Article

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Diagnostic Efficacy of Urinary IGFBP7 and TIMP2 Levels in Early Acute Kidney Injury

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Background: Acute kidney injury (AKI) is an acute renal insufficiency syndrome, often associated with high morbidity and mortality. Currently, there is a lack of early diagnostic biomarkers. Urine Tissue inhibitor of metalloproteinases 2 (TIMP2) and insulin-like growth factor-binding protein 7 (IGFBP7) serve as markers of G1 cell cycle arrest and foretell AKI development. Therefore, this study aimed to investigate the diagnostic efficacy of these two markers in detecting AKI.

Method: We analysed urine and serum samples obtained from the normal control, patients without AKI (NO-AKI), and AKI groups. We assessed the levels of Neutrophil gelatinase-associated lipocalin (NGAL), TIMP2, and IGFBP7 in urine and serum creatinine (Scr) levels utilizing corresponding enzyme linked immunosorbent assay (ELISA) kits. The diagnostic values of urinary NGAL, TIMP2, IGFBP7, and serum Scr were evaluated through receiver operating characteristic (ROC) curve analysis. Moreover, the hypoxia model of renal tubule cells was used to simulate AKI *in vitro*, and the expression levels of TIMP2 and IGFBP7 were determined at different time following hypoxia induction.

Results: There were significant differences in urinary TIMP2, IGFBP7, and NGAL levels as well as Scr levels among the three experimental groups. The urinary TIMP2, IGFBP7, and NGAL levels, as well as Scr levels were significantly higher in the AKI group than those in the normal and the NO-AKI groups ($p < 0.01$). However, the urinary IGFBP7 and Scr levels were elevated in NO-AKI group compared to the normal group. Moreover, there was no substantial difference in urinary TIMP2 and NGAL levels between the NO-AKI and normal groups ($p > 0.05$). Additionally, ROC curve analysis revealed that the urinary IGFBP7 had excellent diagnostic performance for AKI, followed by urinary TIMP2, NGAL, and Scr. Furthermore, the TIMP2 and IGFBP7 levels elevated in a time dependent manner, reaching the peak at 120 minutes after hypoxia induction, followed by a gradual decline ($p < 0.001$).

Conclusions: The present study shows that IGFBP7 and TIMP2 have good diagnostic value for early AKI, which are potential biomarkers for early screening of high-risk AKI patients.

Keywords: acute kidney injury; biomarker; TIMP2; IGFBP7; diagnosis

Introduction

Acute kidney injury (AKI) is an acute renal insufficiency syndrome, often associated with high morbidity and mortality [1]. AKI is a latent risk factor for the development of early chronic kidney disease, cardiovascular disease, and end-stage renal disease (ESRD) [2]. It is primarily caused by ischemia, insufficient circulating blood volume, nephrotoxic drugs, and urinary tract obstruction [3]. Currently, blood purification is the most effective treatment approach for AKI [4]. Fortunately, early-stage AKI is reversible, emphasizing the significance of early diagnosis and appropriate intervention to effectively alleviate its effects [5]. Recently, the diagnosis of AKI relies on the elevation of serum creatinine (Scr) level or a decline in urine volume. However, the specificity of Scr in the diagnosis of AKI is not entirely satisfactory, mainly because of its susceptibility to many non-renal factors, which fail to reflect the glomerular filtration rate [6]. Consequently, there is a need for more specific and sensitive biomarkers to diagnose early AKI and foretell the severity of injury. Presently, significant progress has been made in discovering and validating novel biomarkers for the early diagnosis of AKI [7]. For instance, Neutrophil gelatinase-associated lipocalin (NGAL), a lipocalin, shows increased plasma levels in AKI, partly due to elevated liver production, while urine NGAL serving as a strong marker for AKI [8]. Tissue inhibitor of metalloproteinases 2 (TIMP2) and insulin-like growth factor-binding protein 7 (IGFBP7) are markers of G1 cell cycle arrest [9]. During the early onset of AKI, elevated levels of TIMP2 with IGFBP7 in urine have been confirmed as hav-

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ing good clinical predictive capabilities for AKI [10]. Despite significant improvements in discovery and validation of these biomarkers, numerous uncertainties remain regarding their clinical application to the early diagnosis of AKI.

In this study, we analysed urinary NGAL, TIMP2, and IGFBP7 levels, as well as Scr levels in clinical samples. Subsequently, we examined the diagnostic efficacy of these markers in detecting AKI. Finally, we assessed the appropriate time point for the clinical application of TIMP2 and IGFBP7 detection to predict the early onset of AKI.

