

The levels of urinary joining chain containing IgG immune complexes are associated with disease severity in IgA nephropathy

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Abstract

Background: Circulating levels of aberrantly glycosylated IgA1 and its immune complexes (IgA/IgG-IC) are elevated and correlated with disease severity in IgA nephropathy (IgAN). The pathologic IgA containing immune complexes deposits in the kidney were found to be in dimeric or polymeric forms, suggesting that those deposited IgA immune complexes contain joining chain, which is critical for the multimerization and transportation of IgA. Dimeric IgA and polymeric IgA both can cause renal damage by inducing release of cytokines from mesangial cells. We aimed to investigate the urinary J chain containing IgG immune complexes/creatinine ratio (UJGCR) and analyze its relationship with disease severity in IgAN. **Methods:** The UJGCR were measured by sandwich enzyme linked immunosorbent assay in 26 patients with IgAN, 31 patients with other kidney diseases and 32 healthy volunteers. **Results:** The levels of UJGCR were higher in patients with IgAN than that in non-IgAN patients ($P = 0.006$) and healthy volunteers ($P < 0.0001$). Importantly, receiver operating characteristics curve analysis confirmed that UJGCR had good discrimination between non-IgAN patients and IgAN patients. The levels of UJGCR positively correlated with 24-hour urinary protein excretion ($r = 0.63$, $P = 0.0006$), serum creatinine ($r = 0.55$, $P = 0.003$), and negatively correlated with estimated glomerular filtration rate ($r = -0.61$, $P = 0.0008$) in IgAN. Furthermore, the levels of UJGCR were higher in IgAN patients with IgA mesangial deposition of (+/++) than patients with (+++/++++). And IgAN patients with tubular atrophy/interstitial fibrosis showed higher levels of UJGCR than that without ($P = 0.03$). Similarly, the levels of urinary IgA-IgG/creatinine ratio (UAGCR) were also found to be elevated and associated with clinical and pathological parameters as UJGCR in IgAN. Besides, significant correlations between the levels of UJGCR and UAGCR were shown, suggesting UJGCR were mainly composed of J-IgA-IgG. **Conclusions:** The levels of UJGCR are associated with disease severity in IgAN. UJGCR is a potential biomarker for both glomerular and tubulointerstitial lesions in IgAN.

Background

IgA nephropathy (IgAN) is one of the most common glomerulonephritis worldwide [1, 2]. IgAN is associated with a poor prognosis, over 40% of patients with IgAN progress slowly to end-stage renal failure (ESRD) 30 to 40 years after diagnosis [3]. Regular measurement of kidney injury and disease progression is crucial for patients with IgAN during the follow-up period. Although proteinuria, hypertension, renal dysfunction and a few histological features have been identified as parameters for disease severity of IgAN [4-6], these biomarkers are not always specific for an individual. Thus, non-invasive and more specific biomarkers are still needed to accurately assess the disease severity in IgAN.

IgAN is an immune complexes mediated glomerulonephritis characterized by the presence of IgA containing immune complexes deposits in the mesangial area [7]. Although the pathogenesis of IgAN is still under investigation, it has been suggested that galactose-deficient IgA1 (Gd-IgA1) and its corresponding immune complexes (IgA/IgG-IC) were found to be elevated and in IgAN [8-12]. Berthoux et al. reported that serum Gd-IgA1 and IgA/IgG-IC are associated with progression in IgAN [9]. And Suzuki et al. found that these serum biomarkers are associated with the degree of hematuria and proteinuria in

IgAN [11]. Evidences from recent researches suggest that Gd-IgA1 and IgA/IgG-IC accumulate in the glomerular mesangium to instigate renal damage [13, 14].

