± 16.26	0.223
Total calcium (mg/dl)[a]	9.19 ± 1.03	9.23 ± 0.84	9.14 ± 1.22	0.719
Phosphorus (mg/dl)[a]	3.40 ± 0.86	3.32 ± 0.87	3.48 ± 0.85	0.456
Calcium-phosphorous product (mg^2/dl^2)[a]	30.87 ± 6.66	30.42 ± 6.96	31.34 ± 6.41	0.719
iPTH (pg/ml)[b]	136.35 ± 116.82	146.54 ± 133.13	125.56 ± 97.50	0.698
Insulin (uIU/ml)[a]	9.16 ± 5.26	7.62 ± 3.53	10.79 ± 6.27	0.011*
HOMA-IR[b]	2.67 ± 2.25	1.76 ± 0.9	3.63 ± 2.80	0.002*
FORT (mmol/L H_2O_2)[a]	13.66 ± 5.06	12.23 ± 4.38	15.17 ± 5.35	0.014*
FORD (mmol/L trolox)[a]	0.56 ± 0.49	0.71 ± 0.59	0.40 ± 0.30	0.009*

Data are expressed as mean ± SDs. [a]Data were tested using the Student's *t*-test. [b]Data were tested using the Mann-Whitney *U* test. baPWV, brachial-ankle pulse wave velocity; FORD, free oxygen radicals defense; FORT, free oxygen radicals testing; HDL-C, high density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; iPTH, intact parathyroid hormone; LDL-C, low density lipoprotein cholesterol; KT, kidney transplantation. *$P < 0.05$ by Student's *t*-test or Mann-Whitney *U* test.

the study were classified as having metabolic syndrome 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, TG ≥ 150 mg/dl, and 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 classification. Type 2 diabetes was determined according to World Health Organization criteria [19]. Briefly, a patient 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 or she was using diabetes medications (oral or insulin).

Blood pressure and brachial-ankle pulse wave velocity measurements

After blood sampling, patients were immediately asked for baPWV measurements on the same day. Measurements were performed in a quiet, temperature-controlled room after 10 min at rest, with the patients in a supine position, according to the recommendations for user procedures of clinical applications of arterial stiffness. Blood pressure and heart rate (taken as the mean of three individual readings) were measured with an automatic upper-arm oscillometric device. Pulse pressure was calculated by subtracting diastolic blood pressure (DBP) from systolic blood pressure (SBP). The baPWV was measured in the right or left

Table 2. Baseline characteristics of the 70 kidney transplantation patients with or without arterial stiffness

Characteristic		Low arterial stiffness group (%)	High arterial stiffness group (%)	*P* value
Gender	Male	16 (44.4)	23 (67.6)	0.051
	Female	20 (55.6)	11 (32.4)	
Diabetes	No	31 (86.1)	19 (55.9)	0.005*
	Yes	5 (13.9)	15 (44.1)	
Hypertension	No	26 (72.2)	11 (32.4)	0.001*
	Yes	10 (27.8)	23 (67.6)	
Metabolic syndrome	No	28 (77.8)	18 (52.9)	0.029*
	Yes	8 (22.2)	16 (47.1)	
Transplantation model	Cadaveric	34 (94.4)	28 (82.4)	0.112
	Living	2 (5.6)	6 (17.6)	
Tacrolimus use	No	12 (33.3)	18 (52.9)	0.098
	Yes	24 (66.7)	16 (47.1)	
Mycophenolate mofetil or mycophenolic acid use	No	7 (19.4)	13 (38.2)	0.082
	Yes	29 (80.6)	21 (61.8)	
Steroid use	No	5 (13.9)	7 (20.6)	0.457
	Yes	31 (86.1)	27 (79.4)	
Rapamycin use	No	31 (86.1)	25 (73.5)	0.188
	Yes	5 (13.9)	9 (26.5)	
Cyclosporine use	No	29 (80.6)	24 (70.6)	0.331
	Yes	7 (19.4)	10 (29.4)	

