Kidney injury molecule-1 levels are associated with therapeutic outcomes and renal tubulointerstitial injury severity in idiopathic membranous nephropathy

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Abstract. Kidney injury molecule-1 (KIM-1) has an important role in chronic kidney disease development. The present study aimed to retrospectively analyze patients with idiopathic membranous nephrology (IMN) with different therapeutic outcomes to investigate the association between KIM-1 levels and therapeutic outcomes. A total of 51 patients with IMN and 20 healthy controls were included. Patients were classified into three groups: Spontaneous remission, remission with immunosuppressive therapy (IST) and nonremission with IST. Clinical and biochemical variables were collected. Urinary KIM-1 levels were measured by ELISA and renal KIM-1 expression was evaluated by immunohistochemistry. Patients with IMN were characterized as having elevated urinary and renal KIM-1 levels compared with those in the controls. Significantly increased urinary and renal KIM-1 levels were observed in the nonremission with IST group compared with those in the spontaneous remission group, and the same trend was observed for the plasma anti-podocyte antigen phospholipase A2 receptor antibody levels. Patients with more severe tubular injury (T2 index) presented with significantly higher urinary and renal KIM-1 levels than those with the T0 index. Urinary and renal KIM-1 levels were positively correlated with blood urea nitrogen, serum creatinine, serum cystatin-C, urinary albumin/creatinine ratio, urinary β2-microglobulin and the renal interstitial fibrosis index, and they were negatively correlated with serum albumin. Furthermore, urinary KIM-1 levels were positively correlated with the renal KIM-1 levels. In conclusion, the measurement of urinary and renal KIM-1 levels may be helpful in guiding medication selection and predicting therapeutic outcomes for patients with IMN.

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Introduction

Idiopathic membranous nephropathy (IMN) remains one of the most common causes of nephrotic syndrome (NS) in adults, accounting for ~20% of all NS cases (1). The proportion of patients with MN among patients with primary glomerular disease was increased from 10.77% in 2009 to 32.98% in 2018 in mainland China (2). A major breakthrough was the identification of the podocyte antigen phospholipase A2 receptor (PLA2R) as the target of circulating antibodies in ~70% of patients with IMN, which confirmed that IMN is fundamentally an antibody-mediated autoimmune disease (3). IMN treatment consists of immunosuppressive therapy (IST) and conservative therapy (4). IST has been proven to be effective in increasing the probability of the remission of proteinuria and protecting patients from renal function deterioration (5). Immunosuppressive agents are recommended in patients at high risk of developing end-stage renal disease (ESRD) (6). Patients with a low risk for ESRD are treated with angiotensin-converting enzyme inhibitors and/or angiotensin II receptor blockers, which are referred to as 'conservative therapy' (7). There are still certain patients who do not enter remission after taking different types of immunosuppressive agents for at least 6 months while suffering from numerous side effects. Therefore, novel useful and predictive markers to determine the appropriate therapeutic strategy and predict the prognosis of patients are in high demand.

In recent years, research interests have focused kidney injury molecule-1 (KIM-1). KIM-1, a sensitive and specific marker for the presence of tubular damage (8), is not expressed in the normal kidney, but its expression is induced and markedly increased in proximal tubular epithelial cells after various types of kidney injury (9,10). It has been demonstrated that urinary KIM-1 levels are closely correlated with the severity,

therapeutic response and prognosis of various kidney diseases, including IgA nephropathy, lupus nephritis and diabetic nephropathy (4,11-14).

In the present retrospective study, KIM-1 levels in urine and its expression in renal biopsy tissues from adult patients with IMN and healthy controls were analyzed and the association between KIM-1 and the therapeutic efficacy of IMN was determined. Furthermore, KIM-1 expression levels were compared between patients with different clinical indexes and pathological parameters.

Materials and methods

Patients. Patients were recruited from the Department of Nephrology at Qilu Hospital of Shandong University (Jinan, China) between January 2010 and December 2012. The inclusion criteria were as follows: i) Typical features of membranous nephropathy detected by light and electron microscopy; ii) No clinical and/or laboratory signs of secondary glomerulus nephritis; iii) No previous treatment with corticosteroids or immunosuppressive drugs; and iv) Renal tissue samples were available for immunohistochemistry and urine samples for the measurement of urinary KIM-1. A total of 51 patients with IMN aged between 21 and 53 years were included in this retrospective clinical study. Based on the treatment strategy (6) and curative effect, patients were classified into three groups: Spontaneous remission (n=18), remission with IST (n=20) and nonremission with IST (n=13). Remission included complete remission and partial remission. Complete remission was defined as urinary protein excretion of 0.3 g/day [urine protein creatinine ratio (uPCR) 300 mg/g] based on two values obtained at least 1 week apart accompanied by a normal serum albumin concentration and normal serum creatinine (sCr) levels. Partial remission was defined as urinary protein excretion <3.5 g/day (uPCR, 3,500 mg/g) with a 50% or greater reduction based on the peak values, as indicated by two values obtained at least 1 week apart, accompanied by improvement or normalization of the serum albumin concentration and a stable sCr (6). The movement of the patients in the present study is depicted in Fig. 1.

