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Aberrant miR-219-5p is correlated with TLR4 and serves as a novel biomarker in patients with multiple organ dysfunction syndrome caused by acute paraquat poisoning

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Abstract

This study aimed to investigate the clinical significance of serum microRNA-219-5p (miR-219-5p) in patients with multiple organ dysfunction syndrome (MODS) caused by acute paraquat (PQ) poisoning, and its correlation with Toll-like Receptor 4 (TLR4). Luciferase reporter assay was used to investigate in vitro the correlation of miR-219-5p with TLR4. Serum miR-219-5p levels were evaluated by quantitative real-time polymerase chain reaction. Serum levels of TLR4, IL-1β, and TNF-α were measured by Enzyme-linked immune sorbent assay (ELISA). ROC analysis was performed to assess the diagnostic significance, Kaplan-Meier survival curves and Cox regression analysis were used to evaluate the prognostic value of miR-219-5p in MODS patients. TLR4 was a target gene of miR-219-5p and was increased in MODS patients. Serum miR-219-5p level was decreased and negatively correlated with TLR4 level in MODS patients ($r = -0.660$, $P < 0.001$), which had important diagnostic value and negatively correlated with APACHE II score in MODS patients. The miR-219-5p expression was markedly associated with the WBC, ALT, AST, $PaCO_2$, Lac, and APACHE II score. Non-survivals had more patients with low miR-219-5p expression. Patients with low miR-219-5p expression had shorter survival time. MiR-219-5p and APACHE II score were two independently prognostic factors for 28-day survival. MiR-219-5p was negatively correlated with, while TLR4 was positively correlated with the levels of IL-1β and TNF-α. The serum miR-219-5p level may be a potential biomarker for acute PQ-induced MODS diagnosis and prognosis. Furthermore, miR-219-5p may be associated with the progression of MODS by regulating TLR4-related inflammatory response.

Keywords

diagnosis, microRNA-219-5p, multiple organ dysfunction syndrome, paraquat poisoning, prognosis, TLR4

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Introduction

Paraquat (N, N-dimethyl-4, 4-bipyridinium dichloride, PQ) is a herbicide which is widely used in agriculture, because it has a faster effect and is cheaper compared with other herbicides.[1] Besides, in the developing countries, self-poisoning by pesticides is an important public health problem.[2] PQ is high toxic to humans, has no antidotes and no

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effective treatment, resulting in an individual mortality rate as high as 50–90%.[3] Although clinical comprehensive treatment, including gastric lavage, international diarrhea, diuresis, and blood perfusion, has been adopted, the therapeutic effect is poor and the fatality rate remains high.[4] Studies have found that the concentration of PQ in severely body from low to high was stomach wall (<limit of quantitation, LOQ), cardiac blood (0.11 μg/mL), liver (0.2 μg/g), kidney (0.24 μg/g), brain (0.32 μg/g), and lung (0.49 μg/g).[5] PQ mainly accumulates in the lungs (pulmonary concentrations can be 6 to 10 times higher than those in plasma) and remains in the lungs even as blood levels begin to drop.[6] PQ poisoning can lead to multiple organ dysfunction syndrome (MODS).[7,8]

The development of MODS is wildly believed to be the result of uncontrolled immune system dysfunction and the resulting systemic inflammatory response is characterized by the release of large amounts of inflammatory mediators and extensive genomic activation patterns.[9] MODS is the leading reason of morbidity and mortality in the intensive care units.[10] The lost homeostasis between pro-inflammatory and anti-inflammatory reaction is considered to play a vital role in the progression of MODS.[11] Generally, anti-inflammatory therapy is the major regimen in the early therapy of PQ poisoning.[2] And many genes, including microRNAs (miRNAs), have been found to be differentially expressed in the occurrence and development of inflammation and therefore can be used as candidate targets for gene treatment.[12] Recent study has reported that miR-27a might regulate the MODS process induced by PQ poisoning via regulating the inflammatory response, and IL-10 is involved in this process.[13] Additionally, miR-146a might be involved in the occurrence of lung injury induced by PQ poisoning, which was achieved via IL-6.[14]