Methods

Data Collection

We accessed GSE30718 (GSE: GEO accession number) and GSE227970 datasets from Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/). The GSE30718 dataset included 28 AKI samples and 8 nephrectomy samples. However, the GSE227970 dataset included 9 AKI samples and 3 control samples. Differentially expressed genes (DEGs) were assessed through the gene expression omnibus DESeq2 R package (GEO2R) using the threshold p-value < 0.05 and a $|\log2\text{fold change}| > 1$. Subsequently, DEGs underwent Gene Ontology (GO) enrichment analysis. Furthermore, *TIMP2* and *IGFBP7* expression levels were determined through both the GSE30718 and GSE227970 datasets.

Study Participants

We included 80 study subjects, including 60 patients undergoing percutaneous coronary intervention, nephrotoxic drug use, or hemodynamic instability and 20 healthy controls, in this study. We collected both blood and urine samples from all study participants at The Fourth Affiliated Hospital of Guangzhou Medical University, China, from January 2022 to October 2022. Based on the KIDIGO guidelines [11], clinical specimens of the patients were divided into two groups: the AKI group (n = 26) and the patients without AKI (NO-AKI) group (n = 34). Moreover, the clinical diagnosis, pathologic data, and expression levels of biomarkers of each specimen were independently examined by two pathologists.

Informed consent was obtained from all study participants. This study was approved by the Ethnic Committee of The Fourth Affiliated Hospital of Guangzhou Medical University, China (Approval No: 2019-9), and the study design adhered to the Helsinki Declaration. The included patients satisfied the diagnostic criteria for acute kidney injury, as per the KDIGO guidelines, which define acute kidney injury as fulfilling any of the following criteria: (1) An increase in serum creatinine by $\geq$0.3 mg/dL ($\geq$26.5 µmol/L) within 48 hours, (2) an increase in serum creatinine exceeding 1.5 times the baseline within 7 days, and (3) a urinary output of less than 0.5 mL/(kg·hr) for 6 hours. However, the exclusion criteria for the patients were set as follows: (1) Patients who died within 24 hours of ICU admission, (2) patients diagnosed with chronic renal insufficiency and malignant tumors, and (3) those unwilling to participate in the study. Based on the predefined inclusion and exclusion criteria, all study participants were included within the specified time.

Clinical Specimen Collection

Venous blood (5 mL) and urine samples (5 mL) were collected from each study participant. Venous blood was collected in a coagulant tube and then separate serum after 4 ℃ overnight. The samples were centrifuged immediately, and the resulting supernatants were collected in fresh tubes and stored at –80 °C for subsequent analysis and biomarker assessment.

Renal Cell Hypoxia Model

To stimulate renal hypoxia injury *in vitro*, human renal tubular epithelial cells HK-2 (CL-0109, Procell, Wuhan, China) were cultured in MEM containing NEAA, 10% FBS, and 1% P/S (CM-0109, Procell, Wuhan, China). However, before proceeding with the analysis, the cells underwent STR profiling and mycoplasma verification. The *in vitro* renal cells hypoxia model was established following the previously described method [12]. During this process, the cells were incubated under hypoxic environmental conditions containing 1% O_2, 94% N_2, and 5% CO_2 in modular gas chambers at 37 °C for 0, 30, 60, 120, 180, and 240 minutes. In the control group, cells were cultured under normal O_2 condition. Additionally, the cell culture medium was collected at each time point and stored at –80 °C for subsequent biomarkers assessment.

Measurement of Biomarkers

The corresponding commercially available enzyme linked immunosorbent assay (ELISA) kits were used to determine the levels of NGAL (BMS2202, Novo, Beijing, China), TIMP2 (XG-E990320, Sig Biotechnology, Shanghai, China), IGFBP7 (E-EL-H6176, Elabscience, Wuhan, China), and creatinine (RY3368, Runyu Biotechnology, Shanghai, China) following the manufacturer's guidelines. For this purpose, the whole blood samples were initially incubated overnight at 2–8 °C. The next day, they were centrifuged (1000 $\times g$) for 20 minutes at 2–8 °C, and the supernatants were collected for subsequent analysis. Similarly, urine samples were centrifuged at 1000 $\times g$ for 20 minutes, and the supernatants were collected for further analysis. Subsequently, these samples were processed based on the guidelines provided with each ELISA kit. The OD values within their respective wavelength ranges were assessed using a Multiscan MK3 microplate reader (Thermo Fisher Scientific, Waltham, MA, USA), and the level of each marker was compared with the standard curve.