Researches from the past decades led to an agreement that these IgA deposits in the kidney are in dimeric or polymeric forms [15-17], revealing that these deposited IgA immune complexes contain a small disulfide-linked polypeptide of 15kDa known as the joining chain (J chain). It is generally agreed that J chain regulates the multimerization of IgA and IgM comparing to IgG, forming secretory IgA (sIgA), polymeric IgA (pIgA) and pentameric IgM (pIgM) [18-20]. J chain is also required for the transportation of sIgA across the mucosal epithelium, preventing attachment of bacteria and viruses to mucous membranes [20]. It is well known that the classic manifestation of IgAN is episodic hematuria with or without proteinuria following mucosal infection. It is possible that mucosal immunity dysregulation in IgAN might lead to the increased synthesis of sIgA which is transported across the mucosa with the help of J chain and thus reaches the circulation and deposit in mesangium. Several investigations have revealed that the serum levels of sIgA and pIgA are elevated in IgAN [21-24]. And J chain had been found in renal biopsy specimens of IgAN patients using anti-J chain polyclonal antibody or antisera [15, 16, 25]. Dimeric IgA and pIgA both can cause renal damage by inducing release of cytokines, including interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), and transforming growth factor- β (TGF- β) by mesangial cells [26-29]. It is possible that a fraction of circulating J chain containing immune complexes deposits in the kidneys of IgAN patients are excreted into the urine and thus represent as non-invasive disease-specific biomarkers in IgAN. However, only one previous study found that the levels of urinary IgA-IgG complexes were significantly higher in IgAN patients [30]. The value of urinary IgA-IgG immune complexes for accessing disease severity in IgAN remains unclear. In addition, urinary J chain in IgAN has not been reported and it is not known whether the levels of urinary J chain containing immune complexes are associated with clinical or pathological severity in IgAN.

In this study, we detected urinary J chain containing IgG (J-IgG) immune complexes by sandwich enzyme linked immunosorbent assay (ELISA) with anti-J chain monoclonal antibody (mAb). We analyzed the association between the levels of urinary J-IgG immune complexes and clinicopathological parameters of IgAN at the time of renal biopsy.

Methods

Patients

We enrolled 26 patients with biopsy-proven primary IgAN (IgAN group), 31 patients with other renal diseases (Disease control group, DC group) and 32 healthy volunteers (Healthy control group, HC group). All patients admitted to Peking Union Medical College Hospital between April 2016 and September 2016. The histologic diagnosis of IgAN was based upon the demonstration of mesangioproliferative changes on light microscopy and the concomitant presence of predominant or codominant mesangial deposition of IgA. Patients with lupus nephritis (LN), Henoch–Schönlein purpura, liver cirrhosis and other secondary causes of IgAN were excluded from the study. The diagnoses of DC group included membranous

nephropathy (n=15), minimal change nephropathy (n=3), LN (n=1), diabetic nephropathy (n=2), focal segmental glomerulosclerosis (n=2), chronic pyelonephritis or interstitial nephritis (n=4), hypertensive renal damage (n=1) and sclerosing glomerulonephritis (n=2), obesity-related glomerulopathy (n=1).

Clinical and pathological manifestations

The demographic and clinical parameters of patients, including age, gender, blood pressure (BP), urinary red blood cells count, serum creatinine (SCr), urine creatinine (UCr) and 24-hour urinary protein excretion (24hUPro) were obtained immediately before renal biopsy. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [31].

Medication history, including the usage of cortisone, immunosuppressant and renin-angiotensin system (RAS) blockers such as angiotensin-converting enzymes inhibitors (ACE-Is) and angiotensin II receptor blockers (ARBs) was also recorded.

Renal biopsies

For each patient, the diagnosis of IgAN was based on histologic assessment of renal biopsy tissue with hematoxylin and eosin, periodic acid–Schiff and Masson's trichrome for light microscopy and staining with antibodies against IgA, IgG, IgM, C1q, C3, C4 and fibrinogen for immunofluorescence. IgAN was defined by the presence of at least 1+ (range, 0–4) IgA mesangial deposits as dominant or codominant immunoglobulins on immunofluorescence microscopy performed on frozen tissue. Pathological features were evaluated according to updated Oxford classification [32] and Lee grading [33], which were graded by a pathologist blinded to patients' clinical data.

Urine samples

Spot morning urine samples from patients were collected on the same day of renal biopsy. All samples were centrifuged at 3000rpm/min for 10 min to remove cellular components and the supernatant was kept at -80°C until use. Spot morning urine from healthy controls were also collected and prepared in the same manner as for patients.

Generation of anti-J chain monoclonal antibody

J chain-GST recombinant peptide IGJ-pGEX-4T-1 (NCBI ID for J chain sequence: NM_144646) was synthesized and purified as an antigen to immunize BALB/c mice. Candidate hybridomas were

established from splenocytes (Absea Biotechnology Ltd., Beijing, China). Hybridomas that produce anti-J chain monoclonal antibodies were selected by ELISA with the recombinant antigen peptide, by Dot-blot with the pIgA purified from multiple myeloma patients and saliva that contains J chain-sIgA by Western Blot.