Toll like receptor 4 (TLR4) is a pattern recognition receptor, which is a vital part of the innate immune system and has the regulatory effects on the acute inflammatory response.[15] Liu et al.[16] found that the mRNA level of TLR4 markedly increased in lung tissue treated by PQ, consequently TLR4 might play a vital role in PQ-induced acute lung injury. A study by Dong et al.[17] revealed that the TLR4 might function as a mediator, suggesting the vital role of TLR4 in PQ-induced myocardial injury. Up to now, the key role of miRNAs played in regulating TLR4 has been widely studied. In the study of the role of miR-124 in regulating the TLR4 signaling pathway of microglia, miR-124 could inhibit the TLR4 levels.[18] Additionally, the TLR4 was reported to be a direct target of miR-590, and miR-590 promoted proliferation and blocked ox-LDL-induced apoptosis in HAECs by inhibiting the TLR4/NF-KB pathway.[19] Yu et al.[20] found that long noncoding RNAs (lncRNA) PCAT1 negatively regulated miR-145-5p, which promoted TLR4 expression and promoted osteogenic differentiation by activating the TLR signaling pathway. MiR-219-5p has been known to be associated with the regulation of inflammatory response.[21,22] However, as far as we know, miR-219-5p has not been previously studied in relation to PQ poisoning, nor its relationship with TLR4. For exploring the regulatory mechanism of TLR4, the miRNA molecules that may regulate TLR4 were predicted by bioinformatics prediction method. The complementary binding sequence of miR-219-5p was found in the 3'-UTR of TLR4 (Figure 1a), suggesting that miR-219-5p may be a potential regulator of TLR4. Therefore, we suspected that miR-219-5p might play a potential role in the MODS caused by PQ poisoning by regulating TLR4-related inflammatory response.

In this study, serum miR-219-5p levels and TLR4 levels in MODS patients induced by PQ poisoning were measured, the correlation between TLR4 and miR-219-5p as well as the correlation of them with pro-inflammatory cytokines in MODS patients were discussed, and the clinical value of miR-219-5p was further explored.

Material and methods

Patients and sample collection

A total of 75 patients with MODS caused by acute PQ poisoning were retrospectively analyzed in this study, who visited the emergency department of Qingdao Jiaozhou Central Hospital from 2012 to 2017. The inclusion criteria: (1) all patients arrived at the hospital within 24 h of poisoning; (2) the poisons were all taken orally with the estimated amount of 10–80 mL; (3) all patients were diagnosed following Fry reported diagnostic criteria.[23] As soon as admission to hospital, 10–15 mL venous blood of all patients were collected, serum was separated and stored at –80°C for further using. Besides, 75 healthy subjects who had physical examinations in our hospital during the same period were enrolled in the control group and their fasting venous blood were

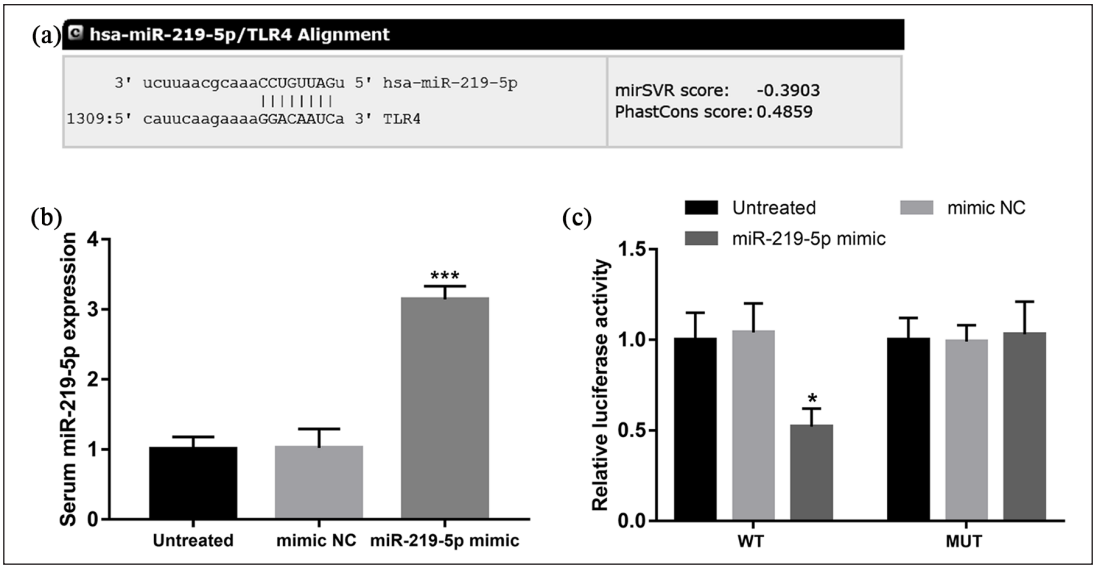

Figure 1. TLR4 is a direct target gene of miR-219-5p. (a**)** Schematic illustration of the miR-219-5p targeting site at the 3'-UTR of TLR4 gene. (b) The serum miR-219-5p expression levels was upregulated by the miR-219-5p mimic (***$P < 0.001$). (c) Luciferase activity was inhibited by overexpression of miR-219-5p in WT group (*$P < 0.001$).