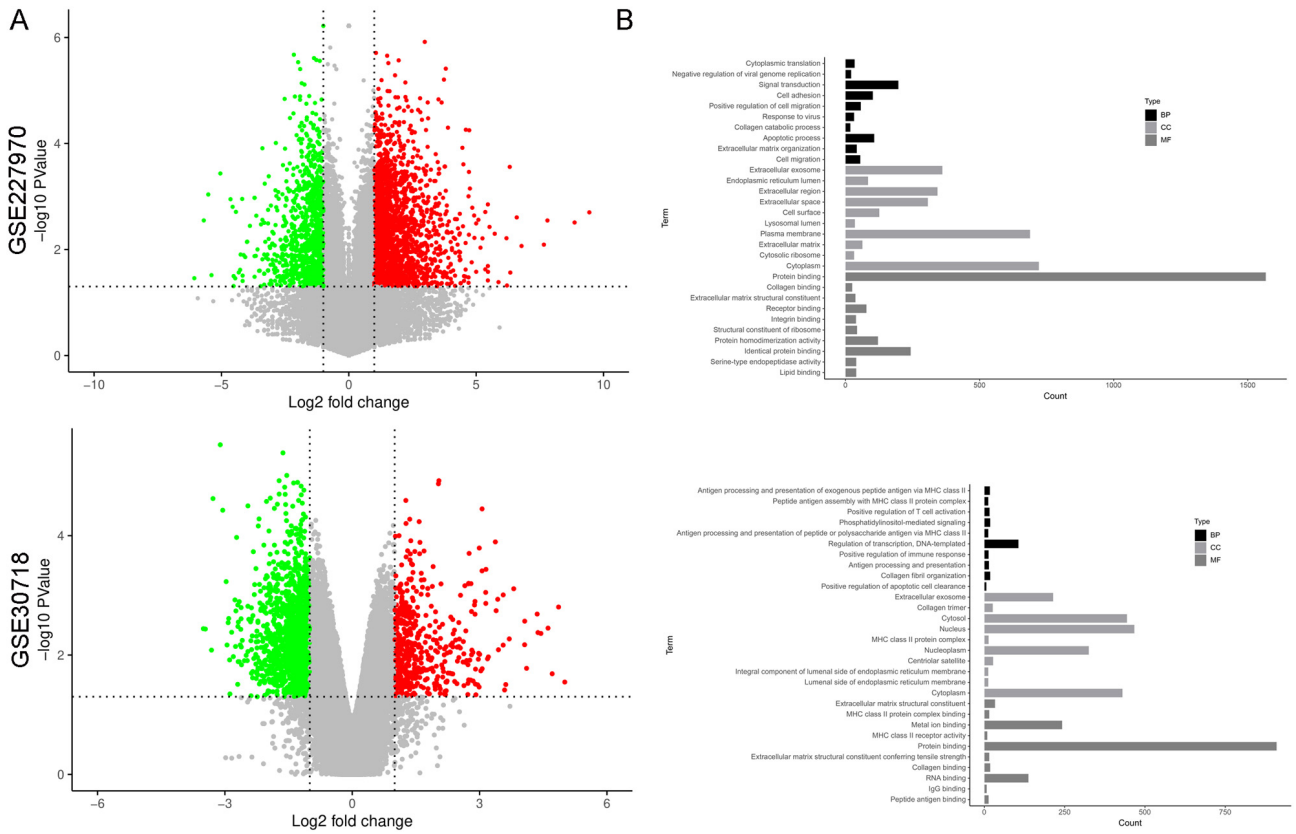

Fig. 1. Differentially expressed genes (DEGs) were identified using gene expression omnibus DESeq2 R package (GEO2R) in the GSE30718 (GSE: GEO accession number) and GSE227970 datasets. (A) The DEGs in the GSE30718 and GSE227970 datasets were shown using a volcano plot. (B) The DEGs in the GSE30718 and GSE227970 datasets were proceeded for Gene Ontology (GO) enrichment analysis. GEO, Gene Expression Omnibus; BP, biological process; CC, cell component; MF, molecular function.

Statistical Analysis

Statistical analyses were conducted using IBM SPSS 22.0 (IBM, Armonk, NY, USA). The data were expressed as the means ± SD. Differences between the two groups were assessed using a *t*-test, and multiple group comparisons were performed through a one-way ANOVA followed by a Tukey test for post hoc analysis. However, the level of each biomarker was determined using a receiver operating characteristic (ROC) curve. A p-value < 0.05 was considered statistically significant.

