Research Paper

Urinary kidney injury molecule-1 as early diagnostic marker of chronic kidney disease in cats

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Abstract Chronic kidney disease (CKD) is a common disorder and cause of death in cats. In the classification proposed by the International Renal Interest Society (IRIS), stage 1 and 2 CKD are difficult to diagnose accurately using markers, in comparison with normal controls. We recently described a simple and highly reproducible tandem mass tag labelling method for identifying potential disease-marker candidates among low-abundance urine proteins. In the current study, urine samples were obtained from 90 normal control cats as the control group and from 50 cats with CKD (stage 1). To identify new urine biomarkers for CKD, two pool urine samples (normal controls and CKD stage 1 cats) were differentially labelled with TMT, subjected to analysis using SDS-PAGE, digested with trypsin and subjected to analysis using LC-MS/MS. Kidney injury molecule-1 (KIM-1) was identified as a protein with higher levels in cats with CKD (stage 1). An ELISA of urine KIM-1 showed within-run (3.2−4.5%) and between-day (3.4−4.8%) reproducibility. Urine KIM-1 levels measured with this assay were significantly greater in CKD (stage 1) cats than in normal cats (63.7±10.7 vs. 35.7±9.7 μg/g Cre, $p<$ 0.001). These results indicate that KIM-1 may be useful as a complementary marker with p-Cre and BUN for detection of CKD (stage 1) in cats.

Key words: cat, chronic kidney disease, kidney injury molecule-1, urine

Introduction

Feline renal diseases are increasingly common in veterinary practice[1)]. It is important to diagnose and identify the pathological basis of renal dysfunction accurately at an early stage, since early diagnosis and treatment can delay progression of renal dysfunction, which leads to prolongation of survival and improvement of QOL[2)]. However, there are only a few reports on this area in clinical veterinary medicine[3)].

In daily clinical practice, plasma creatinine (p-Cre) and serum symmetric dimethylarginine (serum SDMA) are used as markers based on the stage classification criteria of the International Renal Interest Society (IRIS)[4)] (Table 1). However, many of these markers have low specificity for renal function, and there is a blind area without abnormal values despite development of mild renal dysfunction. It is especially important to evaluate the transitional period from normal status to Stage 1 in the IRIS classification for early diagnosis of renal function. However, it is difficult to evaluate a mild decrease of renal function in the early phase

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Table 1. Staging of CKD based blood creatinine and SDMA concentrations

Stage	1	2	3	4
Blood creatinine (mg/dL)	＜1.6	1.6−2.8	2.9−5.0	＞5.0
SDMA (μg/dL)	＜18	18−25	26−38	＞38

using the available markers in clinical practice. In stage 1 there may not be any reduction in GFR or the surrogate markers of GFR despite the fact there is active disease ongoing in the kidney.

In veteri-nary medicine, the application of proteomics techniques is stilllimited, but recently there have been significant efforts to study the urine proteome in cats[5−7].

In the present study, we employed the TMT method to generate comparative protein profiles of urine samples obtained from normal controls and CKD stage 1 cats. Furthermore, we compared the urine levels of candidate proteins to evaluate the ability to discriminate between normal controls and CKD cats. Here, we identify novel urine biomarkers that are potentially superior to classical markers for early and specific detection of CKD stage 1 cats.

Materials and Methods

Animals

Table 2 shows Clinical characteristics and biochemical variables of the control groups and CKD groups. Urine samples from 50 cats with stage 1 and 10 cats with stage 2 chronic kidney disease (CKD) brought to Maeda Veterinary Hospital between January 6, 2017 and February 20, 2018 (American Shorthair: 5, Chinchilla: 2, Mix: 53; 29 males and 31 females aged 2−13 years) were used in the study. Urine samples from 90 cats aged 2−13 years (Scottish Fold: 4, American Shorthair: 2, Mix: 84; 43 males and 47 females) were used as normal controls. Regarding the diagnostic criteria, cats clinically diagnosed as normal based on serum biochemistry and ultrasonography tests were defined as normal cats, and cats showing clinical signs of CKD in blood chemistry were defined as those with stage 1 CKD. Criteria for normal urinalysis were urine specific gravity (USG) ＞1.030, urinary protein to creatinine ratio (UPC) ≤0.2, and a negative bacteriologic urine culture[8]. Diagnosis of CKD was made prior to inclusion in the study, based on clinical and laboratory findings. After stabilization, cats were classified[8] into 4 groups with p-Cre of ＜1.6 and 1.6−2.8 mg/dL in stages 1 and 2, based on the IRIS guidelines. The diagnosis of cats with stage 1 CKD was made if p-Cre was ＜1.6 mg/dL, while urine concentrating ability had been lost, other disorder for decreased urine concentrating ability were excluded, and ultrasonographic changes were consistent with CKD (e.g., small kidney size and poor or absent renal corticomedullary differntiation)[9]. Urine was collected by catheterization. Samples were centrifuged at 1,190×*g* for 10 min (Kubota, Tokyo, Japan) and then stored at −80°C for further use. All owners gave signed informed consent to participation of their animal in the study.

