

Expression of miR-495 and miR-326 in peripheral blood of rheumatoid arthritis patients and its significance

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Abstract. The aim of the present study was to investigate the expression of microRNA (miR)-495 and miR-326 in the peripheral blood of patients with rheumatoid arthritis (RA). A total of 107 RA patients, admitted to the Yidu Central Hospital of Weifang (Weifang, China) from February 2016 to February 2019, and 112 healthy subjects, who underwent physical examination during the same period, were selected as the research subjects for prospective analysis. The RA patients served as the study group and the healthy subjects as the control group. The expression levels of miR-495 and miR-326 in the peripheral blood of the two groups of subjects were compared. The association between miR-495 and miR-326 with RA clinical pathology and the diagnostic value of miR-495 and miR-326 for RA were analyzed. In the study group, miR-495 expression was significantly higher than that in the control group, and miR-326 expression was significantly lower than that in the control group ($P < 0.001$). miR-495 and miR-326 combined diagnosis showed good predictive value for the occurrence of RA ($P < 0.001$) and was closely related to RA clinical pathology ($P < 0.001$). After treatment, miR-495 expression was significantly decreased in the study group, whereas miR-326 expression was significantly increased ($P < 0.001$). Pearson's correlation coefficient analysis showed that rheumatoid factor (RF) was positively correlated with miR-495 expression and negatively correlated with miR-326 expression ($P < 0.001$). In conclusion, miR-495 was highly expressed in patients with RA, whereas miR-326 was poorly expressed in RA patients, and the combined detection of miR-495 and miR-326 has good diagnostic value for RA.

Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease. Currently, RA is considered as an autoimmune disease in clinical practice (1). The main manifestations of RA are those of symmetrical, chronic and progressive polyarthritis. As the development of the disease continues, the articular cartilage and capsule will eventually be destroyed (2). RA is a very common disease with high incidence in clinic, which has been on the rise due to the aging of population in recent years (3,4). RA can be accompanied by various degrees of dysfunction in the early stage and skeletal muscle atrophy in the late stage, which is one of the major causes of labor loss and disability (5). At present, there is no specific treatment for RA in clinical practice, and the main focus has been on the comprehensive treatment of inflammation and sequelae (6). For the treatment of RA, clinical requirements include controlling inflammation of joints and tissue, maintaining normal joint function and repairing the damaged joints. Comprehensive treatment can achieve certain efficacy for most patients in the early stage (7). However, there are still some patients whose bone tissue is irreversibly damaged when the disease develops through the middle and late stages. At this time, only artificial joint replacement, arthroplasty and other operations can achieve the therapeutic purpose, and in more serious cases, only amputation can prevent further necrosis of the bone tissue (8). Therefore, the treatment of RA is particularly important at the early stage of the disease. At present, the diagnosis for RA is relatively complicated in clinical practice. As RA has no special symptoms and the specificity of imaging examination is low in clinical practice, complicated series of examinations are usually required to confirm the occurrence of RA (9), which are not conducive to early screening of RA in clinical practice. Therefore, in order to find an effective and convenient diagnostic method, researchers are constantly exploring various markers of RA (10).

With in-depth research, the clinical application of microRNA (miR) has gradually become a hot research topic in various diseases. mRNA, as a non-coding short-chain RNA with a length of about 22 nt, is mainly used to inhibit the translation and transcription process of target genes by binding the untranslated regions (UTRs) at the 3' end of target gene mRNA downstream, achieving the effect of changing the expression of target genes (11). miR-495 is

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located on chromosome 14q32.31 and was recently discovered to be an extremely important derivative of the miR family (12). Yang *et al* (13) proved that miR-495 activates NF- κ B signaling pathway to inhibit chondrocyte apoptosis in osteoarthritis. miR-326 can inhibit the expression of NP1, an important gene of MAPK signaling pathway, by combining with its 3'UTR, promoting tumor cell apoptosis and inhibiting proliferation, invasion and metastasis (14). Wang *et al* (15) explored the role of miR-326 in osteosarcoma and showed that miR-326 can regulate the role of NOB1 as an oncogene in osteosarcoma. Both miR-495 and miR-326 are closely related to bone tissue growth, metabolism or bone tissue diseases. However, there is no study on the expression and significance of miR-495 and miR-326 in RA. In the present study, it was assumed that miR-495 and miR-326 may also participate in the occurrence and development of RA, and this was confirmed through experimental analysis, providing a new reference for the clinical diagnosis and treatment of RA in the future.

