Changes of Serum miR-202-3p Level and Its Correlation with Prognosis in Patients with Acute Kidney Injury Induced by Sepsis

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To investigate the changes of serum miR-202-3p level and its correlation with prognosis in patients with acute kidney injury (AKI) induced by sepsis. From April 2017 to January 2019, 66 patients with AKI induced by sepsis in our hospital and 70 healthy people in the same period were selected as the research objects. The levels of miR-202-3p, BUN and Cr in serum were tested. Subsequently, cell experiments were carried out to verify the effects of the decrease of miR-202-3p level on BUN, Cr and cell growth and apoptosis. The risk factors were analysed. Compared with healthy volunteers, patients with AKI induced by sepsis had higher levels of miR-202-3p, BUN and Cr in serum. When miR-202-3p level was knocked down, the levels of BUN and Cr were declined, cell growth was improved and apoptosis rate was decreased. The risk factors were analysed, and the results revealed that miR-202-3p, BUN and Cr were independent risk factors for poor prognosis in patients with AKI induced by sepsis. The level of miR-202-3p is elevated in patients with AKI induced by sepsis, which may be a potential biomarker for diagnosis and prognosis of patients with AKI induced by sepsis.

Keywords: sepsis, acute kidney injury, miR-202-3p, prognosis correlation
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INTRODUCTION

Sepsis is a life-threatening organ dysfunction caused by the maladjustment of host response to infection [1]. There are more than 30 million cases with sepsis every year in the world. Due to the high cost of treatment, sepsis poses great challenges to the physical and economic conditions of patients [2]. Systemic inflammatory response syndrome caused by sepsis is the cause of progressive organ failure, which leads to AKI [3]. Sepsis-related AKI is a very common complication in sepsis patients, with a very high mortality rate [4]. Although the current understanding of the pathogenesis and treatment of sepsis has made progress, but it is only an experiment in animal models, and translational studies in clinical aspects have not yet been successful. However, these related experiments have revealed that sepsis-induced AKI has related molecular targets [5,6]. In this experiment, our purpose is to detect whether microRNA-202-3p (miR-202-3p) is a related molecular target of AKI induced by sepsis.

The research on the role of microRNA in diseases has been increasing in recent years. Because miRNA can regulate mRNA by binding to the 3' untranslated region (3'-UTR) of the target mRNA, and then regulate gene expression, the development of disease is associated with abnormal expression level of miRNA [7,8]. In this research, miR-202-3p has been proved to be closely related to the development, progression and expression pattern of many diseases, such as depression and glioma.

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Different diseases have different functions [9-11]. Although miR-202-3p is widely used in the screening of various diseases worldwide, there are relatively few studies on the correlation between AKI induced by sepsis and miR-202-3p. In this research, we will clarify the relationship between them and explore whether it can be used as related risk factors of AKI.

METHODS

Baseline Data

From April 2017 to January 2019, 66 patients who were treated in Danzhou People's Hospital and 70 healthy volunteers were selected as the research objects for study. The research was ratified by the Ethics Committee of our hospital. Patients and their families were informed in advance before study, and affixed the informed consent.

Inclusion criteria: The patient's age was ≥18 years old; The patient was diagnosed as sepsis according to the third edition of International Consensus on Definition of Sepsis and Septic Shock [12]; Patients had no obvious contraindication to hemodialysis; Patients were mentally normal and could express themselves accurately.

Exclusion criteria: The patient suffered malignancy; In the past, the patient suffered chronic renal insufficiency, autoimmune disease, multiple trauma, other blood disorders, acute coronary syndrome, hypovolemic shock, thyroid disease or stroke; Patients received immunosuppressive agent or glucocorticoid for a long time; The patient was in lactation or pregnancy.

Main Reagents, Instruments and Detection Methods

(1) Main reagents and experimental equipment

Human renal tubular epithelial HKC cells and human mesangial HMC cells were from Procell Life Science&Technology Co.,Ltd. DMEM medium was from Hunan Fenghui Biotechnology Co., Ltd. Fetal bovine serum (10%) was from ThermoFisher Scientific Technology (China) Co., Ltd. Bacterial lipopolysaccharide (LPS from esch-erichia coli 055: B5, specification 100 mg) was from Sigma Company, the States. Cell cycle detection kit was from Shanghai Enzyme-linked Biotechnology Co., Ltd. Apoptosis detection kit, transfection reagent and LipofectamineTM3000 were from Sigma-Aldrich (Shanghai) Trading Co., Ltd. Trizol reagent was from Shanghai Yuanye Biotechnology Co., Ltd. The primer sequences and transfection plasmids of miR-202-3p and internal reference were compounded and designed by Sangon Biotech (Shanghai) Co., Ltd. Ultraviolet spectrophotometer was from Beijing Jiayuan Xingye Technology Co., Ltd. CoulterCytoFLEX flow cytometer was from Beckman Coulter, Inc., the States. American ABI7500 fluorescence quantitative PCR instrument was from Beijing Long Jump Biological Science And Technology Development Co.,Ltd. Transwell chamber was from Shanghai SunBio Biomedical technology CO., LTD. Enzyme-labeling instrument was from Beijing Image Trading Co., Ltd.