Detection of urinary J-IgG and IgA-IgG immune complexes by ELISA

To measure urinary immune complexes, ninety-six well microtiter plates were coated with anti-J chain mAb or anti-IgA mAb (Absea Biotechnology Ltd., Beijing, China) in carbonate-bicarbonate buffer, pH 9.6, overnight at 4 °C. The plates were washed three times with phosphate buffered saline (PBS) containing 0.05% Tween 20 (PBST) and then blocked with 200 µL/well of 2% bovine serum albumin (BSA) for 2 h at 37°C. The urine samples were added to the same plate and incubated for 3h at 37 °C. Then the plates were washed and HRP-conjugated mouse anti-human IgG (Absea Biotechnology Ltd., Beijing, China) was added and incubated for 1 h at 37 °C. After washing, the color was developed using TBS (Amresco, Solon, USA) as a substrate, and the absorbance was measured at 450 nm with a microplate reader (BioTek Synergy 4, Winooski, VT, USA).

Levels of urinary J-IgG (UJGCR) and IgA-IgG (UAGCR) immune complexes were standardized to the UCr concentration to adjust for differences of urine flow rate.

Statistical analysis

Statistical calculations were performed using SPSS for Windows, version 20.0 (SPSS, Chicago, IL) and Graph Pad Prism 5.0 (Graph Pad Software Inc., San Diego, CA). Data are presented as mean values ± SD, medians (interquartile range) or frequency in percent according to the types of variables. The Mann-Whitney U-test was used for statistical comparisons between two groups. Receiver operator characteristic curve (ROC) analysis was performed to assess the value of urine markers in differentiating between patients with IgAN and combined controls. Correlation analyses were performed using Spearman's correlation analyses. All tests were two-sided and a P-value < 0.05 was considered statistically significant.

Results

Demographic and clinical characteristics of the participants

Demographic and clinical characteristics of the study subjects are summarized in Table 1. Overall, our study population consisted of 26 patients with IgAN (9 males and 17 females), 31 patients with other renal diseases (16 males and 15 females) and 32 healthy volunteers (22 males and 10 females). The mean age was 41(32-47) years, 46(29-60) years and 46(29-60) years for the groups of IgAN, DC and HC, respectively. Compared to patients with other renal diseases, the values of 24hUPro were significantly

lower [1.32 (0.65-3.48) g/24h versus 4.05 (1.46-5.97) g/24h, $P=0.01$] and serum albumin were higher [39.00 (33.00-43.00) g/L versus 30.00(25.00-40.00)] in patients with IgAN.

A total of 16(61.5%) patients with IgAN were treated with RAS blockers, 8(30.8%) were treated with immunosuppressive therapy and 4(15.4%) received corticosteroids. In DC group, 17(54.8%) were treated with RAS blockers, 15(48.4%) were treated with immunosuppressive therapy and 18(58.1%) received corticosteroids. The utilization rate of corticosteroids in patients with IgAN was significant lower than that in patients of patients with other kidney diseases. However, the distribution of gender, age and treatments (immunosuppressants and ACE-Is / ARBs) did not differ between IgAN group and DC group.

UJGCR were elevated in patients with IgAN compared with controls

The levels of UJGCR were significantly higher in patients with IgAN than that in patients with other renal diseases (0.31 ± 0.20 OD450nm/mmol/L Cr versus 0.19 ± 0.17 OD450nm/mmol/L Cr, $P=0.006$) and healthy subjects (0.31 ± 0.20 OD450nm/mmol/L Cr versus 0.04 ± 0.04 OD450nm/mmol/L Cr, $P<0.0001$). And the levels of UAGCR were also significantly higher in patients with IgAN than that in patients with other kidney diseases ($P=0.004$) and healthy subjects ($P<0.0001$) (Fig.1). In addition, we found that the values of these urinary markers did not differ between IgAN patients with corticosteroid therapy ($n=4$) and those without ($n=22$) (data not shown). We also found that the values of urinary markers did not differ between IgAN patients with immunosuppressant therapy ($n=7$) and those without ($n=19$) (data not shown).

ROC comparing UJGCR in distinguishing patients with IgAN from patients with other renal diseases

ROC analysis confirmed good discrimination between patients with IgAN and patients with other renal diseases for elevated levels of UJGCR (AUC, 0.71; 95%CI, 0.58-0.85; $P=0.006$) and UAGCR (AUC, 0.72; 95% CI, 0.59–0.85; $P=0.04$) (Fig.2). The respective optimal derived cut-off values were 0.1491 OD450nm/mmol/L Cr for UJGCR (Sensitivity: 84.6%; Specificity: 58.1%) and 0.0877 OD450nm/mmol/L Cr for UAGCR (Sensitivity: 84.6%; Specificity: 58.1%).