Subjects and methods

General data. A total of 107 RA patients, admitted to the Yidu Central Hospital of Weifang (Weifang, China) from February 2016 to February 2019, and 112 healthy subjects, who underwent physical examination during the same period, were selected as the research subjects for prospective analysis. The RA patients served as the study group, including 59 males and 48 females, 52-71 years of age, with a mean age of 60.5 \pm 7.7 years. The control group consisted of 64 males and 48 females, 50-70 years of age, with a mean age of 59.2 \pm 8.3 years. The study was approved by the Ethics Committee of Yidu Central Hospital of Weifang and signed written informed consents were obtained from the patients and/or guardians.

Inclusion and exclusion criteria

Inclusion criteria. Patients were 30-75 years of age. The RA clinical diagnostic criteria were as follows: i) Morning stiffness lasted for 1 h (every day) with a course of at least 6 weeks; ii) there were \geq 3 arthroncus for at least 6 weeks; iii) swelling of wrist, metacarpal finger and proximal knuckle lasted for at least 6 weeks; iv) symmetrical joint swelling lasted for at least 6 weeks; v) there were subcutaneous nodules; vi) changes were observed on the hand X-ray scans; and vii) rheumatoid factor (RF) was positive (titer $>$ 1:20). The patients met the above 7 diagnostic criteria and were diagnosed with typical RA. If $>$ 4 criteria were satisfied, the patient was diagnosed with RA. After diagnosis, the patients received follow-up treatment in the Yidu Central Hospital of Weifang. Additionally, in the study were included patients who had not been treated with any antibiotics within 3 months before admission and had a complete medical record. Signed written informed consents were obtained from all patients or their immediate family members.

Exclusion criteria. Patients with tumor, cardiovascular or cerebrovascular diseases, other autoimmune or infectious diseases; patients with liver or renal insufficiency due to organ failure; patients with drug allergy; patients with physical disabilities

who had been bedridden for a long time and could not take care of themselves; patients with low treatment compliance due to mental disorders; transferred patients; pregnant women; patients with history of bone tissue surgery.

Inclusion criteria for the control group. Subjects who underwent physical examination in the Yidu Central Hospital of Weifang, whose all test results were normal, with no medical history, who agreed to cooperate and participate in the study.

Research methods

Treatment methods. In strict accordance with RA clinical treatment guidelines (16), non-steroidal anti-inflammatory drugs were used as the first-line treatment scheme, and slow acting anti-rheumatic drugs were used as second-line drugs. A low dose ($<$ 10 mg/day) of glucocorticoids was given to patients with rheumatoid vasculitis, severe RA or when the treatment was ineffective. Immune purification and hematopoietic stem cell transplantation were also carried out for patients who did not respond to the aforementioned treatment. In cases when the illness and serious joint dysfunction could not be effectively controlled by the medical treatment, the patients were treated by surgery (release of carpal tunnel syndrome, repair after tendon tear, synovial resection, or even joint replacement for severe cases), according to the patient's medical condition.

Detection methods. A total of 4 ml of fasting venous blood were collected from all subjects before treatment (before any treatment course) and after treatment (after all treatment courses were completed), and the samples were placed at room temperature for 30 min. Next, centrifugation was carried out for 10 min (1,050 \times g, 4 $^{\circ}$ C) to obtain the upper serum, which was stored in a refrigerator at -80 $^{\circ}$ C for further analysis. The expression of miR-495 and miR-326 in the serum was detected by RT-qPCR. Total RNA was extracted from the collected serum using TRIzolTM LS reagent (10296010; Invitrogen: Thermo Fisher Scientific, Inc.) and the purity, concentration and integrity of total RNA was detected by UV spectrophotometer and agarose gel electrophoresis. PrimeScriptTM RT reagent (RR036A; Takara Bio, Inc.) and TB Green[®] Fast qPCR Mix (RR430A; Takara Bio, Inc.) were used for the reverse transcription of total RNA, according to the manufacturer's protocol. Next, PCR amplification was carried out. The PCR reaction system was as follows: 1 μ l of cDNA, 0.4 μ l of upstream and downstream primers, 10 μ l of 2X TransTaq[®] Tip Green qPCR SuperMix (AQ141-01; Beijing Transgen Biotech Co., Ltd.), 0.4 μ l of Passive Reference Dye (50X) (75768 500 UL; Shanghai Yanqi Biotechnology Co., Ltd.), and finally ddH₂O was added for a final volume of 20 μ l. The PCR reaction conditions were as follows: Pre-denaturation at 94 $^{\circ}$ C for 30 sec, denaturation at 94 $^{\circ}$ C for 5 sec, annealing at 60 $^{\circ}$ C for 30 sec, with a total of 40 cycles. Each sample was provided with 3 repeated wells, and the experiment was carried out 3 times. U6 was used as internal reference and 2^{- $\Delta\Delta$ C_q} method was used for data analysis (17). The primer sequences of miR-495, miR-326 and U6 are presented in Table I.