collected. The exclusion criteria: patients suffered from immune or immune-related diseases, such as diabetes, tumors, chronic liver disease, chronic kidney disease, or connective tissue disease. All patients were treated according to a standard comical therapy regimens, including gastric lavage, catharsis, hemoperfusion, antioxidants, high-dose intravenous methylprednisolone and cyclophosphamide, and other treatment methods. All procedures of this study were approved by the Ethics Committee of Qingdao Jiaozhou Central Hospital and the written informed consents of each participant were obtained. Each patient was followed up for 28 days and survival data were recorded. As for the selection of sample size, which was decided based on margin of error (set at 10%) and confidence level (set at 90%). Therefore, the minimum sample size should be 68. In addition, we have also referred to other similar published studies for confirming that the sample size in our cohort study is appropriated.

Data collection

All data were collected and recorded on a standard basis for each patient within 24 h after admission, including: (1) Demographic parameter, such as age and gender; (2) Time interval between PQ intake and admission to the emergency department; (3) Estimation of PQ intake; (4) Blood biochemical indexes included white blood count (WBC), blood urea nitrogen (BUN), alanine transaminase (ALT) and aspartate transaminase (AST); (5) Arterial partial pressure of carbon dioxide ($PaCO_2$) and blood lactic acid (Lac) were measured using i-STAT blood gas analyzer (Abbott Laboratories, Abbott Park, USA).

Cell culture and transfection

Human kidney epithelial cells 239 cells were purchased from Shanghai Cell Bank of Chinese Academy of Science (Shanghai, China) for luciferase reporting experiment. The cells were cultured using Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, New York), and sustained in a 5% CO_2 atmosphere at 37°C. MiR-219-5p mimic and corresponding negative control (NC) were used for cell transfection to regulate the miR-219-5p expression in 239 cells, which were purchased from GenePharma (Shanghai, China). The cells were transfected with the miR-219-5p mimic, mimic NC using Lipofectamine 2000 transfection reagent (Invitrogen, Carlsbad, California) following the manufacturer's protocols. Cells were collected 24 h after transfection for subsequent analyzes.

Luciferase reporter assay

The putative binding site of miR-219-5p at the 3'-UTR of TLR4 was predicted using miRanda

(http://www.microrna.org/microrna/home.do). To confirm whether there was a direct interaction between miR-219-5p and TLR4, a luciferase reporter assay was performed. The wild-type (WT) 3'-UTR containing the binding site of miR-219-5p or mutant-type (MT) 3'-UTR were inserted in the pGL-control vector (Promega, Madison, WI, USA). The combined vectors were respectively co-transfected into 239 cells with miR-219-5p mimic or mimic NC using Lipofectamine 3000 (Thermo Fisher Scientific, Waltham, MA, USA) according to the instruction of manufacturer. 48 h after transfection, the activity of firefly luciferase was evaluated by a Dual-Luciferase Reporter Assay System (Promega).

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Trizol Reagent (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA from serum samples following the protocol of manufacturer. MiScript Reverse Transcription Kit (QIAGEN, Germany) was used to perform reverse transcriptions. The miR-219-5p expression was estimated by qRT-PCR, which was carried out with SYBR green I Master Mix kit (Invitrogen, Carlsbad, CA, USA). U6 was used as an internal control. The final miR-219-5p expression was calculated using the $2^{-\Delta\Delta Ct}$ method[24] and normalized to U6.

Enzyme-linked immune sorbent assay

The concentration of serum TLR4 and the levels of pro-inflammatory cytokines (IL-1β and TNF-α) were all measured by ELISA method, which was carried out according to the instructions of manufacturer (Germany, IBL Company). TLR4 concentration and the levels of pro-inflammatory cytokines were calculated according to the standard curves provided by the kits.