Association between clinical parameters and urinary immune complex in patients with IgAN

In patients with IgAN, the levels of UJGCR were correlated positively with the levels of 24hUPro and SCr, and negatively correlated with eGFR ($r=0.63$, $P=0.0006$; $r=0.55$, $P=0.003$; $r=-0.61$, $P=0.0008$, respectively) (Fig.3a-c). Likewise, the significant correlation between the levels of UAGCR and 24hUPro, SCr and eGFR ($r=0.58$, $P=0.002$; $r=0.67$, $P=0.0002$; $r=-0.75$, $P<0.0001$, respectively). (Fig.3d-f) were also observed. There was no significant correlation between UJGCR or UAGCR and U-RBC (data not shown).

A significant correlation between UAGCR and UJGCR ($r=0.80$, $P<0.0001$) was observed in IgAN patients (see Additional file 1).

Association between pathological parameters and UJGCR in patients with IgAN

We examined the association between urinary immune complexes and different histological lesions. The intensity of the immunofluorescence of renal IgA deposition was graded as none (-), trace (\pm), (+), (++) , (+++) and (++++). Surprisingly, the levels of UJGCR [IgA deposition of (+/++): 0.36 (0.22-0.49) nm/mmol/L vs. IgA deposition of (+++/++++): 0.16 (0.08-0.22) OD450nm/mmol/L Cr, $P=0.0007$] were higher in IgAN patients with IgA mesangial deposition of (+/++) than patients with (+++/++++) (see Additional file 1).

Regarding Lee grading, UAGCR and UJGCR were significantly higher in patients of grade IV ($n=18$) than that in grade II and III ($n=8$ in total) (see Additional file 3). We also evaluated the tubular atrophy/interstitial fibrosis grade according to Oxford classification. The levels of UJGCR [T0: 0.16 (0.10-0.31) OD450nm/mg Cr vs. T1/T2: 0.36 (0.21-0.48) OD450nm/mmol/L Cr, $P=0.03$] were significantly higher in patients with tubular atrophy/interstitial fibrosis ($n=11$) than that without ($n=15$ in total). Similarly, the levels of UAGCR were also significantly elevated as UJGCR between two subgroups (see Additional file 3).

However, UJGCR and UAGCR of IgAN patients with different grades of mesangial hypercellularity, segmental glomerulosclerosis and cellular/fibrocellular crescents did not show any significant differences (data not shown).

Discussion

In the present study, our data showed that the levels of UJGCR and UAGCR were markedly elevated in patients with IgAN compared with patients with other kidney diseases and healthy subjects. These elevated urinary immune complexes can distinguish patients with IgAN from patients with other renal diseases, as shown by the AUC in the ROC analysis. Furthermore, we demonstrated for the first time that the elevated UJGCR and UAGCR correlated with clinical and pathological injury in patients with IgAN.

IgAN is characterized by the presence of IgA containing immune complexes deposits in the mesangium [7]. And studies have confirmed that Gd-IgA1 containing IgG complexes exhibited stronger stimulation of mesangial cell proliferation comparing to uncomplexed Gd-IgA1 [34-36]. Gd-IgA1 and IgA/IgG-IC in the circulation were found to be elevated and associated with progression and disease severity in IgAN [8-12]. These evidences strongly suggest that IgAN is a kind of immune complexes mediated glomerulonephritis. It is generally accepted that the IgA deposits in the kidney are in dimeric or polymeric forms [15-17, 23, 37], suggesting that those deposited IgA immune complexes contain J chain. Previous studies had demonstrated that J chain is required for the multimerization of IgA forming sIgA and pIgA [18-20]. And J

chain can also mediate the transportation of sIgA and pIgA through binding to Ig receptor (pIgR) on the basolateral side of epithelial cells [38-40]. It is possible that excessive production of galactose deficient sIgA after mucosal infection might be transported through the epithelium under the help of J chain, forming immune complexes with its glycan-specific IgG or IgA autoantibodies in the circulation, and thus deposit in the mesangium. A part of these immune complexes might pass through the filter barrier of injury glomeruli and thus enter the urine.