Observation indicators

Main indicators. The expression of miR-495 and miR-326 in the peripheral blood of the two groups of subjects, the

Table I. Primer sequences of miR-495, miR-326 and U6.

Genes	Upstream	Downstream
miR-495	5'-TCCGATTCTTCACGTGGTAC-3'	5'-GTGCAGGGTCCGAGGT-3'
miR-326	5'-GCAGCACGCTAGGTAGTTTCC-3'	5'-TATCGTTGTTCTCCACTCCTTGAC-3'
U6	5'-TCCGATCGTGAAGCGTTC-3'	5'-GTGCAGGGTCCGAGGT-3'

miR, microRNA.

diagnostic value of miR-495 and miR-326 for RA, and the changes of miR-495 and miR-326 expression in the study group before and after treatment were observed.

Secondary indicators. The predictive value of miR-495 combined with miR-326 for complications during RA treatment, the expression of miR-495 and miR-326 in RA patients with different clinical pathology, and the correlation between miR-495 and miR-326 with RF were observed.

Statistical analysis. The experimental data were analyzed by SPSS 24.0 statistical software (Shanghai Yuchuang Network Technology Co., Ltd.) and all graphical results were visualized by GraphPad Prism 8 (Shenzhen SoftHead Software Technology Co., Ltd.). Counting data were expressed in the form of rate, and chi-square test was used for their comparison between groups. Measurement data were expressed as the mean \pm SD, and t-test was used for inter-group comparisons, whereas one-way analysis of variance, with LSD post hoc test, for multi-group comparisons. The diagnostic value of miR-495 and miR-326 was determined by ROC curve analysis. Multivariate Logistic analysis was used to calculate the independent variable joint model and then the ROC curve was analyzed. Pearson's correlation coefficient analysis was used for the investigation of the correlation between miR-495 and miR-326 with RF. $P < 0.050$ was considered to indicate a statistically significant difference.

Results

General data comparison. Age, body mass index, white blood cells, red blood cells, platelets, fasting blood glucose, systolic blood pressure, diastolic blood pressure, sex, smoking history, drinking history, exercise, residence, nationality and family history were compared between the two groups and the differences were not statistically significant ($P > 0.050$) (Table II).

Comparison of miR-495 and miR-326 expression between the two groups. The expression of miR-495 in the study group was significantly higher than that in the control group ($P < 0.001$), and miR-326 expression in the study group was significantly lower than that in the control group ($P < 0.001$) (Fig. 1).

Diagnostic value of miR-495 and miR-326 for RA. The results of the ROC curve analysis revealed that when the cut-off value was 6.05, the diagnostic sensitivity and specificity of miR-495 for RA were 55.14 and 90.18%, respectively. When the cut-off

value was 5.11, the diagnostic sensitivity and specificity of miR-326 for RA were 78.50 and 75.89%, respectively. miR-495 and miR-326 were taken as two independent variables, and binary Logistic regression analysis was carried out to obtain the Logistic regression model. $\text{Logit}(P) = 0.753 + (-0.739 \times \text{miR-495}) + (-0.313 \times \text{miR-326})$. When the cut-off value was 0.55, the sensitivity and specificity of the model in diagnosing RA were 77.57 and 87.50%, respectively (Fig. 2 and Table III).

Changes of miR-495 and miR-326 expression in the study group after treatment. After treatment, miR-495 expression was significantly decreased ($P < 0.001$) compared to that before treatment, whereas miR-326 expression was significantly increased ($P < 0.001$), in the study group (Fig. 3).