Statistical analysis

All statistical analyzes were performed by using SPSS 21.0 software (SPSS, Inc., Chicago, USA) and GraphPad Prism 7.0 software (Inc., Chicago, USA). All data were presented as mean ± standard deviation (SD). The measurement data was compared using student's *t* test, and the counting information was compared using chi-square test. Correlation of miR-219-5p and TLR4 levels with APACHE II score were assessed using Pearson's correlation coefficient. Receiver operating characteristic (ROC) curve was applied to assess the diagnostic value of miR-219-5p in MODS caused by PQ poisoning. Kaplan-Meier and Cox regression analysis were used to evaluate the prognostic value of miR-219-5p. A $P < 0.05$ was considered statistically significant.

Results

TLR4 is a direct target gene of miR-219-5p

To confirm whether there was a direct interaction between miR-219-5p and TLR4, a luciferase reporter assay was conducted. A binding site of miR-219-5p was identified in the 3'-UTR of TLR4, indicating that miR-219-5p can directly bind toTLR4 (Figure 1a). By cell transfection, miR-219-5p expression in the 239 cells was significantly upregulated by the miR-219-5p mimic ($P < 0.001$, Figure 1b). Additionally, the results luciferase reporter assay indicated that the miR-219-5p upregulation significantly inhibited the relative luciferase activity in WT group ($P < 0.05$), while there was no change in the MUT group (Figure 1c). These data indicated that miR-219-5p could directly targeted TLR4.

Serum miR-219-5p expression in patients with MODS induced by PQ poisoning

The serum miR-219-5p levels were measured using RT-qPCR in all MODS patients and controls. The results indicated that serum miR-219-5p level was markedly reduced in MODS patients compared with those in the controls ($P < 0.001$, Figure 2).

Association of miR-219-5p expression with clinicopathological characteristics of MODS patients induced by PQ poisoning

To explore the role of miR-219-5p in the development of MODS caused by PQ poisoning, the association between miR-219-5p and the clinicopathological features of all patients were examined. The MODS patients were classified into low (n = 39) and high (n = 36) miR-219-5p expression groups based on the mean value of the expression of miR-219-5p. As shown in Table 1, miR-219-5p expression was clearly associated with WBC ($P = 0.018$), ALT ($P = 0.012$), AST ($P = 0.003$), PaCO2 ($P = 0.002$), Lac ($P = 0.002$), and APACHE II score ($P < 0.001$),

whereas no significant association was observed between miR-219-5p and other clinicopathological features, including gender, age, time to hospital, estimated amount, BUN (all $P > 0.05$).

Diagnostic value of miR-219-5p and its correlation with disease severity in MODS patients

In view of the significant dysregulation of serum miR-219-5p expression in the MODS patients. A ROC curve (Figure 3a) was plotted to assess the diagnostic value of serum miR-219-5p in this study. The area under the curve (AUC) for miR-219-5p was 0.891 with a sensitivity of 89.33% and a specificity of 76% at the cutoff of 0.800, indicating the considerable diagnostic accuracy of the serum miR-219-5p expression in MODS patients. Additionally, we observed a negative correlation between miR-219-5p expression and APACHE II score in MODS patients ($r = -0.403$, $P = 0.003$, Figure 3b), suggesting that miR-219-5p was negatively correlated with the disease severity in patients with MODS induced by PQ poisoning.

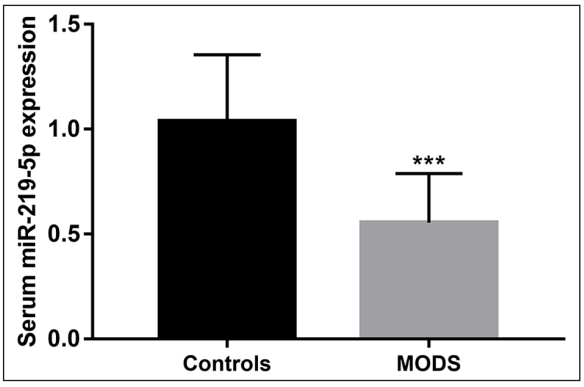

Figure 2. Serum miR-219-5p level was markedly decreased in MODS patients induced by PQ poisoning compared with those in controls (***$P < 0.001$).