In addition, during the same time window as the patients, 20 age- and sex-matched healthy adults from the medical examination center of Qilu Hospital of Shandong University (Jinan, China) were recruited as controls. The present study was approved by the Ethics Committee of Qilu Hospital of Shandong University (Jinan, China).

Clinical examination. Demographic, clinical and biochemical variables were recorded at the time-point of diagnosis. The blood pressure of all subjects was measured in a sitting position using a mercury sphygmomanometer after a 10-min rest. The biochemical parameters were measured in the clinical laboratory department of Qilu Hospital of Shandong University (Jinan, China). sCr, blood urea nitrogen (BUN), serum albumin (sALB), serum cystatin-C (sCys-C), serum complement C3 (C3), serum C4, routine urine values, urinary albumin/creatinine ratio (ACR) and urinary β2-microglobulin (β2-MG) were determined. The estimated glomerular filtration rate (eGFR) was determined according to the Chronic Kidney Disease Epidemiology Collaboration formula (15). Plasma and urine samples were collected. After centrifugation at 867 x g for 10 min, the supernatants separated from the samples were frozen at -80˚C until further analysis.

Measurement of anti-PLA2R antibody (PLA2R-Ab) levels. The levels of PLA2R-Ab in plasma were measured using an ELISA kit (cat. no. EA 1254-9601 G; Euroimmun).

Measurement of KIM-1 levels in urine. The KIM-1 concentration in urine was measured by a commercial ELISA kit (cat. no. DKM100; R&D Systems, Inc.) in accordance with the manufacturer's protocol. The lower limit of the detection of urinary KIM-1 was 0.046 ng/ml. The interassay coefficient of variation (CV) was 6.6% and the intra-assay CV was 4.1%. Urinary KIM-1 levels were normalized to those of urinary creatinine for each sample. The normalized data are expressed as the urinary KIM-1 concentration/creatinine concentration (ng/mg).

Histological parameters. Renal biopsy specimens of all patients were reviewed by a pathologist at Qilu Hospital of Shandong University (Jinan, China). The pathologic stage was determined according to Ehrenreich and Churg and samples were classified as disease stages I-IV (16). The percentage of glomerular sclerosis was calculated for all renal biopsies. The extent of tubular atrophy/interstitial fibrosis was classified according to the proportion of tubular atrophy and interstitial fibrosis as follows: Absent (T0), mild (<25%, T1), moderate (25-50%, T2) and severe (>50%, T3) (17).

Immunohistochemical analysis of KIM-1 in the kidney. Immunohistochemical staining for KIM-1 was performed on 4-μm paraffin sections of formaldehyde-fixed renal biopsy tissues. Normal renal tissues adjacent to neoplastic areas (paraneoplastic) within the nephrectomy specimens used to test for malignancy were taken as controls. In brief, sections were incubated in 3% H_2O_2 in methanol for 15 min at 37˚C to ablate endogenous peroxidase activity after dewaxing and rehydration at room temperature. The sections were directly incubated with mouse anti-human monoclonal antibody against KIM-1 (cat. no. MAB1750; R&D Systems, Inc.; 1:2,000) overnight at 4˚C. Sections were then washed and incubated with polymer helper (cat. no. D02-18; OriGene Technologies, Inc.) and horseradish peroxidase-labeled anti-mouse IgG polymer (cat. no. D02-18; OriGene Technologies, Inc; 1:300) for 20 min at room temperature. The colorimetric reaction of peroxidase was performed in 3,3-diaminobenzidine solution according to the manufacturer's protocol (cat. no. D02-18; OriGene Technologies, Inc.) after washing with PBS three times. The level of KIM-1 expressed in renal specimens was semiquantified by an image analysis system (Image-Pro® Plus version 6; Media Cybernetics, Inc).

Statistical analysis. Values are expressed as the median (25 and 75th percentile) or mean ± standard deviation where appropriate. Differences in quantitative parameters with a normal distribution among groups were assessed by one-way ANOVA. Tukey's multiple-comparisons test was used as post-hoc test after ANOVA. Differences in quantitative parameters with an abnormal distribution among groups were

