Urine clusterin/apolipoprotein J is linked to tubular damage and renal outcomes in patients with type 2 diabetes mellitus

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SUMMARY

Objective—The objective of this study was to evaluate the association of urine clusterin/ apolipoprotein J (Apo J) with the development and/or progression of diabetic kidney disease (DKD) in type 2 diabetes.

Materials and methods—159 type 2 diabetic patients and 20 non-diabetic subjects with estimated glomerular filtration rate (eGFR) 60 mL/min/1.73 m^2 were enrolled. The baseline values of urine clusterin and tubular damage markers were measured. The primary outcome was the annual decline rate in eGFR and secondary outcomes were the development of chronic kidney disease (CKD) stage 3 or greater and the persistence/progression of albuminuria. The median follow-up duration of enrolled patients was 3.0 (1.0–5.9) years.

Results—Baseline clusterin levels in urine were significantly increased in type 2 diabetic subjects compared with those of non-diabetic subjects. The levels of urine clusterin had a significant correlation with urine tubular damage markers. A positive correlation between the annual rate of decline in eGFR and urine clusterin after adjusting for clinical confounding factors was detected. Multivariate analysis further indicated that urine clusterin correlated with the development of CKD stage 3 or greater and persistence/progression of albuminuria. In type 2 diabetic subjects with albuminuria, urine clusterin remained associated with the annual decline rate in eGFR and the progression of CKD stage.

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Conclusions—Urine clusterin reflects tubular damage in the early-stage of DKD. The increase of urine clusterin along with albuminuria could be an independent predictive marker for the progression of DKD in type 2 diabetes.

Keywords

Albuminuria; Clusterin; Diabetic nephropathy; Glomerular filtration rate; Proteinuria

INTRODUCTION

The prevalence of diabetes is increasing worldwide and its complication of diabetic kidney disease (DKD) is a major global healthcare and socioeconomic burden, with serious effects for individual patients.[1–3] DKD is clinically characterized by persistent albuminuria and a progressive decline in glomerular filtration rate (GFR) and is the leading cause of end-stage renal disease (ESRD).[2] To improve the lives of people with DKD, identification of a new marker that can predict the development and progression of DKD at an early stage is urgently needed.

It is clear that the presence of albuminuria is considered to be an early sensitive marker of DKD.[2] However, significant glomerular damage has already occurred when albuminuria is present.[4] In addition, there is a limited value in the use of albuminuria because not everyone with DKD and reduced GFR has increased albuminuria and because the methodology for measuring albuminuria is not well standardized due to individual variability.[2] Thus, the issue of whether albuminuria can be used as an early marker for DKD has been an outstanding subject in the field.

Over the past few years, significant advances have been made in identifying new diagnostic tools and non-invasive biomarkers for DKD, especially by using urine samples.[5,6] Urine biomarkers in DKD patients can be categorized based on the pathogenic features of glomerular or tubular dysfunction.[6] Although glomerular dysfunction has been thought to be a key factor for the development and/or progression of DKD, recent studies demonstrate that involvement of renal tubular damage is crucial in the pathogenesis of DKD.[7,8] In clinical practice, the importance of tubular damage has been underestimated because of the limitation of sensitive tests for this in human subjects.[9]

Clusterin, also known as apolipoprotein J (Apo J), is a glycoprotein expressed ubiquitously in various metabolic tissues and body fluids.[10] Clusterin is shown to be involved in the regulation of remodelling, lipid transport, complement inhibition, and apoptosis.[11] Clusterin also plays an important role in cardiovascular-related diseases, including dyslipidaemia, atherosclerosis, obesity, and type 2 diabetes,[12–14] all of which are pathogenic features of insulin resistance. In response to acute renal injury, including ischaemia/reperfusion injury, toxin-induced kidney injury, and unilateral ureteral obstruction, clusterin is rapidly induced in the kidney and urine.[15] Interestingly, a higher level of clusterin is found in dedifferentiated tubular cells of the kidney.[16] Thus, it is important to know that urine clusterin could be used as a tubular damage marker and is involved in development and/or progression of DKD.

In the current study, we evaluated the clinical implications of urine clusterin on the early development and/or progression of DKD in human subjects with type 2 diabetes. We also determined whether urine clusterin correlates with urine tubular makers and the annual rate of decline in eGFR in type 2 diabetic patients. Finally, we further evaluated whether urine clusterin can predict the persistence/progression of chronic kidney disease (CKD) stage and the persistence or progression of albuminuria.

