



Expression and clinical significance of thrombospondin-1 and plasminogen activator inhibitor-1 in patients with mesangial proliferative glomerulonephritis

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Background: As a common pathological type of glomerular disease in China, mesangial proliferative glomerulonephritis is related to plasminogen activator inhibitor-1 (PAI-1) and thrombospondin-1 (TSP-1). Here, this study aims to investigate the expression and clinical significance of TSP-1 and PAI-1 in patients with mesangial proliferative glomerulonephritis.

Methods: Renal tissue specimens from 46 patients with mesangial proliferative glomerulonephritis admitted to this hospital were selected as the subjects, and 8 specimens of renal tissue from autopsy were used as controls. The expression levels of TSP-1 and PAI-1 were detected by immunohistochemistry and analyzed.

Results: The 24-hour urine protein, triglyceride, and total cholesterol levels of patients with severe mesangial hyperplasia were significantly higher than those of patients with mild and moderate mesangial hyperplasia, and serum albumin was lower than that of patients with mild and moderate mesangial hyperplasia ($P < 0.05$). The 24-hour urine protein level of patients with moderate mesangial hyperplasia was higher than that of patients with mild mesangial hyperplasia while the albumin level was lower ($P < 0.05$), but there was no significant difference in triglyceride and total cholesterol ($P > 0.05$). There was no significant difference in creatinine clearance (Ccr) between the three groups ($P > 0.05$). The 24-hour urine protein and urine alpha-1-microglobulin (A1M) levels in patients with renal interstitial disease were higher than those in patients without renal interstitial disease, while their Ccr level was lower ($P < 0.05$). TSP-1 and PAI-1 were not positively expressed in the glomeruli and renal tubules of specimens of the control group. However, in mesangial hyperplasia patients, the expression of TSP-1 and PAI-1 in mesangial hyperplasia with varying degrees and in different renal tubular damage were as follows: mild degree < moderate degree < severe degree ($P < 0.05$).

Conclusions: The pathological changes of mesangial proliferative glomerulonephritis are related to 24-hour urine protein, triglyceride, total cholesterol level, urine A1M, and Ccr level. The expression of TSP-1 and PAI-1 in the mesenchyme of glomerular and renal tubules significantly increased with the severity of the disease, suggesting that TSP-1 and PAI-1 play an important role in the occurrence and development of mesangial proliferative glomerulonephritis.

Keywords: Thrombospondin-1 (TSP-1); plasminogen activator inhibitor-1 (PAI-1); mesangial proliferative glomerulonephritis; immunohistochemistry

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Introduction

Mesangial proliferative glomerulonephritis is a common pathological type of glomerular disease in China (1), characterized by diffuse mesangial cell proliferation and/or increased mesangial matrix. The etiology of this disease is not clear yet, but some studies have reported that it is a severe immune-mediated inflammatory disease, caused by immune circulation complex deposition in the glomerular mesangial through some antigens to stimulate the body to produce antibodies (2). As a member of serine protease inhibitors family, plasminogen activator inhibitor-1 (PAI-1) is a kind of inflammatory proteins produced by various cells, which can restrain fibrin degradation and promote the deposition of fibrin. PAI-1 can also affect the synthesis and degradation balance of extracellular matrix (ECM) of the glomerulus and promote glomerulosclerosis. Thrombospondin-1 (TSP-1), which is distributed in renal tissues, renal tubules and mesangial cells, is one of the most important extracellular matrix glycoproteins that can lead to fibrosis (3,4). In recent years, a lot of studies have shown that both of the molecules have certain effect on the occurrence of mesangial proliferative glomerulonephritis (5,6). Therefore, to further investigate the clinical significance of TSP-1 and PAI-1 expression in patients with mesangial proliferative glomerulonephritis, the renal tissue specimens from 46 patients with mesangial proliferative glomerulonephritis who were admitted to our nephrology department from May 2013 to May 2018 were collected and studied. We present the following article in accordance with the MDAR reporting checklist (available at <http://dx.doi.org/10.21037/apm-20-1291>).