Expression difference of miR-495 and miR-326 in RA patients with different clinical pathology. The expression of miR-495 had no significant difference among patients with different functional activity ($P > 0.050$). However, miR-495 expression was closely related to the course of the disease, the stage of pathological changes, the disease progression and the clinical manifestations ($P \leq 0.050$). miR-326 expression was closely related to the course of the disease, the pathological activity stage, the disease progression, the functional activity and the clinical manifestations ($P < 0.050$) (Table IV).

Correlation analysis of miR-495 and miR-326 with RF. Pearson's correlation coefficient analysis showed that miR-495 was positively correlated with RF ($r = 0.579$, $P < 0.001$), whereas miR-326 was negatively correlated with RF ($r = -0.533$, $P < 0.001$) (Fig. 4).

Predictive value of miR-495 combined with miR-326 for complications during treatment. In the study group, 21 patients had complications during treatment, including 8 cases of synovitis, 1 case of arteritis, 3 cases of chronic pleural effusion, 1 case of myocarditis, 6 cases of anemia, and 2 cases of glomerulonephritis. The incidence of complications was 19.63%. The patients with complications were regarded as the poor group ($n = 21$), and patients without complications were regarded as the excellent group ($n = 86$). miR-495 and miR-326 were taken as two independent variables and binary Logistic regression analysis was carried out. The Logistic regression model obtained was: $\text{Logit}(P) = 2.638 + (-1.589 \times \text{miR-495}) + (-16.232 \times \text{miR-326})$. When the cut-off value was 0.52, the sensitivity and specificity of the model for predicting complications during the RA treatment were 84.88 and 95.24%, respectively (Fig. 5 and Table V).

Table II. General data comparison between the two groups of subjects [mean \pm SD, n (%)].

Variables	Study group (n=107)	Control group (n=112)	t or χ^2	P-value
Age (years)	60.5 \pm 7.7	59.2 \pm 8.3	1.200	0.231
BMI (kg/cm ²)	23.52 \pm 2.63	23.36 \pm 3.05	0.415	0.679
White blood cells (x10 ⁹ /l)	6.85 \pm 2.54	6.71 \pm 2.26	0.431	0.667
Red blood cells (x10 ¹² /l)	4.87 \pm 1.36	4.61 \pm 1.56	1.312	0.191
Platelets (x10 ⁹ /l)	258.62 \pm 54.21	249.62 \pm 46.26	1.324	0.187
Fasting blood glucose (mmol/l)	18.51 \pm 3.45	17.89 \pm 3.85	1.253	0.212
Systolic pressure (mmHg)	132.59 \pm 15.26	128.97 \pm 14.91	1.776	0.077
Diastolic pressure (mmHg)	76.24 \pm 9.17	75.16 \pm 8.95	0.379	0.882
Course of disease (years)	2.16 \pm 0.86			
Sex			0.089	0.765
Male	59 (55.14)	64 (57.14)		
Female	48 (44.86)	48 (42.86)		
Smoking history			0.853	0.356
Yes	63 (58.88)	59 (52.68)		
No	44 (41.12)	53 (47.32)		
Drinking history			0.344	0.558
Yes	52 (48.60)	50 (44.64)		
No	55 (51.40)	62 (55.36)		
Exercise habit			0.662	0.416
Yes	32 (29.91)	28 (25.00)		
No	75 (70.09)	84 (75.00)		
Residence			1.392	0.239
Urban	62 (57.94)	56 (50.00)		
Rural	45 (42.06)	56 (50.00)		
Nationality			1.474	0.225
Han	102 (95.33)	110 (98.21)		
Minorities	5 (4.67)	2 (1.79)		
Family history			2.307	0.129
Yes	26 (24.30)	18 (16.07)		
No	81 (75.70)	94 (83.93)		

BMI, body mass index.

Discussion

The incidence of RA is on the rise year by year and the clinical challenges are increasing (18). Although RA is not a deadly disease, the complications caused by its development are extremely harmful, such as infarction caused by artery invasion, myocarditis and valvulitis caused by heart invasion, as well as drug-induced gastrointestinal mucosal lesions, and spinal cord lesions in patients with serious illness (19,20). At present, the clinical diagnosis of RA is still based on the diagnostic criteria developed by the American Rheumatology Association in 1987. Thus, not only the RA examination is extremely complicated, but also specific RA cases that may have appeared at present due to the development of the disease cannot be diagnosed based on these diagnostic criteria. Therefore, the exploration of serum markers is particularly important for the clinical diagnosis and treatment of RA.

