Original Article

Levels of Apelin-12, AT1R, and AGT are correlated with degree of renal fibrosis in patients with immunoglobulin A nephropathy

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Background: To explore the relationship between the degree of renal fibrosis in patients with immunoglobulin A nephropathy (IgAN) and their levels of Apelin-12, Average Optical Density of angiotensin II type 1 receptor (AOD_{AT1R}), and angiotensinogen (AGT).

Methods: A total of 156 patients with IgAN diagnosed by renal biopsy in our hospital were selected and divided into a T_0 group (54 cases), T_1 group (49 cases) and T_2 group (53 cases). The levels of Apelin-12, AT1R, and AGT were compared among the three groups, and the relationship between the above three indicators and degree of renal fibrosis was analyzed among patients with IgAN.

Results: The AOD_{AT1R} and AGT level in the T_2 group and T_1 groups were significantly higher than those of the T_0 group, and the Apelin-12 level of patients in the T_2 group and T_1 groups were significantly lower than that in T_0 group. Significances of the same trend were observed among all the above indicators between the T_2 group and T_1 group. ROC curves showed that when the cutoff value of Apelin-12 was 2.36 μg/L, the area under curve (AUC), sensitivity, and specificity of T_0-T_1T_2 were 0.889, 92.00%, and 88.00%, respectively. When the cut-off value of AOD_{AT1R} was 0.065, the AUC, sensitivity, and specificity were 0.706, 76.00%, and 76.00%, respectively, and when the cut-off value of AGT was 47.26 ng/mL, the AUC, sensitivity, and specificity were 0.899, 84.00%, and 88.00%, respectively. When the cutoff value of Apelin-12 was 0.92 μg/L, the AUC, sensitivity, and specificity of T_0T_1-T_2 were 0.819, 84.62%, and 87.50%, respectively, and when the cutoff value of AOD_{AT1R} was 0.079, the AUC, sensitivity, and specificity were 0.699, 76.92%, and 79.17%, respectively. When the cut-off value of AGT was 92.96 ng/mL, the AUC, sensitivity, and specificity were 0.893, 84.62%, and 91.67%, respectively.

Conclusions: Apelin-12 decreased with disease progression, while AT1R and AGT increased. The changes of levels of Apelin-12, AT1R, and AGT have certain significance in judging the degree of renal fibrosis in patients with IgA nephropathy, and the change of level of AGT has the highest correlation with the degree of renal fibrosis.

Keywords: Apelin-12; angiotensin II type 1 receptor; angiotensinogen (AGT); immunoglobulin A nephropathy (IgAN)

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Introduction

Immunoglobulin A (IgA) nephropathy (IgAN) is a kind of immune complex nephritis in which IgA deposits in the mesangial area of the glomerulus (1-3). IgAN is the most prevalent primary glomerular disease worldwide and in China, IgAN patients account for about 45% of primary glomerular disease (4-7). Studies have shown that renal fibrosis is an important factor affecting the progression and prognosis of IgAN (8,9). In the present study, we examined the levels of Apelin-12, angiotensin II Type 1 receptor (AT1R), and angiotensinogen (AGT) in IgAN patients with different degrees of renal fibrosis and discussed the relationship between the above indicators and the degree of kidney fibrosis of IgAN patients.

We present the following article in accordance with the STARD reporting checklist (available at http://dx.doi.org/10.21037/apm-21-1059).

Methods

Patients

One hundred and fifty-six patients with IgAN diagnosed by renal biopsy in our hospital were selected. According to the MEST-C criteria in the updated Oxford Classification of IgA Nephropathy (10), patients were divided into aT_0 group (54 cases), T_1 group (49 cases), and T_2 group (53 cases). This study was approved by the ethics committee of Sichuan Provincial People's Hospital. The approval number was not provided, as this was a retrospective study. Individual consent for this retrospective analysis was waived. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The inclusion criteria were all of the following: Age >18, diagnosed with IgAN via biopsy, and no glucocorticoid treatment before biopsy. The exclusion criteria were any of the following: Secondary nephropathy caused by systemic lupus erythematosus, hepatitis B, or Henoch-Schonlein purpura; renal vascular stenosis; coronary heart disease, pulmonary hypertension, arrhythmia; patients with incomplete clinical data. There was no significant difference in gender, age, and body mass index between the two groups of patients (*Table 1*, P>0.05).

Renal fibrosis

The degree of renal fibrosis was classified by the updated Oxford Classification of IgA Nephropathy based on the degree of cortical tubule atrophy or renal interstitial fibrosis area (10). In the T_0 group cortical tubule atrophy or the renal interstitial fibrosis area was ≤25%. In the T_1 group, cortical tubule atrophy or the kidney interstitial fibrosis was >25% and ≤50%, and in the T_2 group, cortical tubule atrophy or renal interstitial fibrosis were >50%.