Prognostic value of miR-219-5p for the 28-day survival of MODS patients

According to 28-day survival, all 75 MODS patients induced by PQ poisoning were grouped into two groups: survival group (n = 26) and non-survival group (n = 49). Thus, the 28-day survival rate (%) was 34.67 in the 75 MODS patients. As shown in Table 2, non-survivors had markedly high levels of WBC, BUN, ALT, AST, Lac, and markedly low levels of $PaCO_2$ compared with the survivals (all $P < 0.001$). Besides, as for the traditional score between survival and non-survival group, non-survivors had markedly higher APACHE II score ($P < 0.001$). Moreover, the miR-219-5p expression level was markedly different between survival and non-survival group ($P < 0.001$), the expression of miR-219-5p was markedly low in non-survival group.

Moreover, the Kaplan-Meier survival curves (Figure 4) was established to analyze the correlation between miR-219-5p expression and the

Table 1. Association of miR-219-5p expression with the clinicopathological characteristics of MODS patients induced by paraquat poisoning.

Variables	Low miR-219-5p expression ($n = 39$)	High miR-219-5p expression ($n = 36$)	*P* value
Gender			
Female	21	19	0.926
Male	18	17	
Age (years)	29.923 ± 9.359	31.194 ± 8.978	0.551
Time to hospital (h)	11.256 ± 5.632	9.611 ± 4.842	0.181
Estimated amount (mL)	37.414 ± 16.758	36.817 ± 15.308	0.873
WBC	16.180 ± 2.424	14.985 ± 1.762	0.018
BUN	11.928 ± 1.753	11.598 ± 1.527	0.389
ALT	157.703 ± 28.689	142.206 ± 22.424	0.012
AST	147.327 ± 28.456	131.358 ± 13.836	0.003
$PaCO_2$	26.626 ± 2.264	28.659 ± 3.260	0.002
Lac	2.733 ± 0.592	2.304 ± 0.570	0.002
APACHE II	10.037 ± 2.518	7.255 ± 1.883	<0.001

WBC: white blood count; BUN: blood urea nitrogen; ALT: alanine transaminase; AST: aspartate transaminase; $PaCO_2$: partial pressure of carbon dioxide; Lac: arterial blood lactic acid; APACHE II: the acute physiology and chronic health evaluation II score.

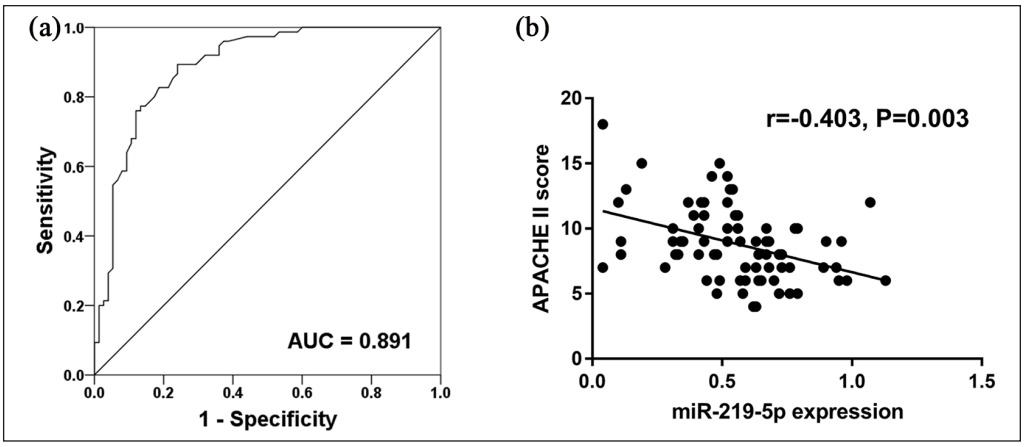

Figure 3. Diagnostic value of miR-219-5p and its correlation with disease severity in patients with MODS induced by PQ poisoning. (a) ROC curve for MODS patients based on serum miR-219-5p, which had an AUC of 0.891 with the sensitivity of 89.33% and specificity of 76%. (b) Correlation between APACHE II score and miR-219-5p expression in MODS patients induced by PQ poisoning ($r = -0.403$, $P = 0.003$).

Table 2. The comparisons between survivors and non-survivors of MODS patients induced by paraquat poisoning.