Data are expressed as number of patients. Analysis performed using the chi-square test (*$P < 0.05$).

brachial artery to the ankle segments using an automatic pulse wave analyzer (VaSera VS-1000, Fukuda Denshi Co. Ltd., Tokyo, Japan) [12-14]. In brief, cuffs were applied to the extremities of the four limbs, and electrocardiographic electrodes were attached to the upper arm. A microphone was placed on the sternal angle for phonocardiography. The subjects then rested in a supine position for 5 min. The baPWV was calculated by dividing the distance from the aortic valve to the ankle artery by the sum of the difference between the time the pulse waves were transmitted to the brachium and the time the same waves were transmitted to the ankle and the time difference between the second heart sound on the phonocardiogram and the notch of the brachial pulse wave. To minimize cuff inflation effects on blood flow dynamics, pulse waves were measured with the cuffs inflated to less than the DBP (50 mmHg). The extremity blood pressure was then measured by oscillometry. SBP and DBP were obtained by measuring the blood pressure at the right brachial artery [12-14]. In this study, left or right baPWV values of > 14.0 m/s were used to define the high arterial stiffness group [12-14].

Statistical analysis

Data were expressed as the mean ± standard deviation (SD) and were tested for normal distribution using the Kolmogorov-Smirnov test. Comparisons between patients 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 with non-normal distribution (TG, fasting glucose, HDL-C, BUN, Cre, iPTH, HOMA-IR). Data expressed as the number of patients were analyzed by the chi-square test. Variables that were significantly associated with arterial stiffness in the KT patients were tested for independence by multivariate logistic regression analysis. Because TG, HDL-C, fasting glucose, BUN, Cre, iPTH, and HOMA-IR were not normally distributed and underwent base 10 logarithmic transformations to achieve normality. Clinical variables that correlated with serum FORD levels in KT patients were evaluated using univariate linear regression analysis. Variables that were significantly associated with FORD in KT patients were tested for independency in multivariate forward stepwise regression analysis. Data were analyzed using SPSS for Windows

Table 3. Odds ratio for arterial stiffness by multivariate logistic regression analysis of FORD among the 70 kidney transplantation patients

FORD (mmol/L trolox)	Unadjusted		Model 1		Model 2		Model 3	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Per 0.1 mmol/L trolox increase	0.849 (0.740-0.973)	0.019*	0.821 (0.702-0.960)	0.013*	0.804 (0.671-0.964)	0.019*	0.671 (0.456-0.987)	0.043*

Model 1 is adjusted for age, diabetes mellitus, FORT, and kidney transplantation duration. Model 2 is adjusted for the Model 1 variables and for systolic blood pressure, diastolic blood pressure, and hypertension. Model 3 is adjusted for the Model 2 variables and for waist circumference, fasting glucose, triglyceride, high-density lipoprotein-cholesterol, and metabolic syndrome. *$P < 0.05$ by multivariate logistic regression analysis. CI, confidence interval; FORD, free oxygen radicals defense; FORT, free oxygen radicals testing; OR, odds ratio.

Table 4. Correlation between FORD levels and variables among the 70 kidney transplantation patients