(2) Detection of miR-202-3p expression level

The expression of miR-202-3p was tested in the serum of patients and healthy volunteers, as well as in the subsequent cell culture. The blood samples were drawn from patients in both groups and tested by qPCR technique. First, total RNA was extracted from the cells. Approximately 50ml of serum was put into 1.5ml RNase-free centrifuge tube, and 0.5ml of Trizol was added. After the shock, 0.5ml of Trizol was added and placed statically. The whole process was about 0.5h. The chloroform (200μl) was put in each 1ml of Trizol, shaken rapidly and mixed for 30s. Then, it was placed on ice for 5min and centrifuged at 1500xg and 4 ° C for 10min. Approximately 400-600μl of supernatant was transferred to a new centrifuge tube with pipet gun, and then 500μl of isopropanol was put in 1ml of Trizol. After covering, the mixture was reversed and mixed repeatedly. After that, it was placed statically for 10min and centrifuged at 1500xg and 4 ℃ for 10min. The supernatant was discarded, and the isopropanol was removed. Then, 75% ethanol (1ml) was added, thoroughly mixed and centrifuged at 1500xg and 4℃ for 10min, and then RNA was washed. The

supernatant was discarded and dried naturally for 5-10min. DEPC water (20μl) was added to dissolve it thoroughly and obtain total RNA. After that, qPCR was performed in a fluorescence quantitative PCR instrument, which was pre-denatured at 95℃ for 5 min, denatured at 95℃ for 15 s, annealed at 60℃ for 30 s, and extended at 60~95℃, with 40 cycles. After that, it was compared with the internal parameters to get the result.

(3) Detection of BUN and Cr expression levels

Western blot was applied to test the relative expression levels of BUN and Cr proteins and the relative expression levels represented by the ratio of BUN/GAPDH and Cr/GAPDH in serum and subsequent cell cultures of patients and healthy volunteers.

(4) Cell culture and transfection

HKC and HMC cells were conventionally subcultured in high glucose DMEM medium comprising 10% fetal bovine serum in a cell culture incubator at 37℃ and 5%CO2, and stimulated with 20ug/ml LPS. Before transfection, the cells were inoculated into 96-hole plates, and then divided into miR-NC group and miR-202-3p inhibitor group. The cells were transfected with Lipofectamine™3000 kit according to the operation specifications, and then the miR-202-3p expression of cells in each group was tested.

(5) Detection of cell growth

In both groups, the transfected HKC and HMC cells were inoculated into 96-hole plates respectively. Three multiple holes were set for each hole, and three time points were set at 24h, 48h and 72h. MTS cell proliferation colorimetric assay kit (CCK8) (20μL) was put in each hole at 2h before the end of culture, and placed in a cell incubator at 37℃ and 5%CO2. After 2 hours, the OD value was measured at 490nm wavelength with an automatic enzyme-labeling instrument to observe the cell growth.

(6) Apoptosis detection

The cell apoptosis was tested by CoulterCytoflex flow cytometry (purchased from Beckman Instruments, Inc., the States). The cells that had been transfected for 48 hours and stained with AnnexinV and PI in the 96-hole plate were tested. The experiment was repeated three times.

Statistical Methods

SPSS 25.0 (Asia Analytics Formerly SPSS China) was applied for statistical analysis of comprehensive data. In this research, the analysis methods included X2 test, One-Way ANOVA test and T test. The difference was obvious and had statistical significance with $P<0.05$.

RESULTS

Baseline Data

There was no obvious difference in age, hypertension, diabetes and other general information among 66 patients and 70 volunteers in our hospital ($p > 0.05$) (Table 1).