Methods

General information

The 46 subject patients with mesangial proliferative glomerulonephritis were selected from our nephrology department from May 2013 to May 2018. Specimens were obtained using renal puncture biopsies, and were then fixed and paraffin-embedded. All patients were subjected to renal biopsy histopathology to confirm the diagnosis. There were 28 males and 18 females, aged 19 to 56 years old, with an average age of 34.75 ± 6.24 years old and a disease course of 1 month to 9 years, with an average of 14.5 ± 6.31 months. Of the 49 patients, 16 showed nephrotic syndrome, 25 showed chronic glomerulonephritis, and 5 showed occult nephritis. There were 8 cases with hematuria, 20 cases with

elevated blood pressure, 37 cases with edema, 6 cases with nausea, and 16 cases with soreness of the loins. There were 16 cases without cause before the onset, 26 cases with upper respiratory tract infection, and 4 cases with urinary tract infection.

For the control group, 8 autopsy kidney tissue specimens from patients who died unexpectedly between May 2013 and May 2018 were selected. The patients had no renal tissue lesions or damage, showed normal renal function, and met medical ethics requirements. There were 5 males and 3 females, aged 23 to 59 years old, with an average age of 36.00 ± 5.96 years old.

Inclusion criteria: (I) complete clinical data; (II) aged between 19 and 59 years old; (III) no diabetes history. Exclusion criteria: (I) impaired renal function and abnormal thyroid metabolism; (II) patients had diabetic ketoacidosis recently; (III) patients who had taken thyroid hormone metabolism or kidney function drugs.

The study was approved by Ningbo Yinzhou People's Hospital Community (No. 2019-046). All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The informed consent was taken from all the patients.

Research methods

Main reagents

Primary antibodies for rabbit anti-TSP-1 antibody, mouse anti-human PAI-1 antibody, and immunohistochemical staining kit (ready-to-use) were purchased from Baoshide Company (Wuhan, China).

Methodology

TSP-1 and PAI-1 immunohistochemical staining was performed by strept avidin biotin-peroxidase complex (SABC) staining method (7). Tissue sections were routinely dewaxed and rinsed, and 3% methanol hydrogen peroxide was added for 20 minutes. Samples from the same patients were divided into two groups: one group was subjected to TSP-1 immunohistochemical staining, and the other group was subjected to PAI-1 immunohistochemical staining. The operations were conducted strictly according to the kit instructions. The results of TSP-1 and PAI-1 staining of known mesangial proliferative glomerulonephritis tissues were used as the positive control, while the product of replacing the primary and secondary antibodies with phosphate-buffered saline (PBS) solution was used the

negative control.

Methods for detecting renal function indicators

Four mL of fasting venous blood were collected from all the patients in the 3 groups at 8 am in the morning to detect serum albumin, triglycerides, and total cholesterol levels. The enzymatic method was used to determine serum Scr level. The 24-hour urine output samples of patients in the 3 groups of need to be collected excluding proteinuria caused by exercise, infection and other factors, and the 24-hour proteinuria was measured with toluene preservation methods. Urine a-1 microglobulin in the urine 1 hour after drinking 5,000 mL of water at 6 am was detected by radioimmunoassay (RIA).

Ccr calculation formula: $[(140 - \text{age}) \times \text{body weight (kg)}] / [0.818 \times \text{serum creatinine } (\mu\text{g/mL})]$.

Criteria of pathological scoring

Following the 1982 classification criteria for glomerular pathology from the World Health Organization (WHO) (8), the scoring assessment of glomerular pathology in our study considered three criteria: mesangial cell proliferation, stroma widening, and changes in sclerosis. These were recorded from 0 to 3 points according to normal, mild, moderate, and severe levels, respectively. A mild level was defined as slight proliferation of mesangial cells, no significant effect on capillary ridges, and glomerular sclerosis <30%. A moderate level was defined as moderate proliferation of mesangial cells, occlusion of capillary narrowing <50%, and glomerular sclerosis of 30–60%. A severe level was defined as severe proliferation of mesangial cells, occlusion of capillary narrowing >50%, and glomerular sclerosis >60%.

The scoring for the mesenchyme of renal tubule pathology included four criteria: renal tubular degeneration, atrophy, interstitial inflammatory cell infiltration, and interstitial fibrosis. A mild level was defined as slightly damaged renal tubules with small focal tubular atrophy, interstitial inflammatory cell infiltration, and interstitial fibrosis. A moderate level was defined as moderately damaged renal tubules and focal tubular atrophy, interstitial inflammatory cell infiltration, and interstitial fibrosis. A severe level was defined as severely damaged renal tubules and diffuse tubular atrophy, interstitial inflammatory cell infiltration, and interstitial fibrosis. These were recorded from 0 to 3 points according to normal, mild, moderate and severe degree, respectively.