Variables	Univariate	
	β coefficient	*P* value
Age (years)	-0.161	0.182
KT duration (months)	-0.077	0.524
Height (cm)	0.031	0.802
Body weight (kg)	0.024	0.841
Waist circumference (cm)	0.065	0.593
Body mass index (kg/m^2)	0.021	0.862
Body fat mass (%)	-0.183	0.128
Systolic blood pressure (mmHg)	-0.090	0.461
Diastolic blood pressure (mmHg)	-0.034	0.777
Pulse pressure (mmHg)	-0.086	0.477
Total cholesterol (mg/dl)	-0.002	0.990
Log-Triglyceride (mg/dl)	0.106	0.381
Log-HDL-C (mg/dl)	0.104	0.393
Low density lipoprotein cholesterol (mg/dl)	0.086	0.478
Log-Glucose (mg/dl)	-0.211	0.080
Log-BUN (mg/dl)	-0.085	0.486
Log-Creatinine (mg/dl)	-0.057	0.637
Glomerular filtration rate (ml/min)	0.026	0.832
Total calcium (mg/dl)	0.007	0.953
Phosphorus (mg/dl)	0.089	0.462
Calcium-phosphorous product (mg^2/dl^2)	0.059	0.628
Log-iPTH (pg/ml)	0.152	0.208
Insulin (uIU/ml)	-0.239	0.046*
Log-HOMA-IR	-0.280	0.019*
FORT (mmol/L H_2O_2)	-0.020	0.872

Data of triglyceride, HDL-C, glucose, BUN, creatinine, iPTH, and HOMA-IR levels showed skewed distribution and were therefore log-transformed before analysis. *$P < 0.05$ was considered statistically significant in the univariate linear regression analyses. BUN, Blood urea nitrogen; FORD, free oxygen radicals defense; FORT, free oxygen radicals testing; iPTH, intact parathyroid hormone; HDL-C, high density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance.

(version 19.0; SPSS Inc., Chicago, IL, USA). A *P*-value < 0.05 was considered statistically significant.

Results

Relationships between clinical and biological characteristics and antioxidant capacity with peripheral arterial stiffness in KT patients

The clinical characteristics of the KT patients with high arterial stiffness compared with patients with low arterial stiffness are presented in **Table 1**. Thirty-four patients (48.6%) were defined as having a high arterial stiffness. The age ($P = 0.007$), KT duration ($P = 0.007$), waist circumference ($P = 0.039$), SBP ($P < 0.001$), DBP ($P = 0.004$), pulse pressure ($P = 0.001$), TG ($P = 0.049$), fasting glucose ($P = 0.003$), insulin ($P = 0.011$), HOMA-IR ($P = 0.002$), and FORT level ($P = 0.014$) were each higher in the high arterial stiffness group compared with the low arterial stiffness group. HDL-C ($P = 0.004$) and FORD ($P = 0.009$) were lower in patients with high arterial stiffness compared with the low arterial stiffness group.

Comorbidity and immunosuppressive drugs for the KT patients

Clinical characteristics and immunosuppressive drugs used are presented in **Table 2**. Comorbid conditions included diabetes ($n = 20$; 28.6%) and hypertension ($n = 33$; 47.1%). Prescribed therapeutic agents included tacrolimus ($n = 40$; 57.1%), mycophenolate mofetil or mycophenolic acid ($n = 50$; 71.4%), steroids ($n = 58$; 82.9%), rapamycin ($n = 14$; 20.0%), and cyclosporine ($n = 17$; 24.3%). Diabetes ($P = 0.005$), hypertension ($P = 0.001$), and KT patients with

metabolic syndrome (P = 0.029) were more frequent in patients with high arterial stiffness compared with those with low arterial stiffness. There were no statistical differences in gender, transplantation model, or in the use of tacrolimus, mycophenolate mofetil or mycophenolic acid, steroids, rapamycin, or cyclosporine medications between the two patient groups.

