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Association between urinary VEGFA and renal pathology of IgA nephropathy patients

Shaozhen Feng[1,2] | **Naya Huang**[1,2] | **Miaorong Xue**[1,2] | **Puhua Zhang**[1,2] | **Zhong Zhong**[1,2] | **Qunying Guo**[1,2] | **Zhijian Li**[1,2]

[1]Department of Nephrology, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China

[2]Guangdong Provincial Key Laboratory of Nephrology, Guangzhou, China

Correspondence
Zhijian Li and Shaozhen Feng, Department of Nephrology, The First Affiliated Hospital of Sun Yat-sen University, 58th Zhongshan Road II, Yuexiu District, Guangzhou, 510000, China.
Emails: lizhjian@mail.sysu.edu.cn (ZL); fengshzh3@mail.sysu.edu.cn (SF)

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Abstract

Background: Renal biopsy remains the golden standard for diagnosing and monitoring IgA nephropathy (IgAN). Vascular endothelial growth factor A (VEGFA) was crucial for the survival of glomerular cells. Our aim was to screen the expression pattern of urinary, circulating and renal VEGFA in IgAN patients to reveal their relationship with renal pathology and outcomes.

Methods: Baseline VEGFA levels were determined with ELISA, real-time PCR and immunohistochemistry. Associations between VEGFA expression and clinical–pathological parameters, and renal outcomes were evaluated.

Results: Compared with healthy controls, urinary VEGFA level was obviously elevated in IgAN patients (76.19 ± 63.67 pg/mg Cr vs 146.67 ± 232.71 pg/mg Cr, $p = 0.0291$) and not correlated with serum VEGFA level. Baseline urinary VEGFA was significantly associated with gender and tubular atrophy/interstitial fibrosis by stepwise multivariate regression analysis. Urinary VEGFA was higher in male patients accompanied with higher serum creatinine, larger proportion of hypertension and recurrent hematuria than in female patients. In the kidney of IgAN patients, VEGFA were robustly expressed in the parietal epithelial cells, podocytes, mesangial cells and tubular epithelial cells. After a follow-up duration of 38.53 ± 27.14 months, IgAN patients with higher urinary VEGFA level were found to have a poorer renal outcome of renal replacement therapy (HR = 1.027, $p = 0.037$) or composite outcome (HR = 1.023, $p = 0.039$) after adjusting for confounders.

Conclusions: Increased urinary VEGFA might reflect certain renal pathology and, although not fully specific, still could be served as a valuable noninvasive indicator in predicting renal progression of IgAN.

KEYWORDS
IgAN, renal outcome, renal pathology, urinary VEGFA

1 | INTRODUCTION

IgA nephropathy (IgAN) is the most common primary glomerulonephritis and is the leading cause of end-stage renal disease (ESRD) in China.[1] Renal biopsy remains the golden standard for diagnosis and monitoring of IgAN. IgAN is characterized by mesangial proliferation, sometimes accompanied by endothelial cell proliferation and podocyte injury. Clinically, IgAN patients display different degree of microscopic hematuria, proteinuria and hypertension.

Recent studies revealed that mesangial cells, endothelial cells, podocytes and parietal epithelial cells cooperate and support the structure and function of the glomerulus.[2] Vascular endothelial growth factor -A (VEGFA) is an essential angiogenic cytokine and found to be crucial for the survival, differentiation and structure maintaining of these glomerular cells.[3,4] Thereby, VEGFA is pivotal for maintaining the glomerular filtration barrier function. Mice with loss of VEGFA in podocyte developed proteinuria.[4,5] A case report indicated that a 68-year-old man with metastatic rectal cancer who treated with bevacizumab ($VEGFA_{165}$ antibody) for months develops IgAN accompanied with hematuria, proteinuria and thrombotic microangiopathy, and proteinuria almost completely resolved and mesangial IgA deposition markedly decreased in more than 8 months after bevacizumab cessation.[6] In Liu and Veron's study, the increased expression of VEGFA in glomeruli was also found to directly cause glomerular hypertrophy in association with proteinuria.[7,8] These studies indicate that VEGFA exert effects on the glomerular injury besides endothelial injury, and the expression balance of VEGFA is essential for preserving the normal glomerular filtration.

However, the relationship of urinary and renal VEGFA in IgAN patients are still not clear. In this study, we evaluated the expression pattern of VEGFA in circulating, urine and kidney of IgAN patients by ELISA, real-time PCR and immunohistochemistry, and further to investigate the relationship of VEGFA expression and clinical parameters, renal pathology lesions (MEST-C score) and renal progression in IgAN patients.

2 | MATERIALS AND METHODS

2.1 | IgAN patients and controls

The study protocol was approved by the Medical Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University and informed written consent was obtained from every participant. Patient's age >14 years old with primary IgAN confirmed by renal biopsy during October 1st, 2014, to March 30th, 2015, were enrolled in the study. Exclusion criteria included patients with diabetes, systemic lupus erythematosus, Henoch–Schonlein purpura, hepatic disease and patients treated with corticosteroids and immunosuppressive drugs in the previous 3 months. A total of 85 IgAN patients were enrolled. And 71 healthy controls without microscopic hematuria, proteinuria, hepatic disease and with normal serum creatinine were recruited in the physical examination center during May 1st to June 30th, 2015 (Table 1).

