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Higher Serum Levels of Osteoglycin Are Associated with All-Cause Mortality and Cardiovascular and Cerebrovascular Events in Patients with Advanced Chronic Kidney Disease

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Patients with chronic kidney disease (CKD) have markedly increased rates of major adverse cardiovascular and cerebrovascular events (MACCEs) and mortality. Therefore, identifying early biomarkers predicting clinical outcomes in patients with CKD is critical. We aimed to determine whether osteoglycin, a basic component of the vascular extracellular matrix, was associated with MACCEs or all-cause mortality, using data from a prospective randomized controlled study, K-STAR (Kremezin STudy Against Renal disease progression in Korea: NCT 00860431). A total of 383 patients (mean age: 56.4 years, men/women = 252/131) with CKD stage 3 to 4 from the original trial were enrolled in the present study. We measured serum osteoglycin level and examined the impact of osteoglycin on clinical outcomes. The mean value of osteoglycin levels was 13.3 ± 9.4 ng/mL (healthy control: 5.3 ± 2.1 ng/mL). In multivariable analysis, lower levels of proteinuria and hemoglobin and higher levels of C-reactive protein were significantly associated with higher osteoglycin levels. Estimated glomerular filtration rate was not related to osteoglycin level. During a mean follow-up period of 56 months, 25 deaths, 61 MACCEs, and 76 composite outcomes (all-cause mortality or MACCEs) occurred. In the non-diabetic group, each 1-ng/mL increase in serum osteoglycin was associated with all-cause mortality and composite outcome (hazard ratio [HR] = 1.058, P = 0.031; HR = 1.041, P = 0.036). However, osteoglycin levels were not associated with mortality, MACCEs, or composite outcome in the diabetic group. Our results indicate that serum osteoglycin is a potential predictor of adverse outcomes in patients with CKD.

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Introduction

Patients with chronic kidney disease (CKD) have markedly increased rates of cardiovascular (Foley et al. 1995; Lai et al. 2015) and cerebrovascular disease (Dad and Weiner 2015) and mortality even before reaching end-stage renal disease (ESRD). Therefore, it is critical to identify early biomarkers predicting major adverse cardiovascular and cerebrovascular events (MACCEs) or survival in patients with CKD.

Osteoglycin (OGN) is a bone-associated glycoprotein (Bentz et al. 1990) and a basic component of the vascular extracellular matrix (Shanahan et al. 1997; Cheng et al. 2014). OGN is a class III member of the small leucine-rich proteoglycan, a distinct group of extracellular proteoglycans that may affect matrix integrity (Iozzo 1999; Van Aelst et al. 2015). Recently, OGN has been shown to be expressed by vulnerable hemorrhagic coronary atherosclerotic plaques, and may predict a MACCE within 1 year after coronary angiography (Cheng et al. 2014). In patients with heart failure, OGN is a biomarker for ischemic heart failure, survival, and cardiac remodeling (Eurlings et al. 2012; Van Aelst et al. 2015). However, OGN has not yet been investigated as a prognostic biomarker of a MACCE or mortality in patients with CKD not undergoing dialysis.

Therefore, we hypothesized that OGN might predict a MACCE or mortality in patients with advanced CKD. We performed the current study to identify whether OGN was associated with MACCEs or all-cause mortality, using data from a prospective randomized controlled study, K-STAR (Kremezin STudy Against Renal disease progression in Korea) (Cha et al. 2016).

Materials and Methods

Study population

K-STAR, a multicenter, prospective, randomized clinical trial, demonstrated that long-term use of AST-120 added to standard treatment did not alter renal disease progression in patients with advanced CKD (Clinicaltrials.gov: NCT 00860431). The detailed study protocol is described elsewhere (Cha et al. 2016). In brief, the study participants with advanced CKD were recruited from March 2009 to August 2010 and underwent follow-up for 36 months. Inclusion criteria for the original study included: age ≥ 18 years; estimated glomerular filtration rate (eGFR) by the Cockcroft–Gault equation of 15-59 mL/min/1.73 m^2 and serum creatinine (SCr) of 2.0-5.0 mg/dL, with a measured or expected eGFR decline of ≥ 2.5 mL/min/1.73 m^2 for 6 months or ≥ 5 mL/min/1.73 m^2 for 12 months (SCr measured two or more times at intervals of ≥ 4 weeks); and controlled blood pressure (BP) (systolic BP ≤ 160 mmHg, diastolic BP ≤ 100 mmHg) (Table 1). The study was approved by the institutional review boards (IRBs) of the participating centers and conducted in accordance with the 2008 Declaration of Helsinki and good clinical practice guidelines.

Of the 579 participants from the K-STAR study, 383 (66.1%) who had available data for baseline OGN were included in the present analyses (Fig. 1). In addition to the original data, we retrospectively collected data on survival status and occurrence of MACCEs as of July 2015 through review of medical records. The retrospective expansion of the follow-up duration was also approved by the IRBs of the participating centers: Seoul National University Bundang Hospital (B-0812/066-006), Seoul National University Hospital

Table 1. Inclusion and exclusion criteria of K-STAR study.