FORD is independently associated with peripheral arterial stiffness

The unadjusted and multivariate logistic regression analysis of the factors significantly associated with peripheral arterial stiffness with serum FORD levels is presented in **Table 3**. The unadjusted serum FORD levels with peripheral arterial stiffness showed that FORD increased per 0.1 mmol/L trolox [odds ratio (OR): 0.849, 95% confidence interval (CI): 0.740-0.973, P = 0.019] decreased the 15.1% risk of peripheral arterial stiffness in KT patients. Multivariate logistic regression analysis adjusted for age, diabetes mellitus, FORT, and KT duration revealed a 17.9% decrease in the risk of peripheral arterial stiffness (adjusted OR 0.821, 95% CI: 0.702-0.960, P = 0.013) for every 0.1 mmol/L trolox increase in FORD (Model 1). After multivariate logistic regression analysis with Model 1 added with SBP, DBP, and hypertension, a decreased 19.6% risk of the peripheral arterial stiffness (adjusted OR 0.804, 95% CI 0.671-0.964, P = 0.019) was observed for every 0.1 mmol/L trolox increase in FORD (Model 2). Multivariate logistic regression analysis using Model 2 with added metabolic factors (waist circumference, fasting glucose, TG, HDL-C, and metabolic syndrome) also revealed a decreased 32.9% risk of peripheral arterial stiffness (adjusted OR 0.671, 95% CI: 0.456-0.987, P = 0.043) for every 0.1 mmol/L trolox increase in FORD (Model 3). Each of these analyses confirmed that serum FORD level is inversely associated with peripheral arterial stiffness in KT patients.

Correlation between serum FORD levels and clinical and biochemical parameters in KT patients

The univariable linear regression analysis of FORD levels with variables in KT patients is presented in **Table 4**. Serum insulin (r: -0.239; P = 0.046) and log-HOMA-IR (r: -0.280; P = 0.019) were negatively correlated with FORD levels in KT patients. Multivariable forward stepwise linear regression analysis of significant variables (insulin and log-HOMA-IR) showed that log-HOMA-IR (β = -0.280, P = 0.019) was independently associated with FORD levels in KT patients (data not shown).

Discussion

The results of our study show that oxidative stress, as indicated by the serum FORT level, is higher and the FORD level is lower in KT patients with high arterial stiffness. KT patients in high arterial stiffness group also noted had higher serum insulin level, HOMR-IR level, and metabolic syndrome than in low arterial stiffness group. Multivariate logistic regression analysis revealed a lower serum FORD level to be an independent predictor of peripheral arterial stiffness in KT patients. Among these KT patients, log-HOMA-IR is negatively associated with serum FORD level.

Metabolic syndrome promotes arterial stiffening and accelerates vascular aging and development of hypertension in humans. Hypertension, hyperglycemia, and dyslipidemia cause vascular endothelial dysfunction and oxidative stress, which activates extracellular matrix metalloproteinases leading to vascular remodeling and arterial stiffness [20]. Furthermore, obesity, insulin resistance, hypertension, dyslipidemia, and type 2 diabetes mellitus are associated with higher prevalence of arterial stiffness and higher risk of cardiovascular events [21]. A previous study noted that baPWV was significantly higher in subjects with metabolic syndrome than in those without metabolic syndrome in a south Chinese population undergoing a health examination [22]. Arterial stiffness has been found to be independently associated with insulin resistance as measured by HOMA-IR in Taiwanese middle-aged adults and also noted baPWV to be associated with waist circumference, SBP, DBP, fasting glucose, and TG [23]. In the present study, diabetes, hypertension, and KT patients with metabolic syndrome were more frequent in the high arterial stiffness group compared with the low arterial stiffness group. Furthermore, metabolic syndrome and its associated hallmarks such as waist circumference, fasting glucose, TG, SBP, and DBP were higher, while serum HDL-C level was lower, in the high arterial stiffness group compared with low arterial stiffness patients in this study. In addition, high TCH level was

observed in high arterial stiffness patients. Our results also noted a higher serum insulin level and HOMA-IR in high arterial stiffness group among KT patients.

Aging of the arterial system is accompanied by progressive structural changes consisting of fragmentation, calcification, and degeneration of elastin; increases in collagen in arterial wall; induces endothelium damage; dilates of the arteries; and increases arterial stiffness [7]. Stiffening of the large conduit arteries results in increased pulse pressure and PWV. As arterial wall stiffness increases, the central SBP increases and DBP decreases, leading to increased pulse pressure, an independent risk factor for future cardiovascular events [10]. Our study showed that age and pulse pressure was greater in the high arterial stiffness group compared with low arterial stiffness patients. A positive correlation was found between increased the KT duration and increased PWV in KT patients [24]. Increased KT duration was also noted in the high arterial stiffness group in our study.

