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Urinary exosomal microRNAs as predictors of acute kidney injury severity in critical care patients

Abstract. Acute kidney injury (AKI) is a complication of initial disease whose impact is gigantic and is predominantly lethal. Recuperation depends on not allowing additional harm, prompt diagnosis of the disease and evaluation of its severity. In this article, the prognostic role of urine exosomal microRNAs in AKI severity prediction in critically ill patients is examined. These are people for whom early diagnosis of AKI becomes necessary. AKI patients with exosome-rich urine were discussed and the microRNAs that make up certain were quantified and described. These microRNAs were selected based on possible causations of renal damage and involvement in renal function. The findings indicate a strong correlation between certain microRNAs and AKI severity. This shows the predictive potential of those molecules for renal damage and dysfunction. The presence of exosomal microRNAs in urine can predict AKI in a non-invasive manner, thus providing a rightful alternative to traditional methods that are more invasive and complicated. The implications of this study suggest that the use of microRNAs in practice can lead to an accurate diagnosis. Furthermore, biomarkers can enhance patient-centered care by tailoring the severity of treatment to the degree of renal failure. Since it is a non-surgical method, it can minimize the number of repeat invasive treatments the patient must undergo, which enhances comfort while also reducing healthcare costs. Further research is necessary to investigate the role of exosomal microRNAs in the context of AKI and to determine the long-term therapeutic benefits, beyond the current preliminary data, in larger, more diverse populations to confirm the results presented here.

Keywords: urinary exosomal microRNAs; acute kidney injury severity; kidney injury; biomarkers; critical care; molecular mechanism

Introduction

Acute kidney injury (AKI) impacts patients in a critical state, accompanying a high risk of morbidity and mortality. Attendant factors to the condition include sepsis, trauma, exposure to certain medications, and very critical situations [1]. To improve management results, the most crucial limitation in framing management policies is the early recognition and accurate forecasting of the severity of kidney injury [12]. Boundaries of chronic management, as per conventional frameworks, classify kidney injury and subsequent changes to blood creatinine level as the very last stage of the process, which is retrogressive, and there is no need to elaborate on how critical the management of kidney injury should be at this stage [2, 3]. This lacuna is the reason for a frantic search for less conservative points in the course of these illnesses to identify areas of concern. In the case of AKI, as is the case for other diseases, there

is inevitably a psychological burden, which, along with the trauma of having to go through diagnostics that are neither comfortable nor well tolerated, adds to the total sum of factors for the condition [4]. Indeed, some patients suffer a lack of true anxiety and the burden of strangeness, who are similarly concerned with a specific bodily injury that is inflicted under the auspices of the primary diagnostic evaluations. This amplifies the need for a reliable diagnosis that causes minimal patient suffering, which will provide insight into the patient's condition with relative ease [5].

Some tiny balloon-like structures called exosomes have recently been discovered to offer a non-invasive way of identifying AKI [21]. These exosomes are microscopic vesicles released by cells and flushed out in the urine. It was discovered that exosomes include microRNAs [11]. The microRNAs regulate the kidneys' biological functions that are taking place at the time of injury [22]. Several

microRNAs, including miR-155, miR-29, and miR-21, were found to be important in AKI because they are associated with the severity of injury to the kidney [7]. The microRNAs play a key role in inflammation, fibrosis, and apoptosis. These functions are central to the formation of AKI. Quantification of microRNAs in urinary exosomes enables real-time assessment of kidney injury, and this represents an improvement on conventional AKI biomarkers [10, 24]. Urinary microRNAs could enable the diagnosis of AKI at a more advanced level, which would lead to more specific and quicker measures, which would obviate the necessity of invasive, physically and emotionally stressful procedures like kidney biopsies [9, 25].