Samples of IgAN patients were obtained at the day of renal biopsy. Serum and morning urine collected from each subject were transferred to a separate vial after centrifuge and stored at −80°C until assayed. For RNA analyses, 5 ml of whole blood was collected in an EDTA Vacutainer™ tubes. Blood pressure and 24-h urine protein excretion at the time of renal biopsy were collected from medical records. Hypertension was defined as the average of two blood pressure readings on a single occasion with a systolic blood pressure of 140 mmHg or more, or a diastolic blood pressure of 90 mmHg or more, or patients who reported use of antihypertensive medication. The estimated glomerular filtration rate (eGFR) was evaluated using the modified glomerular filtration rate estimating equation for Chinese.[9] The renal histopathology from all patients was scored by pathologists blinded to the clinical data using 5 pathological variables according to the Oxford classification: mesangial hypercellularity (M), endocapillary hypercellularity (E), segmental glomerulosclerosis (S), tubular atrophy/interstitial fibrosis (T) and fibrocellular crescents (C).[10]

2.2 | Measurement of serum and urinary VEGFA levels

Serum levels of VEGFA were measured by ELISA from Raybiotech (ELH-VEGF-1), and urine VEGFA levels were measured by ELISA from R&D Systems (QVE00B) according to the manufacturer's instructions. Duplicate determination was carried out. Absorbance was measured at 450 nm using the SpectraMax Plus 384 Microplate reader (Molecular devices). The concentrations of VEGFA were calculated according to the standard curve.

2.3 | Isolation of total RNA and real-time PCR

For isolation of total RNA from peripheral blood mononuclear cells (PBMC), whole blood collected in an EDTA Vacutainer™ tubes were firstly treated with red blood cell lysis buffer (Legend). Remaining leukocytes were recovered by centrifugation. After washing with phosphate buffer saline (PBS), the leukocyte pellet was processed for purification of total cellular RNA with Trizol (Invitrogen). RNA was reverse-transcribed with Superscript II reverse transcriptase (Roche) into cDNA according to manufacturer's instruction. cDNA derived from 1 μg of total RNA served as a template for real-time PCR. The expression levels of VEGFA (Hs00900055_m1) and GAPDH (Hs03929097_g1) were quantified by real-time PCR using Taqman (Lifescience) with a fluorescence detection monitor 7900 Real-time PCR system (Applied Biosystems). Mean C_t values were calculated for each molecule using $2^{-\Delta Ct}$ method, where C_t values of the molecules were normalized to that of GAPDH.

TABLE 1 Clinical data of IgAN patients and healthy controls

	Healthy control ($N = 71$)	IgAN patients ($N = 85$)	p value
Gender (female/male)	40/31	47/38	0.896
Mean age (years SD)	34.69 ± 9.04	36.81 ± 10.06	0.972
eGFR (ml/min/1.73 m^2)	115.04 (97.74, 125.66)	56.9 (27.77, 83.81)	0.000
Serum creatinine (μmol/L)	58.75 (54.52, 70.69)	114 (82, 225.5)	0.000
Hypertension (%)	-	31 (36.47%)	-
Proteinuria range (g/24 h)	0	1.37 (0.78, 2.72)	-
Recurrent hematuria (%)	0	74 (87.06%)	-
Glomerulosclerosis percentage	-	35.5% ± 26.09%	
Oxford classification (%)			
M score			
M0	-	1 (1.18%)	
M1	-	84 (98.82%)	
E score			
E0	-	80 (94.12%)	
E1	-	5 (5.88%)	
S score			
S0	-	30 (35.29%)	
S1	-	55 (64.71%)	
T score			
T0	-	39 (45.88%)	
T1	-	26 (30.59%)	
T2	-	20 (23.53%)	
C score			
C0	-	44 (51.76%)	
C1	-	36 (42.35%)	
C2	-	5 (5.88%)	
Treatment			
ARB and/or ACE-I	-	52/83 (62.65%)	
Corticosteroid	-	34/83 (40.96%)	
Immunosuppressive drug	-	2/83 (2.41%)	

Note: eGFR (ml/min/1.73 m^2) = 175 × (creatinine, mg/dl)$^{-1.234}$ × (age, years)$^{-0.179}$ × (if female, × 0.79).[9] All data are mean ± SD or median (25th, 75th) or number of patients (%, ratio of group). Two groups assessed by *t*-test, Mann–Whitney test or Chi-squared test.

Abbreviations: ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blockers; C, fibrocellular crescents; E, endocapillary hypercellularity; eGFR, estimated glomerular filtration rate; M, mesangial hypercellularity; S, segmental glomerulosclerosis; T, tubular atrophy/interstitial fibrosis.