Results

TIMP2 and IGFBP7 Expression in GSE30718 and GSE227970 Datasets

According to threshold, 2130 DEGs were up-regulated and 1017 DEGs were down-regulated in GSE227970 and 527 DEGs were up-regulated and 1473 DEGs were down-regulated in GSE30718 (Fig. 1A). In addition, these DEGs were explored by GO analysis. The results revealed that in GSE227970, these DEGs were enriched in signal transduction, cytoplasm, and protein binding. In contrast, in GSE30718, they were implicated in the regulation of transcription, nucleus, and protein binding (Fig. 1B). Moreover, the expressions of *TIMP2* and *IGFBP7* were significantly enhanced in both the GSE30718 and GSE227970 datasets ($p < 0.001$, Fig. 2).

Comparison of Biomarkers Levels across Three Groups of Clinical Specimens

We comparatively evaluated the levels of NGAL, TIMP2, IGFBP7, and Scr in urine samples across different groups, including the normal, NO-AKI, and AKI groups utilizing ELISA assay (Fig. 3). The findings revealed that the urinary TIMP2, IGFBP7, NGAL, and Scr levels were significantly higher in the AKI group than in the normal and NO-AKI groups ($p < 0.001$). However, the levels of IGFBP7 and Scr were substantially elevated in the NO-AKI group compared to the normal group ($p < 0.01$). Moreover, there was no significant difference in levels of TIMP2 and NGAL between the NO-AKI and normal groups.

Diagnostic Efficacy of Biomarkers Levels for AKI

Based on the urinary levels of TIMP2, IGFBP7, NGAL, and Scr, the sensitivity and specificity for diagnosing normal or AKI cases were determined through ROC

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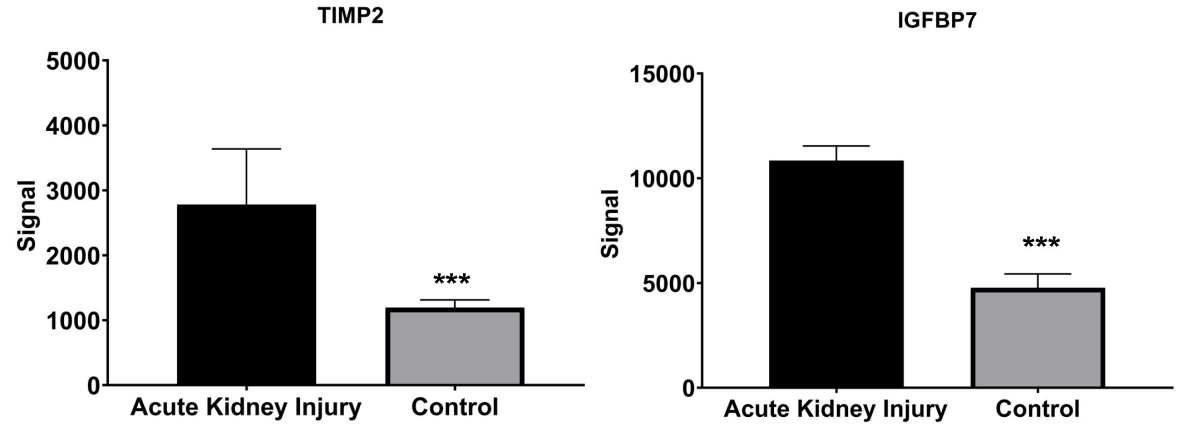

Fig. 2. Tissue inhibitor of metalloproteinases 2 (*TIMP2*) and insulin-like growth factor-binding protein 7 (*IGFBP7*) expressions were significantly elevated in both the GSE30718 and GSE227970 datasets. The expressions of *TIMP2* and *IGFBP7* in both the GSE30718 and GSE227970 datasets were assessed using GEO2R. $^{***}p < 0.001$.