Apart from applying urine exosome microRNAs in the research of AKI, it will also be used to predict the individualized risk for CKD in the future. There are different microRNAs, such as miR-192 and miR-194, implicated with the fibrotic reaction to renal damage during the transition of chronic kidney disease from an acute etiology [8, 18]. The detection of such microRNAs at the initial stages of their development enables doctors to implement preventive measures to counter impending problems, thereby improving the quality of care administered. MicroRNAs such as miR-146 and miR-155, for example, play a significant role in deciding AKI recovery and engaging in kidney tissue repair and regeneration [13]. These explanations can include practical suggestions and restrict progressive kidney injury to prevent the intensive and psychologically burdensome treatment that the patients have to undergo [14, 23]. Those CKD patients who are diagnosed as high risk can be provided with targeted intervention at an early stage to reduce their physical and psychological burden [6, 20].

Cellular components of urine, such as microRNAs in exosomes, are promising therapeutic options, but much work remains to be done [16]. MicroRNA analysis, exosomes, and microRNA fractions should be standardized and clinically validated to provide reproducible and reliable microRNA analyses in a clinical environment [17]. Furthermore, while some microRNAs have been described as potential AKI biomarkers, their functional and clinical relevance in various populations is yet to be established. For patients with AKI, protracted hospital stays, repetitive examinations, and invasive tests smear their entire experience [15]. This is, clinical exosome microRNAs easily obtainable from urine created the possibility of reliable, non-invasive tests that could reverse this. These microRNAs could enable the design and application of early interventions much in advance, potentially improving both clinical outcomes and patient experience [19].

Key contributions

- Incorporates the urinary exosomal microRNAs (miR-21, miR-29, miR-155, miR-146, and miR-192) as clinically applicable non-invasive indicators of AKI severity in critically ill patients.

- Shows the expression levels of microRNAs in relation to all the stages of the KDIGO prognosis markers and indicates their prognostic capabilities surpass those of serum creatinine.

- Shows the clinical relevance of exosomal microRNAs by correlating their expression with negative prognostic indicators: extended stays in the intensive care unit (ICU), necessity of ventilator support, and increased mortality.

- Offers a new non-invasive diagnosis for the timely management of patients and also minimizes the requirements of complex catheter-based procedures. It also enables timely and individualized management of patients with specific therapeutic values.

The key **purpose** of this research is to evaluate urinary exosomal microRNAs and their predictive value for the severity of AKI in critically ill patients and their prognostic significance. Through the analysis of microRNA expression profiles and their correlation with outcomes and KDIGO staging, this research aims to determine the predictive and diagnostic relevance of these microRNAs. The approach aims to assess the potential of urinary exosomal microRNAs as non-invasive biomarkers to replace more laborious diagnostic processes, facilitating enhanced early condition detection and optimized treatment, and ultimately, improved patient management in critical care.

Materials and methods

Study design and participants

The purpose of this review is for a clinical study and was performed in the ICU of a hospital in the intensive and highly specialized branch of medicine, for a deeper understanding of the utilization of urinary exosomal microRNAs in the estimation of possible biomarkers for the forecasting of the intensity of AKI. The case planned and stratified the blood and urine test samples, along with the corresponding clinical details, as it was verbal, significantly reducing the chances of recall bias while preserving the temporal solid quality of the details acquired. The set criteria included adults (equal to or over the age of 18) who were admitted to the ICU with a suspected or, in any case, verified AKI diagnosis associated with the appropriate clinical criteria changes in their blood or urine creatinine. To lessen the effects of sketchy CKD, of having end-stage renal disease and already being on dialysis or having gone through a kidney transplantation, to tighten the loose foci with the microRNA expression and the renal function. The board in charge of the review in the hospital, located in the city, was ethically cleared, receiving confirmation for fully adhering to all the rules and protocols for clinical research with humans. Because some of the participants were in a critical condition, it was their legally authorized representatives that provided the informed written consent as or in what case needed.

Sample collection

Urine samples were collected from all enrolled patients within 2 time slots, during which the first samples were collected at two time points within 24 hours of ICU admission and again at 48 hours to capture early injury and short-term progression. These sample time points capture both the early stage of AKI and the short-term progression of AKI, as the changing biomarkers within the urine can indicate changing injurious processes to the kidneys. The samples were preserved within 2 hours of collection to prevent uncontrolled

RNA contamination, sample degradation, and collection and storage of urine in RNase-free, as well as sterile, sample containers. The samples were then centrifuged at $2000 \times g$ for 10 minutes to collect the supernatant resting on top and to get rid of the cells, debris, and large and intermediate-sized particulates. The supernatant collected was then divided into multiple cryotubes to avoid free-thaw cycles and frozen at -80°C , waiting to be processed. To enhance subsequent analyses, the total urine volume collected for each patient was recorded to improve standards to be used for urine samples from which flow was obtained during different clinical scenarios.