SUBJECTS AND METHODS

Study population and design

A cohort of patients with type 2 diabetes mellitus and eGFR 60 mL/min/1.72 m^2 was monitored for renal impairment through the Diabetic Kidney Disease Study [DKDS]) at Pusan National University Hospital in Busan, Korea. To identify early biological markers for DKD, type 2 diabetic patients were consecutively enrolled from outpatient clinics during two periods that were one year apart (1st DKDS cohort [from February 2010 to January 2011] and 2nd DKDS cohort [from February 2012 to February 2014]). The eligibility inclusion and exclusion criteria were described in a previous study[17]: To establish the early biomarkers for DKD, patients had relatively conserved renal function by meeting the following inclusion criteria: 1) eGFR 60 mL/min/1.73 m^2, and serum creatinine <106 μmol/l, 2) stable renal function without a 2-fold elevation of serum creatinine for at least 5 months, and 3) no history of administration of renin-angiotensin system (RAS) inhibitors. Patients who had acute or chronic conditions that affected their renal function or urinary samples were excluded if they had: 1) a history of renal diseases other than diabetic nephropathy, 2) active urinary infection, 3) neoplastic, inflammatory disorders, 4) severe liver dysfunction, 5) uncontrolled thyroid disorders, 6) were pregnant, and 7) a recent history of acute myocardial infarction, stroke, or occlusive peripheral vascular disease. A total of 159 type 2 diabetic patients were enrolled. Age and sex matched non-diabetic subjects were randomly enrolled (n=20). They also fulfilled the following criteria: 1) no prior history of diabetes, renal disease or cardiovascular diseases, including hypertension, 2) fasting plasma glucose levels of <5.5 mmol/l after an overnight fasting, and 3) eGFR of 60 mL/min/1.73 m^2 and serum creatinine <106 μmol/l. This study was performed in accordance with the Declaration of Helsinki and the protocol was approved by the Institutional Review Board of Pusan National University Hospital (20100024). Each patient provided written informed consent before enrollment. Data are available to all interested researchers on request to the Institutional Review Board of Pusan National University Hospital.

As a part of the DKDS, patients' follow-up is still ongoing. Appropriate diabetes management, according to well-known standard guidelines, was provided by two endocrinologists and one nephrologist through their outpatient clinics. Of the 159 patients, 19 were excluded during follow-up for the following reasons: 12 were hospitalized for other severe acute and chronic diseases; 6 patients were diagnosed with malignancies, and 1 patient died of other causes during the follow-up period. Urine and blood samples for measurement of biological markers were collected at intervals of 12±1 (mean±SD) months during the follow-up period. Our primary outcome was the annual decline rate in eGFR during follow-up. The secondary outcomes were development of CKD stage 3 or greater and

persistence/progression of albuminuria. To follow-up the renal outcomes, serum creatinine (for calculation of eGFR) and albumin-to-creatinine ratio (ACR) were measured using the same methods at intervals of 6±1 (mean±SD) months. For the analysis of this study, the patients were followed up until March 2016.

Metabolic parameter measurements and definitions

Random spot urine and blood samples were collected from each patient at their clinic visit. Medical histories and anthropometric measurements were also recorded the same day. The eGFR was estimated using the following equation derived from the Modification of Diet in Renal Disease (MDRD) formula: MDRD = $175 \times$ (serum creatinine [μmol/L] $\times$ 0.0113L])$^{-1.154} \times$ (age in years)$^{-0.203}$.[18] A correction factor of 0.742 was used for females. The annual decline rate in eGFR was calculated as (eGFR at baseline – eGFR at last visit)/ years of follow-up. CKD stage 3 or greater was defined as having eGFR <60 mL/min/1.73 m^2 in two consecutive measurements from the last follow-up visit. Urinary albumin was measured through the latex turbidimetric immunoassay (Modular P800, Roche, Diagnostics, Mannhein, Germany). Total protein in the urine was measured using the turbidimetric method (Modular DP Hitachi, Roche), which utilizes a chemical reagent for precipitation. The lowest detectable level and the coefficient of variation in our laboratory were as follows: 20 mg/L and <0.48% for total proteinuria and 4 mg/L and <7.4% for albuminuria. The urine nonalbumin protein-to-creatinine ratio (NAPCR) was calculated by using total proteinuria and albuminuria: NAPCR=protein-to-creatinine ratio (PCR) – albumin-to-creatinine ratio (ACR).[19] Albuminuria was measured based on spot urine samples and defined as A2 or A3 category (ACR   3 mg/mmol creatinine) according to Kidney Disease Improving Global Outcome (KDIGO).[20] Several urine markers were measured using a commercial ELISA kit according to the manufacturer's protocols: clusterin (Boster biological technology, Pleasanton, CA, USA), kidney injury molecule (KIM)-1 (R&D systems, Minneapolis, MN, USA), neurophil gelatinase-associated lipoprotein (NGAL) (R&D systems) and liver-type fatty acid-binding protein (LFABP) (CMIC Co. Ltd, Tokyo, Japan). Urine samples were centrifuged for 10 min at 3,000 rpm to remove particulate matter and stored at −70°C until analysis. All samples were analyzed with their duplicates. Values below the detection limit (20 ng/L for clusterin, 0.009 μg/L for KIM-1, 0.012 μg/L for NGAL and 3 μg/L for LFABP) were approximated using the mean value between zero and the lower limit of detection. The intra- and inter-assay coefficients of variation were as follows: for clusterin, 4.2–4.6% and 6.9–7.5%; for KIM-1, 3.9–4.4% and 6.0–7.8%; for NGAL, 3.1–4.4% and 5.6–7.9%; for LFABP, 9.6–11.9% and 3.5–7.9%. The data for urine markers were expressed as ratios of urine marker to urine creatinine (urine marker/Cr) to assess the hydration state and renal function of the patients.