Table 1.
General clinical baseline data of patients and volunteers

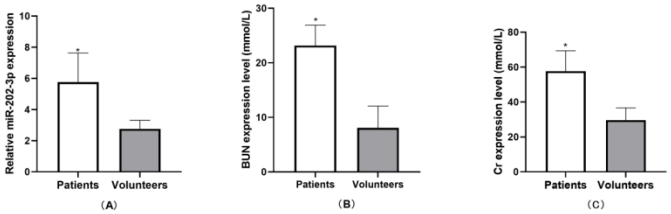
Group	Patients (n=66)	Volunteers (n=70)	t/X^2	p
Gender			0.12	0.724
Male	35（53.03）	35（50.00）		
Female	31（46.97）	35（50.00）		
Age			0.32	0.572
18-50	27（40.91）	32（45.71）		
43	39（59.09）	38（54.29）		
Average age (years old)	53.47±4.92	52.84±5.07	0.73	0.464
Average weight (Kg)	68.23±13.31	67.75±14.12	0.20	0.839
Hyperlipemia			0.06	0.800
Yes	41（62.12）	42（60.00）		
No	25（37.88）	28（40.00）		
Hypertension			0.29	0.588
Yes	37（56.06）	36（54.55）		

No	29（43.94）	34（45.45）		
Diabetes			0.15	0.698
Yes	51（77.27）	56（80.00）		
No	15（22.73）	14（20.00）		

Expression Level of miR-202-3p, BUN and Cr

By comparing the levels of serum miR-202-3p, BUN and Cr between patients and volunteers, it was found that the level of miR-202-3p in patients was higher than that in volunteers, and the levels of BUN and Cr were also obviously higher than those in CG (P<0.05). This indicated that miR-202-3p promoted the development of sepsis (Figure 1).

Figure 1.
Levels of miR-202-3p, BUN and Cr in serum of patients and volunteers



(A) miR-202-3p level: The miR-202-3p level of patients was obviously higher than that of volunteers (P<0.05). (B) BUN level: BUN level of patients was obviously higher than that of volunteers (P<0.05). (C) Cr level: The Cr level of patients was obviously higher than that of volunteers (P<0.05). Note: * means the comparison with the CG, P<0.05.

Relationship between the Expression Levels of miR-202-3p, BUN and Cr and the Characteristics of Different Patients

(1) miR-202-3p

By comparing the levels of serum miR-202-3p in patients with different clinical characteristics, it was found that the miR-202-3p level was not obviously different from that of patients with different clinical characteristics (p<0.05), except that the miR-202-3p level was different in patients with diabetes (P<0.05) (Table 2).

Table 2.
Expression level of miR-202-3p in patients with different clinical characteristics

Clinical parameters	Cases	Expression level	t	p
Gender			0.24	0.809
Male	35	2.31±0.64		
Female	31	2.34±0.59		
Age			0.22	0.829
18-50	27	2.79±0.45		
＞50	39	2.81±0.46		
Hyperlipemia			0.17	0.681
Yes	41	2.67±0.33		
No	25	2.69±0.34		
Hypertension			0.23	0.816
Yes	37	2.32±0.97		
No	29	2.36±0.92		
Diabetes			3.99	<0.001
Yes	51	2.73±0.43		
No	15	2.31±0.46		

(2) BUN and Cr

By comparing the levels of serum BUN and Cr in patients with different clinical characteristics, it was found that the BUN and Cr levels were not obviously different from those of patients with

different clinical characteristics (p<0.05), except that the BUN and Cr levels were different in patients with diabetes (P<0.05) (Table 3, 4).

Table 3.
Expression level of BUN in patients with different clinical characteristics

Clinical parameters	Cases	Expression level (mmol/L)	t	p
Gender			0.03	0.973
Male	35	22.91±2.88		
Female	31	22.89±2.92		
Age			0.24	0.812
18-50	27	24.76±3.08		
＞50	39	24.61±3.14		
Hyperlipemia			0.10	0.920
Yes	41	23.03±3.83		
No	25	23.11±3.78		
Hypertension			0.33	0.743
Yes	37	22.77±2.86		
No	29	22.58±2.89		
Diabetes			4.70	<0.001
Yes	51	26.59±3.65		
No	15	22.42±3.78		

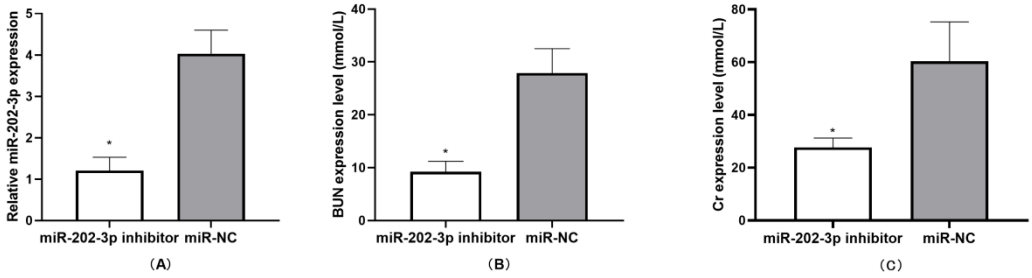
Table 4.
Expression level of Cr in patients with different clinical characteristics