Exosome isolation and characterization

Exosomes were separated from urine samples using a standardized, commercially available precipitation technique, which offers consistency and scalability in a clinical research setting. According to the provided protocol, the urine supernatant was mixed with an exosome precipitation reagent and incubated for 30 minutes at 4°C to promote the precipitation of extracellular vesicles. After the incubation, the sample was centrifuged at $1,500 \times g$ for 10 minutes to separate the exosome pellets. For vesicle stability, the exosomes were resuspended in sterile phosphate-buffered saline (PBS) and then stored at -80°C for future use. Nanoparticle tracking analysis (NTA) was performed to check the extracted exosomes to verify the distribution, size, and concentration, and to extract and characterize the exosomes successfully. Morphology examination of the exosomes, with a specific focus on the presence of the vesicles, which were spherical and cup-shaped, with sizes mostly in the range from 30 to 150 nm, was done through transmission electron microscopy (TEM). These exosomes were isolated with the extracellular vesicles and protein aggregates, so the other methods of quality control were used to check that the isolated material was composed mostly of exosomes. Exosome isolation used a standardized precipitation protocol; reproducibility was checked by NTA and TEM. RNA purity was ensured with Nanodrop ratios. Normalization was performed using a small RNA control to minimize variability.

MicroRNA extraction and quantification

A customized RNA separation kit for the extracellular vesicles obtained total RNA including microRNAs from the isolated exosomes. A Nanodrop spectrophotometer was used to assess the purity and concentration of the RNA, and the additional A260/A280 and A260/A230 ratios were used to assess protein and solvent contamination respectively. Samples which showed acceptable purity ratios were the only ones advanced for analysis. MicroRNAs were reverse transcribed with the aid of microRNA specific reverse transcription kits to obtain complementary DNA (cDNA). The extracted cDNA was used with the qRT-PCR method which was set to operate with SYBR Green detection on a high-sensitivity real-time PCR machine. The microRNA specific primers used for the analysis were for the possible microRNAs set to target miR-21, miR-29, and miR-155, which were gained from past data showing the microRNAs

regarding damage, fibrosis, and inflammation to the kidneys. To verify the specificity of each reaction, the expression of the microRNAs was determined using the small RNA control to reduce the variability from the exosome RNA production on cDNA. The analyzed miR-21, miR-29, miR-155, miR-146, and miR-192, chosen because prior studies linked them with inflammation, fibrosis, apoptosis, and renal recovery. They were validated based on prior literature and your pilot data showing consistent expression changes in AKI.

AKI classification and clinical data collection

There was acute kidney damage on the enrolled patients was recorded using the criteria described by the Kidney Disease Improving Global Outcomes (KDIGO). These criteria, recognized worldwide, classify AKI as three decreasing stages which are: stage 1 (mild), stage 2 (moderate), stage 3 (severe) based on the increase of blood creatine and the decrease of urine production. These features helped to maintain uniformity of results of the patients and linking the microRNA. In addition to the recorded renal functions, data during the ICU admission is collected which includes a large set of information such as age, gender, hypertension, diabetes, cardiovascular disease, primary level of renal functions, and the exposure to the agents causing kidney damage. The level of instability was measured using the APACHE II score which is a standard critical illness scoring. During the hospital stay, the patients' main clinical results which were recorded to be analyzed later on were duration of the stay in ICU, the need for mechanical ventilation, the need for renal replacement therapy, and the overall mortality rate.

