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Predictive Value of Mir-155 and Mir-203 in Children with Chronic Gastritis

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Abstract

This study inquired into the predictive value of miR-155 and miR-203 in children with chronic gastritis. In this study, 86 children with chronic gastritis treated in our hospital from February 2015 to May 2017 were collected as the observation group (OG), and another 73 healthy children who underwent physical examination in our hospital during the same period were collected as the control group (CG). QRT-PCR was employed to detect the profile of related indexes in serum of the two groups. ROC curve was applied to analyze the diagnostic value of miR-155 and miR-203 in children with chronic gastritis and Helicobacter pylori (Hp). Multivariate Logistic regression was adopted to analyze the risk factors of Hp infection in children with chronic gastritis. Pearson test was used to analyze the correlation between serum miR-155 and miR-203 and inflammatory indexes IL-6 and TNF-α in the OG. The miR-155 was remarkably higher while the miR-203 was lower in the OG than in the CG. According to the ROC curve, the area under the curve (AUC) of miR-155, miR-203 and their combination in diagnosing chronic gastritis in children was 0.789, 0.703 and 0.833 respectively, and their AUCs in diagnosing Hp infection in children was 0.776, 0.775 and 0.812 respectively. Family history of digestive tract diseases, IL-6, family size, miR-155 and miR-203 were independent risk factors for Hp infection in children with chronic gastritis in children. A negative correlation was identified between miR-155 and miR-203, between miR-203 and IL-6 expression, and between miR-203 and TNF-α expression, while a positive correlation was found between miR-155 and IL-6 expression and between miR-155 and TNF-α expression. In conclusion, miR-155, miR-203, miR-155 and miR-203 may be potential diagnostic indicators of chronic gastritis and Hp infection in children.

Keywords: miR-155; miR-203; chronic gastritis in children; Helicobacter pylori

Introduction

Chronic gastritis is one of the main causes of abdominal pain in children, which is generally caused by bacteria, toxins and poor diet, but till now, its specific pathogenesis in children remains elusive (Islek et al, 2016; Meliţ and Mărginean,2019). Helicobacter pylori (Hp) infection is believed to be the main cause of most gastritis, and if the infection is not well controlled, it will further develop to gastrointestinal ulcer and gastric cancer (Ali et al, 2018). Worldwide, the prevalence of Hp has exceeded 50%, and it is more pervasive in developing countries. In addition, Hp infection is tricky to detect in the early stage as there are no specific symptoms, while will only show in the later stages when symptoms like abdominal pain, vomiting, dyspepsia and hematemesis may occur (Gheibi et al, 2016; Hagag and Amin,2018). Generally, patients get infected with Hp in their childhood, and it will be a life-lasting disease if the bacteria are not actively treated (Selimoglu et al, 2014). Therefore, the diagnosis of gastritis and Hp infection in children should be conducted as soon as possible, so that the corresponding treatment can be timely carried out to control the disease and prevent its further progression.

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At present, the main clinical detection approaches are gastroscopy and pathological examination, which will cause certain discomfort for patients, plus that these invasive tests are often difficult to implement in children, so the search for some better non-invasive diagnostic indicators are urgent (Meliţ et al, 2019; Kalach and Bontems,2017). MicroRNA is an endogenous non-coding RNA, which can regulate the expression of target mRNA after transcription, and further regulate the occurrence and development of some diseases. Some microRNAs also participate in the regulation of gastrointestinal diseases including chronic gastritis, gastric cancer, and intestinal cancer, and show differential expression compared with the normal population (Vidal et al, 2016). Of these, miR-155 and miR-203 have been found to have a certain relationship with some gastrointestinal diseases (Wan et al, 2016; Ng and Chan,2015). Research by Imaoka et al. (Imaoka et al, 2016) revealed that the decreased miR-203 in gastric cancer patients was significantly associated with higher staging, distant metastasis and poor prognosis, and may be used as a non-invasive biomarker to predict prognosis and metastasis. Liu et al. (Liu et al, 2018) reported that miR-155 could improve the condition of colitis rats. But we still don't know the expression and value of miR-155 and miR-203 in children with chronic gastritis.

Therefore, this study hopes to investigate the diagnostic and predictive value of miR-155 and miR-203 in children with chronic gastritis in children, so as to provide direction and basis for clinical practice.