Inclusion Criteria	Exclusion Criteria
Followed over 6 months by nephrologists	Using ketosteril or AST-120 with the last 2 months
Chronic kidney disease 3 or 4 eGFR by Cockcroft-Gault equation of 15-59 mL/min/1.73 m^2 serum Cr 2.0-5.0 mg/dL	Gastrointestinal disease active ulcer or inflammatory bowel disease
Measured or expected eGFR decline of ≥ 2.5 mL/1.73 m^2 over 6 months or ≥ 5 mL/1.73 m^2 over 12 months	Obstructive uropathy or reversible kidney disease Autosomal polycystic kidney disease Proteinuria ≥ 10 g/d History of kidney transplantation
Controlled blood pressure SBP ≤ 160 mmHg and DBP ≤ 100 mmHg Measured three or more times at intervals of 4 weeks	Heart failure (New York Heart Association classes 3 and 4) Uncontrolled arrhythmia Acute coronary syndrome Cerebral infarction, hemorrhagic infarction within 6 months
No significant changes in CKD treatment	Active infection or uncontrolled inflammatory disease Liver cirrhosis (Child-turcotte Pugh B or C) Progressive malignancy Uncontrolled blood sugar (HbA1C > 10%), Hb < 7.0 g/dL Life expectancy < 12 months Pregnancy, lactating women, planning to pregnancy

eGFR, estimated glomerular filtration rate; Cr, creatinine; SBP, systolic blood pressure; DBP, diastolic blood pressure; CKD, chronic kidney disease; HbA1C, hemoglobin A1.

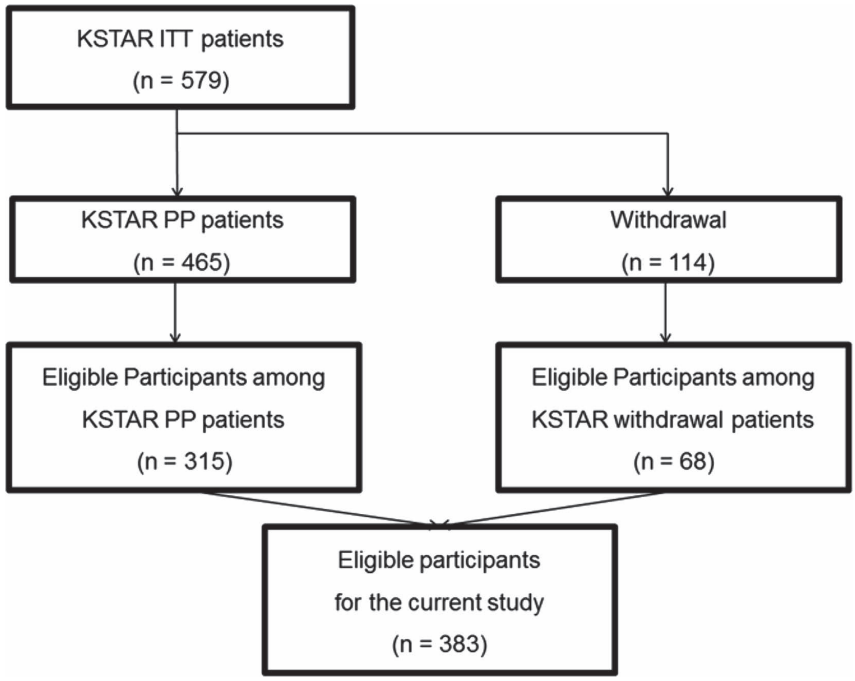

Fig. 1. Study population algorithm.
KSTAR, Kremezin STudy Against Renal disease progression in Korea; ITT, intention to treat; PP, per protocol.

(B-0810/009-259), Seoul National University Boramae Medical Center (06-2009-7), Yonsei University Hospital (4-2008-0586), Korea University Ansan-Hospital (AS0884), Hallym University Sacred Heart Hospital (2009-I004), Inje University Ilsan-Paik Hospital (IB-0811-076), Konkuk University Hospital (KUH1010125), and Gachon University Gil Medical Center (GIRBA2010). The requirement for informed consent for the retrospective expansion was waived by the IRBs of participating centers because the study did not infringe on patient privacy or healthy status. Patients' records and information were anonymized and de-identified prior to analysis.

We further recruited 40 subjects who were not diagnosed with CKD, hypertension, diabetes mellitus, or cardiovascular disease in the healthy control group, and measured levels of OGN in these patients for comparison with those in the K-STAR population. Collection of serum in the healthy population was also approved by the IRB of Seoul National University Bundang Hospital (IRB number: B-1312/230-005) with written consent.