Karel et al have reported that the urinary concentrations of UAGCR were elevated and correlated with the magnitude of proteinuria [30], which is consistent with our study. But neither did they analyze the relationship between UAGCR and pathological lesions in IgAN, nor did they measure urinary J chain in IgAN. In this study, we found that the elevated levels of UJGCR and UAGCR were not only correlated with 24hUPro, but also correlated well with kidney function in IgAN, which are well-known factors in predicting disease severity and renal outcome for the development of ESRD in IgAN patients.

To the best of our knowledge, this is the first study to demonstrate that UJGCR and UAGCR correlate with pathological parameters in patients with IgAN. In this study, we analyzed the associations between urinary immune complexes and histopathological phenotypes with disease predictive significance according to the Oxford classification, which indicated that the elevated levels of UJGCR and UAGCR in IgAN patients correlated with tubular atrophy/interstitial fibrosis. Moreover, surprisingly, we found that UJGCR and UAGCR were all higher in IgAN patients with less renal IgA deposition than patients with a more intense renal IgA deposition, which strongly suggests that these immune complexes deposits in the mesangium could cross the filtration barrier and thus be excreted into the urine. Taken together, it seems most likely that J-IgG and IgA-IgG immune complexes in the urine detected in the present study came from glomerular mesangium and injured the kidney, especially renal tubular epithelial cells.

More interestingly, this current study found significant correlations between UJGCR and UAGCR in IgAN. Based on our observation, we postulate UJGCR were mainly composed of J-IgA-IgG.

In clinical setting, renal biopsy is the only golden procedure to diagnose and evaluate disease activity in IgAN, but this being an invasive procedure which is impractical to repeat. In addition, 24hUPro, urine protein/creatinine excretion (UPCR), SCr and hematuria are often used to evaluate the severity of IgAN which could not always distinguish patients of IgAN from patients with other renal diseases. Thus, UJGCR measurement may be of particular value in diagnosing and evaluating the deterioration of renal injury in IgAN, avoiding more invasive procedures when not necessary.

There were some limitations in our research. First, urinary immune complexes were measured at a single time-point and it is unclear whether the levels of urinary immune complexes may fluctuate on different occasions. It is widely accepted that spot urinary protein/creatinine ratio predicts actual 24UPro with reasonable accuracy [41, 42]. So the levels of UJGCR and UAGCR in our study were all standardized to the UCr to adjust for differences of urine flow rate. Secondly, further prospective studies in a larger population of patients are warranted to measure levels of serum J chain, urinary J chain, renal J chain, and mRNA expression of J chain to support our hypothesis.

Conclusions

In summary, our data demonstrate that UJGCR is potential specific biomarkers in IgAN. Higher levels of UJGCR not only related to relatively severe clinical features, but also pathologic involvement on renal biopsy, especially tubulointerstitial injury. These findings support the notion that pIgA containing immune complexes might play an important role in the pathogenesis of IgAN. In conclusion, UJGCR could be a useful and reliable biomarker for evaluating the severity of renal injury in IgAN.

Abbreviations

ACE-Is: angiotensin-converting enzymes inhibitors; ARBs: angiotensin II receptor blockers; BP: blood pressure; BSA: bovine serum albumin; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; DC group: disease control group; ESRD: end-stage renal failure; Gd-IgA1: galactose-deficient IgA1; HC group: healthy control group; IgAN: IgA nephropathy; IL-6: interleukin-6; J chain: joining chain; sIgA: secretory IgA; mAb: monoclonal antibody (mAb); pIgA: polymeric IgA; pIgM: pentameric IgM; pIgR: Ig receptor; ROC: Receiver operator characteristic curve; RAS: renin-angiotensin system; SCr: serum creatinine; TNF- α : tumor necrosis factor- α ; TGF- β : transforming growth factor- β ; UJGCR: urinary J chain containing IgG immune complexes; UCr: urine creatinine; UAGCR: urinary IgA-IgG immune complexes; UPCR: urine protein/creatinine excretion 24hUPro: 24-hour urinary protein excretion

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Peking Union Medical College Hospital Ethics Committee and the study was conducted in accordance with the World Medical Association Declaration of Helsinki.

Consent for publication

All participants provided written informed consent to participate in this study.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Authors contributions

G.H.L. was involved in the study design, sample collection, biomarkers measurement and analysis and interpretation of the data, as well as drafting the manuscript. C.Y., X.H.F., Y.Q. and G.C. were involved in patient enrollment and clinical data collection. Y.B.W made the pathological confirmations of IgAN. R.T.G. and X.M.L are the corresponding authors who conceived the study and participated in the study design, interpretation of results. All authors critically revised the manuscript as well as reading and approving the final version prior to submission.