1 Methods and materials

1.1 Patient information

Eighty-six children with chronic gastritis treated in our hospital from February 2015 to May 2017 were selected and included in the OG, among which 49 were male and 37 were female, with an average age of (7.3±2.6) years old. In addition, 73 healthy children who underwent physical examination in our hospital during the same period were collected as the CG, including 47 males and 26 females, with an average age of (6.8±2.4) years old. This study was approved by the Medical Ethics Committee, and all patients and their legal guardians were informed about the details and signed the informed consent.

1.2 Inclusion and exclusion criteria

Inclusion criteria: All the patients were diagnosed with chronic gastritis by gastroscopy and pathology(Liu et al, 2018), and a 14C-urea breath test was performed. The participants ranged in age from 1 to 18 years, cooperated with the treatment and follow-up, with complete clinical data.

Exclusion criteria: Children with other inflammatory diseases, innate immune deficiency, or other gastrointestinal diseases.

1.3 Main kits and instruments

PCR instrument (ABI Corporation, 7500), TRIzol reagent (Invitrogen Corporation, 15596018), reverse transcription + PCR kit TransScript miRNA First-Strand cDNA Synthesis SuperMix (TransGen Biotech, Beijing, China, ER601-01, AT351-01). All primers, which are shown in Table 1, were designed and synthesized by Sangon Biotechnology Co., Ltd., Shanghai, China.

1.4 Sample collection and testing

5 mL of venous blood of all the participants was collected and placed in coagulation tubes, and the serum was collected by centrifuge (3000 xg at 4℃ for 10 min). Part of the serum was collected in RNA-free EP tubes and stored at -80℃. miR-155 and miR-203 levels were detected by RT-qPCR. The total RNA was extracted from the serum by TRIzol kit, and its purity, concentration and integrity were examined by UV spectrophotometer and agarose gel electrophoresis. The total RNA was then reverse-transcribed using TransScript ®miRNA RT Enzyme Mix and 2 × TS miRNA Reaction Mix, with the operating procedures strictly following the manufacturer's kit instructions. Then came the PCR amplification, with the reaction system as follows: cDNA: 1 μL, upstream and downstream primers: 0.4 μL each, 2×TransTaq® Tip Green qPCR SuperMix: 10 μL, Passive Reference Dye (50×): 0.4 μL, and finally added ddH2O to achieve 20 μL. PCR reaction conditions: pre-denaturation at 94 °C for 30 s, denaturation at 94 °C for 5 s, annealing at 60 °C for 30 s, totaling 40 cycles. Three replicate wells were set for each sample and the experiment was performed a total of three times. With U6 as the internal reference, $2^{-\triangle\triangle ct}$ was employed to analyze the data.

1.5 Outcome measures

Main outcome measures: miR-155 and miR-203 expression levels were compared between the OG and the CG, and their expression levels in children with Hp infection and those without Hp infection were compared. ROC curve was used to analyze the

diagnostic and predictive value of miR-155 and miR-203 in children with chronic gastritis and those with Hp infection.

Secondary outcome measures: the clinical data of patients and healthy controls were compared. The independent risk factors of Hp infection in children with gastritis were analyzed by multivariate Logistic regression, and the correlation between serum miR-155, miR-203 and inflammatory indexes IL-6 and TNF-α in the OG was analyzed by Pearson test.

1.6 Statistical methods

SPSS20.0 (SPSS, Chicago, USA), a medical statistical analysis software, was employed for statistical analysis of the collected data, and the image rendering was performed by GraphPad Prism 7 (GraphPad Software, San Diego, USA). The counting data were described in percentage (%) and analyzed by a chi-square test (expressed as χ^2). The measurement data, all normally distributed, were expressed as mean ± standard deviation (Mean±SD), and the inter-group comparisons were performed by the independent t-test. Pearson test was applied to analyze the relationship between serum miR-155, miR-203 and inflammatory indexes IL-6, TNF-α in the OG after treatment. ROC evaluated the ability of miR-155 and miR-203 to diagnose and predict Hp infection in children with chronic gastritis. Multivariate Logistic regression analysis was used to analyze the independent risk factors of Hp infection in children with gastritis. P<0.05 was regarded as statistically significant.