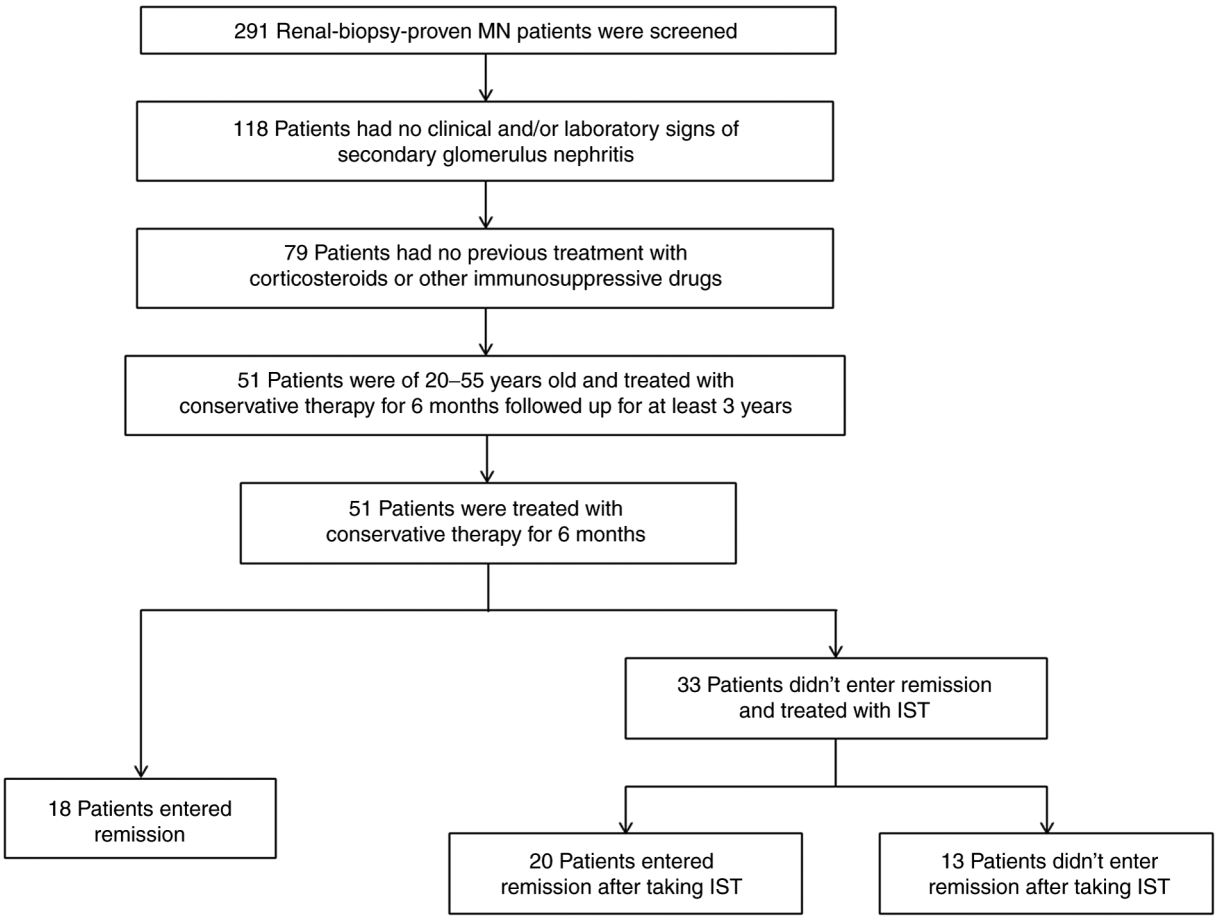

Figure 1. Schematic depicting the movement of the patients in the present study. MN, membranous nephrology; yrs, years; IST, immunosuppressive therapy.

assessed by the Kruskal-Wallis H-test. Dunn's test was used for further comparison after the Kruskal-Wallis analysis. Categorical variables were described as n or n (%) and comparisons among groups were performed using Mantel-Haenszel χ^2 tests for dichotomized variables. Spearman's correlation was used to analyze the correlation between two parameters. The sensitivity and specificity of urinary KIM-1/Cr and plasma PLA2R-Ab as indicators of the therapeutic effect in patients with IMN were compared using receiver operating characteristic (ROC) curves. An AUC value between 0.85 and 0.95 was considered to represent a high predictive value. A two-sided P<0.05 was considered to indicate statistical significance. Analysis was performed with the SPSS statistical software package (version 22; IBM Corp.).

Results

Clinical characteristics of patients with IMN and healthy subjects. The clinical characteristics of patients (n=51) and healthy controls (n=20) are provided in Table I. There was no difference in the sex distribution, age, BMI, diastolic or systolic blood pressure, sCys-C, eGFR, and serum C3 and C4 among the groups. There were more subjects in the IMN patient group and significantly more patients in the nonremission with IST group who smoked and drank than in the control group (P<0.05). sCr in the nonremission with IST group was significantly higher than that in the control group (P<0.05). Serum BUN was significantly higher in the nonremission with IST group than in the control group (P<0.05). The values of sCr and BUN in the patient groups were still within the normal range. Furthermore, the sALB level in all IMN groups was significantly lower than that in the control group (P<0.01). Among the IMN groups, the spontaneous remission group had the highest sALB levels (30.9±5.4), while the nonremission with IST group had the lowest sALB levels (21.3±4.0) and the differences were statistically significant between any two groups (P<0.05). The urinary ACR in all IMN groups was significantly higher than that in healthy subjects, as expected (P<0.01).

Furthermore, the urinary ACR in the nonremission with IST group was significantly higher than that in the spontaneous remission group (P<0.05). Urinary β2-MG in the nonremission with IST group was significantly higher than that in the spontaneous remission group and remission with IST group (P<0.01). PLA2R-Ab was detected in 12 (66.7%), 14 (70%) and 9 (69.2%) of patients at baseline in the spontaneous remission, remission with IST and nonremission with IST groups, respectively (Table I). The plasma PLA2R-Ab levels were significantly higher in the remission with IST group (P<0.05) and nonremission with IST group (P<0.01) than in the spontaneous remission group. There was no significant difference in terms of the histological score or renin-angiotensin system inhibitor therapy among the three patient groups.

Table I. Clinical characteristics of patients with IMN and healthy subjects.