Samples and tests

Blood from the anterior cubital vein (5 mL) of each IgAN patient was collected on an empty stomach, centrifuged at 3,200 r/min to harvest the serum, and stored in a refrigerator at −80 ℃ for later use. On the day of admission, 15 mL of clean midstream urine was collected, cooled at room temperature, centrifuged at 2,800 r/min, then refrigerated at −80 ℃ for later use. Enzyme Linked Immunosorbent Assay (ELISA) was used to detect the levels of Apelin-12 and AGT using the Apelin-12 ELISA kit and human AGT-ELISA kit, respectively, and all operations were performed according to the manufactures' instructions. The expression of AT1R was determined by immunohistochemistry. The kidney biopsy tissues were routinely dehydrated and embedded in paraffin and sections with a thickness of 3 μm were harvested with a paraffin microtome. After stretching, the slides were taken out with anti-removal slides and dried for 1 h at 57 ℃. Dewaxing for 10 min was then applied in xylene solution three times, and hydration for 5 min was applied with gradient alcohol five times. After 3 min of hydration in phosphate buffer solution three times, the slides were then soaked in pure water for 10 minutes, and a high-pressure repair method was performed to expose the antigens. Citric acid buffer was added to the pressure cooker as the antigen retrieval solution and 2 min after the pressure cooker started air blasting, the heating was stopped, and the solution left to cool to room temperature. After blocking with goat serum for 45 min, AT1R antibody was then added dropwise, then incubated overnight at 4 ℃. The slides were then stained with secondary antibody and hematoxylin solution, rinsed with water for 15 minutes, and observed under a microscope at ×400 magnification. Image Pro-plus software was used to analyze the Average Optical Density (AOD) of the images.

Statistics

All data was analyzed using SPSS20.0 software. The measurement data was expressed as mean ± SD and the comparison between the groups was performed using one-way analysis of variance (ANOVA). The operating characteristic

Table 1 Comparison of clinical data of patients

Variant	T_0	T_1	T_2	χ^2/F	P
n	54	49	53	–	–
Gender				0.091	0.956
Male	29	27	30		
Female	25	22	23		
Age (year)	35.11±8.16	35.48±8.55	35.01±8.69	0.043	0.958
Body mass index (kg/m^2)	27.88±2.94	27.79±3.05	27.67±3.11	0.064	0.938

Table 2 Comparison of laboratory indicators among patients with different degrees of fibrosis

Variant	T_0	T_1	T_2	χ^2/F	P
N	54	49	53	–	–
SBP (mmHg)	128.54±11.23	139.51±12.64*	145.28±15.12*#	22.554	<0.01
DBP (mmHg)	81.63±5.33	86.14±6.23*	89.66±8.65*#	18.275	<0.01
MAP (mmHg)	96.15±12.46	104.94±13.75*	110.21±10.53*#	17.906	<0.01
Urine protein (g/24 h)	1.01±0.32	2.52±0.47*	2.96±0.54*#	273.833	<0.01
Serum creatinine (μmol/L)	70.16±10.74	115.84±21.66*	183.25±30.49*#	343.547	<0.01
Blood urea nitrogen (mmol/L)	4.63±1.07	7.52±1.48*	10.46±2.11*#	175.039	<0.01
Blood albumin (g/L)	36.83±3.49	32.17±3.67*	30.22±3.45*#	49.368	<0.01

*, P<0.05 compared with T_0; #, P<0.05 compared with T_1 group. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean central venous pressure.

curve (ROC) was employed to analyze the predictive value of Apelin-12, AGT, AT1R in the diagnosis of renal fibrosis in IgAN patients. P<0.05 was taken as statistically significant.

Results

General pathology of patients

The systolic blood pressure (SBP), diastolic blood pressure (DBP), mean central venous pressure (MAP), urine protein quantification, and serum creatinine levels in the T_1 group were significantly higher than those in the T_0 group. The blood albumin level of patients in the T_1 group was significantly lower than that in the T_0 group. The indexes were more obviously changed in T_2 group, as differences between T_2 and T_1 were significant (*Table 2*, P<0.05).

Comparison of Apelin-12, AOD_{AT1R}, and AGT levels

The level of Apelin-12 in the T_1 group was significantly lower than that in the T_0 group, and the levels of AOD_{AT1R} and AGT were significantly higher than those in the T_0 group. Similarly, the levels of Apelin-12 in the T_2 group were significantly lower than those in the T_1 group, and the levels of AOD_{AT1R} and AGT were significantly higher than those in the T_1 group (*Table 3*, P<0.05).