2 Results

2.1 Clinical data

By comparing the clinical data of the two groups, we found that there were little differences in gender, age, BMI, passive smoking, residence, family history of digestive tract diseases and family size between the two groups, but there were evident differences in IL-6 and TNF-α. (Table 2)

2.2 Diagnostic capacity of miR-155 and miR-203 in children with chronic gastritis

MiR-155 showed remarkably higher level in the OG (1.32±0.43) than in the CG (0.94±0.22), and miR-203 presented notably lower level in the OG (0.81±0.26) than in the CG (1.01±0.28). Through ROC curve analysis of the diagnostic capacity of miR-155 and miR-203 in chronic gastritis in children, it was found that the AUC of miR-155 was 0.789, 95%CI: 0.718-0.861, the AUC of miR-203 was 0.703, 95%CI : 0.621-0.786, and the AUC of the joint detection was 0.833, 95%CI: 0.768-0.897. (Table3, Figure 1)

2.3 Diagnostic capacity of miR-155 and miR-203 in children with Hp infection

Children in the OG were classified into an infection group and a non-infection groups based on whether they had Hp infection or not. By comparing the miR-155 and miR-203 expression between the two groups, it was found that the miR-155 in the infection group (1.49±0.41) was remarkably higher than that in the non-infection group (1.11±0.35), and the miR-203 in the infection group (0.70±0.23) was evidently lower than that in the non-infection group (0.94±0.23). According to ROC curve analysis of the ability of miR-155 and miR-203 to diagnose Hp infection in children with chronic gastritis, the AUC of miR-155 was 0.776, 95%CI was 0.676-0.875, the AUC of miR-203 was 0.775, 95%CI was 0.676-0.874, and the AUC of joint detection was 0.812, 95%CI was 0.721-0.903. (Table 4, Figure 2)

2.4 Univariate analysis of Hp infection in children

The clinical data of children in the infection and non-infection groups were collected for univariate analysis. It turned out that, rather than gender, BMI, passive smoking, residence, TNF-α, epigastric or periumbilical pain, nausea and vomiting, there were marked differences in age, family history of digestive tract disease, IL-6, course of disease, gastrointestinal bleeding, family size, miR-155 and miR-203 between the two groups. (Table 5)

2.5 Multivariate analysis of Hp infection in children

We included the indicators with differences in univariate analysis into the assignment (see Table 6 of the assignment table), and then chose forward: LR for multi-factor logstic regression analysis. The results showed that age, course of disease, and gastrointestinal bleeding were not independent risk factors for Hp infection in children, but family history of digestive tract diseases (OR: 12.761, 95%CI: 2.851-31.72), IL-6 (OR: 2.644, 95%CI: 1.382-5.059), family size (OR: 13.568, 95%CI: 2.638-38.802), miR-155 (OR: 8.778, 95%CI: 1.488-29.213), miR-203 (OR: 0.015, 95%CI: 0.001-0.742) were. (Table 7)

2.6 Correlation between miR-155 and miR-203 and inflammation indicators

The relationship between miR-155, miR-203 and IL-6 and TNF-α of inflammatory indicators in the OG was analyzed by Pearson correlation. It was found that miR-155 was negatively correlated with

miR-203, miR-155 was positively correlated with IL-6, miR-155 was positively correlated with TNF-α, miR-203 was negatively correlated with IL-6, and miR-203 was negatively correlated with TNF-α. **(Figure 3)**

3 Discussion

With a long infection cycle and high infection, Hp infection has always been a health and safety issue that needs to be addressed worldwide, plaguing large populations (Hooi et al, 2017). It will give rise to various gastric diseases, ranging from gastritis to gastric cancer (Jang et al, 2017). In addition, Hp can exacerbate the bacterial load and the degree of gastritis in gastritis patients, promote the generation of inflammatory factors, and inhibit the proliferation of gastric epithelial cells, leading to the aggravation of gastritis (Lv et al, 2018; Melchiades and Zabaglia,2017). Therefore, it is high time to explore the ways for early diagnosis of gastritis and Hp in children, so that the corresponding treatment can be carried out to curb the disease progression.

