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The value of MiR-146a and MiR-4484 expressions in the diagnosis of anti-SSA antibody positive Sjogren syndrome and the correlations with prognosis

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Abstract. – OBJECTIVE: The aim of this study was to investigate the value of micro ribonucleic acid-146a (miR-146a) and miR-4484 expressions in the diagnosis of anti-SSA antibody positive Sjogren syndrome (SS) and their correlations with prognosis.

PATIENTS AND METHODS: 70 patients with anti-SSA antibody positive SS were selected as the observation group, and the non-positive Sjogren syndrome (PSS) subjects were collected as the control group at the same time. Fluorescent quantitative polymerase chain reaction (PCR) method was used to detect serum expressions of miR-146a and miR-4484 in two groups. Expression changes of miR-146a and miR-4484 in patients before and after treatment were compared. After 3 years of follow-up, 70 patients were divided into high expression and low expression subgroups based on the average expressions of miR-146a and miR-4484. Prognosis in each subgroup was compared.

RESULTS: The expressions of miR-146a and miR-4484 in the observation group were significantly up-regulated compared with those in the control group ($p<0.05$). After treatment, the expressions of miR-146a and miR-4484 were significantly down-regulated compared with those before treatment ($p<0.05$). Combined detection of miR-146a and miR-4484 was superior to single index detection in the diagnosis and prognosis of PSS ($p<0.05$). The 3-year follow-up showed that the incidences of renal injury and pulmonary interstitial lesion in patients with low miR-146a and miR-4484 expressions were significantly lower than those with high expressions ($p<0.05$). However, there were no significant differences in the survival rate between the two groups ($p>0.05$).

CONCLUSIONS: Serum expressions of miR-146a and miR-4484 in anti-SSA antibody positive PSS patients are significantly up-regulated. Their detection can improve the diagnostic rate of SS. Expressions of miR-146a and miR-4484 are closely correlated to the prognosis of the patients, which can be used as prognostic predictors.

Key Words
miR-146a, miR-4484, Anti-SSA antibody positive Sjogren syndrome.

Introduction

Sjögren syndrome (SS) is a common autoimmune disease in clinic[1]. The incidence rate of SS is about 0.5% in adults, among which middle-aged people are most vulnerable. Women are more vulnerable than men with a proportion of about 9:1[2]. The clinical manifestations of SS are diverse, which can be manifested as exocrine gland (salivary glands, lacrimal glands, etc.) injury, lymphopoiesis, desiccation of the mouth, nasal cavity, eye and skin, arthralgia and so on. It can even involve viscera system (kidney, lung, nervous system, etc.), which not only brings physical pain, but also seriously influences the life quality of patients due to economic burden of treatment[3,4]. Anti-SSA antibody is a kind of immunoglobulin. It is produced after the immune reaction of the small ribonucleic acid protein in the autologous cells when the immune response is disorganized. Specifically, anti-SSA is the most common autoantibody of SS with a high positive rate[5].

Micro ribonucleic acid (miRNA) is a class of endogenous non-coded RNA, which can inhibit or degrade the translation of messenger RNA (mRNA), and ultimately regulate the expression of gene. It can reflect the basic physiological state of the cells and tissue levels, making it capable of being a SS marker[6].

In this study, the changes of miR-146a and miR-4484 expressions in anti-SSA antibody positive SS patients were observed and analyzed, so as to provide evidence for the diagnosis and treatment of anti-SSA antibody positive SS.

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Patients and Methods

Patients

A total of 70 patients with anti-SSA antibody positive SS treated in our hospital from September 2013 to December 2014 were selected as the observation group by using the simple random sampling method and were compared with 60 non-SS people in the same period as the control group. The patients were in accordance with the SS diagnostic criteria[7]. Exclusion criteria: 1) patients with psychiatric disorders; 2) patients complicated with severe heart, brain, kidney, liver or other viscera disorders; 3) patients with other connective tissue disease, pregnant or lactating women. Both groups signed the informed consent and Ethics Committee approval was granted from our hospital. There were no statistically significant differences in the general information between the two groups (p>0.05) (Table I).

Methods

Sample Collection

3 mL of peripheral venous blood sample (after 8 hours of fasting) of each patient (before and after treatment) were collected and placed into an ethylenediamine tetraacetic acid (EDTA) anticoagulant tube. Next, plasma and cells were separated and 4 mL of lymphocyte separation solution were added. The peripheral blood mononuclear cells (PBMCs) were extracted *via* centrifugation at 500 rpm for 20 min and stored in a refrigerator at -80°C.

