Correlation Between Initial Urine V-ATPase and Pathological Changes in IgA Nephropathy: Implications for Short-Term Outcomes

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Abstract：This study aimed to investigate the alterations in expression and clinical relevance of ATP6V1A and ATP6V1E1 in urine samples from patients with IgA nephropathy(IgAN). The research included a cohort of 55 patients diagnosed with IgAN and a control group of 30 healthy individuals, with no significant differences in age or gender between the groups. The maximum tolerated dose of angiotensin-converting enzyme inhibitors (ACEI) or angiotensin receptor antagonists(ARB) was used for 6 months. Treatment effectiveness was defined as a reduction in urinary protein of more than 50% from baseline. The results demonstrated that the urinary expression levels of ATP6V1A and ATP6V1E1 in patients with IgAN were significantly lower than those observed in the healthy control group. And in IgAN group, the expression of ATP6V1A in urine was significantly lower in patients with Tubular atrophy and interstitial fibrosis. Patients with IgAN were divided into low expression group and high expression group according to the median urinary levels of ATP6V1A and ATP6V1E1, and there was no significant difference in baseline proteinuria between the two groups. ATP6V1A Low-expression groups showed significantly higher proteinuria levels after 6 months of treatment compared to high-expression groups, as well as notably lower treatment efficacy. However, the two ATP6V1E1 groups did not differ significantly in either proteinuria levels or treatment efficacy at 6 months. Accordingly, urinary ATP6V1A may be a useful biomarker for detecting disease severity and therapeutic response in patients with IgAN.

Keywords: V-ATPase; IgA Nephropathy; Proteinuria; ACEI; ARB

1. Introduction

IgA nephropathy(IgAN) is the most common primary glomerular disease, with 30-40% of patients developing end-stage kidney disease within 20 years[1]. Initial symptoms include hematuria and proteinuria, and diagnosis requires a renal biopsy. The well-accepted pathogenesis of IgAN is the increased production of low-glycosylated IgA1(gd-IgA1), which deposits in the kidneys and triggers an immune response[2]. However, the precise mechanism underlying gd-IgA1 generation remains unclear. It has been suggested that protein glycosylation occurs within the Golgi apparatus and is controlled by factors such as Golgi pH and growth factor signaling pathways[3-5]. Additionally, reduced expression of Golgi protein 130 has been associated with gd-IgA1[6].

Vacuolar-type ATPase (v-ATPase), a proton pump with V1 and V0 subunits, is found in cellular compartments like the Golgi apparatus, lysosomes, and endosomes, and is essential for pH regulation[7]. Abnormal ATP6V1A and ATP6V1E1 are associated with congenital glycosylation defects[8]. Notably, urine proteome analysis shows significantly lower V-ATPase expression in IgAN patients compared to healthy individuals. Renin-angiotensin system (RAS) blockers, including angiotensin-converting enzyme inhibitors (ACEI) and angiotensin receptor antagonists (ARB), are the most widely used and effective drugs for treating IgAN. Nearly half of the patients treated with the maximum tolerated dose of ACEI/ARB exhibited significant remission of proteinuria after a duration of 6 months[9]. However, the underlying causes contributing to this mitigation remain unclear. Therefore, this study aims to determine the expression levels of ATP6V1A and ATP6V1E1 in urine and discuss their clinical significance. Moreover, this current study evaluated the

effect of ATP6V1A and ATP6V1E1 levels on the efficacy of ACEI/ARB drugs.

2. Method

2.1 Study Population

A total of 55 patients diagnosed with IgAN were recruited from both the outpatient and inpatient departments of Loudi Central Hospital between January 2020 and January 2022. Additionally, a control group comprising 30 healthy individuals, aged between 18 and 70 years, was selected from the physical examination center of the same hospital. The diagnosis of IgAN was confirmed via renal biopsy, with inclusion criteria specifying an estimated glomerular filtration rate (eGFR) exceeding 30 mL/min and a 24-hour urinary protein level ranging from greater than 1 g/day to less than 3.5 g/day.Exclusion criteria included secondary IgAN, such as IgA vasculitis, rheumatic disease, and systemic lupus erythematosus. Additionally, patients with acute or chronic infectious diseases, liver cirrhosis, malignant tumors, diabetes mellitus, or other significant conditions were excluded. Patients who had received glucocorticoids or other immunosuppressants within six months prior to the initiation of disease treatment were also excluded from the follow-up analysis. Furthermore, patients who discontinued ACEI/ARB medications at six months of treatment due to severe hyperkalemia or a 25% increase in creatinine levels above baseline were excluded.The study was approved by the Institutional Ethics Committee of Loudi Central Hospital.