The diagnostic value of Apelin-12, AOD_{AT1R}, and AGT in T_0 versus T_1T_2 renal fibrosis

The ROC curve shows that when the cutoff value of Apelin-12 is 2.36 μg/L, the area under curve (AUC), sensitivity, and specificity of T_0 versus T_1T_2 (T_0-T_1T_2) are 0.889, 92.00%, and 88.00%, respectively; when the cutoff value for AOD_{AT1R} is 0.065, the AUC, sensitivity, and specificity are 0.706, 76.00%, and 76.00%, respectively; and when the cutoff value for AGT is 47.26 ng/mL, the AUC, sensitivity, and specificity are 0.899, 84.00%, and 88.00%, respectively, as shown in *Table 4* and *Figure 1*.

Table 3 Comparison of levels of Apelin-12, $AOD_{AT1R,}$ and AGT among T_0 group, T_1 group, and T_2 group

Stage	n	Apelin-12 (μg/L)	AOD_{AT1R}	AGT (ng/mL)
T_0	54	3.41±1.02	0.05±0.01	28.27±4.19
T_1	49	1.16±0.33*	0.07±0.01*	64.76±8.56*
T_2	53	0.74±0.21*#	0.09±0.01*#	120.41±20.23*#
F	–	268.088	213.987	689.554
P	–	<0.01	<0.01	<0.01

*, P<0.05 compared with T_0 group; #, P<0.05 compared with T_1 group. AOD_{AT1R}, Average Optical Density of angiotensin II type 1 receptor; AGT, angiotensinogen.

Table 4 Diagnostic value of Apelin-12, AT1R, and AGT on T_0-T_1T_2

Index	Cut-off	AUC	Sensitivity (%)	Specificity (%)	Youden index	95% CI
Apelin-12	≤2.36	0.889	92.00	88.00	0.800	0.768–0.960
$AO_{DAT1}R$	>0.065	0.706	76.00	76.00	0.520	0.561–0.827
AGT	>47.26	0.899	84.00	88.00	0.720	0.781–0.966

AOD_{AT1R}, Average Optical Density of angiotensin II type 1 receptor; AGT, angiotensinogen; AUC, area under curve.

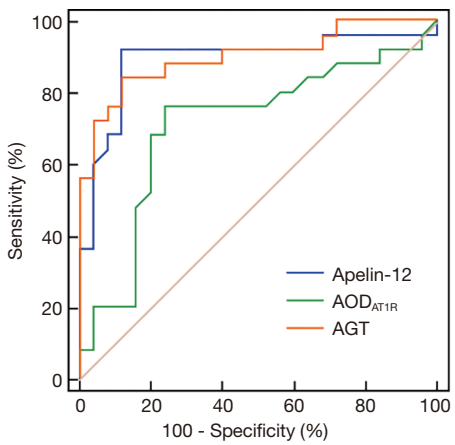

Figure 1 ROC curves of Apelin-12, At1R, and AGT on T_0-T_1T_2. AOD_{AT1R}, Average Optical Density of angiotensin II type 1 receptor; AGT, angiotensinogen.

The diagnostic value of Apelin-12, AT1R, and AGT in T_0T_1 versus T_2 renal fibrosis

The ROC curve shows that when the cut-off value for Apelin-12 is 0.92 μg/L, the AUC, sensitivity, and specificity of T_0T_1 versus T_2 (T_0T_1-T_2) are 0.819, 84.62%, and 87.50%, respectively; when the cut-off value for AOD_{AT1R} is 0.079, the AUC, sensitivity, and specificity is 0.699, 76.92%, and 79.17%, respectively; and when the cut-off value for AGT is 92.96 ng/mL, the AUC, sensitivity, and specificity are 0.893, 84.62%, and 91.67%, respectively, as shown in *Table 5* and *Figure 2*.

Discussion

IgAN is one of the main causes of renal fibrosis and end-stage renal failure. Renal fibrosis is manifested by the accumulation of fibroblasts, myofibroblasts, and extracellular matrix, leading to glomerular interstitial fibrosis and glomerulus sclerosis, eventually leading to the loss of renal function (3,11,12).

In this study, the SBP, DBP, MAP, urine protein quantitative, and serum creatinine levels of patients in the T_2 and T_1 groups were significantly higher than those in the T_0 group, while the blood albumin level of patients in the T_2 and T_1 groups were significantly lower than those in the T_0 group. The SBP, DBP, MAP, urine protein quantification, and serum creatinine level was significantly higher in the T_2 group than in the T_1 group, while blood albumin level was significantly lower than the T_1 group. When the kidney is severely damaged, renal function is significantly reduced and glomeruli are damaged, resulting in proteinuria. Proteinuria can activate the secretion of excessive inflammatory mediators and chemokines from

Table 5 Diagnostic value of Apelin-12, AT1R, and AGT on T_0T_1-T_2

Index	Cut-off	AUC	Sensitivity (%)	Specificity (%)	Youden index	95% CI
Apelin-12	≤0.92	0.819	84.62	87.50	0.721	0.684–0.913
AT1R	>0.079	0.699	76.92	79.17	0.561	0.553–0.820
AGT	>92.96	0.893	84.62	91.67	0.763	0.773–0.962

AT1R, angiotensin II type 1 receptor; AGT, angiotensinogen; AUC, area under curve.