Statistical analysis

Statistical analyses were performed using SPSS version 18.0 for windows (SPSS, Inc., Chicago, IL, USA). Type 2 diabetic patients were divided into quartile groups according to their baseline urine Clusterin/Cr levels. Data were presented as means±SD for normally distributed values and medians (interquartile range) for nonparametric values. Distributions of continuous variables were examined for skewness and kurtosis, and logarithm-transformed values of variables with non-Gaussian distribution were used for analyses.

Geometric means are presented with 95% CI (confidence interval). Differences among the urine Clusterin/Cr quartile groups were analyzed by ANOVA, followed by the Bonferroni's test for normally distributed values or the Kruskal–Wallis test for nonparametric values. Categorical variables were reported as frequencies and proportions. Pearson's χ^2 test was employed to analyze categorical data, when appropriate. Pearson correlation coefficient was used to test the correlation between individual continuous variables. Multivariate regression analyses with annual rates of decline in eGFR as the dependent variables and urine Clusterin/Cr as an independent variable were performed. Several models were conducted to adjust for confounding factors including age, sex, HbA1c, systolic BP, HDL cholesterol, duration of diabetes, and baseline eGFR. Multivariate Cox regression models for development for CKD 3 or greater and persistence of albuminuria, using an enter procedure, were conducted and included factors with a P value of <0.1 in the univariate analyses. A P value of <0.05 derived from the two-tailed Student's t-test was considered to be of statistical significance.

RESULTS

Clinical and metabolic characteristic of study subjects

Table 1 summarizes the clinical and metabolic characteristics of non-diabetic and type 2 diabetes subjects according to the baseline urine clusterin quartiles. The subjects in the non-diabetic control group were well matched with type 2 diabetic patients by age, sex and body mass index (BMI). The mean age of type 2 diabetic patients was 56.9±10.7 years (range, 24–82 years), and there were 72 males and 87 females in this category. Age, sex, BMI, duration of diabetes, glycaemic control and dyslipidaemic status were not significantly different between the urine clusterin quartile groups of type 2 diabetes. The group with the highest urine clusterin level had a higher systolic blood pressure (SBP) (P=0.05). Urine clusterin positively correlated with SBP, diastolic blood pressure (DBP) and low-density lipoprotein (LDL) cholesterol at baseline (r=0.169, P=0.024; r=0.160, P=0.032; r=0.237, P=0.002, respectively) (Table 2). Subjects in both non-diabetic and type 2 diabetic groups showed well-conserved renal function, with eGFRs of 84.6±8.8 mL/min/1.73m^2 and 93.1±29.3 mL/min/1.73 m^2, respectively. The values of eGFR were not significantly different among the diabetic quartile groups according to urine clusterin at baseline (P=0.276). Urine clusterin levels were markedly elevated in type 2 diabetic subjects compared with those of the control (1.47 pg/mmol creatinine [95% CI, 4.47–13.99 pg/mmol creatinine] vs. 1.10 pg/mmol creatinine [95% CI, 1.06–2.36 pg/mmol creatinine], P=0.002). For clinical renal damage markers, there were significant differences among the urine clusterin quartile groups with respect to urine ACR, PCR and NAPCR (P=0.001, P<0.001 and P<0.001, respectively). All values of urine ACR, PCR and NAPCR in the Q4 group increased compared to those in the Q1 group, respectively (all P<0.001). Urine clusterin level notably correlated with urine ACR, PCR and NAPCR, respectively (r=0.369, P<0.001; r=0.398, P<0.001; r=0.403, P<0.001) (Table 2). More patients were administered antihypertensive medications in the highest urine clusterin quartile group than those in other groups (All P<0.05). In addition, diabetic retinopathy was more frequently observed in the Q4 group than in the Q1 group (P=0.004). Significant differences in urinary tubular markers including KIM-1, NGAL, LFABP were noted according to the urine clusterin levels (all

P<0.001). The urine levels of KIM-1, NGAL and LFABP were greatly higher in the Q4 group than in the Q1 group (all P<0.001). Urine clusterin significantly correlated with other tubular markers (urine KIM-1, r=0.530; urine LFABP, r=0.439; urine NGAL, r=0.371; all P<0.001) (Table 3).