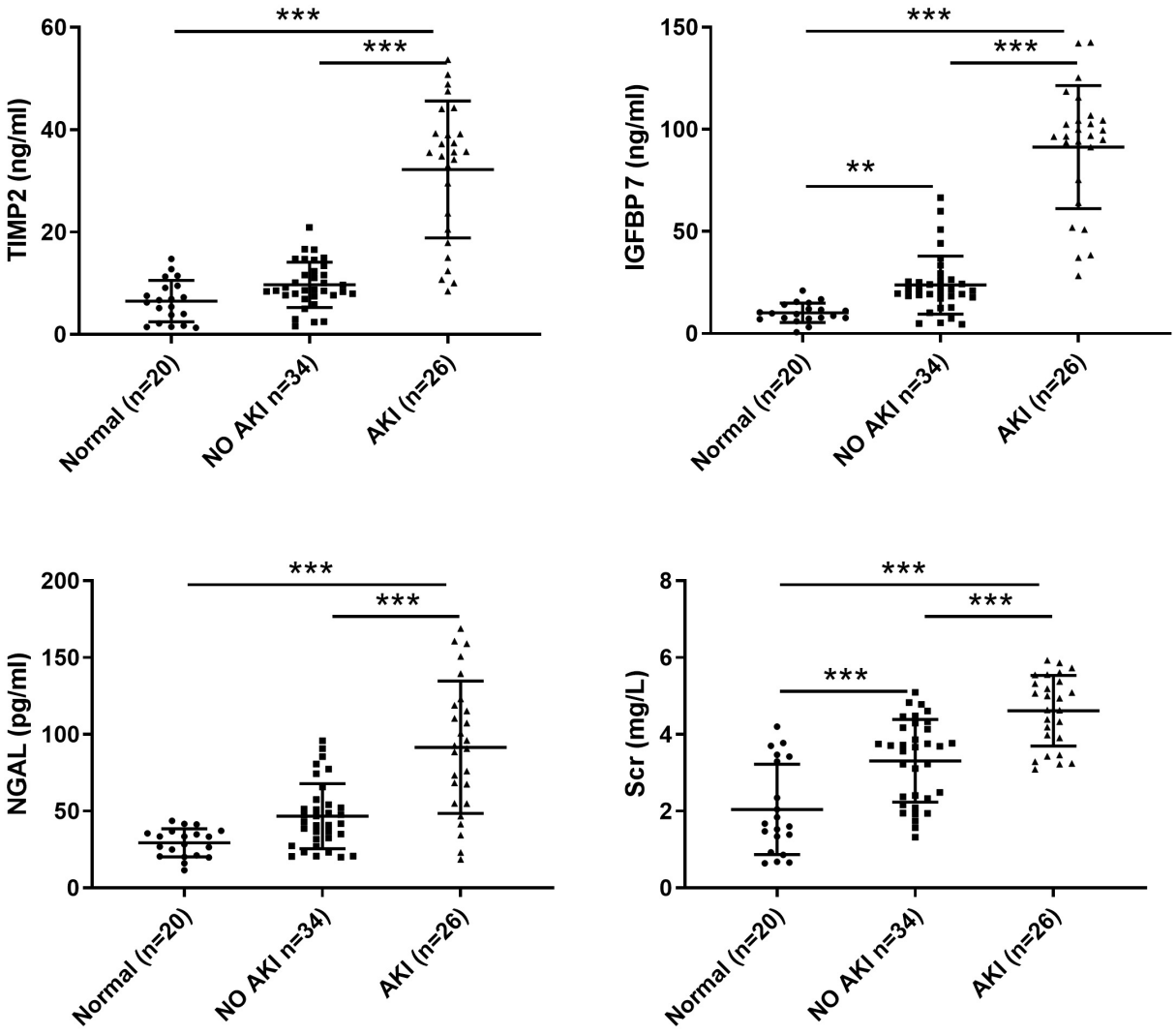

Fig. 3. The urinary TIMP2, IGFBP7, Neutrophil gelatinase-associated lipocalin (NGAL), and serum creatinine (Scr) levels were significantly elevated in the acute kidney injury (AKI) group. TIMP2, IGFBP7, NGAL, and Scr levels in the clinical specimens of the normal, patients without AKI (NO-AKI), and AKI groups were assessed using enzyme linked immunosorbent assay (ELISA). $^{**}p < 0.01$, $^{***}p < 0.001$.