Quantitative Reverse Transcriptase Polymerase Chain Reaction (qRT-PCR)

The expressions of miR-146a and miR-4484 were detected using quantitative reverse transcriptase polymerase chain reaction (qRT-PCR): 1) PBMCs were taken out and thawed; 2) Total RNA was extracted according to the instructions of TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and the RNA purity ratio was controlled in the range of 1.8-2.1; 3) MicroRNA was labeled and miRNA chip hybridization was conducted, followed by scanning and data analysis; 4) MiR-146a and miR-4484 were selected from miRNA chips for the fluorescent quantitative PCR analysis. Reaction mixture was prepared by using the reverse transcription kit (Fermentas Company, Waltham, MA, USA) and circular DNA was synthesized. RT-PCR conditions were as follows: denaturation at 95°C for 10 s, annealing at 60°C for 20 s and extension at 70°C for 10 s, for a total of 40 cycles. Sequences of the related primers were provided by Sangon (Shanghai, China). The CT value of target gene in each sample was normalized by internal reference gene (U6RNA). Finally, the relative expression levels of miR-146a and miR-4484 were calculated using $2^{-\Delta\Delta CT}$.

Evaluation Criteria

The expressions of miR-146a and miR-4484 in two groups of serum were detected by using fluorescent quantitative PCR. Patients were further divided into high miR-146a expression group (n=31) and low expression group (n=39) according to of the average expressions of miR-146a and miR-4484. Patients in high miR-4484 expression group (n=32) and low expression group (n=38) were followed up for 3 years. The prevalence rate of renal injury, pulmonary interstitial lesion rate and survival rate in different groups were recorded.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 19.0 (SPSS Inc., Chicago, IL, USA) statistical analysis software was used, the measurement data were expressed by mean ± standard deviation ($\bar{x}\pm s$) and t-test was used for comparison. The enumeration data were expressed by rate, and analyzed by x^2-test. Receiver operating characteristic (ROC) curve analysis was adopted to evaluate the diagnosis and prognosis, and the test significant level was α =0.05.

Table I. Table of general data of patients.

Items group (n=70)	Observation Control group (n=60)	t/χ^2	p
Sex (male/female)	7/63	5/55	0.006	0.939
Age (years old)	40-60	35~65		
Average age (years old)	48.16±5.49	48.85±5.52	0.320	0.749
Course of disease (years)	1-10	1-12		
Average course of disease (years)	4.36±1.49	4.28±1.52	0.302	0.763
Body mass index (BMI) (kg/m^2)	23.23±1.05	23.46±1.18	1.176	0.242

Table II. Expressions of miR-146a and miR-4484 in two groups.

Group	No.	Relative expression of miR-146a	Relative expression of miR-4484
Observation group	70	16.74±3.42	68.14±3.75
Control group	60	6.12±3.01	10.03±3.04
t		18.645	97.541
p		<0.001	<0.001

Table III. Expressions of miR-146a and miR-4484 in patients before and after treatment.

Group	No.	Relative expression of miR-146a	Relative expression of miR-4484
Before treatment	70	16.74±3.42	68.14±3.75
After treatment	70	9.02±3.02	27.31±3.57
t		14.157	65.978
p		<0.001	<0.001

Results

Expressions of miR-146a and miR-4484 in Two Groups of Serum

The relative expression levels of miR-146a and miR-4484 in the control group were significantly lower than those in the observation group (p<0.05) (Table II).

Expressions of miR-146a and miR-4484 in Patients Before and After Treatment

After treatment, the expressions of miR-146a and miR-4484 were significantly down-regulated compared with those before treatment (p<0.05) (Table III).

Prediction of Diagnosis and Prognosis of PSS via miR-146a and miR-4484

Combined detection of miR-146a and miR-4484 was superior to single index detection in the diagnosis and prognosis of PSS (p<0.05) (Table IV and V, Figure 1A and 1B).

Prognosis of Patients with Different miR-146a and miR-4484 Expressions

The prevalences of renal injury and pulmonary interstitial lesion in patients with low miR-146a and miR-4484 expressions were significantly lower than those with high expressions (p<0.05). However, there was no significant difference in the survival rate between the two groups (p>0.05) (Table VI and VII).

Table IV. Analysis of miR-146a and miR-4484 on the diagnosis of PPS.