Table 2. Clinical characteristics and biochemical variables of the control groups and CKD groups

Variables	Control group	CKD group	
		Stage I	Stage II
Age (Mean±s.d.)	6.8±3.6	6.7±3.3	6.8±3.6
Sex (male, female)	90 (43, 47)	50 (24, 26)	10 (5, 5)
Blood urea nitrogen (mg/dL) (Mean±s.d.)	24.1±5.7	25.6±6.8	26.8±5.9
p-Creatinine (mg/dL) (Mean±s.d.)	0.96±0.23	1.20±0.23	1.99±0.24
Urine specific gravity (Mean±s.d.)	1.053±0.006	1.051±0.005	1.045±0.016
Urinary protein to creatinine ratio (Mean±s.d.)	0.13±0.04	0.15±0.05	0.17±0.06

Urine concentration

Ultrafiltration membrane sample preparation using the Nanosep centrifugal devices (Pall Life Sciences, Ml, USA) were used to process the urine samples. The Nanosep centrifugal devices procedure was prepared according to the manufacturer's instructions. Urine (500 μL) were transferred to a sample reserver (Pall Life Sciences, USA), were centrifuged at 14,000×*g* for 20 min (Kubota, Tokyo, Japan). The final volume was 20 μL.

Tandem mass tag (TMT) labelling

Reduction and alkylation were performed as described previously[10]. TMTsixplex Isobaric Label Reagent Set (Pierce, ID, USA) was used according to the manufacturer's instructions. Tubes containing the different isobaric chemical tags (0.8 mg each) were added to 41 μL of anhydrous acetonitrile (ACN) and dissolved for 5 min with occasional vortexing at room temperature. TMT solution (20 μL) was

added to each tube and allowed to react at room temperature for 60 min. A volume of 4 μL of 5% hydroxylamine was added to each sample, and the mixture was incubated for 15 min to quench the reaction[10]. Samples were finally pooled and lyophilized. Analyses were performed for samples of 20 μL of concentrated pooled urine from normal controls (n=8) (TMT6-126 and TMT6-128) and from stage 1 CKD cats (n=8) (TMT6-127 and TMT6-129).

SDS-PAGE analysis and in-gel digestion of proteins

Lyophilized samples were dissolved in PAGE sample buffer (15 μL) were analyzed by SDS-PAGE (Perfect NT Gel W; DRC Co. Ltd., Tokyo, Japan, 10−20% acrylamide, 20 wells). The gel was stained with Coomassie brilliant blue (PhastGel Blue R; GE Healthcare, Little Chalfont, UK).

The whole lane was cut into 20 pieces. The gel was cut into small pieces, destained in 50% ACN/50 mM NH_4HCO_3 and washed with deionized water. Gel pieces were dehydrated in 100% ACN for 15 min and dried in a SpeedVac Evaporator (Wakenyaku, Kyoto, Japan) for 45 min. The pieces were rehydrated in 10−30 μL of 25 mM Tris-HCl/20% ACN containing 25 ng/L trypsin (Trypsin sequence grade, Roche, Basel, Switzerland) for 45 min. After removal of unabsorbed solution, the gel pieces were incubated in 10−20 μL of 50 mM Tris-HCl/20% ACN for 20 h at 37°C. The solution containing digested protein fragments was transferred to a new tube, and peptides remaining in the gel were extracted with 5% formic acid/50% ACN for 20 min at room temperature[11].