Vascular endothelial cells are important regulators of vascular health and atherogenesis [7]. Endothelial dysfunction is a potential mechanism linked to arterial stiffness [25] and is associated with vascular damages such as vasomotor tone alterations, thrombotic dysfunction, smooth muscle cell proliferation and migration, leukocyte adhesion, progression of atherosclerotic plaques, and subsequent atherosclerotic complications [26]. Another mechanism responsible for age-related endothelial dysfunction is thought to be reduced bioavailability of the endothelium derived relaxing factor, nitric oxide, due to its interaction with reactive oxygen species to form peroxynitrite [10]. Advanced glycation end products are also known to impact endothelial function by quenching nitric oxide and enhancing the generation of reactive oxygen species [27]. Telomere dysfunction and vascular senescence are related to enhanced levels of reactive oxygen species, decreased nitric oxide, and increased proinflammatory molecules [28]. A recent study noted that carotid-femoral PWV is independently and positively associated with age and HOMA-IR and negatively associated with leukocyte telomere length in adults free of cardiovascular diseases [29]. Shoskes et al. found lower trolox equivalent antioxidant capacity levels (TEAC, a marker of oxidative function) in kidney donor's urine that was developed delayed graft function in KT patients [30]. Dalfino et al. found arterial stiffness was inversely correlated with 8-hydroxy deoxyguanosine (8-OHdG, a marker of oxidant stress) in chronic kidney disease patients [31]. In our study in KT patients, serum FORT levels were higher, while serum FORD levels were lower in the high arterial stiffness group compared with the low arterial stiffness group. Furthermore, serum FORD level was an independent predictor and negatively associated with peripheral arterial stiffness among the KT patients.

High levels of reactive oxygen species generated by hypertrophied adipocytes play a significant role in the development of insulin resistance, besides decreasing insulin sensitivity of metabolic organs, promoting inflammation, altering lipid metabolism, and inducing endothelial dysfunction [32]. Adipokines, including leptin, adiponectin, visfatin, resistin, apelin, and plasminogen activator inhibitor type 1, are implicated in physiological and pathological processes involving oxidative stress [33]. Exposure of adipocytes to high levels of reactive oxygen species suppresses adiponectin expression and secretion [34]. In addition, we have previously reported that hyperlipidemia or hypoadiponectinemia correlates with peripheral arterial stiffness in KT patients [13, 14]. In the present study, log-HOMA-IR was seen to be negatively associated with serum FORD levels on multivariable forward stepwise linear regression analysis. Further studies are therefore required to elucidate the relationship between oxidative stress and insulin resistance in KT patients with arterial stiffness.

There are some limitations to the current study. First, this study had a cross-sectional design. Therefore, our findings should be investigated in long-term prospective studies before a causal relationship between serum oxidative stress and peripheral arterial stiffness in KT patients can be established. Second, pharmacologic interventions may influence PWV and oxidant stress in KT patients. Cyclosporine-free regimen based on sirolimus reduces carotid-femoral PWV, plasma endothelin-1, and oxidative stress in KT patients [35]. However, another study did not find a relationship between cyclo-

sporine and carotid-femoral PWV in KT patients [36]. In our study, there was no statistical difference in the use of immunosuppressive drugs in the high arterial stiffness group compared with the low arterial stiffness group. Moreover, some antihypertension drugs or statins may influence PWV [37]. In this study, we did not evaluate the relationship among antihypertension drugs or statins used and baPWV levels in KT patients. Further studies are therefore required to elucidate the relationship between medication and baPWV levels in this patient population.

In summary, we show an independent and inverse association of FORD levels with peripheral arterial stiffness in KT patients.

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Disclosure of conflict of interest

None.

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