Characteristic	Healthy subjects (n=20)	Patients with IMN (n=51)		
		Spontaneous remission (n=18)	Remission with IST (n=20)	Non-remission with IST (n=13)
Demographic/baseline data				
Male sex	11 (55)	9 (50)	11 (55)	12 (92.3)
Age (years)	38.1±10.0	36.7±10.9	36.0±9.7	39.3±8.9
BMI (kg/m^2)	23.4±2.6	23.8±3.3	25.8±4.1	26.2±3.2
SBP (mmHg)	121.8±8.5	121.6±17.0	130.8±26.7	139.2±18.8
DBP (mmHg)	76.7±7.8	77.6±10.5	80.8±12.6	83.4±15.3
Smoking	0	4 (22.2)	5 (25.0)	8 (61.5)[a]
Drinking	0	1 (5.6)	3 (15)	6 (46.2)[a]
sCr (μmol/l)	51.5±11.7	54.7±12.4	56.5±13.9	65.8±7.5[b]
BUN (mmol/l)	4.14±0.97	4.32±1.27	5.00±2.00	5.61±1.77[a]
sALB (g/l)	44.3±3.3	30.9±5.4[b]	26.3±6.0[b,c]	21.3±4.0[b,d,e]
Serum Cys-C (mg/l)	0.83±0.14	0.84±0.16	1.01±0.63	1.06±0.28
eGFR (ml/min/1.73 m^2)	124.5±12.9	122.1±11.9	123.0±10.9	114.6±12.9
Serum C3 (mg/dl)	(-)	1.26±0.19	1.31±0.34	1.20±0.16
Serum C4 (mg/dl)	(-)	0.30±0.09	0.28±0.09	0.30±0.07
Urinary ACR (g/g)	0.01 (0.01-0.03)	2.09 (1.84, 2.79)[a]	2.36 (0.87, 3.91)[a]	4.57 (1.54, 6.51)[a,e]
Urinary β2-MG (mg/l)	NA	0.21 (0.20, 0.22)	0.21 (0.20, 0.22)	0.22 (0.27, 1.48)[d,f]
PLA2R-Ab-positive patients (ELISA)	(-)	12 (66.7)	14 (70.0)	9 (69.2)
PLA2R-Ab titer (ELISA) (RU/ml)[g]	(-)	5.1 (2.4, 28.2)	57.6 (31.7, 131.4)[c]	191.5 (122.8, 406.0)[d]
Histological scores				
Glomerular sclerosis (%)	NA	0 (0,0)	0 (0,5.8)	0 (0,10.5)
Interstitial fibrosis (%)				
Absent	NA	10 (55.6)	7 (35.0)	5 (38.5)
Grade I	NA	8 (44.4)	13 (65.0)	5 (38.5)
Grade II	NA	0	0	3 (23.0)
Grade III	NA	0	0	0
Therapy				
Renin-angiotensin system inhibitors	0	11 (61.1)	12 (60.0)	8 (61.5)
ACEI alone	0	2	9	3
ARB alone	0	7	3	5
Both	0	2	0	0
IST and duration				
Steroids+CYC, 6 months A	0	0	16 (80)	0
A+Tacrolimus, 6 months B	0	0	3 (15)	0
A+B+MMF, 6 months	0	0	1 (5)	13 (100)
Follow-up data				
Follow-up duration (months)		37.1±6.35	37.7±5.37	38.2±6.17
6 months-averaged urinary ACR (g/g)		0.9 (0.3, 2.0)	1 (0.3, 2.3)	4.3 (2.8, 5.1)[c,e]
eGFR declined rate (ml/(min x1.73 m^2) per year		1.86 (-0.96, 4.20)	2.66 (1.6, 4.43)	4.66 (3.33, 6.00)

[a]P<0.05, [b]P<0.01, compared with control; [c]P<0.05, [d]P<0.01, compared with spontaneous remission group; [e]P<0.05, [f]P<0.01, compared with remission with IST group; [g]Median (IQR) of PLA2R-Ab titer only in patients with PLA2R-Ab. Values are expressed as the mean ± standard deviation, n (%) or the median (IQR). Normal ranges of the measurement/laboratory parameters include: BMI, 18.5-23.9 kg/m^2; sCr, 40.0-135.0 μmol/l; BUN, 3.50-6.10 mmol/l; sALB, 35.0-54.0 g/l; Serum Cys-C, 0.60-2.50 mg/l; serum C3, 0.79-1.52 mg/dl; serum C4, 0.16-0.38 mg/dl; Urinary ACR, 0-0.03 g/g; and urinary β2-MG, 0-0.22 mg/l. (-), negative result; NA, not applicable; IQR, interquartile range; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; IMN, idiopathic membranous nephrology; PLA2R-Ab, anti-podocyte antigen phospholipase A2 receptor antibody; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blockers; sCr, serum creatinine; BUN, blood urea nitrogen; sAlb, serum albumin; sCys-C, serum cystatin-C; C3, complement C3; ACR, albumin/creatinine ratio; β2-MG, β2-microglobulin; IST, immunosuppressive therapy; A, accepted steroid+CYC therapy for 6 months; B, accepted Tacrolimus therapy for 6 months; CYC, cyclophosphamide; MMF, mycophenolate mofetil.