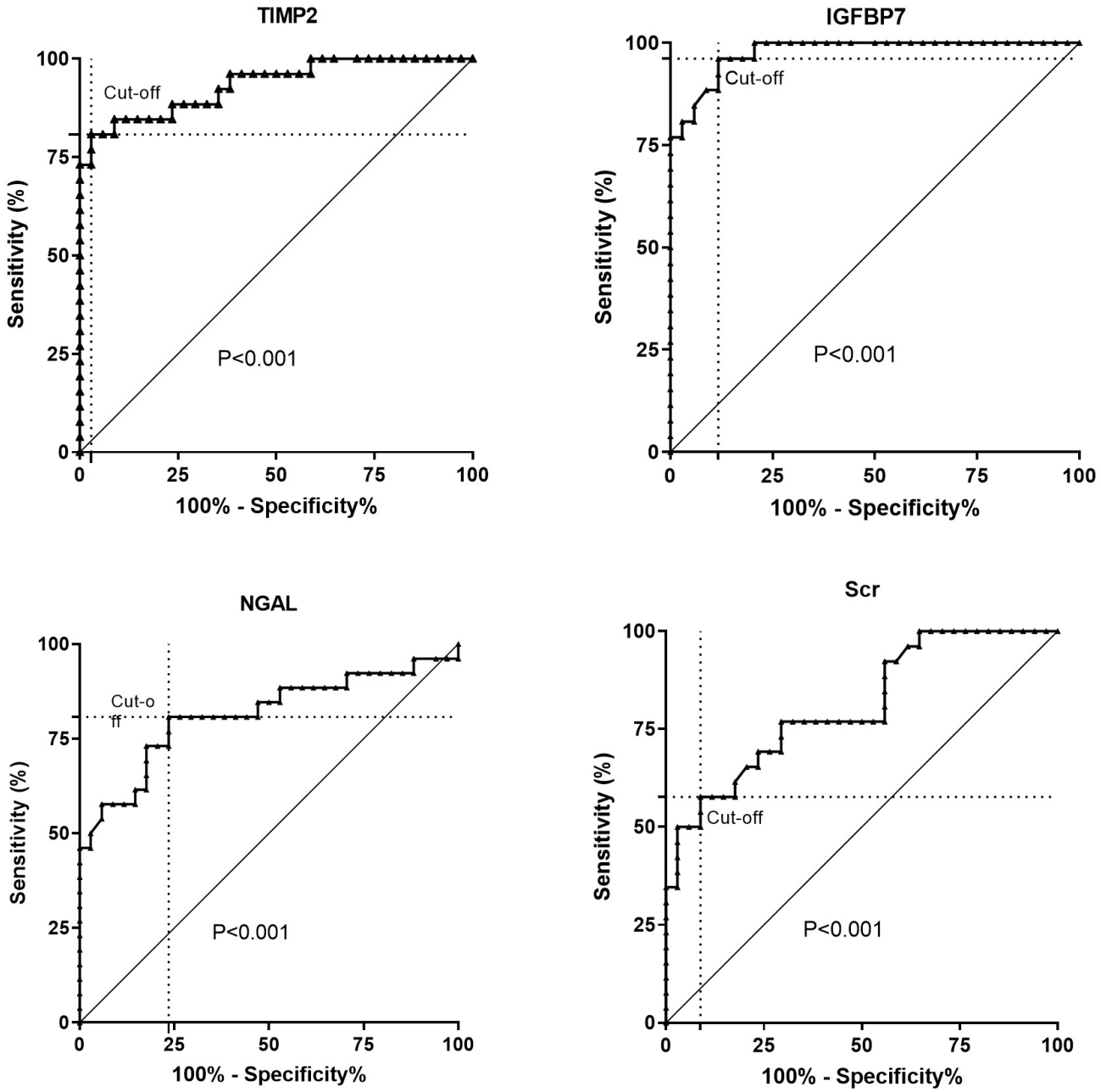

Fig. 4. The receiver operating characteristic (ROC) curve of urinary TIMP2, IGFBP7, NGAL, and Scr levels for AKI.

curve analysis. As shown in Fig. 4 and summarized in Table 1, the diagnostic efficacy of urinary TIMP2, IGFBP7, NGAL, and Scr levels between the normal population and AKI patients was assessed, and the findings revealed that the urinary IGFBP7 exhibited the highest diagnostic accuracy, followed by urinary TIMP2, NGAL and Scr.

Optimal Time for Clinical Detection of Urinary TIMP2 and IGFBP7 Levels in AKI High-Risk Patients

To explore the appropriate time for detecting urinary TIMP2 with IGFBP7 levels in high-risk AKI patients, a renal cell hypoxia model was used to simulate AKI *in vitro*. However, TIMP2 and IGFBP7 levels were monitored at 30, 60, 120, 180, and 240 minutes after hypoxia induction. We observed an association between TIMP2 and IGFBP7 levels and the duration of hypoxia. Furthermore, the TIMP2 with IGFBP7 levels showed a time-dependent increase, reaching a peak at 120 minutes following hypoxia induction, followed by a gradual decline ($p < 0.001$, Fig. 5). These findings demonstrated that detecting urinary TIMP2 and IGFBP7 levels approximately 120 minutes after renal injury holds clinical significance in guiding the early diagnosis of AKI.

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Table 1. The diagnostic efficacy of TIMP2, IGFBP7, NGAL, and Scr for AKI.

Variable	AUC	95% CI	Cut-off value	Sensitivity %	Specificity %
TIMP2	0.934	0.871–0.998	>17.31 (ng/mL)	80.77	97.06
IGFBP7	0.977	0.948–1.005	>37.05 (ng/mL)	96.15	88.24
NGAL	0.813	0.694–0.932	>54.56 (pg/mL)	80.77	76.47
Scr	0.809	0.699–0.919	>4.62 (mg/L)	57.69	91.18

AUC, area under curve; TIMP2, Tissue inhibitor of metalloproteinases 2; IGFBP7, insulin-like growth factor-binding protein 7; NGAL, Neutrophil gelatinase-associated lipocalin; Scr, serum creatinine.