Variables	Survivors ($n = 26$)	Non-survivors ($n = 49$)	*P* value
MiR-219-5p	0.683 ± 0.219	0.486 ± 0.214	<0.001
Gender			
Female	15	25	0.582
Male	11	24	
Age (years)	30.115 ± 9.816	30.755 ± 8.857	0.775
Time to hospital (h)	10.115 ± 6.035	10.653 ± 4.918	0.679
Estimated amount (mL)	31.853 ± 14.305	39.926 ± 16.239	0.036
WBC	13.765 ± 1.323	16.583 ± 1.937	<0.001
BUN	10.362 ± 1.110	12.517 ± 1.375	<0.001
ALT	127.097 ± 19.244	162.557 ± 21.773	<0.001
AST	120.912 ± 13.060	149.611 ± 22.356	<0.001
$PaCO_2$	29.257 ± 2.982	26.723 ± 2.553	<0.001
Lac	2.005 ± 0.434	2.804 ± 0.512	<0.001
APACHE II	6.528 ± 1.546	9.855 ± 2.337	<0.001

WBC: white blood count; BUN: blood urea nitrogen; ALT: alanine transaminase; AST: aspartate transaminase; $PaCO_2$: partial pressure of carbon dioxide; Lac: arterial blood lactic acid; APACHE II: the acute physiology and chronic health evaluation II score.

survival depending on the 28-day survival. From the Kaplan-Meier survival curves, we observed that the patients with low miR-219-5p expression had markedly shorter survival time than the patients with high miR-219-5p expression (log-rank $P < 0.001$). The further multivariate Cox regression analyses indicated that miR-219-5p expression and APACHE II score were two independent prognostic factors for 28-day survival in patients with MODS (all $P < 0.05$, Table 3).

Negative correlation between miR-219-5p and TLR4 in MODS patients

The serum TLR4 expression levels was measured using ELISA in MODS patients and controls. The results indicated that the serum TLR4 level was markedly increased in MODS patients compared with the TLR4 level in the controls ($P < 0.001$, Figure 5a). Furthermore, we observed a negative correlation between serum miR-219-5p and TLR4 in patients with MODS induced by PQ poisoning ($r = -0.660$, $P < 0.001$, Figure 5b).

Correlation of miR-219-5p and TLR4 with pro-inflammatory cytokines in MODS patients

The inflammatory response in MODS patients were determined by measuring the serum pro-inflammatory cytokines, including IL-1β and TNF-α. The results shown in Table 4 indicated the negative correlation of miR-219-5p levels with pro-inflammatory

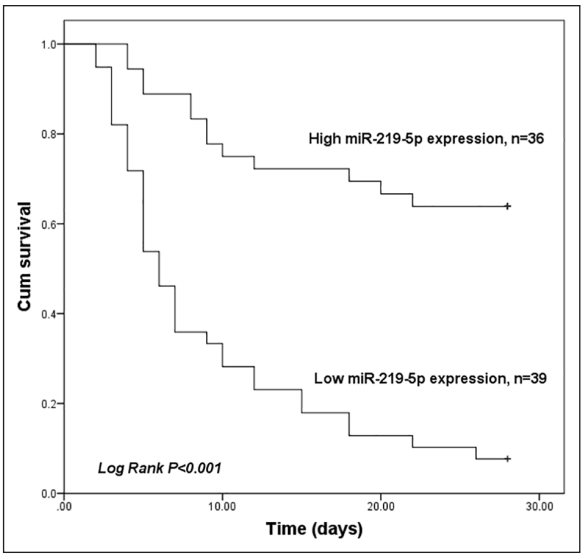

Figure 4. Kaplan-Meier survival curves in the MODS patients induced by PQ poisoning. The survival time of patients with low miR-219-5p expression was shorter than those patients with high miR-219-5p expression (Log-Rank $P < 0.001$).

Table 3. Multivariate cox regression analysis for miR-219-5p in MODS patients induced by paraquat poisoning.

Variables	Multivariate analysis		
	HR	95% CI	*P* value
MiR-219-5p	10.079	4.174–24.342	<0.001
Age	1.440	0.736–2.817	0.287
Gender	1.347	0.700–2.592	0.373
Time to hospital (h)	1.622	0.798–3.297	0.181
Estimated amount (mL)	1.445	0.755–2.766	0.267
WBC	1.279	0.686–2.384	0.438
BUN	1.234	0.622–2.452	0.547
ALT	1.206	0.630–2.310	0.572
AST	1.682	0.796–3.553	0.173
$PaCO_2$	1.266	0.621–2.579	0.517
Lac	1.391	0.622–3.111	0.422
APACHE II	2.269	1.090–4.724	0.028

WBC: white blood count; BUN: blood urea nitrogen; ALT: alanine transaminase; AST: aspartate transaminase; $PaCO_2$: partial pressure of carbon dioxide; Lac: arterial blood lactic acid; APACHE II: the acute physiology and chronic health evaluation II score.

cytokines and positive correlation of TLR4 with pro-inflammatory cytokines, which implied the potential correlation of miR-219-5p and TLR4 with inflammation in MODS progression.