2.4 | Immunohistochemistry analysis of renal tissue sections

Renal biopsies of 27 IgAN patients (Table S1) were randomly selected from enrolled 85 IgAN patients and used for VEGFA testing. Four cases of renal biopsy from kidney donors were served as healthy controls. Both renal biopsies of IgAN and healthy controls were formalin-fixed and subsequently paraffin-embedded. Serial 3 μm sections of tissue were deparaffinized in xylene and rehydrated through a graded ethanol series and washed in PBS (pH 7.4). Antigen retrieval was performed incubating the tissue sections for 15 min in sodium citrate buffer (pH 6.0), in an antigen retriever (121°C). After treating with 3% H_2O_2 and blocking with 3% bovine serum albumin (BSA)/PBS for 1 h, tissue sections were incubated with antihuman VEGFA antibody (Abcam, ab68334) diluted in 1% BSA/PBS overnight at 4°C. After washing with PBS, the immunohistochemical reactions were visualized by using GTvison I DAB kit (Dako, GK5007) with the biotin-linked anti-mouse IgG according to the manufacturer's instructions. The sections were counterstained with hematoxylin. As a negative control for staining, the first antibody was replaced

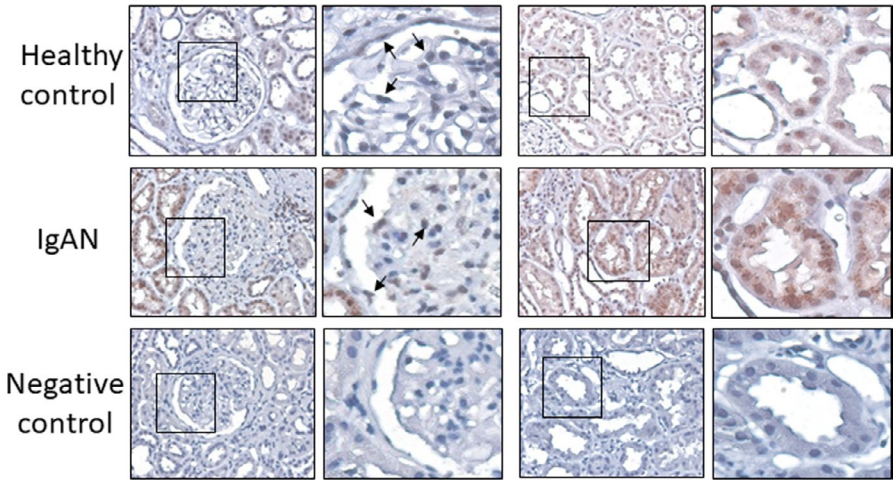

FIGURE 1 VEGFA expression in the kidney of IgAN patients. Immunohistochemistry staining of endogenous VEGFA in renal biopsies of IgAN. VEGFA was stained in the cytoplasm of parietal epithelial cells (arrowheads), podocytes (arrowheads), mesangial cells (arrowheads) and tubular epithelial cells in renal biopsies of IgAN patients. In healthy control renal tissues, VEGFA was weakly immunostained in the cytoplasm of tubular epithelial cells and glomeruli. Tissue without primary antibody was used as negative control

with non-immune mouse immunoglobulin G (Dako). The sections were observed under a microscopy (Carl Zeiss). Measurements were taken at least by five glomeruli and five different tubulointerstitial areas for each section.

2.5 | Follow-up and outcomes

IgAN patients were followed up till August 31st, 2020. The primary renal outcome (renal replacement therapy) included patients requiring hemodialysis, peritoneal dialysis and renal transplantation. The secondary renal outcome (composite renal outcome) included serum creatinine doubling from baseline and progression to ESRD (ESRD means eGFR <15 ml/min/1.73 m^2).

2.6 | Statistical analysis

Data were expressed as the mean ± standard deviation (normal distribution) or medians with the 25th and 75th percentiles (skew distribution). Categorical variables were displayed as frequency and percentage (*n*, %). To calculate statistically significant differences between groups, samples were analyzed by independent-samples *t*-test for continuous variables with normal distribution and by Mann–Whitney test for those with skew distribution or qualitative variables. Relationships between urinary VEGFA level and clinical parameters were calculated using Spearman's rank correlation and stepwise multiple linear regression analysis. Values with skew distribution were log-converted in the Cox regression analysis. Cox regression model was used to assess the impact of multiple covariates for renal outcome. The results of multivariable analysis were expressed by a hazard ratio (HR) and 95% confidence intervals. Data analyses were performed using GraphPad Prism version 8.0 and SPSS 16.0 software.