Protein identification and quantification by LC-MS/MS analysis

The tryptic digested peptides were injected into a trap column (C18, 5 μm, 0.3×5 mm; DIONEX, Sunnyvale, CA, USA) and an analytical column (C18, 3 μm, 0.075×120 mm; Nikkyo Technos, Tokyo, Japan), which was attached to the Ultimate 3000 (DIONEX). The flow rate of the mobile phase was 300 nL min^{-1}. The solvent composition of the mobile phase was programmed to change in 120-min cycles, with varying mixing ratios of solvent A (2% v/v acetonitrile (ACN) and 0.1% v/v formic acid) to solvent B (90% v/v ACN and 0.1% v/v formic acid) as described previously[12]. Purified peptides were introduced from HPLC to the LTQ-Orbitrap XL (Thermo Scientific, San Jose, CA, USA), a hybrid ion-trap Fourier transform mass spectrometer and analyzed with parameters according to previous research[12].

The database search engine (Proteome discoverer; Thermo Scientific) was used to identify and quantify proteins from the mass, tandem mass and reporter ion spectra of peptides. Peptide mass data were matched by searching the International Protein Index database (IPI, European Bioinformatics Institute) and the cat KIM-1 sequence. The database search parameters employed were, peptide mass tolerance=2 p.p.m. and fragment tolerance=0.6 Da. Furthermore, the enzyme parameter was set to trypsin, allowing up to one missed cleavage variable modifications, methionine oxidation, and cysteine alkylation. The minimum criteria of protein identification were filtered with Xcorr vs charge state and set as false discovery rate (FDR)＜1%.

ELISA

Immunogens for development of anti-KIM-1 antibodies: Recombinant KIM-1 was made by Fujifilm Wako Pure Chemical Corp (Osaka, Japan). Synthetic peptides of 20 amino acids (Toray Research Center, Tokyo, Japan) corresponding to the sequences of the KIM-1 N-terminus (KIM-1 N peptide) and C-terminus (KIM-1 C peptide) coupled to keyhole limpet hemocyanin (KLH) were obtained from Sigma-Aldrich Japan (Tokyo, Japan). The peptide-KLH conjugates were dissolved in distilled water and used as antigens for preparation of monoclonal antibodies.

Immunization and establishment of hybridoma cell lines: KIM-1 N and C peptides (50 mg at 1 mg/mL in PBS) were used for immunization of BALB/c mice. Hybridoma cell lines[13] KIM-1 N-02 and C-01 were established and antibody isotypes were determined using a Mouse Monoclonal Antibody Isotyping Test Kit (AbD Serotec, Oxford, UK). To obtain pure monoclonal antibodies on a large scale, BALB/c mice were initially stimulated with 1.0 mL pristine (Sigma Aldrich Japan) and then inoculated 2 weeks later. Monoclonal antibodies were purified as described elsewhere[13].

Western blot analysis using anti-KIM-1 antibodies: To examine the specificity of the antibodies, recombinant KIM-1 (10 ng) and urine samples of cats with CKD stage 1 (5 μL concentrated urine) were separated by SDS-PAGE on a 10−20% gradient gel (DRC, Tokyo, Japan), and then transferred onto a PVDF membrane (Millipore, Billerica, MA, USA). To minimize nonspecific binding, the membranes were incubated with Blocking One (Nacalai Tesque,

Kyoto, Japan). After the membrane was washed three times with PBST (PBS buffer including 0.05% Tween-20), the membranes were incubated with anti-KIM-1 N peptide antibody or anti-KIM-1 C peptide antibody as primary antibodies for 1 h at room temperature. The membrane was washed again three times with PBST and then incubated with a secondary rabbit anti-mouse immunoglobulin/HRP (Dako Japan, Tokyo, Japan) for 1 h at room temperature. The reactive antibodies were visualized by staining with Pierce Western Blotting substrate (Thermo Fisher Scientific).

Immobilization of antibodies on a polystyrene microtiter plate: The anti-KIM-1 C peptide antibody dissolved in PBS buffer was dispensed into a 96-well polystyrene microtiter plate (Thermo Fisher Scientific) at 0.5 mg/well and incubated for 1 day at 4°C. The plate was washed three times with PBS containing 0.05% Tween-20. The microtiter plate was coated with 20% NOF102 containing 10% sucrose for 1 day at 4°C.