Data collection and definitions

Anthropometric measurements including height and weight, and measurements of the resting systolic and diastolic BP were performed in the clinic. All blood samples were collected at baseline according to standardized protocols. SCr values were measured using the alkaline picrate Jaffe kinetic method with an automatic analyzer (Toshiba-200FR; Toshiba Medical Systems Co., Ltd., Tokyo, Japan). Diabetes mellitus was confirmed with a fasting plasma glucose level of ≥ 126 mg/dL, random plasma glucose level of ≥ 126 mg/dL with classic symptoms of hyperglycemia, two-hour plasma glucose ≥ 200 mg/dL during an oral glucose tolerance test, or use of antihyperglycemic agents or insulin.

Biochemistry measurement

Serum OGN level was measured in duplicate using a sandwich enzyme-linked immunosorbent assay method developed by Cloud-Clone Corp. (Houston, TX, USA). The sensitivity of the OGN assay was 12.5 pg/mL and the intra- and inter-assay coefficients of variation of OGN were < 10% and < 12%, respectively.

Study outcomes

We obtained the occurrence of MACCEs and mortality via review of electronic medical records. Outcomes were all-cause mortality, MACCE, or the composite outcome of all-cause mortality or MACCE. MACCEs included acute coronary syndrome (ACS), heart failure, cardiac death, and stroke. ACS was defined as the clinical diagnosis of ST segment elevation myocardial infarction (STEMI), non-STEMI, or unstable angina pectoris. ACS was confirmed by new electrocardiogram changes and/or cardiac troponin and/or typical chest pain. Coronary revascularization was defined as either coronary artery bypass grafting or percutaneous coronary intervention (Cheng et al. 2014; Muhlestein et al. 2014). Heat failure was diagnosed if the patient met the appropriate Framingham criteria. The diagnosis of congestive heart failure required that two major or one major and two minor criteria be present concurrently (Ho et al. 1993). Cardiac death was defined as mortality due to a heart-related cause (death attributable to ACS, heart failure, arrhythmia, or sudden cardiac death) (Muhlestein et al. 2014). Stroke was defined as typical symptoms or signs of remaining neurological deficit lasting > 24 hours with computed tomography or magnetic resonance imaging evidence of cerebral ischemic infarct or intracerebral hemorrhage (Westin et al. 2014).

Statistical analysis

Continuous variables were described as means ± standard deviation (SD) values, and categorical variables were presented as proportions. Differences in continuous variables were analyzed using Student *t*-tests and the Mann-Whitney U test, while chi-square tests were used to analyze categorical variables. The distribution of the

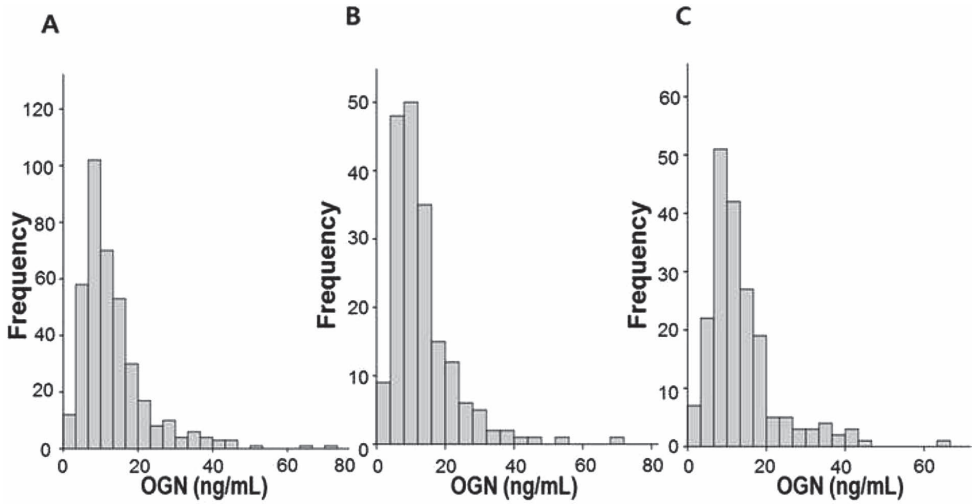

Fig. 2. Distribution of serum osteoglycin levels (ng/mL) according to presence of diabetes mellitus.
Total (A), Non-diabetics (B), and Diabetics (C).
OGN; osteoglycin.