Association of the annual rate of decline in eGFR with urine clusterin levels

The median follow-up duration was 3.0 years (range, 1.0–5.9 years). In all diabetic patients, the median annual rate of decline in eGFR was 3.4 mL/min/1.73m^2/year during the follow-up period. The annual rate of decline in eGFR positively correlated with baseline urine clusterin in univariate analysis (standard β=0.308, P<0.001) (Fig. 1A and Table 4). After adjusting for age, sex and clinical confounding factors including HbA1c, SBP, HDL cholesterol and duration of diabetes, urine clusterin level significantly associated with annual rate of decline in eGFR (standard β=0.283, P=0.004, Table 4). Finally, urine clusterin showed positive correlations with annual rate of decline in eGFR in the final model after adjusting for baseline eGFR (standard β=0.258, P=0.007). To verify the role of urine clusterin on renal function in the patients along with aluminuria, we also analyzed patients with albuminuria (A2 and A3, ACR 30 mg/g). Urine clusterin still correlated with the annual rate of decline in eGFR (standard β=0.341, P=0.004) in this sub-group analysis (Table 4 and Fig. 1B). The positive correlation between urine clusterin and the annual rate of decline in eGFR remained in the final model after adjusting for several clinical factors and baseline eGFR (standard β=0.322, P=0.0015).

Association of development of CKD 3 or greater and persistence/progression of albuminuria with urine clusterin levels

Of 159 patients, CKD progressed to stage 3 or greater in 34 (21.4%) patients during the 3-year follow-up. There was a significant difference in the cumulative incidence of CKD 3 or greater (eGFR <60 mL/min/1.73m^2) in patients according to urine clusterin quartile groups (P=0.032, Table 5 and Fig. 1C). In the multivariate analysis including clinical factors for renal impairment, the two groups with the higher range of urine clusterin (Q3 and Q4) showed a higher cumulative incidence of CKD stage 3 or greater than the lowest quartile group (Q3 vs. Q1: HR, 10.10, 95% CI = 1.18–86.86, P=0.035; Q4 vs. Q1: HR, 11.76, 95% CI=1.43–96.96, P=0.022). Meanwhile, the cumulative incidence of albuminuria persistence or progression was different in the patients according to urine clusterin quartiles (P=0.013, Table 5 and Fig. 1D). Finally, urine clusterin was associated with albuminuria persistence/ progression (Q3 vs. Q1: HR, 9.94; 95% CI=2.02–48.87; P=0.005). When we analyzed clusterin levels of patients in the albuminuria group, there was a marked difference in the cumulative incidence of CKD3 or greater in patients according to urine clusterin quartile groups (P=0.021, Fig. 1E). In addition, the highest quartile group of urine clusterin (Q4) showed a higher cumulative incidence of CKD stage 3 or greater than that of the lowest quartile group (Q4 vs. Q1: HR, 4.85, 95% CI=1.08–21.74, P=0.039).

DISCUSSION

The current study clearly provides new evidence of a relationship between urine clusterin and tubular damage markers, and identifies urine clusterin as a decisive indicator for tubular

damage in type 2 diabetic subjects. We also show that urine clusterin is linked to CKD stage and albuminuria as an indicator of progression of DKD in the early stages of nephropathy in type 2 diabetic patients (eGFR 60 mL/min/1.73 m^2).

Knowledge of urine clusterin's action in the development of acute and chronic kidney disease remains incomplete. It has been reported that increased clusterin gene expression in obstructed rabbit kidneys is related to a decrease in renal concentrating ability and GFR levels.[21] In this model, an elevation of clusterin protein in obstructed kidneys and of urinary clusterin excretion was also observed.[21] Experimental evidence with obese insulin-resistant animals also demonstrated that clusterin mRNA in the kidney positively correlated with urine protein excretion and negatively correlated with creatinine clearance,[22] suggesting that urine clusterin is involved in the renal injury of CKD. Given that clusterin expression was greatly up-regulated in the tubular epithelium of dilated, convoluted proximal tubules,[22] it is conceivable that urine clusterin is a decisive indicator of tubular abnormalities. Together, these observations raised the possibility that clusterin functions as a potential marker during renal injury in both acute and chronic kidney disease.