ELISA conditions: Recombinant KIM-1 was diluted with PBS buffer for calibration. After washing the microtiter plate with PBST, 100-μL aliquots of 10-times diluted urine samples were added in duplicate to wells. The plates were incubated at room temperature for 1 h and then washed three times. HRP-conjugated anti-KIM-1 N antibody in PBST (100 μL) was added to each well and the plate was incubated at room temperature for 30 min. The plate was washed three times, and then 100 μL of TMB solution (Wako Pure Chemical Industries) was added. After incubation at room temperature for 10 min, 100 μL of stop solution was added and the absorbance at 450 nm was measured.

Sensitivity, Precision, and Accuracy: Blank controls at a concentration of 0 ng/mL KIM-1 in 8 replicates were used to determine the sensitivity and the detection limit. Two different concentrations of the KIM-1 (2 ng/mL, 12 ng/mL) in eight replicates were used to determine the within-assay variation. Two different concentrations of the KIM-1 (2 ng/mL, 12 ng/mL) in 5 different days were used to determine the between-assay variation. Precision was evaluated by within/between-assay coefficients of variation (CVs) (%), with within-assay CV ＜10% and between-assay CV ＜20% being acceptable. Accuracy was evaluated by recovery. The observed and expected concentrations of KIM-1 in within-assay were used to analyze the recovery. A recovery of 75−125% was acceptable.

Other procedures

Creatinine was measured enzymatically with creatinine deiminase using a Fuji DRI-CHEM Slide CRE-PIII kit (FujiFilm Medical, Tokyo, Japan). Blood urea nitrogen (BUN) was measured using a N-Assay BUN-L Nittobo D-Type kit (Nittobo Medical Co., Ltd., Tokyo, Japan). Numerical data are presented as the mean±standard deviation (SD).

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 19 (SPSS Inc., Chicago, IL, USA). For non-parametric data, the differences between two groups were analysed using the Mann−Whitney *U*-test. *P*-values＜0.05 were considered statistically significant. Receiver operating characteristic (ROC) curves were constructed to assess the sensitivity, specificity, and respective areas under the curves (AUC). Levels of urinary KIM-1 was expressed in the Creatinine-corrected urinary KIM-1 (μg/g Cre) levels.

Results

Identification of candidate proteins by comparing the urine of normal controls and CKD stage 1 cats using the TMT method

To identify new urine biomarkers for CKD, two pool urine samples (normal controls and CKD stage 1 cats) were differentially labelled with TMT, subjected to analysis using SDS-PAGE, digested with trypsin and subjected to analysis using LC-MS/MS (Fig. 1). Analyzing urinary protein by SDS-PAGE after TMT labeling can accurately determine the molecular weight and eliminate urinary protein degradation products. Of these, normal control pool sample was labelled with TMT with reporter ion at *m/z*=126 or 128, and CKD stage 1 cat pool sample was labelled with TMT with reporter-ion at *m/z*=127 or 129. After comparing the profiles of proteins by LC-MS/MS using an LTQ Orbitrap XL mass spectrometer, 47 proteins with unique peptide sequences were found (Table 3, Table 4). There were five proteins with urine levels in stage 1 CKD cats that were more than two times than the level in normal controls (Table 3). The protein with the largest elevation was identified as KIM-1.