In this study, we first compared miR-155 and miR-203 levels in the OG and the CG, and found that the miR-155 in the OG was dramatically higher while the miR-203 level was noticeably lower compared with the CG. Then we used ROC curve to detect the diagnostic value of the two in children with chronic gastritis. It was found that the AUC of miR-155 was 0.789, and the optimal specificity and sensitivity were 94.52% and 51.50%, while the AUC of miR-203 was 0.703, and the optimal specificity and sensitivity were 74.72% and 60.27%, and the AUC of the joint detection was 0.833, and the optimal specificity and sensitivity were 75.58% and 83.56%. Meanwhile, we noticed that there was a great difference in specificity and sensitivity between them, so we conducted a joint detection to find that the AUC of joint detection was better than that of single detection. Previous studies have shown that gastritis patients infected with Hp are more likely to develop gastric cancer (Lv et al, 2018; Melchiades and Zabaglia,2017). Therefore, according to whether the children in the OG were infected with Hp or not, we divided them into infection group and non-infection groups, and identified that the infection group showed dramatically higher miR-155 level and notably lower miR-203 level compared with the non-infection group. In the study of Cortés-Márquez et al. (Cortés-Márquez et al, 2018), miR-155 expression was also compared between gastritis children infected with Hp with those uninfected, and it was found that the miR-155 level of infected children was 79.4 times that of uninfected ones, which was also similar to our study results? The differential expression between the two groups also suggested that we might be able to predict infection by observing miR-155 and miR-203. To verify our hypothesis, we employed ROC curve to find that the AUC of miR-155 was 0.776, while the AUC miR-203 was 0.775, and that of joint detection was 0.812, further suggesting that miR-155 and miR-203 may be potential diagnostic indicators of chronic gastritis and Hp infection in children.

To further understand the factors that cause children to be infected with Hp, we carried out multivariate logstic regression analysis. The results showed that family history of digestive tract diseases, higher IL-6, more than three family members, higher miR-155 and lower miR-203 were independent risk factors for Hp infection in children with chronic gastritis. In previous studies on Hp infection risk factors, it was mentioned that family history of digestive tract diseases, larger family size and higher IL-6 were risk factors for Hp infection (Smith et al, 2018; Piao and Lee,2016), while in our study, we added higher miR-155 and lower miR-203 in the risk factor list for Hp infection in children.

Then we tested the correlation between miR-155 and miR-203 expression in the OG by Pearson correlation analysis and found that the two were negatively correlated. Inflammatory response is an important response to gastritis, which often reflects the severity of the patient's condition, and the inflammatory response is also related to carcinomatous change. In a study by Raish et al. (Raish et al, 2018), it was found that when the condition of rats with gastric ulcer was alleviated and gastric inflammation was controlled, the inflammation indicators TNF-α and IL-6 decreased accordingly. Therefore, for the sake of understanding the relationship between miR-155 and miR-203 and the degree of inflammatory response, we utilized Pearson correlation analysis to observe the correlation between miR-155 and miR-203 with TNF-α and IL-6. It was found that miR-155 was positively correlated with TNF-α and IL-6, and miR-203 was negatively correlated with TNF-α and IL-6. This also suggests that as the inflammatory response of children increases, miR-155 expression will increase, and miR-203 expression will decrease. Marques-Rocha et al. (Marques-Rocha et al, 2018) mentioned in their study that miR-155 is proinflammatory. While miR-203 has been reported to inhibit inflammation and repair inflammatory damage in many diseases (Wang et al, 2018; Li and Yu,2019).

However, this study also has some shortcomings.

First of all, our study did not include some therapeutic factors for discussion, so the influence of these factors on the study remains unknown. Secondly, the expression of miR-155 and miR-203 of patients was only detected at the time of admission, but had not tested afterwards, so the difference in their expression with illness changes need to be further studied in the follow-up study. Finally, through the correlation analysis, we identified a certain relationship between miR-155 and miR-203, and certain correlations of miR-155 and miR-203 with TNF-α and IL-6, but we have not yet carried out basic experiments to explore the specific relationship and influencing mechanism between them. Therefore, we hope to supplement corresponding basic research in subsequent studies to improve our findings.

To sum up, miR-155, miR-203, miR-155 and miR-203 may be potential diagnostic indicators of chronic gastritis and Hp infection in children, family history of digestive tract diseases, IL-6, family size, miR-155 and miR-203 are independent risk factors for Hp infection in children with chronic gastritis.