The experimental results of the present study showed that miR-495 expression was significantly increased in RA patients, whereas miR-326 expression was significantly decreased, in consistency with the results reported by Clark *et al* and Kefas *et al* (21,22) on cardiomyopathy and glioma. This suggests that miR-495 and miR-326 may be involved in the occurrence and progression of RA. Further analysis of the relationship between miR-495, miR-326 and the clinical pathology of RA patients showed that miR-495 was closely related to the course of the disease, the stage of pathological changes, the disease progression and the clinical manifestations. In addition, miR-326 was closely related to the course of the disease, the stage of pathological changes, the disease progression, the functional activity and the clinical manifestations ($P < 0.050$). These results further confirm the close relationship between miR-495, miR-326 and RA. According to a previous report, miR-495 can inhibit cartilage differentiation process in bone marrow mesenchymal

Table III. Diagnostic value of miR-495 and miR-326 for RA.

Items	miR-495	miR-326	miR-495 + miR-326
Cut-off	6.05	5.11	0.55
Sensitivity (%)	55.14	78.50	77.57
Specificity (%)	90.18	75.89	87.50
AUC	0.787	0.813	0.882
Std. error	0.031	0.029	0.023
95% CI	0.727-0.848	0.756-0.871	0.837-0.928
P-value	<0.001	<0.001	<0.001

miR, microRNA; AUC, area under curve; CI, confidence interval.

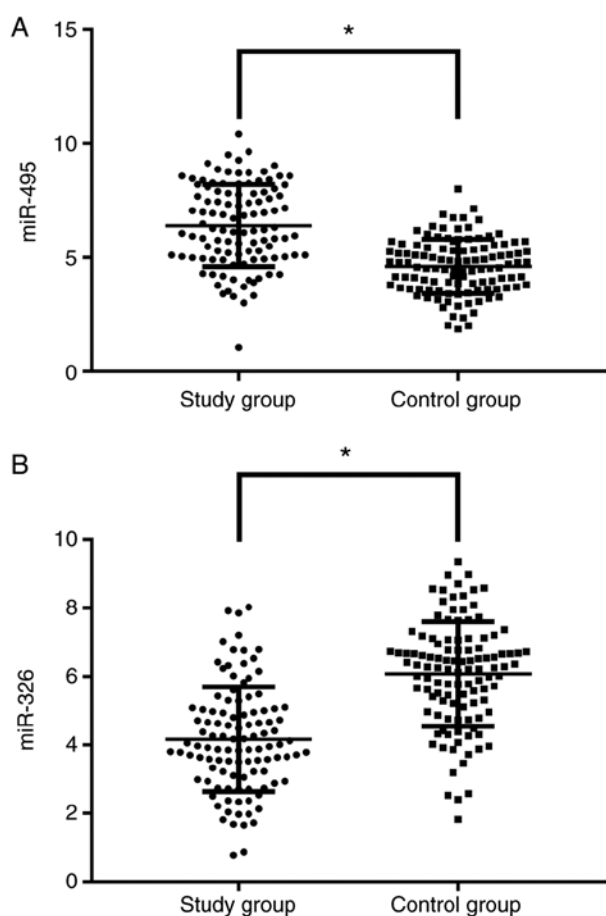


Figure 1. Comparison of miR-495 and miR-326 expression levels between the two groups. (A) The serum miR-495 expression in the study group was significantly higher than that in the control group, * $P < 0.001$. (B) The serum miR-326 expression in the study group was significantly lower than that in the control group, * $P < 0.001$. miR, microRNA.

stem cells (23) by targeting Sox9, and Tanaka (24) has suggested that Sox9 may be an effective tool for future RA joint repair. Therefore, the mechanism of miR-495 on RA may be through the regulation of Sox9 affecting the integrity of cartilage tissue. However, Gao *et al* (25) have suggested that Notch-1 plays an important role in the mediation of RA angiogenesis and Zhang *et al* (26) have shown that the expression of miR-495 is inhibited in retinal neurons. Thus,