Establishment and characterization of ELISA for measuring KIM-1

Anti-KIM-1 N peptide antibody (Fig. 2A) and anti-KIM-1

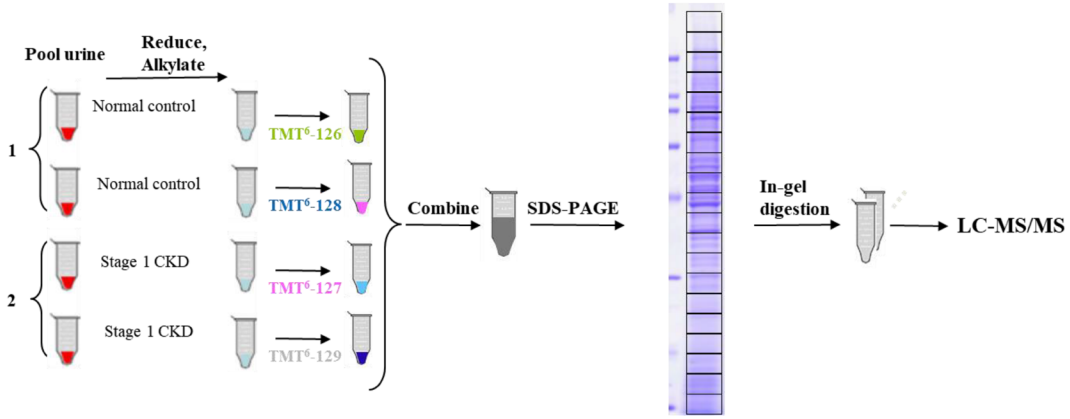

Fig. 1. Identification of candidate proteins using TMT labelling and LC-MS/MS analyses.

Table 3. Urine proteins detected at higher levels in CKD stage 1 cats compared to normal controls in proteome analysis

Database Accession No.	ID	Coverage (%)	Number of matching peptides	TMT rate (127/126)	TMT rate (129/128)
gi 757173465	Kidney injury molecule-1 (Hepatitis A virus cellular receptor 1)	12	5	3.4	3.6
Q29487	Prostaglandin-H2 D-isomerase	14	6	2.6	2.7
Q8I034	Carboxylesterase 5A	9	6	2.5	2.4
O62763	Acetylcholinesterase	12	6	2.3	2.2
E3UTY9	Haptoglobin	16	2	2.2	2.0
Q56H28	Angiotensin-converting enzyme 2	14	2	1.8	2.0
Q9N2I7	Dipeptidyl peptidase 4	8	5	1.6	1.9
P21664	Beta-lactoglobulin-2	10	3	1.6	1.9
P07405	Hemoglobin subunit alpha	15	4	1.6	1.8
P49064	Serum albumin	12	4	1.5	1.7
O97524	Beta-glucuronidase	10	4	1.5	1.7
D5MTH1	Cystatin-C	9	2	1.5	1.6
P79171	Aminopeptidase N	11	5	1.4	1.6
P13369	Macrophage colony-stimulating factor 1 receptor	10	2	1.4	1.6
O62657	Stromal cell-derived factor 1	9	3	1.4	1.5
O19015	Beta-galactosidase	13	2	1.4	1.5
Q95ND4	Pro-epidermal growth factor	15	6	1.3	1.5
Q8WNR9	Cystatin-A	13	4	1.3	1.4
Q76B49	CD63 antigen	13	5	1.3	1.4
Q5MGS7	Beta-2-microglobulin	15	6	1.3	1.4
O46432	Lysosomal alpha-mannosidase	14	2	1.3	1.4
Q6SA95	Coagulation factor IX	15	2	1.2	1.4
Q2WGK2	Junctional adhesion molecule A	9	4	1.2	1.3
Q2MHN1	Ferritin light chain	15	2	1.2	1.3
P58727	Toll-like receptor 4	7	4	1.2	1.3
P49614	Beta-hexosaminidase subunit beta	12	5	1.2	1.3
P40239	CD9 antigen	9	2	1.2	1.3
M5AXY1	Retinol binding protein 4	5	3	1.2	1.3
M3W955	Apolipoprotein A-IV	8	2	1.2	1.3
Q7YRU4	Malate dehydrogenase, cytoplasmic	6	3	1.1	1.3
P33687	Beta-lactoglobulin-1	10	5	1.1	1.2
P14450	Fibrinogen alpha chain (Fragment)	9	2	1.1	1.1

Table 4. Urine proteins detected at lower levels in CKD stage 1 cats compared to normal controls in proteome analysis

Database Accession No.	ID	Coverage (%)	Number of matching peptides	TMT rate (127/126)	TMT rate (129/128)
M3W551	Antioxidant 1 copper chaperone	14	8	0.9	0.8
M3W8S0	Ezrin	7	4	0.9	0.8
A0A2I2U4B5	Nucleoside diphosphate kinase	9	8	0.9	0.7
A0A2I2UZE5	Peroxiredoxin 1	11	5	0.9	0.7
M3X5I6	SH3 domain-binding glutamic acid-rich-like protein	9	4	0.9	0.7
M3X9A1	Triosephosphate isomerase	5	5	0.9	0.7
M3WM96	Annexin	11	3	0.8	0.7
M3VWL2	Fructose-bisphosphate aldolase	7	4	0.8	0.7
Q9N2D5	Glyceraldehyde-3-phosphate dehydrogenase	9	3	0.8	0.7
P37155	Lysozyme C	6	3	0.8	0.7
M3X166	Phosphoglycerate kinase	2	3	0.8	0.7
A0A2I2UPH5	Superoxide dismutase [Cu−Zn]	10	3	0.8	0.7
M3WPG6	Apolipoprotein A-I	3	2	0.7	0.7
M3VZP6	Gelsolin	7	2	0.7	0.6
A0A2I2UJB3	Radixin	4	2	0.7	0.6