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Tables and Figures

Table 1. **Primer sequences**

Gene	Upstream primer	Downstream primer
miR-155	5'-TTAATGCTAATCGTGATAGGGGT-3'	5'-CCTTAAAACTCCACTAGAAGCA-3'
miR-203	5′-GTATTCGCACTGGATACGACCGACC-3′	5′-TGCGCTAACAGTCTACAGCCA-3′
U6	5′-CTCGCTTCGGCAGCACA-3′	5′-AACGCTTCACGAATTTGCGT-3′

Table 2. **Clinical data**

		OG (n=86)	CG (n=73)	χ^2/t	P
Gender					
	Male	49 (56.98)	47 (64.38)		
	Female	37 (43.02)	26 (35.62)	0.905	0.341
Age (years old)		7.3±2.6	6.8±2.4		
BMI (kg/m^2)		17.33±2.39	17.22±2.15		
Passive smoking					
	Yes	21 (24.42)	18 (24.66)		
	No	65 (75.58)	55 (75.34)	0.001	0.972
Place of residence					
	Urban	72 (83.72)	60 (82.19)		
	Rural	14 (16.28)	13 (17.81)	0.065	0.798
Family history of digestive tract diseases					
	Yes	21 (24.42)	13 (17.81)	1.026	0.311
	No	65 (75.58)	60 (82.19)		
Family size					
	>3	31 (36.05)	21 (30.14)		
	≤3	55 (63.95)	52 (69.86)	0.621	0.431
IL-6 (ng/L)		5.83±1.54	4.14±0.83	8.396	<0.001
TNF-α (μg/L)		54.14±6.03	26.36±4.26	32.984	<0.001
Course of disease (month)		15.7±7.3			
Clinical symptoms					
	Epigastric or periumbilical pain	31 (36.05)			
	Nausea and vomiting	27 (31.40)			
	Gastrointestinal bleeding	24 (27.91)			
Hp infection					
	With	48 (55.81)			
	Without	38 (44.19)			

Note: BMI: Body Mass Index; IL-6: Interleukin-6; TNF-α: Tumor Necrosis Factor α

Table 3. **ROC data for diagnosis of gastritis in children**

Indicators	AUC	95%CI	Specificity	Sensitivity	Youden index	Cut-off
miR-155	0.789	0.718-0.861	56.98%	94.52%	51.50%	<1.253
miR-203	0.703	0.621-0.786	74.72%	60.27%	34.99%	>0.960
Joint detection	0.833	0.768-0.897	75.58%	83.56%	59.14%	> 0.453

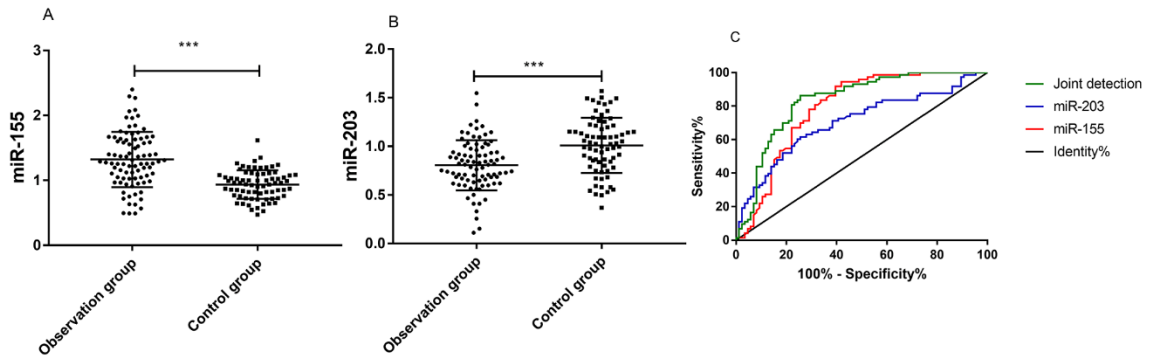

Figure 1. **Expression and diagnostic ROC curve of miR-155 and miR-203 in children with chronic gastritis**

A. The miR-155 in the OG was dramatically higher than that in the CG (t = 6.828, P<0.001). **B.** The miR-203 in the OG was obviously lower than that in the CG (t = 4.666, P<0.001). **C.** The AUC of miR-155 was 0.789, when the cutoff value was 1.253, the optimal specificity and sensitivity were 94.52% and 51.50%, and the Youden index was 51.50%; the AUC of miR-203was 0.703, when the cutoff value was 0.960, the optimal specificity and sensitivity were 74.72% and 60.27%, and the Youden index was 34.99%; the AUC of the joint detection was 0.833, when the cutoff value was taken to 0.453, the optimal specificity and sensitivity were 75.58% and 83.56%, and the Youden index was 59.14%. *** indicated P<0.001.