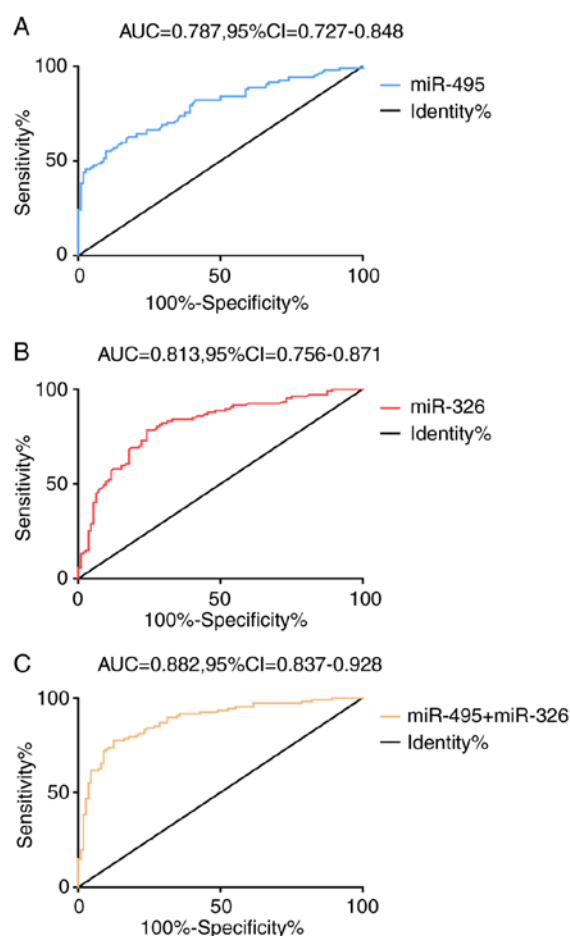


Figure 2. Diagnostic value of miR-495 and miR-326 for RA. ROC curve analysis of (A) miR-495, (B) miR-326 and (C) miR-495 combined with miR-326 for the diagnosis of RA. miR, microRNA; RA, rheumatoid arthritis; AUC, area under the curve; CI, confidence interval.

miR-495 could be a potential therapeutic target for RA and its therapeutic mechanism may be through the upregulation of Notch-1. Mao *et al* (27) and Formosa *et al* (28) have shown that miR-495 is expressed at low levels in esophageal and prostate cancer. The differences with the results of the present study suggest that miR-495 may have specific expression and play different roles in different tissues and cells, which needs further investigation. In addition, miR-326 has

Table IV. Comparison of miR-495 and miR-326 expression in RA patients with different clinicopathology.

Variables	n	miR-495	t or F	P-value	miR-326	t or F	P-value
Course of disease, years			4.261	<0.001		4.188	<0.001
<2.16	42	6.10±1.26			4.03±1.35		
≥2.16	65	7.34±1.59			3.14±0.85		
Stage of pathological changes			5.321	0.007		23.551	<0.001
Acute active period	26	5.42±1.05			4.58±0.52		
Subacute active period	30	5.86±1.26			3.87±0.62		
Chronic delay period	16	6.38±1.58			3.05±1.08		
Stationary phase	35	7.83±1.12			2.62±1.20		
Disease progression ^a			9.323	<0.001		13.184	<0.001
Stage I	21	5.31±1.32			3.86±1.05		
Stage II	29	6.03±1.52			2.68±1.26		
Stage III	39	6.35±1.05			2.26±1.24		
Stage IV	18	7.48±1.41			1.62±1.06		
Functional activity ^b			2.101	0.058		3.179	0.027
Grade I	9	5.84±1.52			4.05±1.52		
Grade II	42	6.24±1.26			3.23±1.42		
Grade III	42	6.30±1.48			2.86±1.26		
Grade IV	14	7.21±1.84			2.52±0.58		
Clinical manifestations ^c			7.082	0.001		52.152	<0.001
Grade 0	8	5.41±1.24			5.42±1.21		
Grade I	34	6.25±1.82			4.27±1.54		
Grade II	65	7.05±1.16			2.52±0.54		

^aDisease progression: Stage I, soft tissue swelling around joint with mild osteoporosis around joint; Stage II, narrowing and blurring of joint space and obvious osteoporosis; Stage III, in addition to osteoporosis there were obvious cartilage and bone destruction, and joint deformities, such as subluxation and ulnar deviation; Stage IV, the same characteristics as in stage III with bony ankylosis. ^bFunctional activity: Grade I: No obstacle in general activities; grade II: Joint discomfort or obstacles in general activities; however, the patient was still able to complete general activities; Grade III, functional activity was obviously limited; however, most patients could take care of themselves; Grade IV, patients were unable to take care of themselves or stayed in bed. ^cClinical manifestations: Grade 0, no pain, tenderness, swelling or morning stiffness; Grade I, no activity, slight pain during activity, pain during compression, swelling of joints (not exceeding the bone protrusion near the joints) and morning stiffness within 1 h; Grade II, pain occurred when the patient was inactive, and the pain increased when the patient was active. Under stress, the patient not only complained of pain, but also had an expression of fear or retracted the joint. The swelling was apparently equal to the bone protrusion, the soft tissue depression was disappeared, and the morning stiffness time was within 1-2 h. miR, microRNA; RA, rheumatoid arthritis.