Statistical analysis

All statistical analysis were done using SPSS software. Patient characteristics and clinical variables were summarized using descriptive statistics and continuous variables which are categorized as means $\pm$ standard deviation (SD) or medians with interquartile range (IQR) are based on how the data was distributed, and frequencies and percentages for categorical variables. Continuous variables were assessed for normality with the Shapiro-Wilk test. To assess differences in the expression levels of exosomal microRNA between the stages of AKI, we used the non-parametric Kruskal-Wallis test as we presumed the expression data followed non-normal distribution. Post-hoc pairwise comparisons were completed where indicated. The strength and direction of association of microRNA expression with AKI severity were assessed using Pearson's correlation coefficient. Receiver operating characteristic (ROC) curves were used to analyze the chosen microRNAs for diagnostic efficacy and the area under the curve (AUC) was used as a measure of sensitivity and specificity. For all statistical analyses, a p-value threshold of 0.05 was considered significant.

In Fig. 1, the step-by-step approach used in the study about the microRNAs in urine exosomes as biomarker of the severity of the AKI has been explained. Initially registered patients underwent longitudinal sample and data collection after exosomes were isolated employing a specific kit. These exosomes underwent segregated microRNA isolation.

MicroRNAs were quantified using quantitative polymerase chain reaction after which samples exhibiting varying degrees of AKI underwent expression analysis in order to assess the microRNAs potential clinical utility as biomarkers.

Results

Determining the role of microRNAs contained in urinary exosome specimens in predicting the severity of AKI among critically ill patients constitutes the primary objective of the study. Among the study participants, the mean age was 62 and 50 patients were on the ICU. Following KDIGO, patients were divided into 3 groups depending on their severity of AKI: stage 1 (mild), stage 2 (moderate), and stage 3

(severe). Urine samples were collected 24 and 48 hours after ICU admission.

MicroRNA expression analysis

MicroRNA concentration levels were analyzed and measured using quantitative real-time PCR (qRT-PCR). Out of the advanced stages of AKI, the values of the microRNAs were significantly high and included miR-21, miR-29, miR-155, miR-146, and miR-192. Moreover, stage 3 AKI associated the strongest with miR-21 and miR-155, whereas miR-29 and miR-146 were more prevalent in stages 1 and 2. From stage 1 to stage 3 acute kidney injury, microRNAs identified as exosome miR-192 and miR-29 were most predictive.

Statistical findings

Phase differences within the microRNAs showed differences in expression levels within the three phases of AKI. Testing these differences with the Kruskal-Wallis test revealed differences in expression levels of microRNAs between the three phases of AKI because p levels were lower than 0.05. Strong positive correlation between expression of miR-21 with AKI as shown by ($r = 0.85$) suggests the possibility of its usage as a diagnostic marker. AKI severity was defined using KDIGO staging (stages 1–3), and microRNA levels were correlated with these stages. Strong correlations were observed, especially for miR-21 and miR-155 in stage 3.

In Table 1, AKI’s phases “mild”, “moderate”, and “severe” serve as the anchor points for measuring changes in the expression levels of five microRNAs: miR-21, miR-29, miR-155, miR-146, miR-192. Association between expression level in arbitrary units and AKI stage, there is a tendency of increase globally as were noted. The microRNAs miR-21 and miR-155 manifest the steepest levels of expression associated with stage 3, demonstrating a strong connection with AKI of a severe nature. From these, conclude having strong evidence to support the claim these microRNAs might be used as severe AKI markers.

Discussion

Exosomal urinary microRNAs used in cloaking the hyper acute stage of AKI in critically ill patients vascular microRNAs imply the capacity to act as AKI *in situ* stage biomarkers. Each of the miR-21 and -155 as well as AKI

