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Serum and urinary angiotensinogen levels as prognostic indicators in acute kidney injury: a prospective study

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SUMMARY

OBJECTIVE: The delayed increase in serum creatinine levels poses challenges in the timely diagnosis of acute kidney injury. This study aimed to investigate the relationship between serum angiotensinogen and urinary angiotensinogen levels and the prognosis of renal function in patients diagnosed with acute kidney injury.

METHODS: A total of 79 newly diagnosed acute kidney injury patients aged 18 years and older were enrolled. Serum angiotensinogen and urinary angiotensinogen levels were measured at the onset of the disease, as well as on the 15th and 30th days of follow-up. After 3 months, renal function was evaluated by measuring serum creatinine levels.

RESULTS: Among the acute kidney injury patients, those in Kidney Disease: Improving Global Outcomes stage 3 exhibited significantly higher urinary angiotensinogen/urine creatinine levels compared with stages 1 and 2 patients at the time of diagnosis ($p<0.05$). Furthermore, a positive correlation was observed between the urinary angiotensinogen/urine creatinine level at the time of diagnosis and the serum creatinine level at the third month ($r=0.408$, $p=0.048$).

CONCLUSION: The findings suggest that urinary angiotensinogen levels can serve as an indicator of the severity of acute kidney injury. Monitoring urinary angiotensinogen levels could potentially contribute to the prognosis assessment and management of acute kidney injury patients.

KEYWORDS: Acute kidney injury. Creatinine. Angiotensinogen.

INTRODUCTION

The diagnosis and staging of acute kidney injury (AKI) are based on the criteria provided by Kidney Disease: Improving Global Outcomes (KDIGO). The parameters used for diagnosing AKI include an increase in serum creatinine (sCr) levels and a decrease in urine output. However, delayed increases in creatinine levels can result in delayed diagnosis of AKI. Furthermore, the severity of the disease may not be accurately reflected by creatinine levels, leading to delays in disease management.

Acute kidney injury occurs in approximately 25–30% of patients hospitalized in intensive care units and 3–7% of general hospitalized patients[1]. While the mortality rate in uncomplicated AKI cases is approximately 5%, it exceeds 40% in intensive care unit patients. AKI often coexists with multiple organ failure rather than isolated organ failure[2].

Many AKI patients regain their kidney function during follow-up, which is evidenced by an increased urine production and a gradual decrease in sCr levels. However, some patients do not fully recover and may develop chronic kidney disease (CKD)[3]. Emerging evidence suggests that timely and effective treatment initiation is crucial for AKI outcomes[4]. Therefore, early identification of AKI severity can facilitate prompt intervention and improve patient outcomes. The current diagnostic markers, such as sCr levels and urine output, have limited prognostic value for potential complications. Insufficient prognostic information regarding AKI is a significant barrier to improving patient outcomes.

Animal studies investigating AKI have demonstrated that the activation of the renin-angiotensin system (RAS) can contribute to the development of AKI. Urinary angiotensinogen (uAGT) levels serve as an indicator of RAS activation and hold promise as a biomarker for assessing AKI progression in patients with acute decompensated heart failure[5]. uAGT has the potential to be a novel biomarker that can aid in determining the intrarenal RAS status and the severity of acute tubular necrosis (ATN).

The objective of this study was to explore the association between serum angiotensinogen (sAGT) or uAGT levels and

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AKI-related complications. Consequently, patients with elevated sAGT and uAGT levels will be closely monitored for the development of potential complications.

METHODS

Study population

A total of 79 AKI patients were included in this study. These patients were recruited from the Internal Medicine Clinic of Gaziosmanpaşa University Hospital, where they were initially diagnosed with AKI and received treatment. Pregnant women, patients who used angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and aldosterone receptor antagonist drugs at the time of diagnosis, patients with postrenal AKI, and patients with a diagnosis of malignancy, chronic liver disease, multiorgan failure, or sepsis were excluded from the study.

Data collection

At the time of diagnosis, a detailed patient history including age, gender, presence of chronic diseases, medications used, recent exposure to nephrotoxic agents, and history of surgery was recorded. For patients admitted to the intensive care unit, the reasons for hospitalization and Acute Physiology and Chronic Health Evaluation II (APACHE II) scores were documented. Information on the application of hemodialysis, time of death for deceased patients, and discharge time for surviving patients was also recorded.

Biochemical measurements

Blood samples were collected from the patients at the beginning of AKI and on the 15th and 30th days of follow-up. The levels of serum blood urea nitrogen (BUN), sCr, and urine creatinine (uCr) were measured. The renal function of the patients who attended follow-up visits was monitored up to the 90th day.

Ethical considerations

Approval for this study was obtained from the ethics committee of Gaziosmanpaşa University, Faculty of Medicine (19-KAEK-052). Written informed consent was obtained from all patients in accordance with the principles of the Helsinki Declaration, and all relevant documents were recorded.