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Tables

Table 1. Demographic and clinical characteristics of patients with IgAN and controls

	IgAN (n=26)	DC (n=31)	HC (n=32)	<i>P</i> value
Male, n (%)	9 (34.6)	16 (51.6)	22 (68.6)	0.66
Age (years)	41 (32-47)	46 (29-60)	30 (26-52)	0.29
Cigarette smoking	6(23.08)	13(41.94)		0.17
Systolic BP (mmHg)	127.81±15.98	135.94±17.08		0.10
Diastolic BP (mmHg)	80.42±11.84	82.74±12.66		0.52
MAP (mmHg)	96.22±12.51	100.47±13.34		0.31
U-RBC (/uL)	67 (32-200)	34 (14-87)		0.11
24-hour urine protein excretion(g/24h)	1.32 (0.65-3.48)	4.05 (1.46-5.97)		0.01
Serum creatinine (mg/dL)	1.10 (0.92-1.71)	0.93 (0.71-1.18)		0.11
eGFR (mL/min•1.73m ²) ^a	70.61±34.75	84.93±35.04		0.13
Albumin (g/L)	39.00 (33.00-43.00)	30.00 (25.00-40.00)		0.001
Treatments, n (%)				
Corticosteroids	4 (15.4)	18 (58.1)		0.001
Immunosuppressant	8 (30.8)	15 (48.4)		0.12
ACE-Is/ARBs	16 (61.5)	17 (54.8)		0.61
ACE-Is	1 (3.8)	3 (9.7)		0.76
ARBs	15 (57.7)	15 (48.4)		0.48

Data are present as mean±SD or median (interquartile range) , or frequency in percent.

24hUPro: 24-hour urinary protein excretion; eGFR: estimated glomerular filtration rate; Urinary RBC: urinary red blood cell; SBP: systolic

blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; 1mmHg=0.133Kpa; ACE-Is: angiotensin converting enzyme

inhibitors; ARBs: angiotensin II receptor blockers

^a eGFR was calculated according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [31]