Table 4. **ROC data for diagnosis of Hp infection**

Indicators	AUC	95%CI	Specificity	Sensitivity	Youden index	Cut-off
miR-155	0.776	0.676-0.875	68.75%	81.58%	50.33%	<1.313
miR-203	0.775	0.676-0.874	70.83%	71.05%	41.88%	>0.821
Joint detection	0.812	0.721-0.903	72.92%	78.95%	51.87%	>0.423

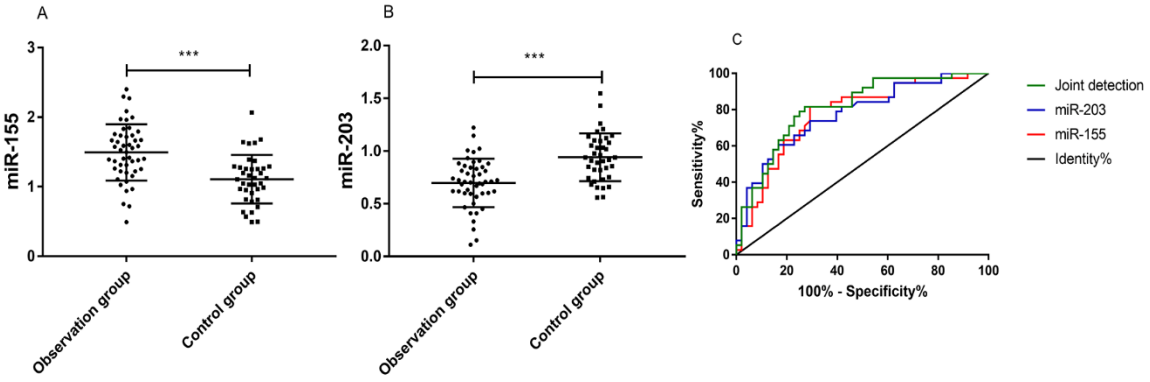

Figure 2. **Diagnostic capacity of miR-155 and miR-203 in children with Hp infection**

A. miR-155 showed markedly higher expression in the infection group than in the non-infection group (t = 4.549, P<0.001). **B.** miR-203 presented notably lower expression in the infection group than in the non-infection group (t = 4.806, P<0.001). **C.** The AUC of miR-155 was 0.776, and the optimal specificity and sensitivity were 68.75% and 81.58%, with the Youden index of 50.33% when the cutoff value was 1.313; the AUC of miR-203was 0.775, when the cutoff value was 0.821, the optimal specificity and sensitivity were 70.83% and 71.05%, and the Youden index was 41.88%; the AUC of the joint detection was 0.812, when the cutoff value was taken to 0.423, the optimal specificity and sensitivity were 72.92% and 78.95%, and the Youden index was 51.87%. * indicated P<0.001.

Table 5. **Univariate analysis**

		Infection group (n=48)	Non-infection group (n=38)	χ^2/t	P
Gender					
	Male	29 (60.42)	20 (52.63)		
	Female	19 (39.58)	18 (47.37)	0.524	0.498
Age (years old)		7.9±2.8	6.4±2.0	2.786	0.007
BMI (kg/m^2)		17.47±2.54	17.14±2.19	0.635	0.527
Passive smoking					
	Yes	14 (29.17)	7 (18.42)		
	No	34 (70.83)	31 (81.58)	1.327	0.249
Place of residence					
	Urban	39 (79.59)	33 (89.19)		
	Rural	9 (20.41)	5 (10.81)	0.487	0.485
Family history of digestive tract diseases					
	Yes	17 (35.42)	4 (10.53)	7.120	0.008
	No	31 (64.58)	34 (89.47)		
IL-6 (ng/L)		6.41±1.27	5.11±1.56	4.261	<0.001
TNF-α (μg/L)		54.69±7.27	53.43±3.91	0.963	0.338
Course of disease (month)		18.37±6.52	12.42±6.83	4.115	<0.001
Clinical symptoms					
	Epigastric or periumbilical pain	12 (25.00)	9 (23.68)	0.020	0.888
	Nausea and vomiting	13 (27.08)	14 (36.84)	0.938	0.333
	Gastrointestinal bleeding	18 (37.50)	6 (15.79)	4.969	0.026
Family size (cases)					
	>3	23 (47.92)	8 (21.05)		
	≤3	25 (52.08)	30 (78.95)	6.640	0.010
miR-155		1.49±0.41	1.11±0.35	4.549	<0.001
miR-203		0.70±0.23	0.94±0.23	4.806	<0.001