It is likely that increased levels of clusterin in urine are more closely associated with tubular damage than with glomerular damage. For example, de novo clusterin synthesis was elevated in the renal tubular epithelium after renal injury, and then excreted in the urine.[23] In addition, induction of clusterin primarily occurred in the tubular epithelial cell but not in the glomerulus of the kidneys of the nephrotic animal model, and ultimately precedes the development of tubular disease.[16] Moreover, urine clusterin showed a better diagnostic performance compared with serum blood urea nitrogen (BUN) and creatinine for detecting proximal tubular injury, whereas urine total protein, cystatin C and β2-microglobulin displayed a better diagnostic performance for detecting glomerular injury.[24] These observations, combined with our findings that urine clusterin correlates with tubular damage markers in type 2 diabetic subjects, suggest that elevation of urine clusterin could be a tubular damage marker in DKD. Among two models for kidney injury leading to CKD, the "fibrosis hypothesis" suggests that a variety of initial kidney insults result in tubular injury, eliciting further inflammation and damage to the tubulointerstitium that proceeds to CKD.[25] In this study, the finding that clusterin correlated with stage 3 CKD or progression of albuminuria might reinforce that at this stage tubular damage may be more evident.

We propose the hypothesis that up-regulation of urine clusterin along with albuminuria may reflect proteinuria-induced renal injury/apoptosis. In support of this, an in vitro study demonstrates that treatment of cultured mouse proximal tubule cells with albumin leads to increased clusterin levels in the media, which in turn inhibits the NF-kB signalling pathway. As a result, albumin-stimulated apoptosis in tubule cells is increased.[26] Although albuminuria has been thought to be a sensitive marker for DKD, there is a concern about the pathogenic nature of DKD's progression. Interestingly, diabetic patients who have albuminuria sometimes showspontaneous remission to normal albumin levels while others progress kidney failure during the course of a long-term follow-up.[26,27] Thus, it appears that the use of only albuminuria as a predictor of progression of the disease could provide incorrect information in DKD patients. Combined with information from urine clusterin, however, the progression of DKD can correctly be predicted in diabetic patients.

It has been reported that glomerular clusterin is variably expressed in human membranous nephritis, a major cause of nephrotic syndrome; it has also emerged as a factor influencing proteinuria and was associated with a reduction of proteinuria after a follow-up of 1.5 years.[28,29] In this regard, mice lacking clusterin developed a progressive glomerulopathy characterized by the deposition of immune complexes in the mesangium.[30] These results support the notion that clusterin may have a protective role for CKD and can modify immune complex metabolism and disposal. However, it is uncertain whether this glomerular clusterin is correlated to the emergence and increase of urine clusterin in the development and/or progression of DKD.

Evidence revealed that RAS may affect renal clusterin expression during the renal injury.[31–33] Clusterin attenuates angiotensin II-induced renal fibrosis through the inhibition of NF-κB signalling and the downregulation of angiotensin II type I receptor (AT1R) signalling, indicating that there is a link between clusterin and angiotensin II signalling in the context of renal disease.[34] A study with the nephrectomized rat model demonstrated that nephrectomy caused a progressive increase in clusterin mRNA levels in the remnant kidney and that an angiotensin-converting enzyme (ACE) inhibitor prevented the injury-induced increase in clusterin mRNA and proteinuria.[32] Collectively, these data suggest that the induction of clusterin is sensitive to renal injury, along with proteinuria, and is modulated by the renin-angiotensin system. In this respect, we exclude the possibility that urine clusterin levels in our patients are reflected in the renin-angiotensin system as the patients taking RAS inhibitors had a sufficient washout period for these drugs before the study.

The potential limitations of the current study come from the fact that urine clusterin is also increased by acute renal injury such as ischaemia/reperfusion injury, toxicant-induced kidney injury, and unilateral ureteral obstruction,[15,24] all of which are often found in diabetic patients. Thus, if urine clusterin is applied as a biomarker of renal injury in the clinical setting, it is difficult to discriminate between acute renal injury and chronic kidney disease. Given that increased levels of urine clusterin after acute injury can decline,[36] it would be important to know the kinetics of the short-term changes in the context of acute damage, which could provide an important clue to overcome this limitation. Even though we excluded patients with several acute illnesses which might affect the natural course of DKD at baseline and during the follow-up period, it is unlikely that our subjects could distinguish between AKI and DKD.

The cohort size is relatively small and the follow-up period was short. As the development and progression of DKD has a variable course and requires a longer time frame, a large cohort study will be needed to further confirm the concept of this study. As the MDRD equation is less accurate, especially in a cohort of patients, at levels above 60 mL/min/1.73 m^2, it might lead to misdiagnosis or misclassification of CKD in individuals with mild renal insufficiency.[35] Measured GFR using urinary or plasma clearance of exogenous filtration markers, rather than eGFR, could have provided the best measure of renal decline. The concentration of urine clusterin was derived from a single point urine sample. Serial samples during the natural course of type 2 diabetes could help to further elucidate the role of clusterin on the pathophysiological mechanisms involved in the development and progression of DKD.

In conclusion, an increase in urine clusterin is considered to be an important indicator of irreversible renal tubular injury, which is linked to the progressive decline of renal function in the early stage of DKD. In a clinical setting, increased levels of urine clusterin and albuminuria in diabetic patients may be informative in predicting the progression of CKD. The clinical use of urine clusterin as a diagnostic and therapeutic target in the development and progression of DKD will require further investigation.