3 | RESULTS

3.1 | Clinicopathological manifestations of IgAN patients

A total of 85 IgAN patients were enrolled and 71 healthy donors were recruited in the study as controls. The baseline clinicopathological manifestations of IgAN patients and healthy controls are summarized in Table 1. Healthy controls ranged in age 20 from 53 years (mean 35 years), 31 (43.66%) were male. The control group had normal renal function without hematuria or proteinuria. For IgAN patients, the average age at renal biopsy was 36.81 ± 10.06 years old, 38 (44.7%) of patients were male and 31 (36.47%) of IgAN patients presented with hypertension. The proteinuria levels were 1.37 (0.78, 2.72) g/d. The eGFR levels of patients were 56.9 (27.77, 83.81) ml/min/1.73 m^2. The mean proportion of glomerulosclerosis is 35.5% (range 0–90%). According to the Oxford classification, 98.82% (84/85) of the patients were grade M1, 5.88% (5/85) were grade E1, 64.71% (55/85) were grade S1, 30.59% (26/85) were grade T1, 23.53% (20/85) were grade T2, 42.35% (36/85) were grade C1, and 5.88% (5/85) were grade C2. A percentage of 62.65% IgAN patients were treated with angiotensin-converting enzyme inhibitors (ACEIs) and/or angiotensin II receptor blockers (ARBs).

3.2 | VEGFA level increased in glomeruli, tubules and urine in IgAN patients

Results of immunohistochemistry analysis manifested that in control kidney, the immunostaining of VEGFA in tubular epithelia and glomeruli were relatively weak in paraffin sections (Figure 1 & Figure S1). The signals of VEGFA were increased with varying degree in the glomeruli including cytoplasm of parietal epithelial cells, podocytes and mesangial cells in all tested IgAN patients compared with

control kidney tissue (Figure 1 & Figure S2). Upregulated expression of VEGFA was also found in the cytoplasm of tubular epithelia including proximal and distal tubules in IgAN patients.

VEGFA levels in urine and peripheral blood were also determined. Compared with healthy controls, markedly increased level of urinary VEGFA was detected in IgAN patients (76.19 ± 63.67 pg/mg Cr vs 146.67 ± 232.71 pg/mg Cr, $p = 0.0291$) (Figure 2A), which was consistent with the higher glomerular and tubular expression of VEGFA in IgAN patients. However, serum level of VEGFA was not statistically different between IgAN patients and healthy controls (158.3 ± 141.3 pg/ml, and 166.5 ± 182.8 pg/ml, $p = 0.7538$, Figure 2B). And urinary VEGFA secretion in IgAN patients were not statically correlated with serum VEGFA level ($r = 0.082$, $p = 0.456$, Figure 2C). VEGFA is secreted from various cells such as lymphocytes. Lymphocytes are the most numerous components of peripheral blood cells (70–90% of PBMCs). To investigate a potential contribution of VEGFA from PBMC to the progress of IgAN, VEGFA mRNA level in PBMCs was detected. We found that there was also no significant difference of VEGFA mRNA levels in PBMC between IgAN patients and healthy controls (0.037 ± 0.026 vs 0.037 ± 0.02, $p = 0.9332$, Figure 2D).

3.3 | Correlation of urinary VEGFA with clinicopathological manifestations in IgAN patients

Relationship of urinary VEGFA level and clinicopathological parameters of IgAN patients was further investigated. Spearman rank correlation test demonstrated that baseline urinary VEGFA level was significantly correlated with gender ($r = -0.383$, $p < 0.001$), prevalence of hypertension ($r = 0.320$, $p = 0.003$), systolic blood pressure (SBP, $r = 0.284$, $p = 0.009$), diastolic blood pressure (DBP, $r = 0.235$, $p = 0.032$), proteinuria ($r = 0.343$, $p = 0.002$), serum creatinine ($r = 0.426$, $p < 0.001$), eGFR ($r = -0.339$, $p = 0.002$), proportion