*Table 6***. Assignment table**

Factors	**Assignment**
Age	Raw data analysis for continuous variables.
Family history of digestive tract diseases	Yes=1, no=0
IL-6	Raw data analysis for continuous variables.
Course of disease	Raw data analysis for continuous variables.
Gastrointestinal bleeding	Yes=1, no=0
Family size	>3=1，≤3 =0
miR-155	<1.435=1, ≥1.435=0
miR-203	<4.245=1, ≥4.245=0
Hp infection	With=1, without=0

Table 7. **Multivariate analysis of survival**

Factors	B	S.E,	Wals	Sig.	Exp (B)	Exp (B) 95% C.I.	
						Lower bound	Upper bound
Family history of digestive tract diseases	3.125	1.06	8.693	0.003	12.761	2.851	31.72
IL-6	0.972	0.331	8.621	0.003	2.644	1.382	5.059
Family size	3.201	1.138	7.907	0.005	13.568	2.638	38.802
miR-155	2.82	1.236	5.204	0.023	8.778	1.488	29.213
miR-203	-4.205	1.993	4.452	0.035	0.015	0.001	0.742

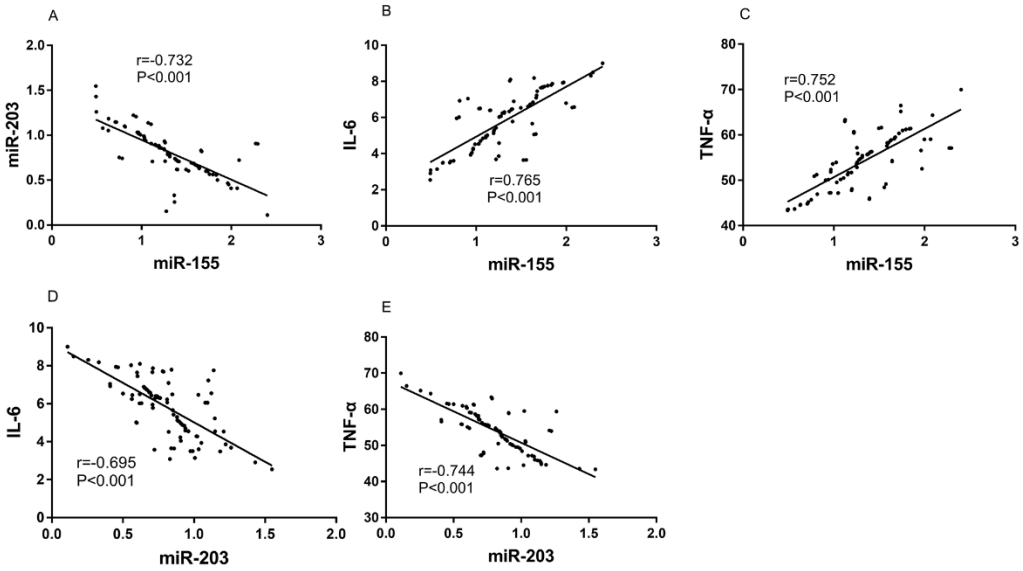

Figure 3. **Correlation of miR-155 and miR-203 with inflammation indicators**

A. miR-155 was negatively correlated with miR-203 (r = -0.732, P<0.001). **B.** miR-155 was positively correlated with IL-6 (r = 0.765, P<0.001). **C.** miR-155 was positively correlated with TNF-α (r = 0.752, P<0.001). **D.** miR-203 was negatively correlated with IL-6 (r = -0.695, P<0.001). **E.** miR-203 was negatively correlated with TNF-α (r = -0.744, P<0.001).