Index	Sensitivity	Specificity	AUC	Youden index
MiR-146a	82.26%	71.26%	0.832	0.53
MiR-4484	81.75%	70.18%	0.825	0.51
MiR-146a combined with miR-4484	91.43%*	86.36%*	0.913*	0.78*

Note: There are statistical significance compared with miR-146a and miR-4484, *p<0.05.

Table V. Analysis of miR-146a and miR-4484 on the prognosis of PPS.

Index	Sensitivity	Specificity	AUC	Youden index
MiR-146a	80.23%	64.56%	0.802	0.45
MiR-4484	79.82%	66.38%	0.795	0.46
MiR-146a combined with miR-4484	90.33%*	82.36%*	0.901*	0.73*

Note: There are statistical significance compared with miR-146a and miR-4484, *p<0.05.

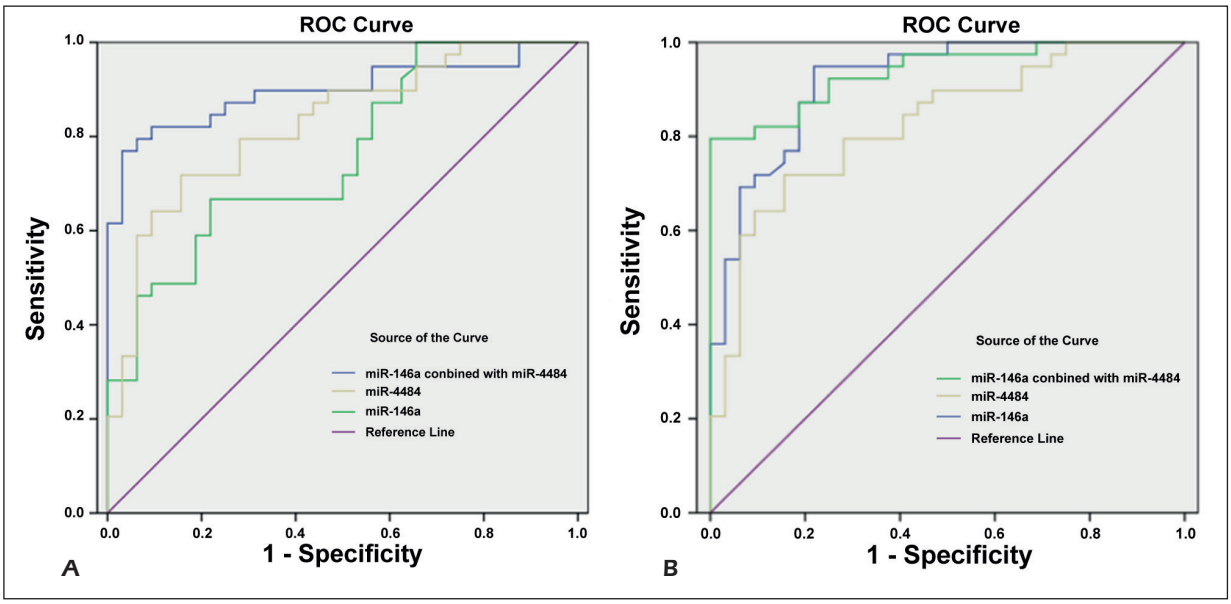

Figure 1. A, ROC curves of diagnosis via miR-146a, miR-4484 and combination of the two. **B**, ROC curves of prognosis estimation via miR-146a, miR-4484 and their combination of the two.

Discussion

SS is a common chronic inflammatory autoimmune disease in clinic. In SS patients, highly activated B cells, a large number of lymphocyte infiltration and multiple autoantibodies can be seen in the affected tissues. Commonly, cryoglobulinemia and hyper-immunoglobulinemia are usually accompanied[8]. There are many causes of SS, including sex hormone abnormality, virus infection, immune dysfunction, heredity and so on. The research data showed that the positive rate of anti-SSA antibody in SS patients can be as high as 60%, and the pathogenesis is closely correlated to the dysfunction of lymphocyte[9]. The long-term immune disorder in SS patients influences metabolites in central nervous system, ultimately resulting in the anxiety and depression of the patients.

Table VI. Comparisons of the prognoses in different miR-146a groups [*n* (%)].