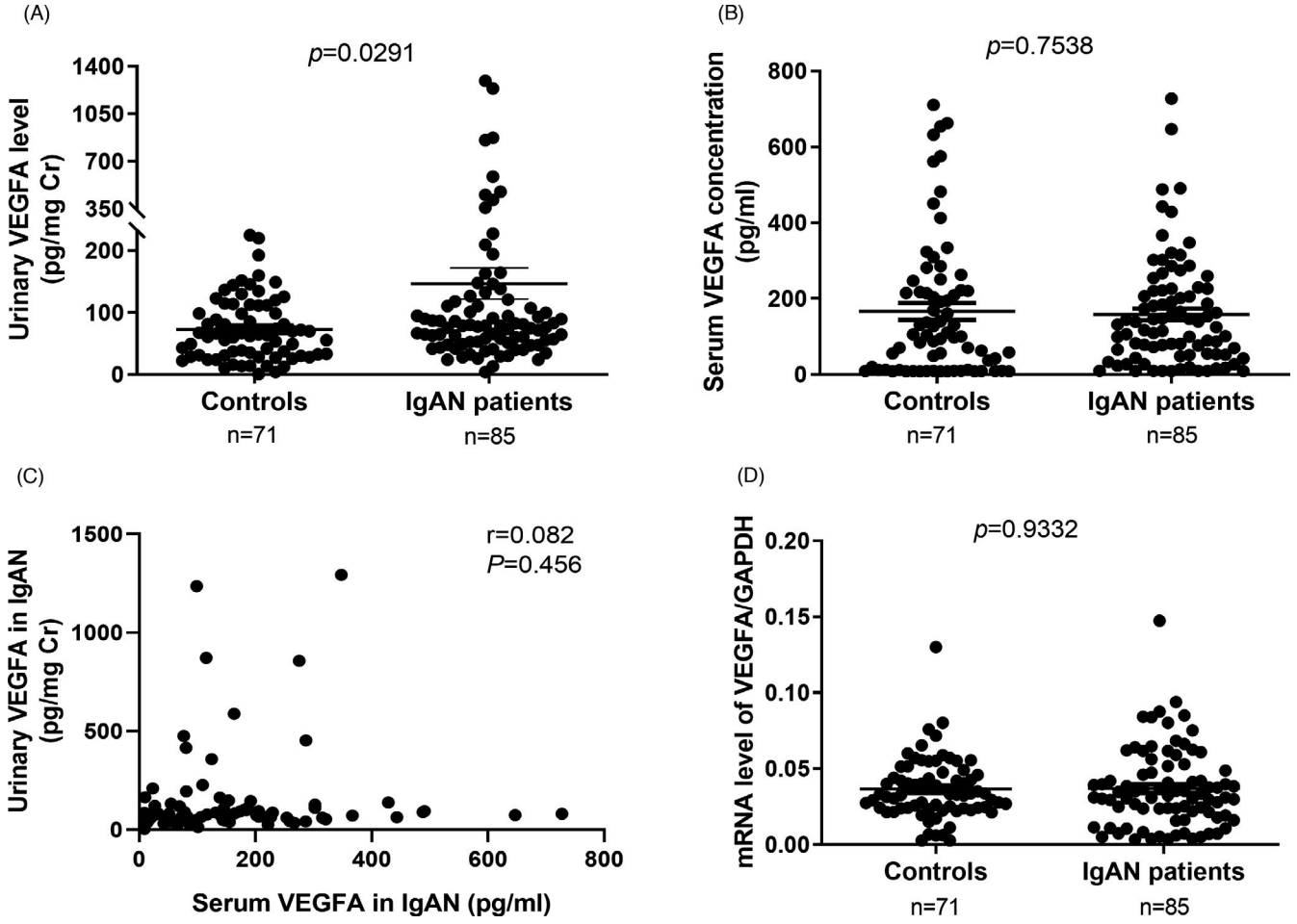

FIGURE 2 Expression of VEGFA in urine and circulation of IgAN patients. A. Urine levels of VEGFA in IgAN patients and healthy controls were determined by ELISA. Urinary VEGFA levels were normalized with urinary creatine (Cr). Spots on the dot plot represent individual mean value of ratio of VEGFA concentration to urinary creatinine. B. Serum levels of VEGFA in IgAN patients and healthy controls were determined by ELISA. Spots on the dot plot represent individual mean value of VEGFA concentration. C. Correlation analysis of urinary VEGFA and serum VEGFA in IgAN patients by Spearman's rank correlation. D, mRNA level of VEGFA in PBMC. Real-time PCR was performed using Taqman and mRNA level of VEGFA was normalized using GAPDH as the reference gene. Data are presented as mean ± SEM. *p* value analysis: nonparametric test (Mann–Whitney U), *t*-test

of glomerulosclerosis ($r = 0.290$, $p = 0.007$) and tubular atrophy/interstitial fibrosis ($r = 0.323$, $p = 0.003$) in IgAN patients (Table 2). In the forward stepwise multivariate regression analysis with age, gender, eGFR, log-transformed 24 h proteinuria, hypertension, glomerulosclerosis and tubular atrophy/interstitial fibrosis, only gender and tubular atrophy/interstitial fibrosis were associated with urinary VEGFA (total adjusted $R = 0.421$) (Table 3).

Interestingly, the mean value of urinary VEGFA in male with IgAN (215.11 ± 305.03 pg/mg Cr) was significantly higher than that in female (91.33 ± 130.56 pg/mg Cr), while there was no obvious difference of urinary VEGFA between male (78.98 ± 66.29 pg/mg Cr) and female (74.03 ± 59.77 pg/mg Cr) in healthy controls (Figure 3A,B). The urinary VEGFA in males was statistically higher in IgAN patients than in healthy controls (Figure 3C), while there was no big difference in females between two groups (Figure 3D).

TABLE 2 Correlation between urinary level of VEGFA and clinical–pathological parameters in IgAN patients at baseline

Factor	Correlation coefficient	*p*-value
Age (year)	0.001	0.990
Gender (female)	**−0.383**	**<0.001**
eGFR (ml/min/1.73 m^2)	**−0.339**	**0.002**
Serum creatine (μmol/L)	**0.426**	**<0.001**
Hypertension	**0.320**	**0.003**
SBP (mm Hg)	**0.284**	**0.009**
DBP (mm Hg)	**0.235**	**0.032**
Albumin (g/L)	−0.162	0.140
CHOL (mmol/L)	0.122	0.290
TG (mmol/L)	0.088	0.451
Proteinuria (g/24 h)	**0.343**	**0.002**
Recurrent hematuria	0.194	0.081
Glomerulosclerosis percentage	**0.290**	**0.007**
Oxford classification		
M	−0.089	0.418
E	−0.114	0.298
S	0.105	0.337
T	**0.323**	**0.003**
C	0.119	0.281

Note: Correlation is significant at the 0.05 level (Spearman). *p* value <0.05 was indicated bold.