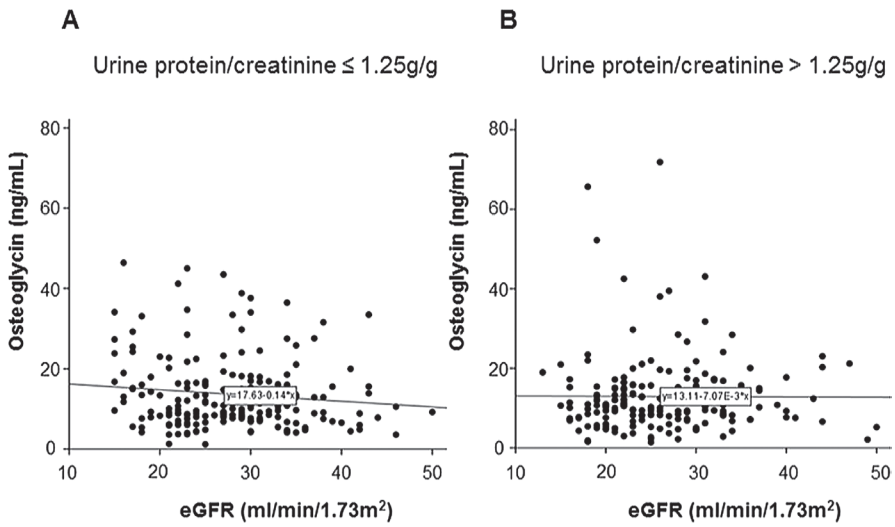

Fig. 3. Two-way scatter plot of osteoglycin levels and estimated glomerular filtration rate stratified by median proteinuria level.
Protein/creatinine ratio ≤ 1.25 g/g (A), Protein/creatinine ratio > 1.25 g/g (B).

OGN levels stratified by diabetes mellitus was expressed by histogram (Fig. 2) and the relationship of OGN levels and eGFR stratified by median proteinuria level was described by two-way scatter plot (Fig. 3). Correlation analysis was conducted using Spearman's correlation analysis. The factors associated with serum OGN level were also evaluated via multiple linear regression. We conducted the test of proportional hazard assumptions. The Cox proportional hazards model was used to depict the relationship between OGN and clinical outcomes. These relationships were assessed with the restricted cubic spline curves and the linearity assumption was met (Fig. 4). Backward stepwise multivariable analysis was used to prevent co-linearity among the variables (Fig. 5). $P < 0.05$ was considered statistically significant. All analyses were conducted using SPSS Statistics V21.0 (IBM Corp., Armonk, NY, USA) and Stata (Stata version 14.0, StataCorp LP, College Station, TX, USA).

Results

Characteristics of the study population

Of the subjects, 383 patients with CKD from the K-STAR study (mean age 56.4 ± 13.1 years, 65.8% men) and 40 healthy controls (nondiabetic, nonhypertensive, and non-CKD, mean age 47.7 years, 50% men) were enrolled in the present analysis. Among the patients with CKD, diabetic nephropathy was reported in 162 (47.1%) subjects. The mean values of baseline SCr, eGFR calculated by the Cockcroft-Gault equation, and urine protein to creatinine ratio in patients with CKD were 2.82 mg/dL, 26.8 mL/min/1.73m^2, and 2.11 g/g, respectively (Table 2). The mean baseline values for body mass index, systolic/diastolic BP, SCr, and hemoglobin in healthy controls were 23.0,

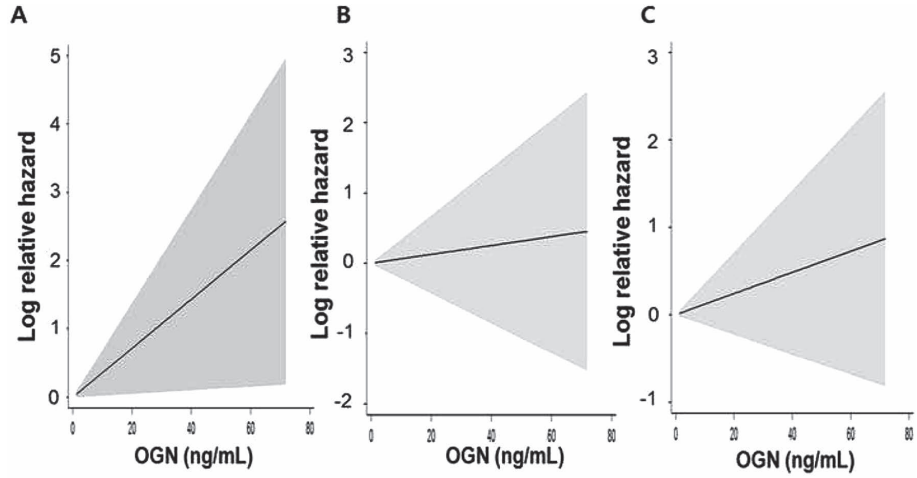

Fig. 4. Restricted cubic spline curves of osteoglycin.
Restricted cubic spline curves of osteoglycin for all-cause mortality (A), major cardiovascular and cerebrovascular events (B), and composite outcomes (C).