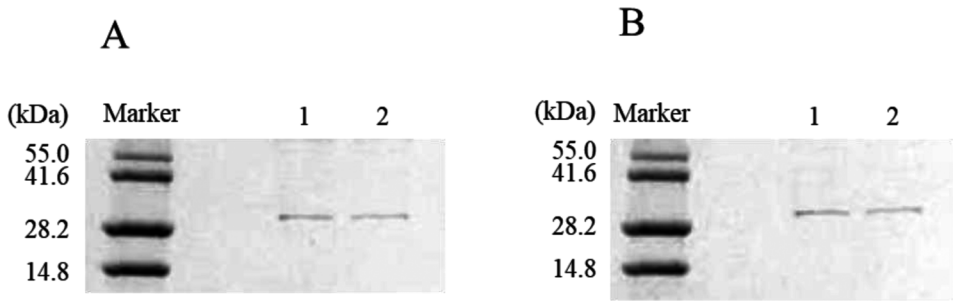

Fig. 2. Western blotting analysis.
Immunoreactive bands were observed for KIM-1 when recombinant and urine KIM-1 were incubated with anti-KIM-1 N peptide antibody (A) or anti-KIM-1 C peptide antibody (B). Lane 1: recombinant KIM-1 (10 ng). Lane 2: urine samples of cats with CKD stage 1 (5 μL concentrated urine).

C peptide antibody (Fig. 2B) recognized recombinant KIM-1 and urine samples from CKD stage 1 cats, respectively.

Range, *dilution analysis and detection limit:* A standard curve was drawn based on the colorimetric intensity of diluted recombinant KIM-1 to establish the relationship of intensity with KIM-1 concentration (Fig. 3). The working range of the assay was 0−50 ng/mL and the assay gave linear results from 0 to 50 ng/mL (y=1.8292x+0.0498, r2= 0.9999, p<0.0001). The detection limit was estimated by assaying the zero concentration eight times, and was defined as the KIM-1 "zero" concentration+3SD. The limit was found to be 0.32 ng/mL.

Within-run and between-run reproducibility: The precision of the assay was determined using two KIM-1 concentrations of 2.0 and 12.0 ng/mL. Within-assay CVs were determined with eight replicates of each sample. Between-assay CVs were determined based on assays performed on 5 different days (two replicates of each sample per day). The within-run CV was 3.2−4.5% and the between-run CV was 3.4−4.8%.

Interference: Interference was assessed in samples containing 5.0 ng/mL KIM-1. Potential interference materials were added to urine at various concentrations. There was no substantial interference from hemoglobin (up to 5,000 mg/L), free bilirubin (up to 207 mg/L), ditaurobilirubin (up to 204 mg/L), chyle (up to 1,400 formazine turbidity units, equal to 1,176 mg/L triglyceride), ascorbic acid (up to 500 mg/L), and rheumatoid factor (up to 500 U/L).

Recovery test: To evaluate recovery in the ELISA, 0.5 and 5.0 ng/mL of synthetic KIM-1 were added to pooled urine (2.8 ng/mL). The percentage recovery ranged from

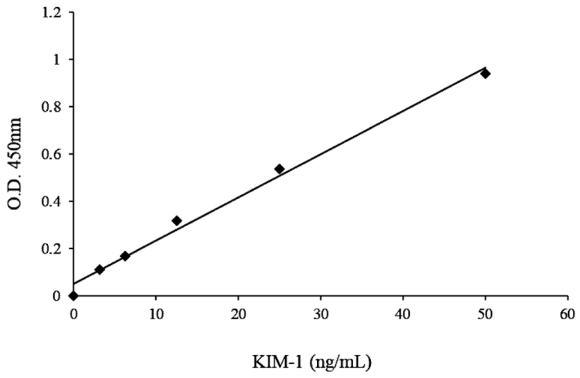

Fig. 3. Standard curves for urine KIM-1 in the ELISA.
Colorimetric intensity and urine KIM-1 concentration were related in the range of 0−50 ng/mL. Six concentrations of urine KIM-1 were determined by ELISA.