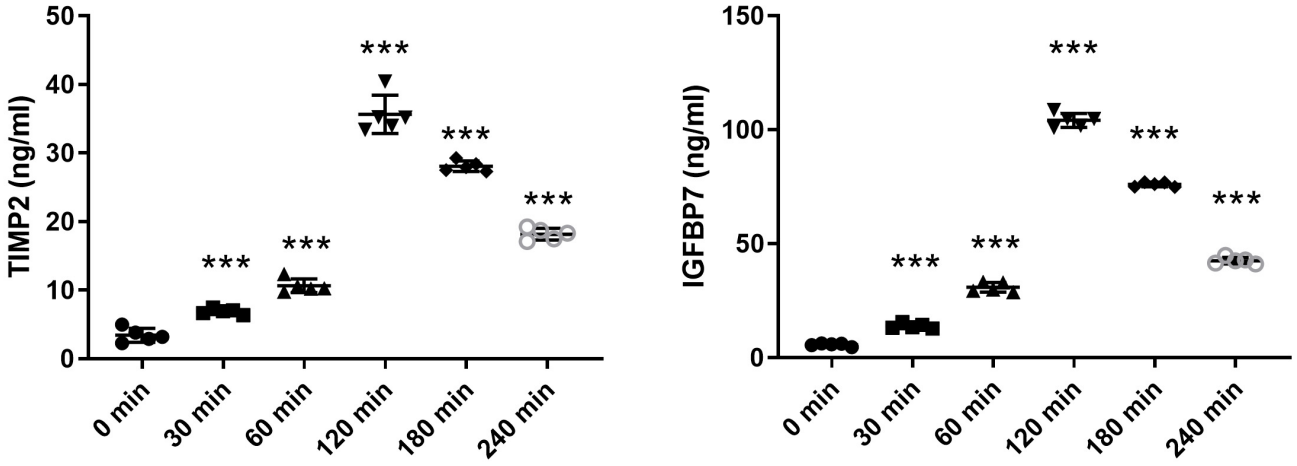

Fig. 5. TIMP2 and IGFBP7 levels in *in vitro* renal cell hypoxia model at different time following hypoxia induction. n = 5, $^{***}p <$ 0.001.

Discussion

Our study revealed that the urinary TIMP2, IGFBP7, NGAL, and Scr levels in the AKI group were significantly higher than in the normal and NO-AKI groups. Furthermore, the urinary IGFBP7 and Scr levels were elevated in the NO-AKI group compared to the normal group, while there was no substantial difference found in the levels of TIMP2 and NGAL between the NO-AKI and normal groups. These findings indicated that the urinary IGFBP7 exhibited the highest diagnostic significance for AKI, followed by TIMP2, NGAL, and Scr. Moreover, we observed that the TIMP2 with IGFBP7 levels elevated in a time-dependent manner, reaching the peak at 120 minutes following hypoxia induction followed by a gradual decline. These results indicated that detecting urinary TIMP2 with IGFBP7 levels around 120 minutes after renal injury holds clinical significance for guiding the early AKI diagnosis. Our observations provide theoretical support for using IGFBP7 and TIMP2 levels in early screening of high-risk AKI patients.

Despite extensive research and significant progress in clinical treatment methods, the pathogenesis of AKI remains largely unclear [13]. Early diagnosis and intervention continue to be effective in improving the prognosis of AKI patients [14]. Therefore, identifying and screening effective diagnostic biomarkers for early AKI detection have gained considerable focus in the research community [15]. Traditional renal function biomarkers, Scr and Cystatin-C, exhibit certain diagnostic accuracy for damaged renal function, but their specificity and sensitivity in diagnosing AKI remain unsatisfactory [16,17]. Previous research has demonstrated a significant increase in the levels of NGAL after kidney injury, with substantially elevated levels among critically ill children diagnosed with AKI [18]. Additionally, two new biomarkers, TIMP2 and IGFBP7, have recently been identified and validated for their ability to foretell the development of AKI in high-risk patients [19]. In our study, the urinary TIMP2, IGFBP7, NGAL, and Scr levels were significantly elevated in the AKI group compared to those in the normal and NO-AKI groups, which is consistent with findings observed by Sakyi *et al.* [20]. Subsequently, we assessed the diagnostic efficacy of urinary TIMP2, IGFBP7, NGAL, and Scr levels for diagnosing AKI. TIMP2, at the optimal cutoff value of 17.31 (ng/mL), showed diagnostic sensitivity and specificity values of 80.77% and 97.06%, respectively, with an area under curve (AUC) of 0.934 (95% CI: 0.871–0.998). Moreover, IGFBP7, at the optimal cut-off value of 37.05 (ng/mL), indicated diagnostic sensitivity and specificity values of 96.15% and 88.24%, respectively, with an AUC of 0.977 (95% CI: 0.948–1.005). Similarly, NGAL,