Discussion

PQ, an agent highly toxic to humans and animals, is a widely used herbicide.[25]Acute PQ poisoning can cause a strong inflammatory reaction.[26] Activated TLR4 is a receptor of the innate immune system that has key effect on the regulation of inflammation.[27] A study indicated that the inflammation mediated by TLR4 played a key role in the occurrence of kidney damage and fibrosis in cyclosporine nephrotoxicity.[28] And Nrf2 could protect lung cells by regulating the TLR4 and Akt signaling pathways, suggesting TLR4 mediated inflammatory-related lung injury.[29] These studies suggested that TLR4 was important in inflammatory response. In addition, some studies has suggested that TlR4 played an important role in PQ poisoning.[17,18]

MiRNAs are widely involved in a variety of pathophysiological processes, including cancer, cardiovascular and metabolic diseases.[30–32] According the results of bioinformatics, miR-219-5p may be a potential regulator of TLR4, suggesting the potential role of miR-219-5p in PQ poisoning. Thus, in this study, a total of 75 patients with PQ poisoning MODS and 75 healthy controls were collected, and serum miR-219-5p levels were compared between control group and case group. The results showed that the serum miR-219-5p level of PQ poisoning MODS patients was significantly lower than the serum miR-219-3p level of health control group. Clinical data analysis revealed that serum miR-219-5p was significantly associated with WBC, ALT, AST, $PaCO_2$, Lac and APACHE II score. We knew that patients with PQ poisoning develop multiple organ dysfunction, including lung, kidney, liver and heart. ALT and AST are indicators of liver function, while $PaCO_2$ and Lac are important indicators of lung function and respiratory function, respectively. Chen et al.[33] found that miR-219-5p prevented cirrhosis through increasing keratinocyte growth factor (KGF). The study by Gao et al.[34] indicated that miR-219-5p was differentially expressed in liver cirrhosis, suggesting that miR-219-5p might affect the process of liver cirrhosis. The above evidence indicated the important role of miR-219-5p in liver injury. Thus, we speculate that miR-219-5p may be involved in organ damage in MODS patients cause by PQ poisoning, which supported our present results.

We further analyzed the diagnostic and prognostic significance of miR-219-5p in patients with PQ poisoning MODS. In this study, serum miR-219-5p level was downregulated in patients compared with the controls. According to the ROC curve, miR-219-5p may be a diagnostic biomarker for MODS caused by PQ poisoning. Besides, we observed a significantly negative correlation between APACHE II score and miR-219-5p expression in MODS patients, suggesting that

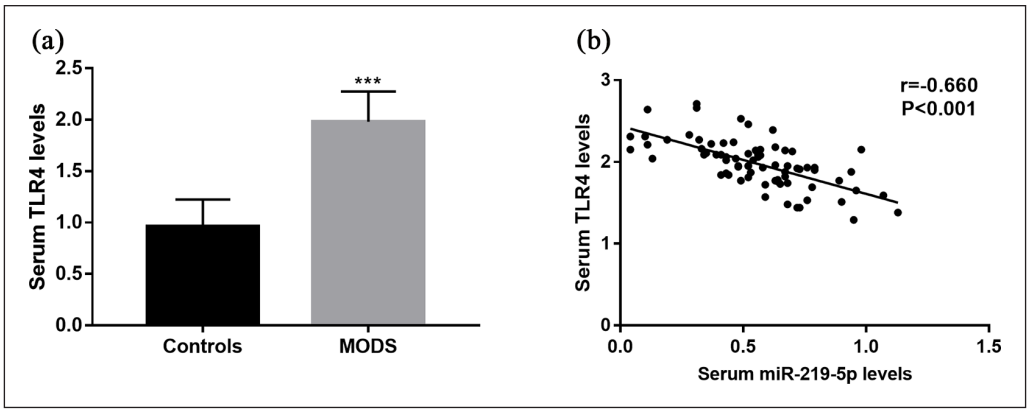

Figure 5. The correlation between serum miR-219-5p level and TLR4 level in MODS patients induced by PQ poisoning. (a) Serum TLR4 level was markedly increased in MODS patients compared with those in controls. (b) Serum miR-219-5p expression levels was negatively correlated with serum TLR4 levels in MODS patients induced by PQ poisoning ($r = -0.660$, $P < 0.001$).