All patients were followed up for ~36 months. The 6-month average urinary ACR in the nonremission with IST group was significantly higher than that in the spontaneous remission group and remission with IST group (P<0.05). There was no significant decrease in the rate of eGFR among the three groups (Table I).

Urinary KIM-1 levels in patients with IMN. The levels of urinary KIM-1/Cr were significantly higher in patients with IMN than in healthy subjects (P<0.05, Fig. 2A). Of note, urinary KIM-1/Cr levels were significantly increased in the nonremission with IST group compared with those in the spontaneous remission group (P<0.05, Fig. 2A).

Urinary KIM-1 levels were also analyzed according to the tubular atrophy and interstitial fibrosis index based on renal biopsy (T0, T1 and T2). The urinary KIM-1/Cr levels gradually increased as tubular atrophy and interstitial fibrosis became more severe. Patients with both T1 and T2 changes had significantly higher urinary KIM-1/Cr levels than those with T0 (P<0.05, Fig. 2B). However, there was no significant difference between patients with T1 and T2 changes (P>0.05, Fig. 2B). Consistently, there were no patients with T2 in any groups other than the nonremission with IST group (Fig. 2C).

Renal KIM-1 expression in patients with IMN. KIM-1-positive staining was present in proximal tubule epithelial cells of patients with IMN, while no KIM-1-positive cells were observed in normal renal tissue (Fig. 3A). Furthermore, KIM-1 expression was only detected at a very low level in the tissues from the spontaneous remission group. KIM-1 expression was significantly increased in the other two groups of patients compared with that in the spontaneous remission group (P<0.01), but there was no significant difference in KIM-1 expression between the two IST groups (P>0.05, Fig. 3B).

Renal KIM-1 levels were analyzed again according to the tubular atrophy and interstitial index. Renal tissues with T2 had significantly higher KIM-1 expression than those with T0 (P<0.01, Fig. 3C). Although KIM-1-positive staining appeared to be deeper in renal tissues with T2 than those with T1 (Fig. S1), there was no significant difference in quantitative analysis between these tissues (P>0.05; Fig. 3C). This may be due to there being a greater proportion of patients with T2 in the nonremission with IST group than in the remission with IST and spontaneous remission groups.

Correlation of KIM-1 and plasma PLA2R-Ab levels with clinical parameters. As presented in Table II, urinary KIM-1/Cr was positively correlated with BUN (r=0.282, P=0.045), sCr (r=0.311, P=0.026), sCys-C (r=0.433, P=0.001), the urinary ACR (r=0.306, P=0.029), urinary β2-MG (r=0.410, P=0.003) and the tubular atrophy and interstitial fibrosis index (r=0.572, P<0.001). Furthermore, it was negatively correlated with sALB (r=-0.343, P=0.014) at the time of renal biopsy. However, it was not correlated with the eGFR, Ehrenreich-Churg stage or glomerular sclerosis index in renal tissues.

Renal tissue KIM-1 expression levels were positively correlated with BUN (r=0.361, P=0.009), sCr (r=0.315, P=0.024), sCys-C (r=0.321, P=0.022), the urinary ACR (r=0.396, P=0.004), tubular atrophy and interstitial fibrosis index (r=0.498, P<0.001) and urinary β2-MG (r=0.497, P<0.001) and negatively correlated with sALB (r=-0.433, P=0.002).

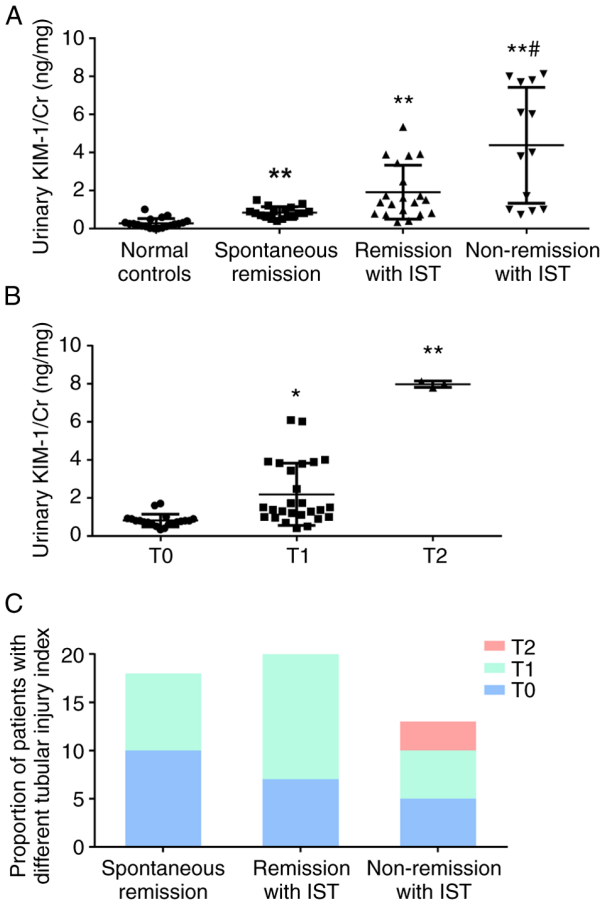

Figure 2. (A) Urinary KIM-1 levels in patients with idiopathic membranous nephrology and normal controls. **P<0.01 vs. control; #P<0.05 vs. spontaneous remission group. (B) Urinary KIM-1 levels in patients with different tubular injury indexes. *P<0.05, **P<0.01 vs. T0. (C) The proportions of patients with different tubular injury indexes in the three different groups. The T0 group included 22 patients, the T1 group included 26 patients and the T2 group included 3 patients. KIM-1, kidney injury molecule-1; Cr, creatinine; IST, immunosuppressive therapy.