at the optimal cut off value of 54.56 (pg/mL), exhibited diagnostic sensitivity and specificity values of 80.77% and 76.47%, respectively, with an AUC of 0.813 (95% CI: 0.694–0.932). Furthermore, Scr at the optimal cut off value of 4.62 (mg/L), showed diagnostic sensitivity and specificity values of 57.6% and 91.18%, respectively, with an AUC of 0.809 (95% CI: 0.699–0.919). Consistent with the findings of Sakyi *et al*. [20], who reported a higher AUC of 0.91 for IGFBP7than AUC of 0.87 for TIMP2 in severe AKI, our study showed that the urinary IGFBP7 showed the highest diagnostic performance, followed by urinary TIMP2, NGAL, and Scr. We further confirmed that IGFBP7 and TIMP2 exhibited excellent diagnostic value for early AKI detection. Importantly, the diagnostic efficacy of TIMP2 and IGFBP7 is higher than certain other commonly used diagnostic proteins, such as serum procalcitonin and CHI3L1 [21,22]. AKI develops rapidly and often cannot be ruled out solely based on a doctor's clinical judgment in patients with acute diseases [23]. Therefore, it is crucial to conduct examinations to detect high-risk groups for AKI development during the early stages of kidney injury. In our study, we aimed to assess the appropriate time for diagnosing urinary TIMP2 and IGFBP7 levels in high-risk AKI patients. For this purpose, we used a renal tubular cell hypoxia model to simulate AKI *in vitro*. We found that the TIMP2 with IGFBP7 levels increased in a time-dependent manner, reaching the peak at 120 minutes after hypoxia induction, followed by a gradual decline. Therefore, the detection of urinary TIMP2 and IGFBP7 levels around 120 minutes after renal injury holds certain clinical guiding significance for the early diagnosis of AKI. Therefore, the urinary markers TIMP2 and IGFBP7 offer several crucial advantages as biomarkers for AKI. Firstly, they show the potential to increase in the early stages of AKI. Secondly, their non-invasive collection through urine makes TIMP2 and IGFBP7 convenient and readily accessible biomarkers in clinical practices. However, the reliability of urine output as a measure may be affected by treatments. Furthermore, additional validation through larger patient cohorts and multicentred trials is needed to determine whether urinary TIMP2 and IGFBP7 can be established as diagnostic markers. In these validation studies, the inclusion of multivariate analysis can further confirm the correlation between TIMP2, IGFBP7, and AKI.

Nevertheless, this study has several limitations. Firstly, the sample size is not sufficiently large, and it is a single-centre study, limiting its universality to the broader population. Secondly, we did not perform biomarker tests at different time points during the progression of AKI patients. Instead, we used *in vitro* cell assay simulations, which prevented us from providing standardized time points and cut-off values for diagnosing AKI outcomes. Additionally, this study only examines the effect of TIMP2, IGFBP7, NGAL, and Scr, lacking a comprehensive discussion on the statistical power of the study, potential confounding factors, and how they were addressed. A more comprehensive statistical analysis will address these limitations in our future investigation.

Conclusions

Our study indicates that IGFBP7 and TIMP2 have excellent diagnostic significance for early AKI, which are potential biomarkers for early screening of high-risk AKI patients.

Availability of Data and Materials

All experimental data included in this study can be obtained by contacting the corresponding author if needed.

Author Contributions

Study conception and design: HZ, YH, and JX; data collection: HZ, YH, LZ, and WY; analysis and interpretation of results: HZ, YH, QH, XL, FL, and JL; draft manuscript preparation: HZ and YH. All authors reviewed the results and approved the final version of the manuscript. All authors contributed to editorial changes in the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Obtain written informed consent from all participants. This study was through ratification of the Ethnic Committee of The Fourth Affiliated Hospital of Guangzhou Medical University Hospital (Zengcheng District People's Hospital of Guangzhou, Approve No: 2019-9) and accorded with Helsinki statement.

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Conflict of Interest

The authors declare no conflict of interest.

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