Figures

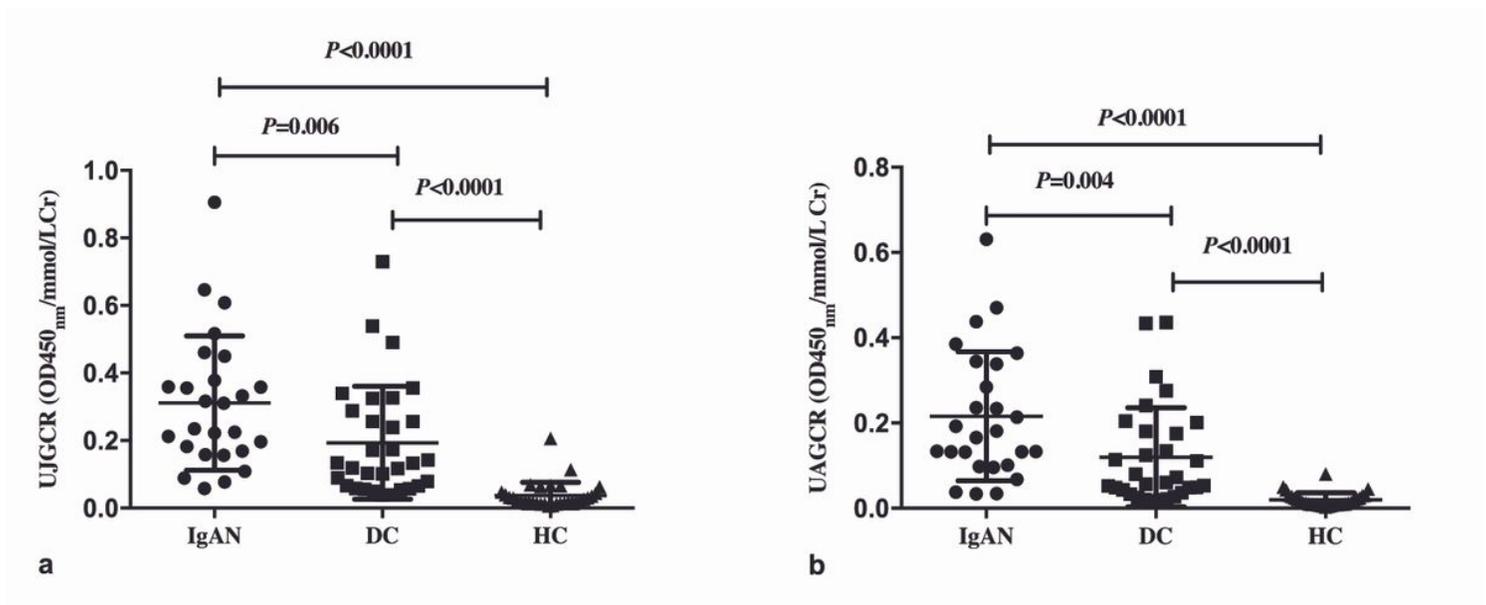


Figure 1

The levels of urinary joining chain containing IgG immune complexes/creatinine ratio and IgA-IgG immune complexes/creatinine ratio (OD450nm/mmol/L Cr) in patients with IgA nephropathy, patients with other kidney diseases and healthy subjects. The levels of (a) UJGCR and (b) UAGCR were significantly higher in IgAN group than HC group and DC group. UJGCR: urinary joining chain containing IgG immune complexes/creatinine ratio; UAGCR: urinary IgA-IgG immune complexes/creatinine ratio; IgAN: IgA nephropathy; DC: patients with other kidney diseases; HC: healthy subjects

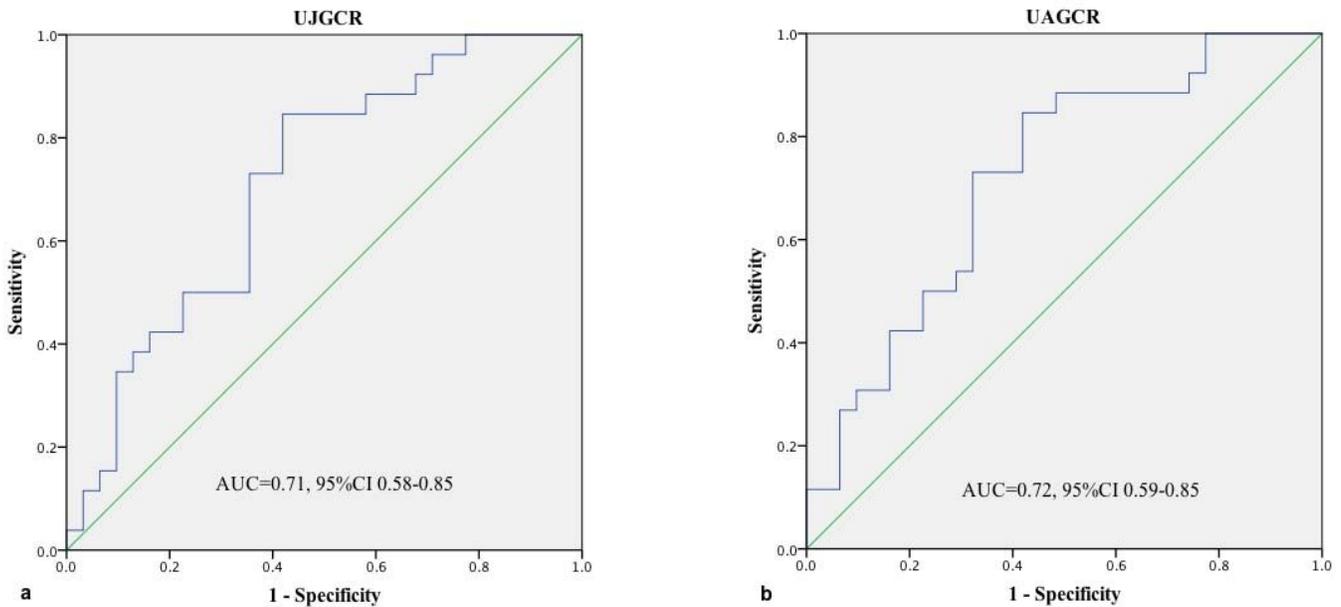


Figure 2

Receiver operating characteristic curve analysis of the prediction values of urinary joining chain containing IgG immune complexes/creatinine ratio and IgA-IgG immune complexes/creatinine ratio (OD450nm/mmol/L Cr) in patients with IgA nephropathy. ROC curve revealed good discrimination of elevated levels of (a) UJGCR and (b) UAGCR between non-IgAN patients and IgAN patients. ROC: receiver operating characteristic; AUC: area under the curve; 95% CI, 95% confidence interval; UJGCR: urinary joining chain containing IgG immune complexes/creatinine ratio; UAGCR: urinary IgA-IgG immune complexes/creatinine ratio; IgAN: IgA nephropathy Estimated glomerular filtration rate (eGFR) was calculated according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [31]