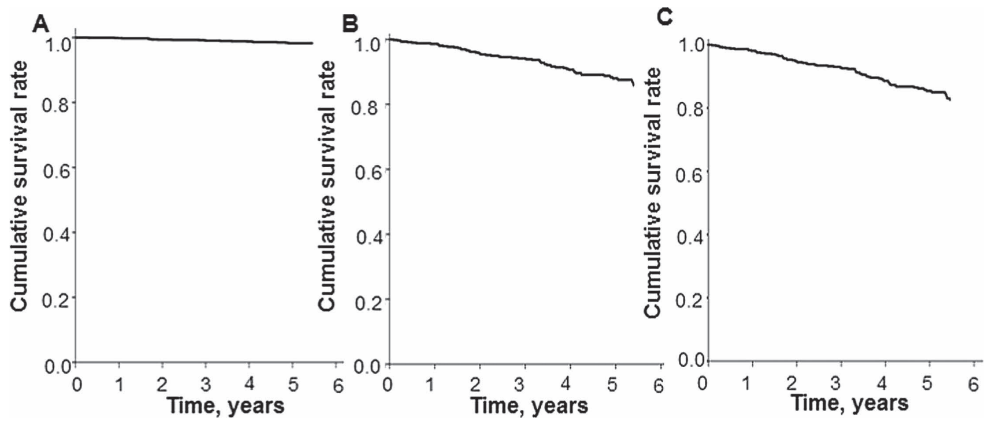

Fig. 5. Survival curves of osteoglycin.
Survival curves of osteoglycin for all-cause mortality (A), major cardiovascular and cerebrovascular events (B), and composite outcomes (C).

114.8/73.0 mmHg, 0.76 mg/dL, and 14.0 mg/dL, respectively. Patients with CKD had significantly higher serum OGN levels than the controls did (13.3 ± 9.4 [range 1.19-71.9] vs. 5.3 ± 2.1 [range 2.5-11.1] ng/mL, P < 0.001). The distributions of OGN in a total and according to the presence of diabetes mellitus are shown in Fig. 2.

Factors associated with serum OGN level

In univariable analysis, hemoglobin was found to have the strongest correlation with serum OGN levels (r = −0.180, P < 0.001). In multivariable linear regression, higher serum OGN was associated with lower urine protein to creatinine ratio (r = −0.225, P = 0.001), lower hemoglobin (r = −0.150, P = 0.018), and higher level of C-reactive protein (r = 0.152, P = 0.012) (Table 3).

We divided the patients into two groups stratified by median proteinuria levels (≤ 1.25 g/g or > 1.25 g/g) because serum OGN levels were not associated with eGFR but proteinuria. Two-way scatter plots for OGN and eGFR stratified by median proteinuria levels are shown in Fig. 3. Serum OGN levels are not associated with eGFR stratified by median proteinuria levels adjusted for relevant covariates (age, sex, systolic BP, body mass index, hemoglobin, albumin, low density lipoprotein, C-reactive protein, calcium, phosphorus, uric acid, and diabetes mellitus) (Fig. 3).

OGN and outcomes

During a mean follow-up period of 56 months, there were 25 deaths, 61 MACCEs, and 76 composite outcomes. Using serum OGN as a continuous variable, we found linear relationships between OGN and all-cause mortality/MACCEs/composite outcome, respectively in total (Fig. 4). Almost half of the patients had diabetes, and we analyzed the diabetic and nondiabetic groups separately. Among the nondiabetic group, serum OGN in the occurrence of outcomes tended to be higher than that in the nonoccurrence of outcomes (Table 4). In univariable analysis, each 1-ng/mL increase in serum OGN level was associated with higher

Table 2. Baseline characteristics at initiation of study.

Baseline characteristics	Total N	Total	Male	Female
Age (years)	383	56.4 ± 13.1	56.5 ± 13.4	56.3 ± 12.5
Sex (Male) (%)	383	252 (65.8)		
ESRD cause (n (%))	383			
Diabetic		195 (50.9)	126 (50.0)	69 (52.7)
non-diabetic		188 (49.1)	126 (50.0)	62 (47.3)
Body mass index (kg/m^2)	383	24.6 ± 3.6	24.5 ± 3.4	24.9 ± 4.1
Systolic blood pressure (mmHg)	382	129.5 ± 14.6	129.0 ± 14.2	130.2 ± 15.4
Diastolic blood pressure (mmHg)	382	75.7 ± 9.9	75.6 ± 9.8	75.9 ± 9.9
Serum Cr (mg/dL)	383	2.82 ± 0.67	2.89 ± 0.71	2.67 ± 0.56
eGFR (mL/min/1.73m^2)	383	26.84 ± 7.19	27.96 ± 7.45	24.67 ± 6.13
Urinary protein (g/g Cr)	375	2.11 ± 2.32	2.07 ± 2.13	2.18 ± 2.66
Hemoglobin (g/dL)	382	11.4 ± 1.8	11.8 ± 1.8	10.6 ± 1.2
Albumin (g/dL)	379	4.0 ± 0.4	4.0 ± 0.5	4.0 ± 0.4
Uric acid (mg/dL)	382	8.3 ± 1.9	8.6 ± 1.9	7.9 ± 1.8
LDL (mg/dL)	327	92.4 ± 34.6	89.6 ± 35.6	97.6 ± 32.1
C-reactive protein (mg/dL)	365	0.7 ± 2.7	0.8 ± 3.2	0.4 ± 1.4
Osteoglycin (ng/mL)	383	13.3 ± 9.4	12.7 ± 8.8	14.4 ± 10.3

ESRD, end stage renal disease; Cr, creatinine; eGFR, estimated glomerular filtration rate; LDL, low density lipoprotein.