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; CHOL, cholesterol; TG, triglycerides.

Furthermore, higher serum creatinine, larger proportion of hypertension and recurrent hematuria were seen in male patients with IgAN than in female patients (Table S2).

3.4 | Urinary VEGFA level associated with renal outcome in IgAN patients

A total of 63 IgAN patients were followed up (Table 4). After a mean follow-up period of 38.53 ± 27.14 months, there were 39 patients (61.9%) with microscopic hematuria. The median proteinuria of IgAN patients were 1.13 (0.55, 2.72) g/day. A total of 21 (33.33%) patients progressed to ESRD and 11 of them had renal replacement therapy, 16 (25.4%) patients developed serum creatine doubling, and 23 patients reached composite renal outcome composing serum creatine doubling and progression to ESRD. Cox regression analysis revealed that raised urinary VEGFA was significantly associated with the increased risk either of renal replacement therapy (HR 1.027, 95% CI 1.002–1.054, $p = 0.037$) or composite renal outcome (HR 1.023, 95% CI 1.001–1.046, $p = 0.039$) after adjustment for age, gender, hypertension, baseline eGFR, log-transformed proteinuria (24 h), glomerulosclerosis and tubular atrophy/interstitial fibrosis (Table 5). IgAN patients with high level of urinary VEGFA at baseline had a poor renal outcome.

4 | DISCUSSION

In the present study, we found that urinary VEGFA was significantly elevated in IgAN patients compared with healthy controls, and the level of urinary VEGFA in IgAN was correlated with gender, prevalence of hypertension, proteinuria, eGFR, proportion of glomerulosclerosis and tubular atrophy/interstitial fibrosis. VEGFA is around 27 KDa, which could be freely filtered through the glomerulus and leaked from serum into urine. It was reported that plasma VEGFA was increased in chronic kidney diseases (CKD) patients defined as eGFR <60 ml/min/1.73 m^2 or presence of albuminuria.[11] However, serum VEGFA levels in IgAN patients were not significantly different with that in healthy controls in our study, which was consistent with previous reports.[12-14] No significant correlation between urinary and serum VEGFA level was found in IgAN patients, indicating that VEGFA excretion was probably independent of the serum level of VEGFA. It is speculated that the increased urinary VEGFA might be derived from the kidney.[15] In line with this interpretation, we observed an increased

TABLE 3 Forward stepwise multivariate regression analysis for predictors of urinary VEGFA in IgAN patients ($n = 85$)

Dependent	Variable	B ± SE	*t*	*p*-value	*R*
Urinary VEGFA	Gender	−98.311 ± 48.588	−2.023	0.046	0.421
	Tubular atrophy/interstitial fibrosis	98.985 ± 30.2	3.278	0.002	

SE: Standard error. Model is adjusted for age, gender, eGFR, hypertension, log-transformed 24 h proteinuria, glomerulosclerosis and tubular atrophy/interstitial fibrosis (T).