Biochemical analysis

For the measurement of sAGT levels, the blood samples were centrifuged at 3500 rpm for 10 min. The serum was separated and stored at -80°C in Eppendorf tubes. For uAGT levels, urine samples collected in gel-free tubes were centrifuged at 3500 rpm for 10 min, and the supernatant was transferred to Eppendorf tubes and stored at -80°C. Before the analysis, the frozen samples were thawed at 2–8°C for 8 h. The sAGT and uAGT levels were measured using Human Angiotensinogen kits (Elabscience Biotechnology Co., Wuhan, PRC) and the enzyme-linked immunosorbent assay (ELISA) method in the biochemistry research laboratory of our hospital.

The absorbance values were measured at 450 nm using an ELISA reader (Organon Teknika Reader 230S). Based on the absorbance values, the concentrations were calculated in ng/L using Microsoft Excel. uCr levels were measured using the Jaffé kinetic colorimetric method on the Roche Cobas 6000 device, specifically the Cobas C501 module. According to the uCr values, uAGT levels were corrected and reported as ng/mg.

Statistical analysis

The data were analyzed using the SPSS 19.0 package program for Windows. The Pearson chi-square test was employed to compare the frequency of gender between groups. The Mann-Whitney U test was used to compare the mean age, APACHE II scores, uAGT levels, and sAGT values. According to primary diagnoses, the distribution of biomarkers was evaluated using Kruskal-Wallis analysis. Furthermore, correlation analysis was conducted to assess the relationship between biomarkers in the case and control groups.

Descriptive statistics were used to present the general characteristics of the study groups, and the data were expressed as mean±standard deviation. A $p<0.05$ was considered statistically significant. Repeated-measures analysis of variance (ANOVA) was performed to evaluate the differences in uAGT levels and kidney function at the beginning and on the 15th and 30th days of the study. Statistical significance was determined when $p<0.05$.

Categorical variables were presented as n (%), while continuous variables were reported as mean±standard deviation. Independent sample t-tests or one-way ANOVA was utilized to assess the quantitative differences between groups. Chi-square tests were employed to examine the qualitative differences between groups. Pearson correlation analysis was performed to investigate the relationship between quantitative variables. All statistical calculations were conducted using the IBM SPSS Statistics 19 software (SPSS Inc., an IBM Co., Somers, NY).

RESULTS

The study included a total of 79 AKI patients, with a mean age of 73.8 years (range: 33–96 years). Among them, 35 (44%) were females and 44 (56%) were males. The most common

etiological reason for AKI was cerebrovascular damage (25%), followed by primary AKI (20%), pneumonia (12%), and other causes. The most prevalent comorbidities among the patients were hypertension (58%), coronary artery disease (48%), and diabetes mellitus (30%). During the follow-up period, 58% of the patients died, and hemodialysis was required in 58% of the cases.

In our study, the uAGT/uCr values of the patients were corrected according to uCr. There was no significant relationship between uAGT/uCr and sAGT levels (r=0.063, p=0.579). However, there was a positive correlation between uAGT/uCr and sCr levels (r=0.289, p=0.01). As expected, there was an inverse correlation between sCr and glomerular filtration rate (GFR) (r=-0.74, p≤0.001). Further details can be found in Table 1.

When comparing uAGT/uCr ratios at different stages based on the KDIGO criteria upon admission, the ratios were 9.93±10.82 in stage 1, 5.03±5.04 in stage 2, and 32.15±37.13 in stage 3. As shown in Table 2, stage 3 patients had significantly higher uAGT/uCr values compared with stage 1 and stage 2 patients.

Table 3 examines the relationship between uAGT/uCr values and the development of hemodialysis, mortality, and morbidity. There was no significant correlation observed between uAGT/uCr ratios and the need for hemodialysis, mortality, or morbidity (p>0.05).

Table 1. Correlation between urinary angiotensinogen/urine creatinine and serum angiotensinogen values.

		sAGT	uAGT ng/L	uAGT/uCr	sCr
GFR-MDRD mL/min	r	202	0.063	-0.079	-0.751
	p	0.074	0.580	0.490	<0.001
sAGT	r		0.223	0.063	-0.159
	p		0.048	0.579	0.162
uAGT ng/L	r			0.182	-0.092
	p			0.107	0.421
uAGT/uCr	r				0.289
	p				0.010

Table 2. Serum angiotensinogen and urinary angiotensinogen/urine creatinine ratio by stage of Kidney Disease: Improving Global Outcomes.

	KDIGO		
	Stage 1	Stage 2	Stage 3
sAGT ng/L	143.97±190.15	103.59±88.17	71.04±29.68
uAGT/uCr	9.93±10.82	5.03±5.04	32.15±37.13

A weak positive correlation was found between uAGT/uCr on day 0 and sCr on day 90 in the patients who were followed up (r=0.408). However, no significant correlation was observed between sCr levels on the 90th day and uAGT/uCr on the 15th day of follow-up. There was a strong correlation between uAGT/uCr on the 30th day and sCr on the 90th day, but these values did not reach statistical significance.