been proven to bind 3'UTR of helper T cell 17 differentiation inhibitor Ets-1 to inhibit Ets-1 expression (29). A previous study has shown that Ets-1 can be used as one of the markers of RA, and can promote the progression of diseases in RA by supplying blood to inflammatory tissue and recruiting immune ability and inflammatory cells (30). Therefore, the mechanism of miR-326 may be through the upregulation of Ets-1 expression to enhance inflammatory factors in RA patients. Another study has also pointed out that miR-495 affects the occurrence of mandatory spondylitis through the targeted inhibition of DVL-2 (31) and the study by Tomofuji *et al* (32) has also confirmed that miR-495 may be a potential marker of periodontitis. Similarly, a previous study has suggested that miR-326 may be closely related to RA in diabetic patients (33). All the aforementioned studies have shown that miR-495 and miR-326 are closely related to bone diseases and therefore, it is of great significance to continue to explore miR-495 and miR-326 in RA.

ROC curve analysis showed that the combined diagnosis of miR-495 and miR-326 has good predictive value for the occurrence of RA and complications during treatment, suggesting that miR-495 and miR-326 can be used as effective diagnostic indicators for RA in the future. At present, there is still much room for improvement in the clinical diagnosis of osteoarthritis. The detection of inflammatory factors, although it has a very high sensitivity, the specificity is not enough to accurately determine whether RA occurs in patients. Regarding imaging techniques, the testing process is very complex and cannot be used for extensive early screening. Therefore, it is very important to find blood markers that can accurately and rapidly reflect RA. Compared with the traditional RA diagnostic method, the detection advantages of miR-495 and miR-326 in peripheral blood are as follows: i) The detection is convenient and short in cycle, and can be completed only by extracting peripheral blood; ii) the detection results are relatively intuitive, and no

Table V. Diagnostic value of miR-495 combined with miR-326 for complications during treatment.

Items	miR-495 + miR-326
Cut-off	0.52
Sensitivity (%)	84.88
Specificity (%)	95.24
AUC	0.957
Std. error	0.020
95% CI	0.917-0.997
P-value	<0.001

miR, microRNA; AUC, area under curve; CI, confidence interval.

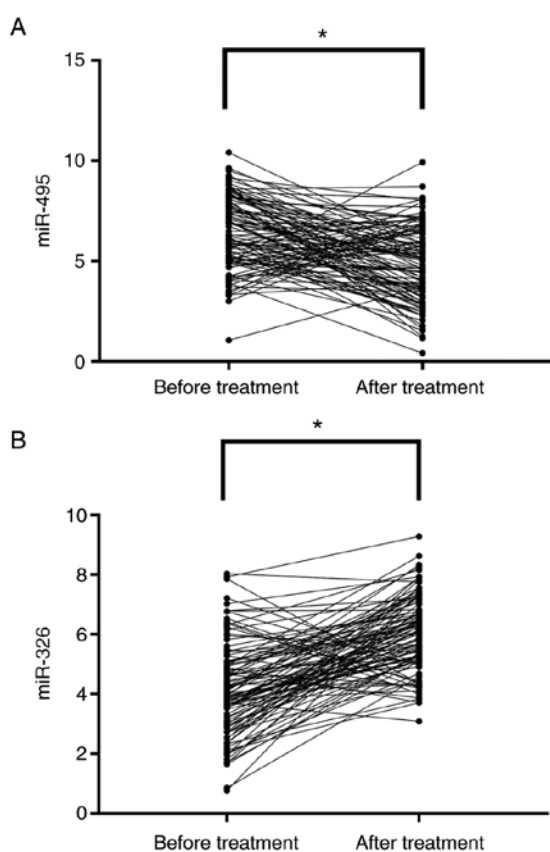


Figure 3. Comparison of miR-495 and miR-326 expression in the study group before and after treatment. (A) After treatment, the miR-495 expression in the study group was significantly lower than that before treatment, * $P < 0.001$. (B) After treatment, the miR-326 expression in the study group was significantly higher than that before treatment, * $P < 0.001$. miR, microRNA.

artificial re-interpretation of results is required compared with the imaging techniques; and iii) the samples are convenient to store and beneficial for the long-term treatment of the patients.