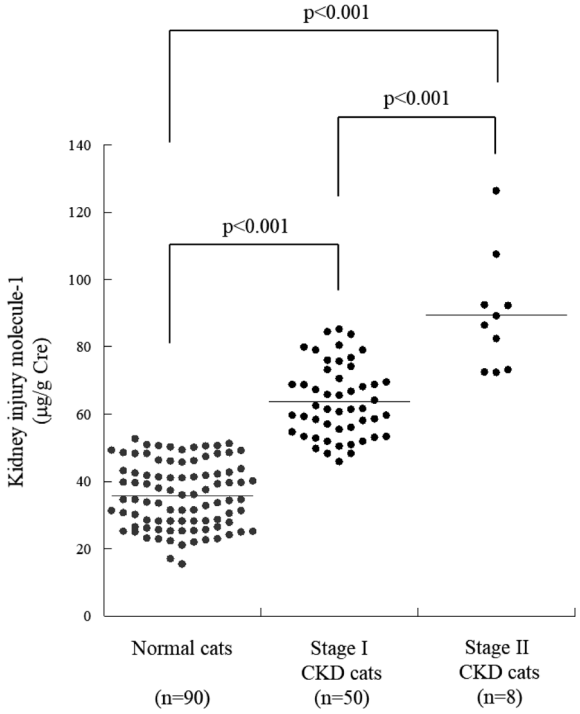

Fig. 4. Urine KIM-1 levels in normal controls and CKD stage 1 cats.
Urine KIM-1 was significantly higher in CKD stage 1 cats (63.7±10.7 μg/g Cre) compared with normal cats (35.7±9.7 μg/g Cre). $p<0.001$ by Mann−Whitney *U*-test. Urine KIM-1 was significantly higher in stage 2 CKD cats than in stage 1 CKD cats (89.1±19.3 vs. 63.7±10.7 μg/g Cre, $p<0.001$; Mann−Whitney *U*-test).

96.2 to 103.9%.

KIM-1 levels are increased in the urine of cats with CKD

Urine KIM-1 levels were measured in stage 1 CKD cats (n=50), stage 2 CKD cats (n=10) and normal cats (n=90). As shown in Fig. 4, urine KIM-1 was significantly higher in stage 1 CKD cats than in normal cats (63.7±10.7 vs. 35.7±9.7 μg/g Cre, $p<0.001$; Mann−Whitney *U*-test). Urine KIM-1 was significantly higher in stage 2 CKD cats than in stage 1 CKD cats (89.1±19.3 vs. 63.7±10.7 μg/g Cre, $p<0.001$; Mann−Whitney *U*-test). Receiver operator characteristic (ROC) curves were constructed to evaluate the urine KIM-1, serum creatinine, and serum BUN cut-offs for distinguishing stage 1 CKD cats from normal cats. The respective areas under the ROC curve (AUCs) were 0.964 for urine KIM-1, 0.698 for serum creatinine, and 0.554 for serum BUN. These results suggest that urine KIM-1 may be a better diagnostic stage 1 CKD biomarker than serum creatinine and BUN (Fig. 5). The ROC curves showed that the optimal diagnostic cut-off value was 49.4 ng/mL for urine KIM-1. Moreover, the AUC for urine KIM-1 was 0.964, with a sensitivity of 90.0% and a specificity of 90.5%. The Pearson correlation coefficients of urinary KIM-1were 0.096 for BUM, and 0.322 for p-Cre, respectively.