Acknowledgments

Abbreviations

ACR	albumin-to creatinine ratio
ACE	angiotensin-converting enzyme
AT1R	angiotensin II type I receptor
BMI	body mass index
CKD	chronic kidney disease
DKD	diabetic kidney disease
DBP	diastolic blood pressure
ESRD	end-stage renal disease
eGFR	estimated glomerular filtration rate
HDL	high-density lipoprotein
KDIGO	Kidney Disease Improving Global Outcome
KIM	kidney injury molecule
LDL	low-density lipoprotein
LFABP	liver-type fatty acid-binding protein
MDRD	Modification of Diet in Renal Disease
NAPCR	nonalbumin protein-to-creatinine ratio
NGAL	neurophil gelatinase-associated lipoprotein
PCR	protein-to-creatinine ratio
Q	quartile

RAS renin-angiotensin system

SBP systolic blood pressure

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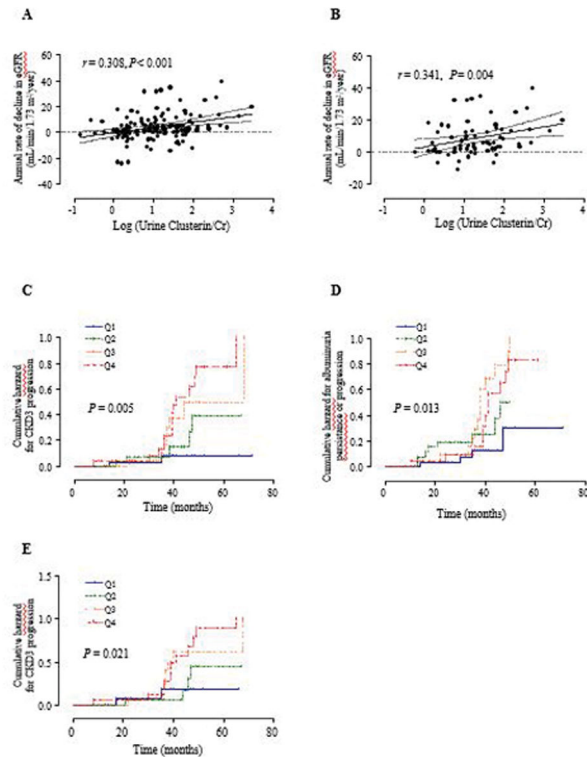
Figure 1.
Single regression analysis of the annual rate of decline in eGFR using urine clusterin in patients with all type 2 diabetic patients (A) and those with albuminuria (B). Cumulative incidences of CKD stage 3 or greater (eGFR <60mL/min/1.73 m^2) (C) and persistence/ progression for albuminuria (D) using the Kaplan-Meier method and the log-rank test in all type 2 diabetic patients according to urine Clusterin/Cr quartile (Q1 to Q4). Cumulative incidences of CKD stage 3 or greater (eGFR <60mL/min/1.73 m^2) in those with albuminuria (E) according to urine Clusterin/Cr quartile. Logarithm-transformed value of urine Clusterin/Cr was used for analysis. Cr, creatinine; eGFR, estimated glomerular filtration rate. CKD, chronic kidney disease; Q, quartile.

Table 1

Baseline characteristics of laboratory and metabolic variables in patients with type 2 diabetes according to urine clusterin quartiles