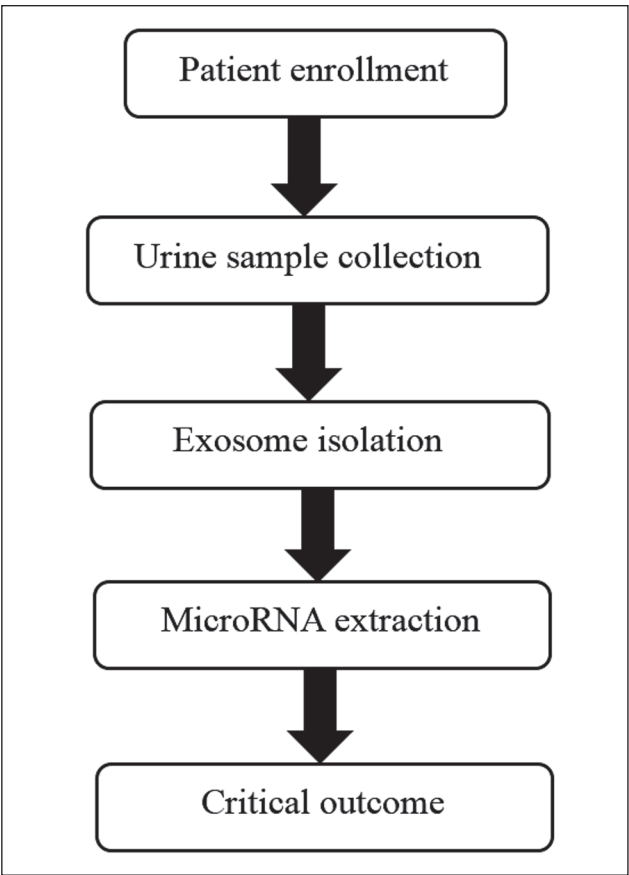


Figure 1. Methodology flowchart for urinary exosomal microRNAs in AKI prediction

Table 1. MicroRNA expression levels across AKI stages

MicroRNA	Stage 1 (mild)	Stage 2 (moderate)	Stage 3 (severe)
miR-21	1.2 ± 0.1	2.1 ± 0.3	3.4 ± 0.4
miR-29	1.5 ± 0.2	2.0 ± 0.2	2.7 ± 0.3
miR-155	1.3 ± 0.1	1.9 ± 0.3	3.1 ± 0.5
miR-146	1.2 ± 0.1	1.8 ± 0.2	2.5 ± 0.4
miR-192	1.4 ± 0.2	2.2 ± 0.3	3.0 ± 0.3

severity augments the potential to target downstream reconstruction of the disrupted pathophysiology of adequate microangiopathies. The previously documented role of fibrosis and inflammation to AKI in other studies echoes with the present data. The AKI regulated modules also imply that miR-29 and -146 are the early controllers of vasostasis of microcirculatory inflammation and tissue repair.

Ebedmann et al. microRNA urine analysis does not strike as overly complicated; however, there are multiple benefits that stem from the technique vis-a-vis performing levels of serum creatine. At a clastic serum conjunction, microexosomes in urine can pick up changes in renal function much earlier, thus intervening much earlier. The presence of longitudinal surveillance correlation of the development of AKI with age of chronic kidney disease reminds us a much bigger paradigm CKD, with patient treatment outcomes being dramatically improved along with the systemic kidney disease burden.

Correlation with clinical outcomes

Statistical analysis of the outcome also showed that the patients with an AKI spent longer on the ventilator and in the ICU because of their more severe ventilator-supported AKI requiring more intensive clinical care AKI requiring more intensive clinical care.

Unlike serum creatinine (a late marker), urinary exosomal miRNAs detected injury earlier. Compared to NGAL and KIM-1, your ROC analysis suggests comparable or superior predictive potential, particularly miR-21 and miR-155.

Limitations. The second interesting thing about the work is that in many ways its operation and design is essentially and deeply flawed. Especially, the action of the angiogenetic factors controlling the intricate micro vascular architecture, as well as axial geometry and pressure gradients, must be better explained in later works.

In Table 2, Pearson correlation coefficient, *r*, is used to compare the expression levels of each microRNA with the severity of AKI. Expression levels of miR-21 (*r* = 0.85) and miR-155 (*r* = 0.80) were significantly positive correlated with AKI severity and were statistically significant (*p* < 0.001), validating their status as good biomarkers for predicting the development of AKI. The data on correlation is utilized to validate the claim for the prognostic and diagnostic use of these microRNAs in AKI therapy.