Table 3. Factors associated with osteoglycin (ng/mL) as a continuous variable in patients with chronic kidney disease not undergoing dialysis.

Parameters	Standardized beta	P-value
Univariable Analysis		
Age (years)	0.085	0.097
Sex (Male) (%)	−0.087	0.089
Diabetic vs non-diabetics	0.030	0.565
Body mass index (kg/m^2)	−0.030	0.552
Systolic blood pressure (mmHg)	0.006	0.903
Diastolic blood pressure (mmHg)	−0.018	0.722
Serum Cr (mg/dL)	−0.032	0.539
eGFR (mL/min/1.73m^2)	−0.052	0.307
Urinary protein (g/g Cr)	−0.095	0.065
Hemoglobin (g/dL)	−0.180	< 0.001
Albumin (g/dL)	−0.046	0.372
C-reactive protein (mg/dL)	0.105	0.045
Multivariable analysis		
Urinary protein (g/g Cr)	−0.225	0.001
Hemoglobin (g/dL)	−0.150	0.018
C-reactive protein (mg/dL)	0.152	0.012

Cr, creatinine; eGFR, estimated glomerular filtration rate.

Table 4. Serum osteoglycin level (ng/ml) according to outcomes.

Outcomes			P-value
All-cause mortality	**Death**	**No Death**	
Total	**N = 25**	**N = 358**	
	16.6 ± 11.7	13.1 ± 9.1	0.155
Non DM	**N = 8**	**N = 180**	
	21.6 ± 17.1	12.7 ± 9.0	0.185
DM	**N = 17**	**N = 178**	
	14.2 ± 7.7	13.5 ± 9.3	0.759
MACCE	**MACCE**	**No MACCE**	
Total	**N = 61**	**N = 322**	
	13.5 ± 8.0	13.3 ± 9.6	0.858
Non DM	**N = 13**	**N = 175**	
	17.1 ± 9.7	12.7 ± 9.5	0.110
DM	**N = 48**	**N = 147**	
	12.5 ± 7.3	13.9 ± 9.7	0.351
Composite outcome*	**Composite outcome**	**No Composite outcome**	
Total	**N = 76**	**N = 307**	
	14.0 ± 9.2	13.2 ± 9.4	0.477
Non DM	**N = 19**	**N = 169**	
	17.5 ± 12.6	12.5 ± 9.0	0.115
DM	**N = 57**	**N = 138**	
	12.8 ± 7.5	13.9 ± 9.8	0.468

DM, diabetes mellitus; MACCE, major adverse cardiovascular and cerebrovascular event.
*Composite outcomes are defined as the occurrence of MACCE or death.

all-cause mortality, MACCEs, and composite outcome in the nondiabetic group only (hazard ratio [HR] = 1.068, P = 0.004; HR = 1.048, P = 0.036; HR = 1.048, P = 0.009, respectively). After adjustment for all confounders including age, sex, body mass index, systolic BP, diabetic nephropathy, eGFR, urine protein to creatinine ratio, hemoglobin, phosphorus, previous randomization, and albumin, serum OGN remained significantly associated only with all-cause mortality and composite outcome (HR = 1.058, P = 0.031; HR = 1.041, P = 0.036). However, the relationship between serum OGN and MACCE lost statistical significance in the adjusted analysis (HR = 1.048, P = 0.085). Among patients with diabetes, serum OGN level was not associated with mortality, MACCEs, or composite outcome in univariable or multivariable analysis (Table 5 and Fig. 5). We additionally adjusted information about drugs, including renin angiotensin aldosterone receptor blockade, beta blockers, calcium channel blockers, diuretics, antiplatelet agents, and lipid modifiers to multivariable model 2. Drug use once during the study period was regarded as taking the drug. The results showed similar trends with adjustment for medication (Table 6).

Discussion

The present study investigated whether OGN can function as a predictor in patients with CKD. The main finding of this study was that OGN was a predictor of clinical outcomes including all-cause mortality and composite outcome in nondiabetic patients with CKD, but not in diabetic patients with CKD.

OGN is a bone-associated glycoprotein (Bentz et al. 1990) and has been involved in a variety of biological processes such as bone formation (Bentz et al. 1990), cochlear development (Williamson et al. 2008), preterm labor (Romero et al. 2010), and tumor biology (Hu et al. 2005). OGN belongs to the family of small leucine-rich proteoglycans and is a basic component of the vascular extracellular matrix (Shanahan et al. 1997; Iozzo 1999; Cheng et al. 2014; Van Aelst et al. 2015). Recently, serum OGN has been shown to be released by vulnerable hemorrhagic carotid and coronary atherosclerotic plaques (Cheng et al. 2014; Malaud et al. 2014) and may also have prognostic value in patients with coronary artery disease (Cheng et al. 2014). Furthermore, OGN level was found to be increased in the adventitia and neointima after balloon injury, suggesting a role in vessel matrix remodeling (Shanahan et al. 1997). In patients with heart failure, OGN was associated with ischemic heart failure, survival, and cardiac remodeling (Van Aelst et al. 2015).