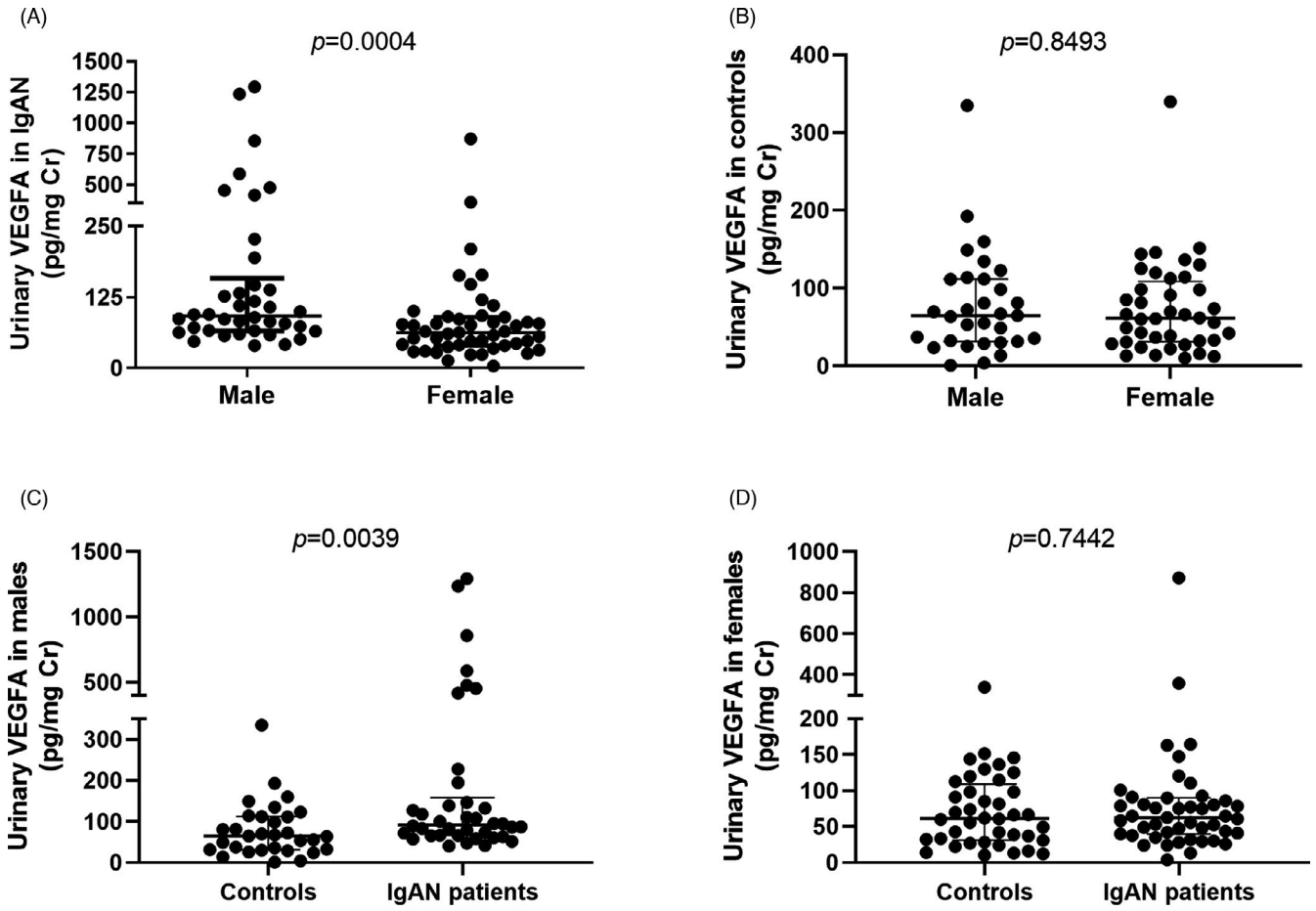

FIGURE 3 Differences in urinary VEGFA level between females or males. Urine levels of VEGFA were determined by ELISA (as Figure 2A). The difference of urinary VEGFA levels between females and males were compared in IgAN patients (A) or healthy controls (B). The urinary VEGFA in IgAN and healthy groups were also compared by sex (C & D). Urinary VEGFA levels were normalized with urinary creatine (Cr). Spots on the dot plot represent individual mean value of ratio of VEGFA concentration to urinary creatinine. Data are presented as median (interquartile range). *P* value analysis: nonparametric test (Mann–Whitney U)

TABLE 4 Outcomes of IgAN patients

	Total (*N* = 63)
Follow-up duration (month)	38.53 ± 27.14
Proteinuria (g/24 h)	1.13 (0.55, 2.72)
Microscopic hematuria (%)	39 (61.90%)
Renal replacement therapy (*n*, %)	11 (17.46%)
Composite renal outcome (*n*, %)	23 (36.51%)
Scr doubling (*n*, %)	16 (25.40%)
eGFR (ml/min/1.73 m^2)	43.57 (5.23, 90.43)
eGFR decline/year (ml/min/1.73 m^2/year)	−3.48 (−9.58, 3.45)
ESRD (n, %)	21 (33.33%)

Note: Renal replacement therapy included patients had hemodialysis, peritoneal dialysis and renal transplantation. The composite renal outcome included serum creatinine doubling from baseline and progression to ESRD (ESRD means eGFR <15 ml/min/1.73 m^2).
Abbreviations: Scr, Serum creatine; ESRD, End-stage renal disease.

VEGFA expression in glomeruli and tubular epithelial cells of IgAN patients. IgAN patients clinically display different degree of proteinuria. The increase in the level of urinary protein would aggravate glomerular injury and stimulate secretion of VEGFA by renal intrinsic cells to repair glomerular injury. Altered VEGFA level in glomeruli could induce glomerular filtration barrier structural and functional abnormalities, which was confirmed in rodent experimental renal disease models.[7,16] In glomerulonephritis such as IgAN, excessive secretion of VEGFA either in glomeruli or in tubules probably aggravates injury of filtration barrier.