2.2 Specimen Collection and Treatment

Urine and blood samples were collected from the first morning void prior to renal biopsy. Neither glucocorticoids nor ACEI/ARB drugs were administered.ACEI/ARB medications were initiated following renal biopsy, with the maximum dosage being gradually titrated over a period of one month. 24-hour urine and serum samples were collected from the enrolled patients at the 1, 3, and 6 months following the administration of the maximum dosage of ACEI/ARB. Urine samples were collected from patients prior to the kidney biopsy, centrifuged at 2500 rpm for 20 minutes at 4°C, and the supernatant was stored in aliquots at -80°C until analysis. Serum was obtained from clotted blood samples by centrifugation at 3,000 × g for 10 minutes at 4°C.The 24-hour urinary protein quantification (24 h-UP), the serum creatinine (Scr) levels were measured using an automatic biochemical instrument of Loudi Central Hospital. Using the modified diet in kidney disease (MDRD) equation, we calculated the glomerular filtration rate (eGFR)[10].

2.3 Measurement of ATP6V1A and ATP6V1E1 Levels in Urine

The levels of ATP6V1A and ATP6V1E1 in urine were quantified using an enzyme-linked immunosorbent assay (ELISA) technique, following the manufacturer's instructions provided with the ELISA kit(KEVINO，Tianjin，China).To summarize, the samples and standard were added to a 96-well ELISA plate coated with a mouse monoclonal antibody specific for human ATP6V1A or ATP6V1E1, and incubated overnight at 4℃. After three washes with PBST, horseradish peroxidase-conjugated goat anti-human ATP6V1A and ATP6V1E1 were added. Following a 60-minute incubation at 37℃, the reaction was terminated by adding 100 μL of 1.0 M sulfuric acid, and the absorbance was measured at 450 nm using a micro-ELISA plate reader. The levels of ATP6V1A and ATP6V1E1 were determined based on the standard curve.

2.4 Pathological Classification of IgAN

In all renal biopsies, two pathologists confirmed the pathological results based on the MEST-C scoring system proposed by the IgAN classification working group[11]. There are two types of mesangial hypercellularity (M) scores: M0 for scores ≤ 0.5 and M1 for scores > 0.5.It was identified that there was endocapillary hypercellularity (E1) and segmental glomerulosclerosis (S1). Tubular atrophy and interstitial fibrosis (T) were classified semiquantitatively as T0 for 0–25%, T1 for 26–50%, and T2 for more than 50%.. C0 indicates no cellular or fibrocellular crescents, C1 indicates crescents less than 25% of glomeruli, and C2 indicates crescents of 25% or more.

2.5 Statistical Analysis

Continuous variables following a normal distribution were presented as mean ± standard deviation (SD) and compared using unpaired Student's t-test. For continuous variables with a non-normal distribution, median and

interquartile range (IQR) were reported and compared using the Mann-Whitney U test. Categorical data were analyzed using the Chi-square test. We used IBM SPSS version 20, and significance was determined based on a P-value of less than 0.05.

3. Results

3.1 Clinical Data of the Included Patients

As delineated in Table 1, the proportion of males within the IgAN cohort was 54.5%, with a median age of 40 years. The prevalence of hypertension among these patients was 34.5%, which did not exhibit significant differences when compared to the control group. For the IgAN cohort, the median 24-hour urine protein excretion was 2.34 grams/day, compared to all control participants excreting less than 150 mg/day. Additionally, patients with IgAN had median serum albumin concentrations of 37 g/L and eGFR of 74.8 ml/min/1.73 m2, which were significantly lower than their respective controls. Furthermore, ELISA results demonstrated that ATP6V1A and ATP6V1E1 were markedly reduced in urine samples from patients with IgAN compared to controls.