It is well known that the main cause of mortality in

Table 5. Multivariable cox analysis predicting all-cause mortality and major adverse cardiovascular and cerebrovascular events stratified by diabetes mellitus.

	Univariable		Multivariable[1]		Multivariable[2]	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
All-cause mortality						
Total	1.037 (1.003 to 1.072)	0.034	1.035 (0.998 to 1.074)	0.063	1.020 (0.979 to 1.063)	0.337
Non-DM	1.068 (1.021 to 1.117)	**0.004**	1.065 (1.013 to 1.119)	0.014	1.058 (1.005 to 1.114)	**0.031**
DM	1.010 (0.960 to 1.063)	0.690	1.008 (0.953 to 1.067)	0.773	0.973 (0.905 to 1.046)	0.459
MACCEs						
Total	1.006 (0.979 to 1.035)	0.652	1.002 (0.973 to 1.031)	0.906	1.004 (0.974 to 1.035)	0.775
Non-DM	1.048 (1.003 to 1.095)	**0.036**	1.041 (0.992 to 1.092)	0.104	1.048 (0.994 to 1.105)	0.085
DM	0.986 (0.951 to 1.022)	0.441	0.982 (0.946 to 1.020)	0.359	0.988 (0.950 to 1.027)	0.530
Composite*						
Total	1.012 (0.989 to 1.036)	0.311	1.008 (0.983 to 1.033)	0.539	1.008 (0.982 to 1.035)	0.546
Non-DM	1.048 (1.012 to 1.085)	**0.009**	1.041 (1.001 to 1.081)	0.043	1.041 (1.003 to 1.081)	**0.036**
DM	0.991 (0.960 to 1.023)	0.570	0.987 (0.954 to 1.021)	0.440	0.988 (0.954 to 1.024)	0.502

HR, hazard ratio; DM, diabetes mellitus; MACCE, major adverse cardiovascular and cerebrovascular event.
Multivariable[1], age, sex; Multivariable[2], age, sex, body mass index, systolic blood pressure, diabetic nephropathy; estimated glomerular filtration rate, urine protein to creatinine ratio, hemoglobin, phosphorous, previous randomization, and albumin.
*Composite outcomes are defined as the occurrence of MACCE or death.

Table 6. Medication-adjusted-multivariable cox analysis predicting all-cause mortality and major adverse cardiovascular and cerebrovascular events stratified by diabetes mellitus.

	Multivariable[3]	
	HR (95% CI)	P-value
All-cause mortality		
Total	1.021 (0.979 to 1.066)	0.332
Non-DM	1.055 (1.000 to 1.113)	**0.048**
DM	0.986 (0.913 to 1.065)	0.727
MACCEs		
Total	1.007 (0.977 to 1.037)	0.664
Non-DM	1.036 (0.971 to 1.104)	0.285
DM	0.996 (0.958 to 1.034)	0.820
Composite*		
Total	1.009 (0.983 to 1.035)	0.513
Non-DM	1.040 (1.000 to 1.081)	**0.049**
DM	0.993 (0.958 to 1.029)	0.696

HR, hazard ratio; DM, diabetes mellitus; MACCE, major adverse cardiovascular and cerebrovascular event.
Multivariable[3], age, sex, body mass index, systolic blood pressure, diabetic nephropathy, estimated glomerular filtration rate, urine protein to creatinine ratio, hemoglobin, phosphorous, previous randomization, albumin, and drug information including renin-angiotensin aldosterone system blockade, beta blocker, calcium channel blocker, diuretics, anti-platelet agents, and lipid modifier.
*Composite outcomes are defined as the occurrence of MACCE or death.

patients with CKD is cardiovascular disease (Fried et al. 2003; Menon et al. 2005). The increased cardiovascular risk in patients with CKD is multifactorial, related to traditional risk factors and CKD-associated factors such as CKD-mineral bone disease and vascular calcification. Although medial calcification contributes to cardiovascular mortality in patients with CKD (Neven and D'Haese 2011), the atherosclerotic plaque burden in the intimal layer is higher in patients with CKD compared to that in the normal population (Lindner et al. 1974; Savage et al. 1998; Schwarz et al. 2000). Because OGN is known to be released by vulnerable hemorrhagic atherosclerotic plaques, we suggest that OGN reflects the traditional risk factors and atherosclerotic plaques but not nontraditional risk factors and medial calcification.