In addition, plasma PLA2R-Ab levels were positively correlated with sCr (r=0.403, P=0.016), the urinary ACR (r=0.390, P=0.020), urinary β2-MG (r=0.378, P=0.025) and the glomerular sclerosis index (r=0.419, P=0.012). It was negatively correlated with sALB (r=-0.454, P=0.006) and the eGFR (r=-0.369, P=0.029). However, it was not correlated with BUN, sCys-C, the Ehrenreich-Churg stage or the interstitial fibrosis index in renal tissues.

Correlation of urinary KIM-1 and renal KIM-1. Next, it was investigated whether there was a correlation between the urinary and renal KIM-1 levels. As presented in Fig. 4, there was a significant correlation between the urinary KIM-1/Cr and renal KIM-1 levels (Spearman r=0.910, P<0.001).

Predictive power of urinary KIM-1 and plasma PLA2R-Ab for the therapeutic effect. ROC curve analysis was performed in PLA2R-Ab-positive individuals to compare the predictive power of urinary KIM-1 and plasma PLA2R-Ab as biomarkers

Table II. Correlation of urinary or renal KIM-1 expression with serum PLA2R-Ab and clinical or histological indexes in patients with idiopathic membranous nephrology.

	Urinary KIM-1/Cr		Renal KIM-1 expression		Plasma PLA2R-Ab	
Item	r-Value	P-value	r-Value	P-value	r-Value	P-value
Clinical data						
BUN (mmol/l)	0.282	0.045	0.361	0.009	0.227	0.190
sCr (umol/l)	0.311	0.026	0.315	0.024	0.403	0.016
sCys-C (mg/l)	0.433	0.001	0.321	0.022	0.143	0.412
eGFR (ml/min x 1.73 m^2)	-0.146	0.306	-0.185	0.194	-0.369	0.029
sAlb (g/l)	-0.343	0.014	-0.433	0.002	-0.454	0.006
Urinary ACR (g/g)	0.306	0.029	0.396	0.004	0.390	0.020
Urinary β2-MG (mg/l)	0.410	0.003	0.497	<0.001	0.378	0.025
Histology parameters						
Ehrenreich-Churg stage	0.274	0.052	0.175	0.220	0.161	0.356
Glomerular sclerosis (%)	0.149	0.296	0.199	0.161	0.419	0.012
Tubular atrophy and interstitial fibrosis index	0.572	<0.001	0.498	<0.001	0.055	0.753

The correlation of PLA2R-Ab and clinical or histological indexes titer only in patients with PLA2R-Ab. KIM-1, kidney injury molecule-1; PLA2R-Ab, anti-podocyte antigen phospholipase A2 receptor antibody; sCr, serum creatinine; BUN, blood urea nitrogen; sAlb, serum albumin; sCys-C, serum cystatin-C; ACR, albumin/creatinine ratio; β2-MG, β2-microglobulin; eGFR, estimated glomerular filtration rate.