Clinical parameters	Cases	Expression level (mmol/L)	t	p
Gender			0.20	0.84
Male	35	57.36±11.77		
Female	31	56.89±12.11		
Age			0.09	0.921
18-50	27	56.93±12.52		
＞50	39	57.18±12.41		
Hyperlipemia			0.18	0.861
Yes	41	57.99±13.23		
No	25	57.51±13.01		
Hypertension			0.14	0.890
Yes	37	57.86±13.15		
No	29	57.49±13.31		
Diabetes			2.44	0.017
Yes	51	59.61±14.57		
No	15	51.42±11.28		

Expression Levels of miR-202-3p, BUN and Cr after Inhibition of miR-202-3p

By comparing the levels of miR-202-3p, BUN and Cr in miR-202-3p inhibitor group and miR-NC group, it was found that the level of miR-202-3p in miR-202-3p inhibitor group was lower than that in volunteers, and the levels of BUN and Cr were obviously lower than those in CG (P<0.05). The results indicated that the expression levels of BUN and Cr could be declined after miR-202-3p was inhibited (Figure 2).

Figure 2.
Levels of miR-202-3p, BUN and Cr of cells in both groups

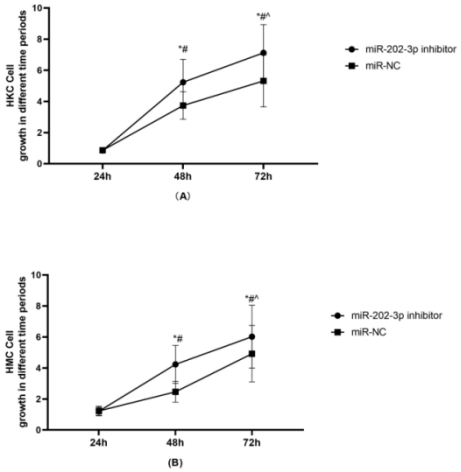


(A) miR-202-3p level: The level of miR-202-3p in miR-202-3p inhibitor group was obviously lower than that in miR-NC group (P<0.05). (B) BUN level: The level of BUN in miR-202-3p inhibitor group was obviously lower than that in miR-NC group (P<0.05). (C) Cr level: The level of Cr in miR-202-3p inhibitor group was obviously lower than that in miR-NC group (P<0.05).
Note: * means the comparison with the CG, P<0.05.

Level of Cell Growth

By comparing the cell growth in both groups, it was found that there were changes in both groups at 48-72h, and the growth of HKC and HMC cells in miR-202-3p inhibitor group was obviously better than that in miR-NC group (P<0.05) (Figure 3).

Figure 3.
Growth of cells in both groups

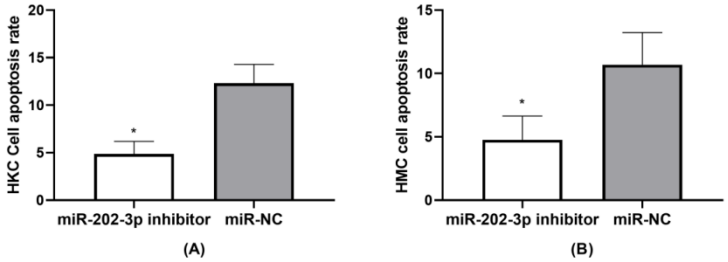


(A) HKC cells: The growth level of HKC cells in miR-202-3p inhibitor group was obviously better than that in miR-NC group at 48-72h (P<0.05). (B) HMC cells: The growth level of HMC cells in miR-202-3p inhibitor group was obviously better than that in miR-NC group at 48-72h (P<0.05). Note: * means the comparison with the CG, P<0.05.

Apoptosis Level

By comparing the apoptosis levels in both groups, it was found that the apoptosis levels of HKC and HMC cells in miR-202-3p inhibitor group were obviously lower than those in miR-NC group (P<0.05) (Figure 4).

Figure 4.
Apoptosis in both groups



(A) HKC cells: The apoptosis rate of HKC cells in miR-202-3p inhibitor group was obviously lower than that in miR-NC group (P<0.05). (B) HMC cells: The apoptosis rate of HMC cells in miR-202-3p inhibitor group was obviously lower than that in miR-NC group (P<0.05). Note: * means the comparison with the CG, P<0.05.