Table 1. Clinical and Laboratory Parameters in the Iga Nephropathy and Controls

Characteristics	Healthy control（n=30）	IgA nephropathy（n=55）	P
Gender(n,M%)	15(50%)	30(54.5%)	0.688
Age(years)	38(32,49.5)	40(36,47)	0.387
24h-Upro(g/d)	0.1(0.09,0.12)	2.34(1.99,3.02)	0.000
ALB(g/L)	40.9(39.4,43.4)	37(32,40)	0.000
eGFR(mL/min/1.73 m2)	94.85(87.85,103.6)	74.8(55.6,95.5)	0.000
hypertension	10(33.3%)	19(34.5%)	0.910
ATP6V1E1(ug/ml)	227.9(199.7,300.8)	142.7(109.3,183.6)	0.000
ATP6V1A(ug/ml)	229.7(205.8,300.0)	148.6(107.4,188.5)	0.000

3.2 Correlation of Urine Atp6v1a and Atp6v1e1 Levels with Clinico-pathological Parameters

In patients with IgAN, no significant differences were observed in the urine levels of ATP6V1A and ATP6V1E1 between genders, nor between hypertensive and normotensive groups. A median urinary protein level of 2.34 g/day was used to stratify patients into high and low proteinuria groups, but there were no notable differences between the two groups in terms of ATP6V1A and ATP6V1E1 levels.Among patients with an eGFR≥ 60 ml/min/1.73m², no significant differences in urinary levels of ATP6V1A and ATP6V1E1 were observed when compared to controls(eGFR <60 ml/min/1.73m²). However, a significant reduction in urinary levels of ATP6V1A was identified in patients with higher degrees of tubulointerstitial fibrosis(T1-2) compared to those without fibrosis(T0). Additionally, other histopathological classifications—such as mesangial proliferation, endothelial cell proliferation, focal segmental sclerosis, or crescentic formations—did not affect the urinary concentrations of ATP6V1A and ATP6V1E1(Table 2)

Table 2. ATP6V1A and ATP6V1E1 Concentration In Urine of IgA Nephropathy Patients Compared With Clinical and Histopathological Parameters

Group	ATP6V1A(ng/ml)	P	ATP6V1E1(ng/ml)	P
Gender				
Male(n=30)	165.1(104.0,199.4)	0.25	154.7(109.0,197.0)	0.272
Female(n=25)	138.5(107.9,181.0)		138.4(109.0,172.3)	
Hypertension				
Yes(n=19)	169.9(108.5,188.5)	0.276	168.3(116.3,187.9)	0.111
No(n=36)	134.1(103.2,188.3)		136.9(105.5,173.8)	
Proteinuria				
low(n=28)	157.4(107.7,189.9)	0.662	150.4(111.4,182.9)	0.893
high(n=27)	148.6(104.5,184.6)		138.4(108.5,185.2)	
eGFR(mL/min/1.73m2)				
<60 (n=17)	129.4(105.1,166.8)	0.105	137.5(109.7,179.3)	0.524
≥60 (n=38)	166.5(107.5,194.6)		151.5(107.1,193.7)	

Oxford classification, n (%)				
Mesangial hypercellularity				
M0(n=8)	173.1(114.0,188.9)	0.599	154.4(104.7,189.6)	0.886
M1(n=47)	139.6(107.4,188.5)		138.5(109.4,185.2)	
Endocapillary hypercellularity				
E0(n=40)	157.7(107.7,187.7)	0.558	145.0(108.7,181.3)	0.880
E1(n=15)	138.5(104.5,188.6)		138.4(109.4,193.0)	
Segmental sclerosis				
S0(n=19)	168.9(104.5,201.3)	0.159	155.8(109.3,198.6)	0.203
S1(n=36)	139.0(107.6,183.4)		138.0(108.7,179.4)	
interstitial fbrosis				
T0(n=31)	167.6(129.6,198.7)	0.004	155.1(116.3,195.8)	0.064
T1-2(n=24)	118.4(98.3,168.4)		128.6(102.8,173.8)	
crescents				
C0(n=50)	150.0(104.1,188.5)	0.558	145.0(109.4,183.3)	0.977
C1-2(n=5)	148.6(128.7,200.5)		138.4(101.1,236.1)	