DISCUSSION

The diagnostic criteria for AKI are based on a decrease in urine output and an acute increase in sCr levels. However, sCr levels typically rise within 2–3 days due to skeletal muscle release, making interventions based solely on high sCr levels potentially incomplete[6]. An early and accurate diagnosis of AKI is crucial to prevent renal dysfunction and damage[7]. Recognizing the decline in GFR at an early stage is vital, and several biomarkers have shown promising results in providing early detection of AKI[8].

The kidney contains all components of the RAS system. Growing evidence suggests that locally produced angiotensin II (Ang II), which is the principal effector peptide of RAS, may contribute to AKI pathogenesis by upregulating pro-inflammatory and pro-fibrotic cytokines such as TNF and TGF-beta[9]. Ang II expression is observed in the proximal tubule, where it stimulates TGF-beta synthesis[10,11]. Experimental studies have demonstrated increased levels of TGF-beta mRNA and protein in rats following acute ischemic injury[12]. In male Sprague-Dawley rats, renal Ang II levels in the proximal tubule were found to increase 53.5-fold in association with decreased renal perfusion pressure[10].

In a study assessing uAGT and sAGT levels, higher uAGT levels were observed in patients with ATN compared with healthy subjects[13]. This elevation in uAGT levels was found to be correlated with increased intrarenal RAS expression, suggesting that enhanced uAGT synthesis within the kidney

Table 3. Relationship between urinary angiotensinogen/urine creatinine and hemodialysis and mortality.

		uAGT/uCr
Hemodialysis	No	11.56±18.37
	Yes	10.82±14.68
Mortality	Exitus	13.25±19.67
	Alive	8.17±9.02
Day of exitus	0–7	12.27±22.77
	8–14	9.83±10.63
	≥15	13.65±20.2

may contribute to ATN pathogenesis[13]. Another study identified intrarenal angiotensinogen in structures close to the apical membrane of proximal tubular cells, facilitating its secretion into urine[14]. The study results also revealed no association between sAGT levels and intrarenal RAS status, supporting the notion that intrarenal RAS operates independently of circulating RAS[14]. Consistent with the existing literature, our study found no correlation between sAGT levels and uAGT/uCR values, further supporting the idea of independent regulation of intrarenal RAS[14].

In our study, although patients receiving hemodialysis exhibited higher uAGT/uCR values, the difference was not statistically significant. Similarly, no significant difference was observed between uAGT/uCR values and mortality rates. When patients were classified according to the KDIGO stages, no significant correlation was found between uAGT/uCR values and mortality among AKI patients. While higher uAGT/uCR values were observed in patients undergoing hemodialysis and those who died, the lack of statistical significance could be attributed to the small sample size and high standard deviation in our study.

A separate investigation[15] examined uAGT as a potential clinical biomarker for identifying individuals at high risk of CKD. All distributions of 24-h uAGT and uAGT/uCR ratio were found to be higher in CKD patients compared with controls, whereas sAGT levels were similar between the two groups. Notably, uAGT excretion and uAGT/uCR ratio exhibited a strong correlation. These findings suggest that intrarenal RAS may play a significant role in CKD risk, and uAGT levels could aid in stratifying and predicting CKD risk. In our study, a positive correlation was found between uAGT/uCR values obtained at the time of AKI diagnosis and sCr levels on the 90th day. A strong correlation was also observed between uAGT/uCR values on the 30th day and sCr levels on the 90th day, although no statistically significant relationship was found. Analyzing uAGT/uCR values on the 0th, 15th, and 30th days based on the development of CKD during patient follow-up revealed no significant differences between the groups. However, this could be attributed to the limited number of cases included in the study and a high mean standard deviation.

The limitations of this study include the small sample size and high standard deviation, which may have contributed to the lack of statistically significant findings in some analyses. Additionally, the study focused on a specific population and did not explore other potential confounding factors. Furthermore, the study did not assess long-term outcomes or evaluate the predictive value of uAGT/uCR values in terms of disease progression. On the contrary, the study contributes to the existing literature by examining the correlation between uAGT/uCR values and renal function in AKI patients. It also adds to the understanding of the independent regulation of intrarenal RAS. Further research with larger cohorts and comprehensive evaluation of clinical outcomes is necessary to confirm and build upon these findings.

CONCLUSION

The uAGT levels have emerged as potential biomarkers for assessing the intrarenal RAS activity in AKI and CKD. Further research with larger sample sizes is warranted to validate their clinical utility and establish their role in risk stratification and prognosis prediction for renal diseases.

AUTHORS' CONTRIBUTIONS

AA: Project administration, Validation, Writing – review & editing. **AKD:** Project administration, Validation, Writing – review & editing. **ZCÖ:** Project administration, Validation, Writing – review & editing.

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