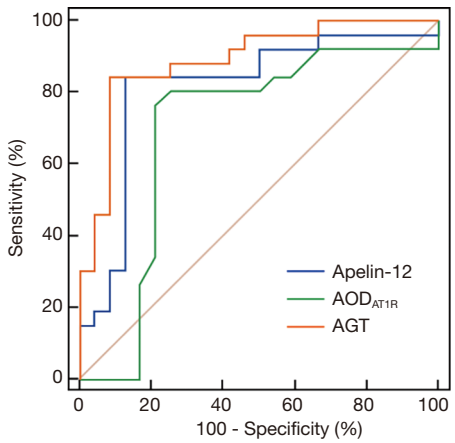

Figure 2 ROC curves of Apelin-12, AT1R, and AGT on T_0T_1-T_2. AOD_{AT1R}, Average Optical Density of angiotensin II type 1 receptor; AGT, angiotensinogen.

tubular cells, causing inflammation and a pro-fibrotic reaction, which possibly leads to an increase in the degree of renal fibrosis. In this study, the levels of Apelin-12 in the T_2 and T_1 groups were significantly lower than those in the T_0 group, and the levels of AOD_{AT1R} and AGT were significantly higher than those in the T_0 group. The levels of Apelin-12 in the T_2 group were significantly lower than those in the T_1 group and the levels of AOD_{AT1R} and AGT were significantly higher than those in the T_1 group. It seems that as the degree of renal fibrosis in patients increased, the level of Apelin-12 decreased, and the expression of AT1R and AGT increased. Apelin-12 is a newly discovered biologically active peptide that can participate in the regulation of normal physiological functions of the kidney and bind to orphan G protein-coupled receptor proteins such as angiotensin. AT1R is distributed in blood vessels, kidneys, adrenal glands, liver, brain, and other tissues and organs, and induces smooth muscle contraction. Angiotensin II is activated by AT1R to exert its cellular and molecular effects, which can cause changes in vasoconstriction and renal fibrosis. Activated AT1R may also promote mesangial cell proliferation and the formation of crescents, and cause glomerular sclerosis.

The renin-angiotensin system (RAS) is an important regulatory system in the human body. As the sole substrate of RAS, angiotensin activation can predict the condition of the kidney. The Apelin-12 peptide is a vasodilator which promotes the synthesis of nitric oxide, and inhibits the proliferation of vascular smooth muscle cells. By activating the classical Smads signaling pathway, Apelin-12 inhibits the epithelium-mesenchyme transition (EMT) of renal tubules. A decrease in the level of Apelin-12 indicates the decline of kidney function and EMT of renal tubular cells, and progression of fibrosis in the interstitium (13-16). The ROC curve results in this study show that the peptides Apelin-12, AT1R, and AGT are all sensitive to the judgment of T_0-T_1T_2, and the AUC is greater than 0.7 in predicting T_0-T_1T_2 for Apelin-12, AT1R, and AGT. Among them, Apelin-12 and AGT are good indicators for T_0-T_1T_2, as they have higher AUC and higher sensitivity. The ROC curve results also show that Apelin-12 and AGT have good sensitivity for predicting T_0T_1-T_2, both of which have an AUC greater than 0.8. AGT has higher AUC and sensitivity for judging T_0T_1-T_2, indicating that AGT has the highest value for judging T_0T_1-T_2. This may be because AGT increases with the progress of tubular interstitial fibrosis or tubular atrophy, and angiotensin II can stimulate the secretion and synthesis of AGT. A high expression of angiotensin II can increase inflammatory factors and active oxidative factors, induce the production of crescents in kidney tissue, and aggravate the degree of renal tissue fibrosis. Therefore, the expression of AGT in patients with severe renal fibrosis is increased the most significantly (17-19).

In summary, changes in the levels of Apelin-12, AT1R, and AGT have significance in determining the degree of renal fibrosis in patients with IgA nephropathy, and changes in the level of AGT have the highest correlation with the degree of renal fibrosis. Anyway, the accuracy and specificity

possibly could be improved when other serum indicators were introduced.

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Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist Available at http://dx.doi.org/10.21037/apm-21-1059

Data Sharing Statement: Available at http://dx.doi.org/10.21037/apm-21-1059

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/apm-21-1059). The authors have no conflicts of interest to declare.

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. This study was approved by the ethics committee of Sichuan Provincial People's Hospital. The approval number was not provided, as this was a retrospective study. Individual consent for this retrospective analysis was waived. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013).

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