In the present study, OGN levels were higher in patients with CKD than in the control group (healthy controls vs. CKD patients: 5.3 [range 2.5-11.1] vs. 13.3 [range 1.19-71.9] ng/mL), and were similar to those reported by Cheng et al. (2014) (healthy controls vs. patients who experienced a major cardiovascular event: 9.6 [7.2-13.7 vs. 13.7 [8.0-18.4]]. In the Trial of Intensified vs. Standard Medical Therapy in Elderly Patients with Congestive Heart Failure (TIME-CHF) study, the mean value of OGN was approximately 50 ng/mL, and was 2.5 times higher than in the present study (heart failure without MI vs. heart failure with MI: 44.7 [32.2-58.8] vs. 53.3 [36.3-71.3] ng/mL). We cannot provide obvious explanations for the increased OGN levels in patients with CKD. In general, serum biomarkers tend to be elevated because renal clearance is reduced in patients with CKD. We presume that the same is true of

OGN, to some extent. However, OGN levels were not associated with eGFR in the current study. This finding suggests that an increase in production also affects the elevation of OGN levels in patients with CKD. Further studies that monitor the urinary excretion of OGN in order to determine whether the elevated levels in patients with CKD are caused by increased production or reduced renal clearance will be needed.

Two studies have been performed that have investigated the relationship between circulating OGN and clinical outcomes. A previous study reported that OGN was positively associated with MACE, which was defined as all-cause mortality, ACS, unplanned coronary revascularization, and stroke, in a matched cohort of 264 patients who underwent coronary angiography for ACS or stable angina pectoris (Cheng et al. 2014). Because the MACE outcome in the previous study was equivalent to the composite outcome in our study, the finding that OGN was positively associated with the composite outcome was consistent with our data. Another previous study performed in patients with heart failure (TIME-CHF study) reported that higher levels of OGN were associated with increased 18-month overall mortality (Van Aelst et al. 2015). Our results are consistent with the findings of Van Aelst et al. (2015), who demonstrated a positive relationship between OGN and all-cause mortality. In contrast, our results differ from those of the earlier two studies, in that OGN was positively associated with clinical outcomes including all-cause mortality and composite outcome in the nondiabetic patients with CKD, but not in the diabetic patients with CKD. Because drugs prescription such as statin and anti-platelet agents may affect stabilization of atherosclerotic plaque, we additionally analyzed multivariable cox model including medication information as covariates. Although the confidence intervals were not statistically significant factors, the results showed similar trends with adjustment for medication (Table 6). We thought that it is resulted from the small number of outcome variables in the non-diabetic group.

No previous study showing whether OGN can be a predictor according to diabetes mellitus has been reported. We cannot provide obvious explanations for why OGN is a predictive marker of clinical outcomes in the nondiabetic group, but not in the diabetic group of patients with CKD. Our rationale for the difference in predictive value according to diabetes mellitus is as follows: first, as expected, diabetes mellitus was the most powerful predictor for MACCEs or composite outcome in our study. In other words, diabetes mellitus might overshadow biomarkers including OGN as a predictor of clinical outcomes, as presented in other studies (Pateinakis et al. 2013; Scialla et al. 2014). Second, CKD (Nakano et al. 2013) and diabetes mellitus (Michel et al. 2011) are risk factors for intraplaque hemorrhage in coronary arteries, related to the physiology of OGN. Considering the previous studies performed on patients without CKD (Cheng et al. 2014; Van Aelst et al. 2015), the predictive value may vary depending on the subject groups, such as non-CKD vs. CKD or nondiabetic vs. diabetic groups.

Our study has several strengths. To our knowledge, this is the first study to examine the clinical usefulness of OGN in patients with CKD. We demonstrated that OGN was a novel prognostic biomarker for clinical outcomes including all-cause mortality and composite outcome in the nondiabetic patients with CKD, but not in the diabetic patients with CKD. In our study population, as shown in Table 1, we excluded patients with histories of severe heart failure (New York Heart Association classes III-IV), uncontrolled arrhythmia or ACS, and cerebral infarct within 6 months, who were expected to experience outcomes of death or MACCE.

Our study also has several limitations. As with any observational study, no direct causality for adverse outcomes could be shown. Second, our sample size and number of outcomes were relatively small because we analyzed the relationship between OGN and outcomes according to the presence of diabetes mellitus. Third, adding OGN to conventional mortality risk factors, including age, sex, diabetes mellitus, diastolic BP, eGFR, and urine protein to creatinine ratio did not improve the discriminatory ability of the model (data not shown).

In conclusion, we found that OGN levels were indicative of adverse outcomes in patients with CKD, especially in nondiabetic patients with CKD, but not in diabetic patients with CKD during a 56-month follow-up period. Higher OGN levels correlated with increased mortality and composite outcome, and OGN is a potential biomarker in patients with CKD. Further studies are required to determine the precise pathophysiological role of OGN in patients with CKD and to confirm its role as a prognostic biomarker of mortality and composite outcome.

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Author Contributions

Study concept and design: SHB, RC, YSK, KYN.

Acquisition of data: RC, SWK, CWP, DRC, SGK, SAY, SK, SH, JHP, JHC, CSL, YSK, KYN.

Analysis and interpretation of data: SHB, RC, KYN.

Drafting of the manuscript: SHB, KYN.

Critical revision of the manuscript for important intellectual content: All authors

Administrative, technical, or material support: RC, SWK, CWP, DRC, SGK, SAY, SK, SH, JHP, JHC, CSL, YSK, KYN.

Conflict of Interest

The authors declare no conflict of interest.

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