	Nondiabetic control	Type 2 diabetic patients				P value
		Q1	Q2	Q3	Q4	
Number	20	40	40	40	39	-
Range (pg/mg)	1.2–35.4	0.1–2.5	2.6–12.2	12.3–51.1	51.3–2779.6	-
Sex (male/female)	10/10	20/20	11/29	26/14	15/24	0.06
Age (years)	55.6 ± 4.7	56.5 ± 10.6	55.4 ± 11.6	58.5 ± 11.7	59.3 ± 8.9	0.618
BMI (kg/m^2)	23.4 ± 3.0	23.7 ± 3.4	23.4 ± 3.3	23.7 ± 3.9	23.3 ± 3.0	0.955
Duration of diabetes (years)	-	5.9 ± 5.7	8.7 ± 6.9	8.7 ± 7.1	8.5 ± 6.3	0.241
SBP (mmHg)	119 ± 15	124 ± 12	126 ± 14	121 ± 15	130 ± 17[#]	0.050
DBP (mmHg)	74 ± 9	76 ± 8	78 ± 12	79 ± 10	80 ± 12	0.426
HbA1c (%)	-	7.4 ± 1.5	8.0 ± 1.9	8.4 ± 2.1	8.0 ± 2.0	0.106
HbA1c (mmol/mol)	-	56.9 ± 15.9	63.4 ± 20.7	67.7 ± 23.0	64.1 ± 21.9	0.106
Total cholesterol (mmol/l)	5.01 ± 0.85	4.56 ± 0.97	4.63 ± 0.91	4.62 ± 1.05	4.91 ± 1.16	0.602
LDL cholesterol (mmol/l)	3.31 ± 0.80	2.50 ± 0.76	2.87 ± 0.92	2.73 ± 0.90	3.07 ± 1.34	0.106
Triglyceride (mmol/l) [*]	.68–1.92	1.03–2.97	1.08–2.49	0.87–2.14	1.28–3.84	0.269
HDL cholesterol (mmol/l)	1.42 ± 0.36	1.28 ± 0.39	1.25 ± 0.36	1.29 ± 0.47	1.29 ± 0.48	0.979
CRP (mg/l) [*]	0.3–0.9	0.2–1.3	0.4–1.9	0.2–1.6	0.3–1.7	0.151
Uric acid (µmol/l)	309 ± 59	291 ± 89	297 ± 77	268 ± 65	286 ± 119	0.553
eGFR (mL/min/1.73 m^2)	84.6 ± 8.8	86.9 ± 16.7	98.2 ± 30.3	90.1 ± 23.8	97.0 ± 40.8	0.276
Urine ACR (mg/mmol) [*]	-	0.54–3.91	0.98–12.55	1.29–20.53	1.62–103.78[†]	0.001
Urine PCR (mg/mmol) [*]	-	7.36–15.59	10.74–33.64	11.22–53.68	12.09–326.49[†,§]	<0.001
Urine NAPCR (mg/mmol) [*]	-	7.09–13.03	9.27–19.91	8.19–21.54	96.8–139.76[†,‡,§,//]	<0.001
Urine KIM-1/Cr (ng/mg) [*]	-	0.6 (0.3–0.9)	1.0 (0.5–1.8)	1.2 (0.7–2.1)[‡]	2.7 (2.0–4.7)[†,‡,§,#]	<0.001
Urine NGAL/Cr (ng/mg) [*]	-	4.2 (1.6–10.5)	8.9 (4.2–17.0)	13.0 (3.3–59.5)[‡]	26.4 (8.2 – 41.9)[†,§]	<0.001
Urine LFABP/Cr (ng/mg) [*]	-	1.5 (1.2–3.1)	1.9 (1.5–7.4)	2.2 (1.5–5.6)	6.5 (2.0–19.3)[†,§,#]	<0.001
Antihypertensive medication, n (%)	-	7 (17.5)	5 (12.5)	9 (23.1)	20 (50.0)[‡,§,#]	0.001
Lipid lowering agents, n (%)	-	17 (42.5)	21 (52.5)	12 (30.8)	13 (39.6)	0.169

	Nondiabetic control	Type 2 diabetic patients				P value
		Q1	Q2	Q3	Q4	
Diabetic retionopathy, n (%)	-	7 (17.5)	15 (37.5)[‡]	14 (35.9)	19 (47.5)[‡]	0.040

Data are expressed as mean ± SD, number of patients (%), median (interquartile range), and geometric means (95% CI) unless otherwise indicated.

[*]
logarithm-transformed values were used for comparison. Comparison was performed among diabetic patients.

[†]$P < 0.001$ vs. Q1;

[‡]$P < 0.05$ vs. Q1;

[§]$P < 0.05$ vs. Q2;

[||]$P < 0.001$ vs. Q3.

[#]$P < 0.05$ vs. Q3.

ACR, albumin-to-creatine; BMI, body mass index; CRP, C-reactive protein; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; KIM-1, kidney injury molecule-1; LFABP, liver-type fatty acid-binding protein; LDL, low-density lipoprotein; NAPCR, nonalbumin protein-to-creatinine ratio; NGAL, neutrophil gelatinase-associated lipocalin; PCR, protein-to-creatinine ratio; SBP, systolic blood pressure.

Table 2

Correlations between urine clusterin and other clinical variables

Variable	Urine Clusterin/Cr[*]	
	r	*P* value
Age	0.038	0.616
BMI	−0.062	0.421
Systolic BP	0.169	0.024
Diastolic BP	0.160	0.032
HbA1c	0.129	0.106
Total cholesterol	−0.075	0.324
LDL cholesterol	0.237	0.002
Triglyceride [*]	0.062	0.416
HDL cholesterol	0.057	0.448
CRP [*]	0.065	0.410
Uric acid	−0.026	0.735
eGFR	0.078	0.300
Urine ACR [*]	0.369	<0.001
Urine PCR [*]	0.398	<0.001
Urine NAPCR [*]	0.403	<0.001

ACR, albumin-to-creatine; BMI, body mass index; Cr, creatinine; CRP, C-reactive protein; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NAPCR, nonalbumin protein-to-creatinine ratio; PCR, protein-to-creatinine ratio; SBP, systolic blood pressure.

[*] logarithm-transformed values were used for comparison.