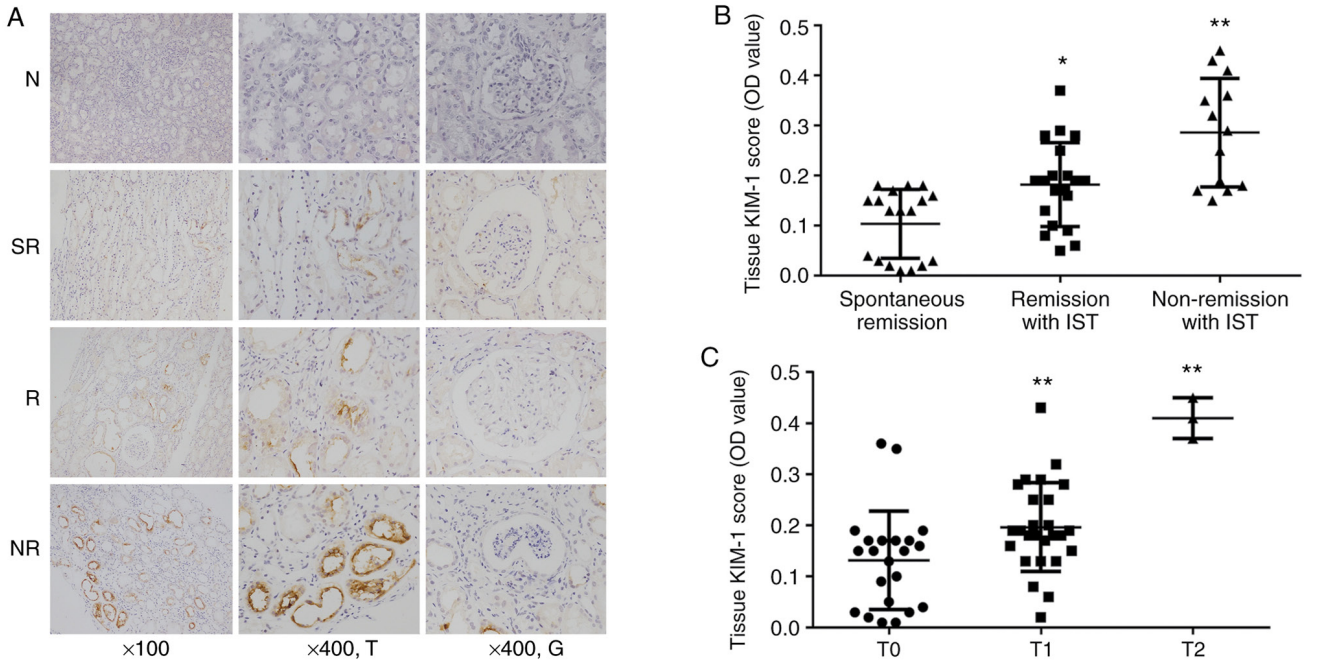

Figure 3. (A) Representative histology images of renal KIM-1 expression in the control and IMN groups (magnification x100 or x400; T and G indicate tubules and glomeruli, respectively. Samples were counterstained with hematoxylin with brown areas indicating positive staining). Groups: N, normal control group; SR, spontaneous remission group; R, remission with IST group; NR, nonremission with IST group. (B) Semiquantitative analysis of tissue KIM-1 expression levels in different groups of patients with IMN. *P<0.05, **P<0.01 vs spontaneous remission group. (C) Semiquantitative analysis of tissue KIM-1 expression levels in patients with IMN with different tubular injury indexes. **P<0.01 vs T0. KIM-1, kidney injury molecule-1; IMN, idiopathic membranous nephrology; OD, optical density; IST, immunosuppressive therapy.

of the therapeutic effect in patients with IMN. The area under the ROC curve (AUC) was measured to evaluate the sensitivity and specificity of urinary KIM-1/Cr, PLA2R-Ab and their combination. As presented in Fig. 5, the AUC for urinary KIM-1/Cr in predicting the therapeutic effect was 0.715 (95% CI, 0.533-0.898) and the AUC of PLA2R-Ab was 0.775 (95% CI, 0.560-0.990). The predictive power of their combination was the highest, with an AUC of 0.852 (95% CI, 0.721-0.983).

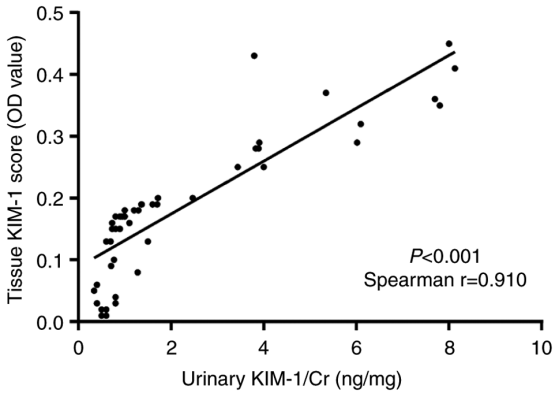

Figure 4. Positive correlation between the urinary KIM-1/Cr levels and tissue KIM-1 expression. KIM-1, kidney injury molecule-1; Cr, creatinine; OD, optical density.

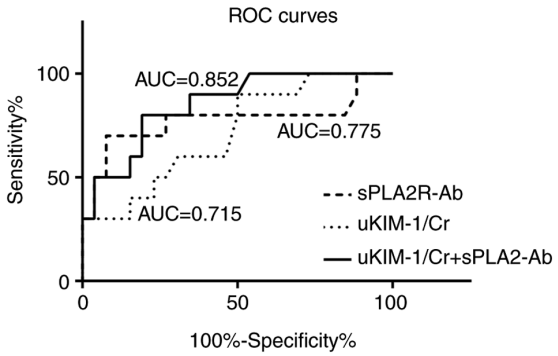

Figure 5. ROC curves for uKIM-1 and sPLA2R-Ab to predict the therapeutic effect in patients with IMN. Urinary KIM-1, urinary kidney injury molecule-1; Cr, creatinine; ROC, receiver operating characteristic; AUC, area under the ROC curve; sPLA2R-Ab, serum anti-podocyte antigen phospholipase A2 receptor antibody.

Discussion

The presence and severity of membranous nephropathy are generally indicated by the degree of podocyte injury. High urinary concentrations of proteins that reflect tubular or glomerular damage precede a decline in the eGFR in patients with IMN (18,19). Urinary KIM-1 is a novel biomarker of tubular damage. There is increasing evidence of the prognostic value of KIM-1 in patients with glomerular disease, including diabetic kidney disease and IMN (20,21). However, the influence of the levels of KIM-1 in urine and renal tissue on the therapeutic efficacy for IMN has remained to be determined. In the present study, KIM-1 expression levels were analyzed according to the therapeutic outcomes for patients with IMN. The results suggested that patients with IMN had elevated urinary and renal KIM-1 levels compared with those in normal control subjects. Significantly increased urinary and renal KIM-1 levels were observed in the nonremission with IST group compared with those in the spontaneous remission group. The present study suggested that higher KIM-1 levels in both urine and tissue at the time of renal biopsy are associated with poorer treatment outcomes for IMN. The present study also confirmed the link between urinary and tubular KIM-1 expression in IMN, since urinary KIM-1 levels are strongly correlated with tubular KIM-1 expression in experimental and human renal disease in both acute kidney injury and ESRD (22). Due to the correlation between urinary and tubular KIM-1 expression, the levels of KIM-1 may be detected directly from urine without necessitating an invasive kidney biopsy in the future.