Scores from glomerular pathology assessment were totaled, and specimens were defined as mild (0–4 points), moderate (5–8 points), and severe (9–12 points). Scores for renal interstitial pathological damage were also totaled, and specimens were defined as mild (0–4 points), moderate (5–8 points), and severe (9–12 points).

Statistical methods

All data were performed using SPSS 18.0 software. Measurement data are described as mean \pm standard deviation ($\bar{x} \pm S$), and were analyzed by *t*-test or variance test. Count data and expressed as rate/composition ratio, and the comparison between groups was assessed by χ^2 test. Results with a P value <0.05 were considered statistically significant.

Results

Relationship between mesangial hyperplasia and clinical indicators

The 24-hour urine protein, triglyceride, and total cholesterol levels of patients with severe mesangial hyperplasia were significantly higher than those of patients with mild and moderate mesangial hyperplasia, while the serum albumin was lower than that of patients with mild and moderate mesangial hyperplasia ($P < 0.05$). The 24-hour urine protein level of patients with moderate mesangial hyperplasia was higher than that of patients with mild mesangial hyperplasia, while the albumin level was lower than that ($P < 0.05$), but there was no significant difference in triglyceride and total cholesterol ($P > 0.05$). There was also no significant difference in creatinine clearance (Ccr) between the three groups ($P > 0.05$) (Table 1).

Relationship between renal interstitial lesion and renal function

The 24-hour urinary protein and urine AIM levels of patients with renal interstitial lesions were higher than those without renal interstitial lesions, while Ccr levels were lower ($P < 0.05$) (Table 2).

Comparison of TSP-1 expression level in mesangial proliferative glomerulonephritis and normal renal tissue specimens

TSP-1 was not positively expressed in the glomeruli and

Table 1 Relationship between mesangial hyperplasia and clinical indicators ($\bar{X} \pm s$)

Group with mesangial hyperplasia	Cases	24-h urine protein (g)	Ccr (mL/min)	Serum albumin (g/L)	Triglyceride (mmol/L)	Total cholesterol (mmol/L)
Mild	23	1.19±0.87	124.05±29.96	37.23±6.35	1.65±0.88	5.61±2.58
Moderate	13	2.26±0.94	109.94±27.56	30.14±6.32	1.93±1.05	6.11±2.04
Severe	10	4.25±1.79	97.52±30.83	15.84±5.92	3.31±1.69	8.96±3.75
F	-	25.09	3.02	40.81	7.56	5.37
P	-	<0.001	0.059	<0.001	0.002	0.008

Ccr, creatinine clearance.

renal tubules of the control group, while its expression in mesangial hyperplasia patients increased with the severity of renal tubular damage ($P<0.05$) (Table 3).

Comparison of PAI-1 expression levels between the mesangial proliferative glomerulonephritis and normal renal tissue specimens

There was no positive expression of PAI-1 in the glomeruli and tubules of the control group, while its expression in mesangial hyperplasia patients increased with the severity of tubular damage ($P<0.05$) (Table 4).

Discussion

Mesangial proliferative glomerulonephritis is a pathological subtype of glomerulonephritis and is common in China and Australia, particularly in adolescents (9). Although most patients have a good prognosis, renal function decline occurs in some patients and may even progress to end-stage renal failure (10). This disease is mostly induced by upper respiratory tract infections with diverse clinical manifestations, most of which are primary nephrotic syndrome (11), asymptomatic hematuria, proteinuria, and other non-specific symptoms, so clinical diagnosis and prognosis are problematic. At present, the etiology, pathology, and pathogenesis of the disease are not clear and there are few studies on the expression of PAI-1 and TSP-1 in mesangial proliferative glomerulonephritis. Thus, in order to guide clinical diagnosis and treatment, clinicians rely on finding sensitive and specific protein molecules as biomarkers of pathological changes. Most studies have shown that glomerular capillary endothelial injury and coagulopathy are important factors in the occurrence of this disease (12-14), which causes diffuse thrombosis in the glomerulus where ECM deposition arising from an imbalance of the plasmin system is a key precipitator of glomerulosclerosis (15-17).