Table 4. Correlation of miR-219-5p and TLR4 with pro-inflammatory cytokines in MODS patients induced by paraquat poisoning.

Variables	IL-1β		TNF-α	
	r value	*P* value	*r* value	*P* value
MiR-219-5p	–0.482	<0.001	–0.501	<0.001
TLR4	0.646	<0.001	0.679	<0.001

miR-219-5p was negatively correlated with the severity in patients with MODS induced by PQ poisoning. All patients were then divided into survival and non-survival groups based on 28-day survival. Compared with the surviving patients, the levels of WBC, BUN, ALT, AST and Lac were higher and levels of $PaCO_2$ was lower in non-surviving patients, reflecting the loss of kidney, liver and lung injury. We also found that there were more patients with low expression of miR-219-5p in the non-survival group, suggesting that the expression of miR-219-5p was associated with 28-day survival in patients with MODS caused by PQ poisoning. And the results of KM curves also showed that the patients with low expression of miR-219-5p had shorter survival time. In addition, multivariate Cox regression analysis suggested that miR-219-5p level and APACHE II score were two independent prognostic factor for 28-day survival. However, the APACHE II score has been shown to assess the prognosis of acute PQ poisoning.[35] This study indicated the significant correlation of serum miR-219-5p level with APACHE II score, suggesting that miR-219-5p was significantly correlated with PQ poisoning severity. Therefore, we believe that serum miR-219-5p may be a valuablel clinical tool for MODS patients by PQ poisoning.

In this study, bioinformatics analysis showed that miR-219-5p may be a potential regulator of TLR4. The results of this study indicated that serum TLR4 expression was increased and serum miR-219-5p was decreased in MODS patients caused by PQ poisoning, and was negatively correlated with TLR4. Finally, the double luciferase reporter gene experiment proved that TLR4 was a direct target gene of miR-219-5p. Therefore, we speculate that miR-219-5p may play a potential role in the MODS patients caused by PQ poisoning by regulating TLR4. Furthermore, considering the differentially expression of miR-219-5p in the progression of inflammation and the regulatory effects of TLR4 on the inflammation, we explored the correlation of miR-219-5p and TLR4 with pro-inflammatory cytokines in MODS patients. Negative correlation of miR-219-5p levels with pro-inflammatory cytokines and positive correlation of TLR4 with pro-inflammatory cytokines were found. Thus, we considered that miR-219-5p might be involved in the MODS progression by regulating TLR4-related inflammatory response.

There are still some limitations in this study. The clinicopathological parameters of the included patients were not comprehensive enough and the data collection was incomplete. Other clinicopathological parameters can be added to better examine the association between miR-219-5p levels and different organ dysfunction, such as serum cardiac troponin I (cTnI), serum creatinine (SCr), and so on. Moreover, the sample size is relatively small, which needs to be further increased in the further research. Furthermore, recent studies have found that activation of Sirtuin 1 plays important role in protecting lung injury induced by PQ and Sirtuin 1 is now strongly linked to MODS,

thus, its activation is important to prevent lung injury and MODS.[36–39] Thus, plasma Sirtuin 1 levels may be relevant to the diagnosis of PQ-induced MODS. We suspected that the relevance of Sirtuin 1 and miR-219-5p may be relevant to PQ poisoning. However, it remains to be determined if Sirtuin 1 analysis is more sensitive to PQ-induced MODS when compared with miR-219-5p, which needs to be further study in the research.

Conclusion

In summary, the results of this study indicated that serum miR-219-5p expression level was decreased, serum TLR4 expression level was increased, and a negative correlation was observed between them in MODS patients induced by acute PQ poisoning. Additionally, serum miR-219-5p level might be a new diagnostic and prognostic factor for MODS patients induced by PQ poisoning. Moreover, TLR4 was demonstrated as a direct target gene of miR-219-5p and significant correlation of miR-219-5p and TLR4 with inflammatory cytokines was found, indicating that miR-219-5p may be involved in the pathogenesis of MODS by regulating the TLR4-related inflammatory response.

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Ethics approval and consent to participate

A signed written informed consent was obtained from each patient and the experimental procedures were all in accordance with the guideline of the Ethics Committee of Qingdao Jiaozhou Central Hospital (#JZCHh20111025).

Consent for publication

Written informed consent for publication was obtained from each participant.

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Availability of data and material

All data generated or analyzed during this study are included in this published article.

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