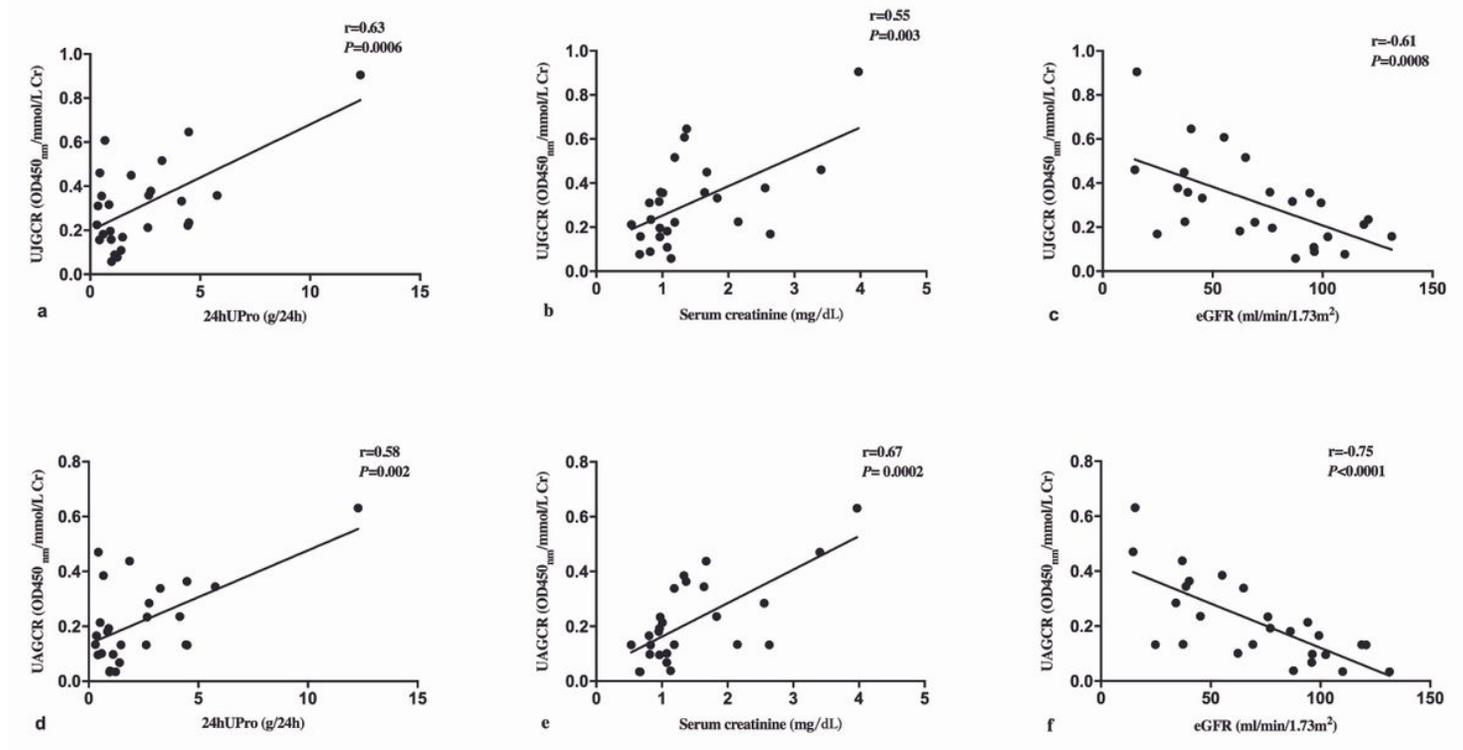


Figure 3

Correlation between the levels of urinary immune complexes and clinical parameters of patients with IgA nephropathy. (a, b and c) Correlation between the levels of UJGCR and 24hUPro, serum creatinine and eGFR; (d, e and f) correlation between the levels of UAGCR and 24hUPro, serum creatinine and eGFR. UJGCR: urinary joining chain containing IgG immune complexes/creatinine ratio; UAGCR: urinary IgA-IgG immune complexes/creatinine ratio; 24hUPro: 24-hour urinary protein excretion Estimated glomerular filtration rate (eGFR) was calculated according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [31]

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