The aim of the present study was to investigate the expression of miR-495 and miR-326 in the peripheral blood of RA patients. Due to limited experimental conditions, the study presents some deficiencies. Because of the lack of basic experimental support, the relevant mechanisms of miR-495 and miR-326 involved in RA disease progression are still at

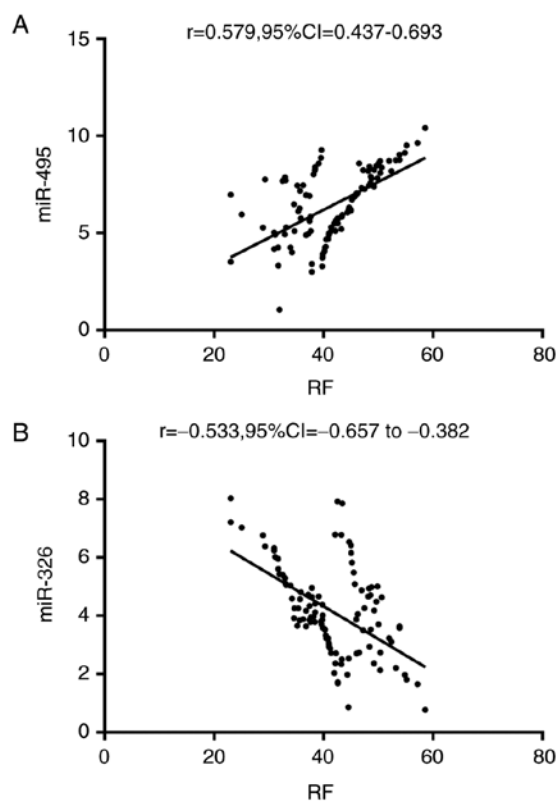


Figure 4. Correlation analysis of miR-495 and miR-326 with RF. (A) miR-495 was positively correlated with RF. (B) miR-326 was negatively correlated with RF. miR, microRNA; RF, rheumatoid factor.

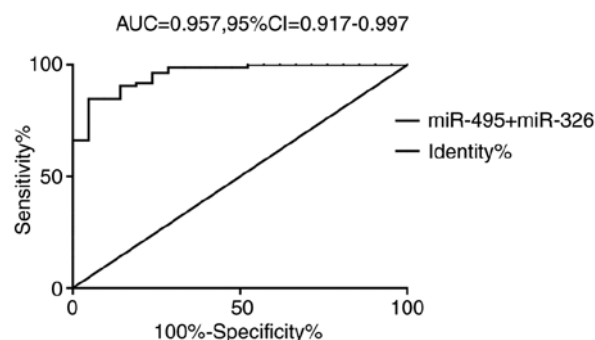


Figure 5. ROC curve analysis of miR-495 combined with miR-326 in predicting complications during treatment. miR, microRNA; AUC, area under the curve; CI, confidence interval.

the stage of speculation. Thus, further experimental research is needed in this direction to confirm the results presented. Moreover, due to the short experimental period, it is still not clear whether miR-495 and miR-326 have significant impact on the prognosis of RA patients. This will be one of our future research directions. In addition, due to incomplete preservation of case data, the relationship between miR-495, miR-326 and DAS28 score was not investigated. More representative patient data will be collected as soon as possible for a more comprehensive analysis. For the specific expression of miR-495 and miR-326 in different osteoarticular diseases, meta-analysis will be conducted in our future research in order to improve the experimental results.

In conclusion, miR-495 was highly expressed in RA patients, whereas miR-326 was expressed at low levels. The combined detection of miR-495 and miR-326 was shown to have a good diagnostic value for RA.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

XS and HL conceived and designed the study, and drafted the manuscript. XS, HL and YZ collected, analyzed and interpreted the experimental data. YZ revised the manuscript for important intellectual content. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Yidu Central Hospital of Weifang (Weifang, China). Signed written informed consents were obtained from the patients and/or guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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