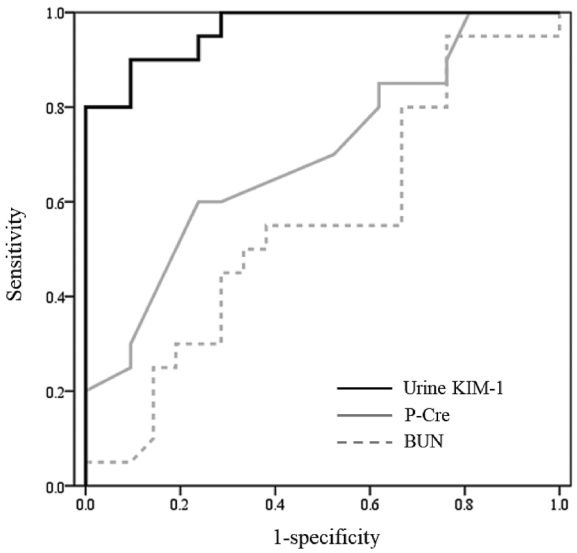

Fig. 5. Urine KIM-1 is more effective than p-Cre and BUN for early detection of stage 1 CKD.
ROC analyses were performed for the serum levels of urine KIM-1, p-Cre and BUN in stage 1 CKD and normal cats. The AUCs were 0.964 for urine KIM-1, 0.698 for p-Cre, and 0.554 for BUN.

Discussion

In this study, SDS-PAGE and proteomics analyses were used to detect Beta-2-microglobulin and KIM-1 in urine from CKD stage 1 cats and normal controls. The protein with the largest elevation was identified as KIM-1. KIM-1, which is also known as hepatitis A virus cell receptor 1 (HAVCR1) and T cell immunoglobulin 1, is a renal tubular

transmembrane glycoprotein that is thought to function in cell-to-cell or cell-to-matrix adhesion[14,15]. KIM-1 is known to be undetectable in normal kidney tissue but is expressed at very high levels in dedifferentiated proximal tubule epithelial cells in human and rodent kidneys after ischemic or toxic injuries[16−17]. The urine KIM-1 concentration is correlated with acute kidney injury (AKI) severity and decreases as kidney repair progresses[18].

Vaidya et al., have already constructed a sandwich ELISA kit for KIM-1 detection in rat urine using two mouse monoclonal antibodies[17]. The sandwich Kim-1 ELISA was to develop a sensitive quantitative urinary test to identify renal injury in the rodent. Two mouse monoclonal antibodies were made against the purified ectodomain of Kim-1, and these were used to construct a sandwich Kim-1 ELISA. The assay range of this ELISA was 50 pg/mL to 5 ng/mL, with inter- and intra-assay variability of ＜10%. In addition, previous reports have shown that three expressed feline KIM-1 transcript variants comprising 894, 810, and 705 bp were identified in renal tissue, and the intensity of KIM-1 expression is increased proportionally with the severity in kidney-damaged cats[19,20]. Immunohistochemistry of kidney sections identified KIM-1 in proximal tubular cells of cats with positive urine immunoassay results[20,21], but a quantitative method has not previously been established. We have constructed a sandwich ELISA kit for KIM-1 detection in cat urine using KIM-1 N peptide and KIM-1 C peptide antibodies. We developed an ELISA that was satisfactory in terms of recovery (96.2−103.9%) and within-run (3.2−4.5%) and between-day (3.4−4.8%) reproducibility. This assay was used to measure urine KIM-1 as a potential biomarker to distinguish CKD stage 1 cats from normal cats.

KIM-1 was identified by proteomics analysis, and such analyses have resulted in identification of many new biomarkers, but most are not appropriate for clinical application. The procedures for developing biomarkers include comparison of disease and control groups by semiquantitative analysis (discovery phase) and selection of marker candidates (validation phase), followed by ELISA validation of markers found with proteomics. Use of proteomics allows a comprehensive analysis of protein levels in vivo and relationships with disease.

Urine KIM-1 in CKD stage 1 cats (63.7±10.7 μg/g Cre) was significantly higher than that in normal cats (35.7± 9.7 μg/g Cre) (p＜0.001). This suggests that KIM-1 is a new marker for CKD that is complementary to conventional markers. The simple ELISA developed in this study was useful for establishing the diagnostic significance of urine KIM-1 in CKD stage 1 cats.

Conclusion

Our results suggest that KIM-1 may be used as a marker complementary to p-Cre and BUN for detection of CKD (stage 1) in cats. We note that the study is limited by the number of samples, and evaluation of more samples from multiple facilities is required in a future study. The fact that longitudinal follow-up of cats in stage 1 CKD would have helped to determine those that have true CKD which is progressive vs. those still recovering from AKI when the samples were taken.

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Conflict of Interest

There are no conflict of interest relevant to this article.

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