Prognostic Analysis of Risk Factors

Multivariate logistic regression analysis revealed that the levels of miR-202-3p, BUN and Cr could be used as prognostic factors of AKI induced by sepsis (Table 5, 6).

Table 5.
Logistic regression analysis variable assignment

Factors	Variable	Assignment
Gender	X1	Male=1, Female=0
Diabetes	X2	Yes=1, No=0
miR-202-3p level	X3	Continuous variable
BUN level	X4	Continuous variable
Cr level	X5	Continuous variable

Table 6.
Prognostic factors that affected disease

Risk factors	β value	SE value	Wald value	p	OR	95%CI
Gender	1.997	2.034	2.103	0.323	1.781	1.559-2.943
History of diabetes	2.871	2.557	3.281	0.078	2.617	0.986-3.519
miR-202-3p level	1.378	0.432	3.221	0.007	1.454	0.553-2.218
BUN level	1.328	0.891	6.133	0.029	2.608	0.762-2.544
Cr level	2.391	0.741	4.783	0.014	2.138	1.281-3.527

DISCUSSION

Although the treatment of AKI has made progress in recent years, the disease still has high severity and mortality, among which AKI induced by sepsis accounts for a large proportion [13]. Some related studies have revealed that miRNA has great research value in the diagnosis and prognosis of sepsis [14-16]. In this research, we examined the relationship between miR-202-3p and AKI induced by sepsis. In this part we discussed its application value in sepsis.

In this research, we found that the expression level of miR-202-3p in sepsis-induced patients was higher than that in healthy volunteers, and the levels of BUN and Cr were also higher. However, there are few studies on the role of miR-202-3p in kidney injury and sepsis. In AKI, BUN and Cr are risk factors, which are used to detect glomerular filtration and renal tubular absorption function in serum. The increase of both levels means that the function of renal organs is damaged [17-19]. It could also explain why BUN and Cr are important

prognostic factors in the subsequent analysis of prognostic factors. Combined with these results, the level of miR-202-3p in patients increased, and the levels of BUN and Cr also increased, which indicated that miR-202-3p was positively related to AKI caused by sepsis, and the increase of miR-202-3p level led to the damage of kidney function. Therefore, we can preliminarily guess that miR-202-3p plays a vital role in AKI caused by sepsis.

Through the experimental results on cells, we can guess whether reducing the level of miR-202-3p can effectively alleviate the AKI of patients. According to our detection results, the expression levels of BUN and Cr declined, while the cell growth level increased and the apoptosis level declined in the miR-202-3p inhibitor group (knocking down miR-202-3p). From the researches of miR-202-3p in some tumours, it can be found that miR-202-3p plays a vital role in the development and progress of tumours, and its increased level can suppress the growth and invasion of tumour cells and improve the apoptosis [20,21]. In other diseases, such as preeclampsia, miR-202-3p can suppress cell growth and improve apoptosis, thus preventing the development of placenta and causing damage to placenta [22]. In AKI, the inflammatory reaction caused by injury will lead to oxidation-reduction imbalance and DNA damage, thus causing apoptosis of glomerulus and renal tubules and damage of kidney function [23-25]. Combined with the results of this study, the growth level of the cultured glomerulus and renal tubular cell line stimulated by LPS increased, the apoptosis rate decreased, and the levels of BUN and Cr related to glomerulus and renal tubular function also decreased after reducing the level of miR-202-3p, which revealed that miR-202-3p was indeed closely related to acute renal failure induced by sepsis. From this, we can draw a conclusion: miR-202-3p has the function of inhibiting growth and improving apoptosis in cancer, and this function can lead to the related functions of glomerulus and renal tubules in AKI, and then aggravate kidney injury, which is similar to its role in preeclampsia. Therefore, it is a pathogenic factor in AKI induced by sepsis, so it can be a potential biomarker.

There are still some shortcomings in this research. In this study, we only detected miR-202-3p and kidney injury-related factors, but did not further explore its specific molecular mechanism and molecular pathway. At the same time, we also failed to detect the expression level of inflammatory factors, and failed to investigate the specific effect of miR-202-3p on inflammatory reaction. In the future research, we will constantly improve these deficiencies and further explore the deeper molecular mechanism to better analyze the specific pathological mechanism of the disease.

To sum up, miR-202-3p is highly expressed in patients with AKI induced by sepsis, which is a pathogenic factor and can be used as a potential biomarker for diagnosis and prognosis in clinic.

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