Table 3

Correlations between urine clusterin and other urine tubular markers.

	Urine Clusterin/Cr*	Urine KIM-1/Cr*	Urine LFABP/Cr*	Urine NGAL/Cr*
Urine Clusterin/Cr*	–	0.530[†]	0.439[†]	0.371[†]
Urine KIM-1/Cr*	0.530[†]	–	0.504[†]	0.614[†]
Urine LFABP/Cr*	0.439[†]	0.504[†]	–	0.459[†]
Urine NGAL/Cr*	0.371[†]	0.614[†]	0.459[†]	–

*
Logarithm-transformed values were used for analysis.

[†]
$P < 0.001$

Cr, creatinine; KIM-1, kidney injury molecule-1; LFABP, liver-type fatty acid binding protein; NGAL, neutrophil gelatinase-associated lipocalin.

Table 4

Multivariate regression of the annual rate of decline in eGFR as a dependent variable in type 2 diabetic patients

Model	All (n=159)		Albuminuria (n=87)	
	Standard β	P value	Standard β	P value
Model 1	0.308	<0.001	0.341	0.004
Model 2	0.305	<0.001	0.328	0.005
Model 3	0.283	0.004	0.344	0.010
Model 4	0.258	0.007	0.322	0.015

Model 1, crude; Model 2, adjusted for age and sex; Model 3, adjust for significant clinical parameters for eGFR decline including HbA1c, SBP, HDL cholesterol and duration of diabetes; Model 4, adjusted for baseline eGFR. eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; SBP, systolic blood pressure.

Table 5

Univariate and multivariate analysis for development of CKD 3 or greater (eGFR <60 mL/min/1.73 m^2) and persistence/progression for albuminuria in the patients with type 2 diabetes.

Development of CKD 3 or greater

	Univariate analysis			Multivariate analysis		
	Log-rank statistics	df	P	HR	95% CI	P
Sex, male	2.97	1	0.085	-	-	-
Age, 65 years	0.21	1	0.644	-	-	-
BMI, 25 kg/m^2	0.71	1	0.400	-	-	-
Duration of diabetes, 10 years	5.37	1	0.020	1.28	0.55–3.01	0.571
HbA1c, 7% (53 mmol/mol)	0.40	1	0.528	-	-	-
LDC cholesterol, 2.6mmol/l	0.11	1	0.746	-	-	-
HDL cholesterol, 1.0 (M), 1.3mmol/l (F)	3.28	1	0.070	1.98	0.73–5.40	0.183
Triglycerides, 3.0mmol/l	1.29	1	0.257	-	-	-
eGFR, <90 mL/min/1.73 m^2	4.85	1	0.028	2.81	1.10–7.17	0.030
Antihypetensive medication, yes	7.25	1	0.007	2.48	0.93–6.57	0.069
Lipid lowering agents, yes	2.64	1	0.105	-	-	-
Urine Clusterin/Cr, pg/mg	12.77	3	0.005			0.032
Q1				1.00		
Q2				3.35	0.34–32.83	0.299
Q3				10.10	1.18–86.86	0.035
Q4				11.76	1.43–96.96	0.022

Persistence/progression for albuminuria

	Univariate analysis			Multivariate analysis		
	Log-rank statistics	df	P	HR	95% CI	P
Sex, male	0.18	1	0.668	-	-	-
Age, 65 years	2.84	1	0.092	5.78	1.28–26.22	0.023
BMI, 25 kg/m^2	0.65	1	0.419	-	-	-

Development of CKD 3 or greater

	Univariate analysis			Multivariate analysis		
	Log-rank statistics	df	P	HR	95% CI	P
Duration of diabetes, 10 years	6.19	1	0.013	2.29	1.01–5.21	0.047
HbA1c, 7% (53 mmol/mol)	4.05	1	0.044	1.55	0.60–4.01	0.362
LDC cholesterol, 2.6mmol/l	1.16	1	0.281	-	-	-
HDL cholesterol, 1.0 (M), 1.3mmol/l (F)	0.76	1	0.382	-	-	-
Triglycerides, 3.0mmol/l	2.37	1	0.123	-	-	-
eGFR, <90 mL/min/1.73 m^2	2.42	1	0.120	-	-	-
Antihypetensive medication, yes	0.83	1	0.361	-	-	-
Lipid lowering agents, yes	4.54	1	0.033	0.47	0.20–1.08	0.075
Urine Clusterin/Cr, pg/mg	10.81	3	0.013			0.032
Q1				1.00		
Q2				4.75	0.94–23.91	0.059
Q3				9.94	2.02–48.87	0.005
Q4				4.73	0.95–23.57	0.058

BMI, body mass index; CI, coefficient interval; CKD, chronic kidney disease; Cr, creatinine; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HR, hazard ratio; LDL, low-density lipoprotein; Q, quartile.