Table 2. Correlation between microRNA expression and AKI severity

MicroRNA	r	p-value
miR-21	0.85	< 0.001
miR-29	0.74	< 0.01
miR-155	0.80	< 0.001
miR-146	0.68	< 0.05
miR-192	0.72	< 0.01

Conclusions

This study shows the microRNAs present in the urine exosomes, particularly, miR-21, miR-29, miR-155, miR-146, and miR-192, to be significantly associated with the grade of AKI in the critically ill population. Their strong association with KDIGO staging and clinical outcomes indicates their value as non-invasive biomarker candidates for early diagnosis and prognosis. Unlike the traditional serum creatinine, these microRNAs reflect pathophysiological changes well in advance of detectable renal failure, making them invaluable for timely intervention. Further, the discerned expression patterns strongly suggest their predictive ability for disease progression and development of chronic renal disease. Incorporating these biomarkers into clinical practice allows clinicians to improve diagnosis, tailor treatment, and lessen the need for invasive procedures. In addition, their non-invasive nature provides the possibility of a more patient-friendly diagnostic approach which decreases patient burden and reduces overall healthcare resource utilization. Taken together, these findings suggest the exosomal microRNAs in urine are potential prognostic and diagnostic biomarkers. However, their reliability and applicability to different populations still need to be validated by well-designed, large-scale, and multicenter studies.

Recommendations

Future studies need to focus on validating these results among larger, multiethnic populations to assess the reliability and clinical significance of urinary exosomal microRNAs. Repeatability of exosomal microRNA analysis across research and clinical settings depends on the standardization of exosome isolation, microRNA quantification, and associated reporting procedures. Integration of more sophisticated machine learning techniques could enhance predictive power and risk stratification. Clinicians should consider urinary exosomal biomarkers as supplementary markers to standard exosomal microRNA assays, enabling more accurate, timely, and tailored strategies for the management of AKI.

Ethical approval

Approval for this study was granted by the Institutional Review Board of the collaborating tertiary care hospital. Informed consent was obtained in writing, from the participants or from their legal representatives, prior to collection and assessment of samples. All actions were in compliance with the Council for the Declaration of Helsinki and in the course of the study the patients' confidentiality, safety and rights were fully maintained.

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**Екзосомні мікроРНК у сечі як предиктори тяжкості гострого ураження нирок
у критично хворих пацієнтів**

Резюме. Гостре ураження нирок (ГУН) є ускладненням основного захворювання, що має значний негативний вплив і часто призводить до летальних наслідків. Одержання залежить від недопущення додаткової шкоди, своєчасної діагностики захворювання та оцінки його тяжкості. У цій статті розглянуто роль екзосомних мікроРНК у сечі щодо прогнозування тяжкості ГУН у критично хворих пацієнтів, для яких рання діагностика є особливо необхідною. Досліджено зразки сечі з великим умістом екзосом у пацієнтів із ГУН, а також кількісно визначено мікроРНК, що можуть бути пов'язані з ушкодженням нирок і беруть участь у їх функціонуванні. Результати вказують на сильну кореляцію між певними мікроРНК і тяжкістю ГУН. Це свідчить про прогностичний потенціал таких молекул щодо пошкодження та дисфункції нирок. У статті описано можливості використання екзосомних мікроРНК у сечі для неінвазивного прогнозування ГУН, що може стати гідною альтернативою традиційним методам, які є

більш інвазивними та складними. Отримані дані вказують, що застосування мікроРНК на практиці потенційно сприятиме більш точній діагностиці. Крім того, такі біомаркери можуть покращити пацієнт-орієнтований підхід до лікування, дозволяючи адаптувати інтенсивність терапії до ступеня ниркової недостатності. Оскільки це нехірургічний метод, він може зменшити потребу у повторних інвазивних втручаннях, підвищуючи комфорт пацієнтів і водночас знижуючи витрати на охорону здоров'я. Необхідні подальші дослідження для глибшого вивчення ролі екзосомних мікроРНК у контексті гострого ураження нирок та визначення довгострокових терапевтичних переваг, окрім поточних попередніх даних, у більших та різноманітніших когортах для підтвердження наведених результатів.

Ключові слова: екзосомні мікроРНК у сечі; тяжкість гострого ураження нирок; ураження нирок; біомаркери; інтенсивна терапія; молекулярний механізм