3.3 Urine ATP6V1A and ATP6V1E1 levels influenced the efficacy of ACEI/ARB in IgAN

We employed the median expression levels of ATP6V1D and ATP6V1E1 in urine as a cutoff to categorize patients with IgAN into high and low expression cohorts. Following a 6-month regimen of the maximum tolerable doses of ACEI/ARB, we observed a significant reduction in 24-hour urinary protein quantification in the high expression group of ATP6V1A compared to the low expression group. Importantly, there was no statistically significant difference in baseline urinary protein levels between these two groups. In contrast, within the high expression group for ATP6V1E1, a trend towards lower average 24-hour urinary protein levels relative to the control group was observed; however, this difference did not achieve statistical significance (Fig 1A). Remission was defined as a reduction in proteinuria by at least 50% from baseline. Approximately 20% of patients achieved remission at three months, with this proportion increasing to 52% at six months. At the six-month mark, patients exhibiting high ATP6V1A expression demonstrated a significantly higher remission rate compared to those with low ATP6V1A expression (72% vs. 32%, P<0.05). However, no significant difference in remission rates was observed between these groups at the three-month interval. In contrast, at three months, patients with elevated ATP6V1E1 expression showed a significantly greater remission rate relative to their low-expression counterparts (28% vs. 12%, P<0.05), although this difference was not sustained at the six-month evaluation.

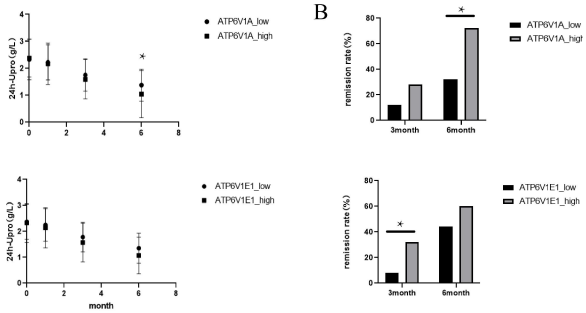

Figure1. Comparison of Treatment Effects of Different Groups

Patients with IgAN were divided into the high and low expression groups according to median urine ATP6V1A and ATP6V1E1 levels. ACEI/ARB were administered for 6 months at maximum tolerated doses.A.the 24-hour urine protein levels in different groups .B. effective rate of treatment in different groups.*,P<0.05.

4. Disscussion

V-ATPase is the primary protein structure responsible for mediating the acidification function of the Golgi apparatus, and its expression or dysfunction has been found to impact normal protein glycosylation[12]. Aberrant glycosylated proteins have been implicated in the development of diseases such as neurodegeneration, congenital glycosylation deficiency, and tumors[13-16]. Extensive evidence supports a link between abnormally high production of low-glycosylated IgA1 and the onset of IgAN[2, 17]. Therefore, we hypothesize that V-ATPase may play a role in the pathogenesis of IgAN. Previous studies have demonstrated significantly lower expression levels of V-ATPase family members in urine samples from IgAN patients compared to

healthy individuals. In this study, ELISA analysis confirmed significantly reduced expression levels of ATP6V1A and ATP6V1E1 in urine samples from IgAN patients compared to healthy individuals. Further analysis revealed that patients with high tubulointerstitial fibrosis in IgAN exhibited lower urinary expression levels of ATP6V1A and ATP6V1E1. Tubulointerstitial fibrosis is an independent risk factor for progression to end-stage renal disease in IgAN patients. Follow-up observations showed that approximately 50% of patients treated solely with ACEI/ARB at maximum dosage achieved a 50% reduction in proteinuria levels after 6 months, consistent with previous studies' findings[9]. However, the effective rate was significantly lower among patients with low urine ATP6V1A levels compared to those with high levels; although not statistically significant, a similar trend was observed for ATP6V1E1 as well. Thus, we propose that baseline levels of ATP6VIA and ATVP61VEI in urine may serve as markers for predicting poor prognosis in IgAN cases.Also, further research into the specific mechanism of ATPase in IgAN may provide new targets and treatment ideas.

5. Conclusion

ATP6VIA levels in urine were associated with tubulointerstitial fibrosis and poor treatment outcomes in IgAN.It is suggested that ATP6VIA in urine could be used to predict prognosis for IgA nephropathy as a prognostic marker.

Ethical Approval and Consent to Participate

The Institutional Review Board of the Loudi Central Hospital (protocol no. 2020-007). All the human subjects were adults and provided written informed consent.

Availability of Supporting Data

The data are available free on reasonable request from the corresponding author.

Acknowledgments

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