KIM-1, which is located on epithelial cells, mediates the phagocytosis of apoptotic and necrotic cells by binding to phosphatidylserine and oxidized lipid epitopes on the apoptotic cell surface (23). Various experimental and clinical studies suggested that KIM-1 reflects tubulointerstitial injury and repair (24). In the present study, increased tubular KIM-1 expression was indicated to be positively associated with BUN, sCr, sCys-C, the urinary ACR and urinary β2-MG at the time of renal biopsy, which suggested that KIM-1 provides additional information on tubular processes involved in progressive renal failure.

MN is the major cause of nephrotic syndrome with special pathological features resulting from the formation of immune complexes in the space between podocytes and the glomerular basement membrane. PLA2R-Ab production is common and is detected in 70-80% of patients with IMN (25). Autoantibodies against M-type PLA2R are specific markers of IMN. The PLA2R-Ab level was reported to be an independent predictor of the risk of remission in proteinuria and to be of prognostic value in IMN (26). The strong correlation between the clinical conditions and PLA2R-Ab levels allowed the prediction of the distribution of the prevalence in patients with active disease and partial and complete remission (27). In the present study, plasma PLA2R-Ab levels were positively correlated with proteinuria. A significantly higher plasma PLA2R-Ab level was observed in the nonremission with IST group than in the spontaneous remission group. Furthermore, significantly higher urinary KIM-1 levels were observed in the nonremission with IST group than in the spontaneous remission group. Therefore, KIM-1 has prognostic value for tubular injury and may be considered a promising biomarker to evaluate the prognosis of patients with IMN together with PLA2R-Ab, which is of prognostic value for glomerular injury.

The patients of the present study were biopsied at an early stage of the disease and subjected to conservative treatment or IST. Patients who were treated with IST but who did not enter remission displayed much more highly elevated urinary KIM-1 levels than those in the spontaneous remission group. This may aid the identification of the IST group at the early stage of the disease. Children with steroid-resistant idiopathic nephrotic syndrome were likely to present with high urinary KIM-1 excretion and had a higher risk of tubulointerstitial fibrosis (22). The primary finding of the report is that chronic KIM-1 expression in renal epithelial cells directly causes interstitial inflammation followed by progressive fibrotic renal disease (28). The present study also indicated that urinary KIM-1 levels exhibited a gradually increasing trend as tubular atrophy and interstitial fibrosis became more severe. KIM-1 excretion rates correlated with the excretion rates of other tubular damage markers, such as urinary α1 microglobulin and urinary β2-MG, and predicted the outcome for patients with IMN. In the present study, it was indicated that urinary KIM-1 levels were positively associated with the Ehrenreich-Churg stage, but this correlation was not observed for renal KIM-1 expression.

The urinary and renal KIM-1 expression levels increased as tubular atrophy and interstitial fibrosis became more severe, which confirmed previous observations.

The major limitation of the present study is that it is a single-center descriptive study with a small sample size, which may limit the broader applicability of the results. Trends regarding differences in KIM-1 expression levels between the two groups of patients treated with IST may be significantly different in a study with a more adequate sample size. All patients included in the present study were at the early stage of IMN with an Ehrenreich and Churg stage of I or II. However, histology scoring could have been performed instead of measuring the OD value when analyzing the expression of KIM-1 in renal tissues. In addition, long-term follow-up of the patients is required to perform a more comprehensive analysis. The dynamic change of KIM-1 levels may provide more values associated with therapeutic treatment and outcomes. Earlier data for urinary KIM-1 levels were not gathered, since the present study had a retrospective design, and a prospective study that dynamically describes the relationship between KIM-1 levels and outcome for patients with IMN will be performed in the near future. Furthermore, the present study mainly focused on the relationship of KIM-1 levels with therapeutic outcomes for IMN and without considering the impact of smoking and drinking. It may also be interesting to compare the non-smoking/drinking subjects to the non-smoking/drinking subjects in the same group if the group sizes were decent in a future prospective study.

In conclusion, urinary and renal KIM-1 levels may be used as biomarkers of tubulointerstitial damage and to evaluate the therapeutic effects of IMN treatment. Further studies are required to elucidate the exact role of KIM-1 in IMN and the correlation between tubulointerstitial injury and the treatment effect.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ZH and XYX were responsible for the study design. YDZ, CHX, LYG, YYZ and JHZ contributed to acquiring and analyzing the data. YDZ and CHX were involved in writing the manuscript. All authors read and approved the final version of the manuscript. All authors have confirmed the authenticity of the raw data.

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee at the institution in which the study was conducted and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The present study was approved by the Ethics Committee of Qilu Hospital of Shandong University [Jinan, China; institutional review board approval no. KYLL-2018(KS)-234]. Written informed consent was obtained from all subjects and/or their guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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