Urinary VEGFA was still significantly associated with tubular atrophy/interstitial fibrosis by the forward stepwise multivariate regression analysis. Although the precise mechanism underlying this correlation remains unknown, this function of VEGFA may be based on the finding that VEGFA was upregulated in renal tubular epithelial cells, even in the damaged tubules in IgAN patients with interstitial injury, which was consistent with previous report.[17] IgAN is characterized by variation of pathological features, especially variable tubulointerstitial lesions from almost normal to diffuse tubular atrophy and interstitial fibrosis. Some evidence from the experimental obstructive nephropathy model suggests that VEGFA may be involved in the early response to tubular injury, mediating neoangiogenic processes.[18,19] Additional VEGFA may worsen renal lesions by

TABLE 5 Cox regression of urinary VEGFA and renal outcomes

	Renal replacement therapy		Composite renal outcome	
	HR (95% CI)	*p*	HR (95% CI)	*p*
Unadjusted	1.019 (1.007, 1.032)	0.002	1.017 (1.005, 1.029)	0.005
Adjusted				
Model 1	1.021 (1.006, 1.035)	0.004	1.019 (1.005, 1.034)	0.006
Model 2	1.028 (1.013, 1.044)	0.000	1.027 (1.012, 1.042)	0.001
Model 3	1.027 (1.002, 1.054)	0.037	1.023 (1.001, 1.046)	0.039

Abbreviation: CI: confidence interval.

Model 1 was adjusted for age and gender. Model 2 was adjusted as Model 1 plus hypertension. Model 3 was adjusted as Model 2 plus baseline eGFR, log-transformed proteinuria (24 h), glomerulosclerosis and tubular atrophy/interstitial fibrosis.

Note: per 10 pg/mg Cr changes in urinary VEGFA.

The composite renal outcome included serum creatinine doubling from baseline and progression to ESRD (ESRD means eGFR <15 ml/min/1.73 m^2).

aggravating the interstitial lesions.[20] Increased renal tubular VEGFA production in transgenic mouse system has been shown to lead to fibrosis and glomerular disease.[16] Furthermore, in patients with glomerular diseases, renal VEGFA level was correlated positively with the extent of renal fibrosis.[21] More urinary VEGFA excretion were seen in IgAN patients with severe tubular atrophy/interstitial fibrosis. Because VEGFA is derived from tubules much more than from glomeruli, urinary levels of VEGFA may reflect tubulointerstitial lesions that could reduce the glomerular filtration rate.

Not only tubular atrophy/interstitial fibrosis, gender was also found to be associated with urinary VEGFA in the stepwise multivariate regression analysis. Urinary VEGFA in male with IgAN was significantly higher than that in female. Higher serum creatinine, proportion of hypertension and recurrent hematuria were seen in male patients with IgAN in our study. Several recent reports also revealed that males with IgAN had worse renal function than females, such as greater proteinuria, lower eGFR or poorer renal progression.[22-25] Sex hormones may be important determinants of the greater susceptibility of males to progressive kidney injury. Male animals progress more rapidly than females in most experimental models of CKD, and modulation of the hormonal milieu can replicate the effects of gender on the progression of CKD.[26] Sex hormones, such as estrogen, affect transforming growth factor-β signal transduction and the renin–angiotensin system which may contribute to kidney disease progression.[27]

After adjusting the well-known predictors of outcome in IgAN patients in Cox regression analysis, such as gender, proteinuria, eGFR, glomerulosclerosis and tubular atrophy/interstitial fibrosis, the urinary VEGFA/creatinine ratio was significantly correlated with the renal outcome of IgAN patients, either defined as renal replacement therapy or composite with serum creatinine doubling and ESRD. IgAN patients with higher urinary VEGFA level at baseline had a significantly worse renal outcome. Kikuchi et al. demonstrated association between lower urinary levels of VEGFA and renal dysfunction in CKD patients,[28] while another study by Avguštin et al. revealed that higher urinary VEGFA was an independent factor predicting worsening renal outcome in CKD patients.[29] We found that increased urinary VEGFA excretion at baseline related to not only the degree of renal pathology lesions, but also renal progression to a certain extent in IgAN patients.

The strengths of our study include the length of follow-up (median 43.37 months, interquartile range 10.6–62.27) and high cumulative number of end-stage renal disease and serum creatinine doubling, which helped to augment statistical power. However, some limitations also need to be addressed. Firstly, the sample size of IgAN patients was relatively small which reduced the power. Secondly, the association of urinary VEGFA with tubular atrophy/interstitial fibrosis in IgAN was needed to be estimated in animal model.

In conclusion, the present study demonstrated an obvious expression of VEGFA in the kidney in IgAN patients, including parietal epithelial cells, podocytes, mesangial cells and tubular epithelial cells, and a significant association between elevated urinary VEGFA level with gender and tubular atrophy/interstitial fibrosis, and poorer renal outcome of renal replacement therapy or composite outcome of ESRD and serum creatinine doubling. Increased urinary excretion of VEGFA might reflect certain renal pathology. Although not fully specific, urinary VEGFA probably is a noninvasive indicator in predicting renal progression of IgAN patients.

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CONFLICTS OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Shaozhen Feng and Zhijian Li had contributed to the conception of the study. Shaozhen Feng, Miaorong Xue, Puhua Zhang, Zhong

Zhong organized the samples, collected the data and performed the experiments. Shaozhen Feng and Naya Huang performed the data and result analysis. Qunying Guo was involved in result discussion. Shaozhen Feng and Zhijian Li wrote the article.

DATA AVAILABILITY STATEMENT

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

ORCID

Shaozhen Feng https://orcid.org/0000-0002-0086-842X

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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