Group	No.	Prevalence rate of renal injury	Pulmonary interstitial lesion rate	Survival rate
Low miR-146a group	39	5 (12.82)	1 (2.56)	39 (100.00)
High miR-146a group	31	20 (64.52)	7 (22.58)	29 (93.55)
χ^2		17.951	5.002	0.787
p		<0.001	0.025	0.375

Table VII. Comparisons of the prognoses in different miR-4484 groups [*n* (%)].

Group	No.	Prevalence rate of renal injury	Pulmonary interstitial lesion rate	Survival rate
Low miR-4484 group	38	4 (10.53)	1 (2.63)	38 (100.00)
High miR-4484 group	32	21 (65.63)	7 (21.88)	30 (93.57)
χ^2		20.633	4.596	0.712
p		<0.001	0.032	0.399

As time goes on, the degree of involvement becomes higher and the patients' physical function is seriously damaged. As result, life quality of affected patients gradually decreases[10]. Therefore, it is of great significance to improve the diagnosis and treatment of SS in order to improve the prognosis and the life quality patients.

miRNA is a key factor in regulating gene expression, which plays an important role in the development of the immune system, immune response and autoimmunity of the body. It can reflect the basic physiological state of cells and tissue levels of the body and can participate in the occurrence and development of autoimmune diseases[11,12]. miRNA can inhibit the degradation of RNA enzymes through the formation of lipoprotein complex, and one miRNA can regulate the expressions of a variety of mRNAs[13]. Therefore, the whole gene should be analyzed in the case of limited number of miRNAs. At the same time, miRNA is relatively stable, which can be detected through repeated purification of blood and body fluids (saliva and urine), so as to avoid invasive inspection. Therefore, the simple, fast and effective detection of miRNA is suitable for supplementary diagnosis of immune disease and tumor[14,15]. PBMC is a collective of immune cells, including mononuclear cells and lymphocytes[16]. Through the detection and analysis of miRNA in the PBMC in research objects, the results showed that the expressions of miR-146a and miR-4484 in the observation group were significantly up-regulated compared with those in the control group. Besides, the expressions of miR-146a and miR-4484 were significantly down-regulated after treatment compared with those before treatment ($p<0.05$). Our data suggested that miR-146a and miR-4484 are differentially expressed in patients with anti-SSA antibody positive SS, which can be used as a novel marker for diagnosing SS and evaluating therapeutic effect.

The results of this study showed that the area under the curve (AUC) of combined diagnosis and prognosis prediction *via* miR-146a and miR-4484 was larger than AUC of diagnosis and prognosis prediction *via* miR-146a or miR-4484 individually. Besides, the combined diagnosis was significantly superior to single index detection ($p<0.05$), indicating that the implementation of the combined detection of the expressions of miR-146a and miR-4484 in SSA positive SS patients can improve the diagnostic efficiency of SS. Combined detection reduces the misdiagnosis rate, monitors the activity of the patients and predicts their prognosis. Therefore, it has a positive guiding significance in choosing and adjusting the appropriate treatment plan.

MiR-146a can play a role of negative feedback regulation on inflammatory response and can be used as a new biological marker for monitoring the activity of autoimmune diseases[17]. MiR-4484 was discovered in 2010, and its specific mechanism and function have not been reported yet. This study demonstrated that the occurrences of renal injury and pulmonary interstitial lesion in patients with low miR-146a and miR-4484 expressions were significantly lower than those with high expressions ($p<0.05$). However, there was no significant difference in the survival rate between the two groups ($p>0.05$). The reason may be that the high expression of miR-146a can induce a large number of inflammatory factors, such as TNF-α and IL-lβ, leading to inflammatory reaction, and multiple organs involvement, especially kidney and lung[18]. MiR-4484 may be involved in the pathophysiological process of SS by regulating various proteins, such as amyloid precursor protein and MSRB3 protein. Overexpressed miR-4484 participates in the regulation of inflammatory pathways, thereby damaging the viscera and leading to poor prognosis of the patients. The two are closely correlated to the life quality of the patients.

Conclusions

We showed that the expressions of miR-146a and miR-4484 in patients with anti-SSA antibody positive PSS are significantly up-regulated, and the combined detection has a positive significance in improving the diagnostic rate of SS. The expressions of miR-146a and miR-4484 are closely correlated to the prognosis of patients, and can be used as indicators for prognosis estimation. Besides, it has the advantages of convenient extraction of serum from patients, small trauma and high repeatability, which is worthy of clinical popularization and application.

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Conflict of Interest:

The Authors declare that they have no conflict of interests.

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