The results of this study showed that the 24-hour urinary protein, triglycerides, and total cholesterol levels in patients with severe mesangial hyperplasia were significantly higher than those in patients with mild and moderate mesangial hyperplasia, while the serum albumin level was lower. The 24-hour urine protein in patients with moderate mesangial hyperplasia was higher than that in patients with mild mesangial hyperplasia, while the albumin levels were lower, but there was no significant difference in triglyceride and total cholesterol levels. Furthermore, there was no

Table 2 Relationship between renal interstitial lesions and renal function ($\bar{x} \pm s$)

Group	Cases	24-h urinary protein quantification (g)	Urine A1M (mg/L)	Ccr (mL/min)
Renal interstitial lesion	25	1.87±1.07	286.79±154.31	125.35±28.66
No renal interstitial lesion	21	3.56±1.58	576.61±204.77	89.74±37.98
t	–	4.305	5.469	3.621
P	–	<0.001	<0.001	0.001

Ccr, creatinine clearance; A1M, alpha-1-microglobulin.

Table 3 Comparison of TSP-1 level in mesangial proliferative glomerulonephritis and normal renal tissue specimens ($\bar{x} \pm s$)

Variable	Group with mesangial hyperplasia		Group with renal interstitial lesion	
	Cases	TSP-1 level ($\mu\text{g/mL}$)	Cases	TSP-1 level ($\mu\text{g/mL}$)
Control	8	0	8	0
Mild	23	0.38±0.08	10	0.76±0.18
Moderate	13	0.49±0.10	5	1.05±0.19
Severe	10	0.97±0.14	6	2.10±0.25
F (last three groups)	–	121.48	–	83.03
P (last three groups)	–	<0.001	–	<0.001

TSP-1, thrombospondin-1.

Table 4 Comparison of PAI-1 levels between mesangial proliferative glomerulonephritis and normal renal tissue specimens ($\bar{x} \pm s$)

Variable	Group with mesangial hyperplasia		Group with renal interstitial lesion	
	Cases	PAI-1 level ($\mu\text{g/mL}$)	Cases	PAI-1 level ($\mu\text{g/mL}$)
Control	8	0	8	0
Mild	23	0.15±0.10	10	0.70±0.18
Moderate	13	0.23±0.12	5	0.94±0.20
Severe	10	0.41±0.09	6	1.42±0.24
F (last three groups)	–	21.75	–	23.70
P (last three groups)	–	<0.001	–	<0.001

PAI-1, plasminogen activator inhibitor-1.

significant difference in Ccr between the three groups. The expression levels of urinary protein quantification and urine A1M in patients with renal interstitial disease were higher than those without renal interstitial disease, while the Ccr level was lower. This indicates that the more severe the glomerular pathological changes in patients is, the greater the difference between clinical indicators like 24-hour urine protein quantification, triglycerides, total cholesterol, and serum albumin from the normal range will be. Patients with

renal interstitial disease may even suffer impaired renal function, which suggests that the degree of pathological changes could reflect the degree of the patient's condition. Therefore, it is of great importance to assess the degree of pathological changes of the glomeruli and renal interstitium in clinic (18,19).

Previous studies have shown that highly expressed PAI-1 and TSP-1 can be found in the mesangial region and renal tubulointerstitium of this disease, suggesting that

they are closely related to the pathogenesis of mesangial proliferative glomerulonephritis (20). In this study, TSP-1 and PAI-1 were not positively expressed in normal renal tissue specimens, and the expression level of them in different degrees of mesangial hyperplasia and different degrees of renal tubular injury was mild < moderate < severe. Therefore, we can perhaps conclude that TSP-1 and PAI-1 are inextricably linked to pathological changes of mesangial hyperplasia and tubulointerstitial lesions; they may promote the progression of mesangial proliferative glomerulonephritis, and their expression levels could thus indirectly reflect the disease progression.

In summary, the pathological changes of mesangial proliferative glomerulonephritis are related to clinical indicators of 24-hour urine protein quantification, triglycerides, total cholesterol, urine A1M, Ccr levels, and others. In mesangial proliferative glomerulonephritis patients, the expression levels of PAI-1 and TSP-1 in the mesenchyme of glomerular and renal tubules increased with the severity of the disease, suggesting that TSP-1 and PAI-1 are critically involved in the occurrence and development of mesangial proliferative glomerulonephritis.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was approved by Ningbo Yinzhou People's Hospital Community (No. 2019-046). All procedures performed in this study involving human participants were in accordance with the

Declaration of Helsinki (as revised in 2013). The informed consent was taken from all the patients.

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