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Diagnostic values of serum IL-10 and IL-17 in rheumatoid arthritis and their correlation with serum 14-3-3η protein

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Abstract. – OBJECTIVE: This study aimed to investigate the diagnostic values of serum IL-10 and IL-17 in rheumatoid arthritis (RA) and their correlation with serum protein.

PATIENTS AND METHODS: A retrospective analysis was performed on 116 RA patients admitted to the Yantaishan Hospital (the RA group) and 116 healthy subjects (the control group). Enzyme-linked immunosorbent assay was used to detect the expression levels of serum interleukin (IL)-10, IL-17 and 14-3-3η protein. Pearson analysis was performed to analyze the correlation between the expression levels of serum IL-10, IL-17 and 14-3-3η protein of patients in the RA group, and ROC curve analysis was conducted to measure the diagnostic values of IL-10, IL-17 and their combination in RA.

RESULTS: Patients in the RA group had significantly lower serum IL-10 level and markedly higher IL-17 and 14-3-3η protein levels than those in the control group ($p<0.001$). Serum IL-10 level was negatively correlated with 14-3-3η protein level in RA patients (r=-0.582, $p<0.001$). Serum IL-17 level was positively correlated with 14-3-3η protein level in RA patients (r=0.482, $p<0.001$). Serum IL-10 level was negatively correlated with IL-17 level in RA patients (r=-0.468, $p<0.001$). The AUC of IL-10 for diagnosing RA was 0.671, with a 95% confidence interval of 0.602-0.741 and a cut-off value of 87.315. The AUC of IL-17 for diagnosing RA was 0.856, with a 95% confidence interval of 0.807-0.905 and a cut-off value of 87.844. The AUC of IL-10 combined with IL-17 for diagnosing RA was 0.887.

CONCLUSIONS: RA patients had remarkably lower serum IL-10 level and significantly higher IL-17 and 14-3-3η protein levels than healthy people. IL-17 has better sensitivity and specificity than IL-10 for diagnosing RA. IL-10 combined with IL-17 is beneficial to improve the diagnostic level of RA, which provides the reference for the diagnosis, treatment and pathogenesis of RA.

Key Words:
IL-10, IL-17, Rheumatoid arthritis, Diagnostic value, Serum 14-3-3η protein.

Introduction

Rheumatoid arthritis (RA) is a chronic, disabling autoimmune disease with inflammatory lesions in the synovial tissue of joint[1]. RA may be the result of the interaction between genetic and environmental factors, and among environmental factors, smoking has the strongest correlation with RA susceptibility[2]. At present, the specific pathogenesis of RA has not yet been clarified. However, previous studies[3-5] have shown that both cytokines and inflammatory mediators are involved in the pathogenesis of RA.

Interleukin (IL)-17 is a novel inflammatory factor, and cytokines are important mediators between RA inflammation and joint injury[6]. In the pathogenesis of RA, IL-17 interacts with other cytokines to promote inflammatory responses[7,8]. Metawi et al[9] have shown that IL-17 is highly expressed in the synovial fluid of RA patients. In addition, it can up-regulate the expressions of various angiogenesis factors to promote angiogenesis. However, inflammatory cells can be transmitted to the synovium through neovascularization, which may be one of the important causes of inflammation recurrence in RA patients[10].

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According to reports[11], IL-10, an anti-inflammatory cytokine in RA, plays a protective role in the pathogenesis of RA, inhibits the activity of natural killer cells and promotes the differentiation of B cells, thereby producing antibody[12].

As an acidic chaperone family widely expressed in eukaryotic cells, 14-3-3η protein can bind to more than 200 ligandins and is involved in the occurrence and development of various diseases[13,14]. It has been reported[15] that serum 14-3-3η protein, a co-derivative biomarker, up-regulates cytokines and enzymes and allows local and systemic inflammation to persist, leading to the joint injury. Maksymowych et al[16] believe that serum 14-3-3η, a new RA mechanism marker with high specificity related to the severity of the disease, is an addition to the existing markers and is more accurate in the diagnosis of RA.

At present, there are a few studies on the correlation between serum IL-10, IL-17, and 14-3-3η protein in RA. Therefore, this work aimed at investigating the diagnostic values of serum IL-10 and IL-17 in RA and their correlation with 14-3-3η protein to provide the reference for the diagnosis, treatment and pathogenesis of RA.

Patients and Methods

General Data

A retrospective analysis was performed on 116 RA patients admitted to the Yantaishan Hospital from October 2015 to October 2017 (the RA group); there were 42 males and 74 females aged (35-70) years. Another 116 healthy subjects in the same period were used as the control group, aged (28-70) years, including 54 males and 62 females. There was a significant difference in the BMI of subjects between the two groups ($p<0.05$), but no significant difference in gender, age, the course of the disease, smoking, RA family history, work and living environment ($p>0.05$). All RA patients met the RA classification criteria by the American College of Rheumatology (ACR) in 2010[17]. All subjects with incomplete clinical data, pregnancy, lactation, malignant tumors, autoimmune diseases, cardiovascular and cerebrovascular diseases, diabetes mellitus or coagulation disorder were excluded. The study has been approved by the Medical Ethics Committee. Patients were informed, agreed to participate in the clinical study and they and their families signed an informed consent form. (Table I)

Detection Methods

A total of 3 mL of fasting venous blood was extracted from all subjects, agglutinated for about 20 min at room temperature. The blood was centrifuged at 3000 rpm for 10 min, and the serum was collected. ELISA was conducted to detect the levels of serum IL-10 (Cat No. BE53101, Tecan Trading Co., Shanghai, China), IL-17 (Cat No. BE45171, Tecan Trading Co., Shanghai, China) and 14-3-3η protein (Cat No. JK-SJ-32763, JK Bioengineering Co., Ltd., Shanghai, China) of subjects in the two groups. The experimental procedure was carried out in strict accordance with the instructions. 100 μL of standard sample and 100 μL of sample to be tested were added to ELISA plates, respectively. They were gently shaken, incubated at 37°C for 2h, and dried. 100 μL of antibody working solution was added and then dried. ELISA plates were washed 3 times, then washed 5 times with enzyme. 90 μL of stop solution was added to develop in the dark, and another 50 μL was added to terminate the reaction. The optical density (OD) value was detected immediately at a wavelength of 450 nm with an automatic microplate reader (Beijing Image Trading Co., Beijing, China), and the concentrations of serum IL-10, IL-17 and 14-3-3η protein were calculated according to the standard curve.

Statistical Analysis

SPSS 20.0 statistical software (SPSS IBM, Armonk, NY, USA) was used for analysis. Chi-square test was conducted to analyze count data and t-test was performed to analyze measurement data. LSD test was the post-hoc test. Pearson analysis was carried out to analyze the correlation between IL-10, IL-17, and 14-3-3η protein, and ROC curve analysis was used to analyze the diagnostic values of IL-10 and IL-17. When $p<0.05$, the difference was statistically significant.

Results

Expression Levels of Serum IL-10, IL-17 and 14-3-3η Protein in Two Groups of Patients

Patients in the RA group had significantly lower serum IL-10 level and markedly higher IL-17 and 14-3-3η protein levels than those in the control group, with statistically significant differences ($p<0.001$). See Figure 1, Table II.

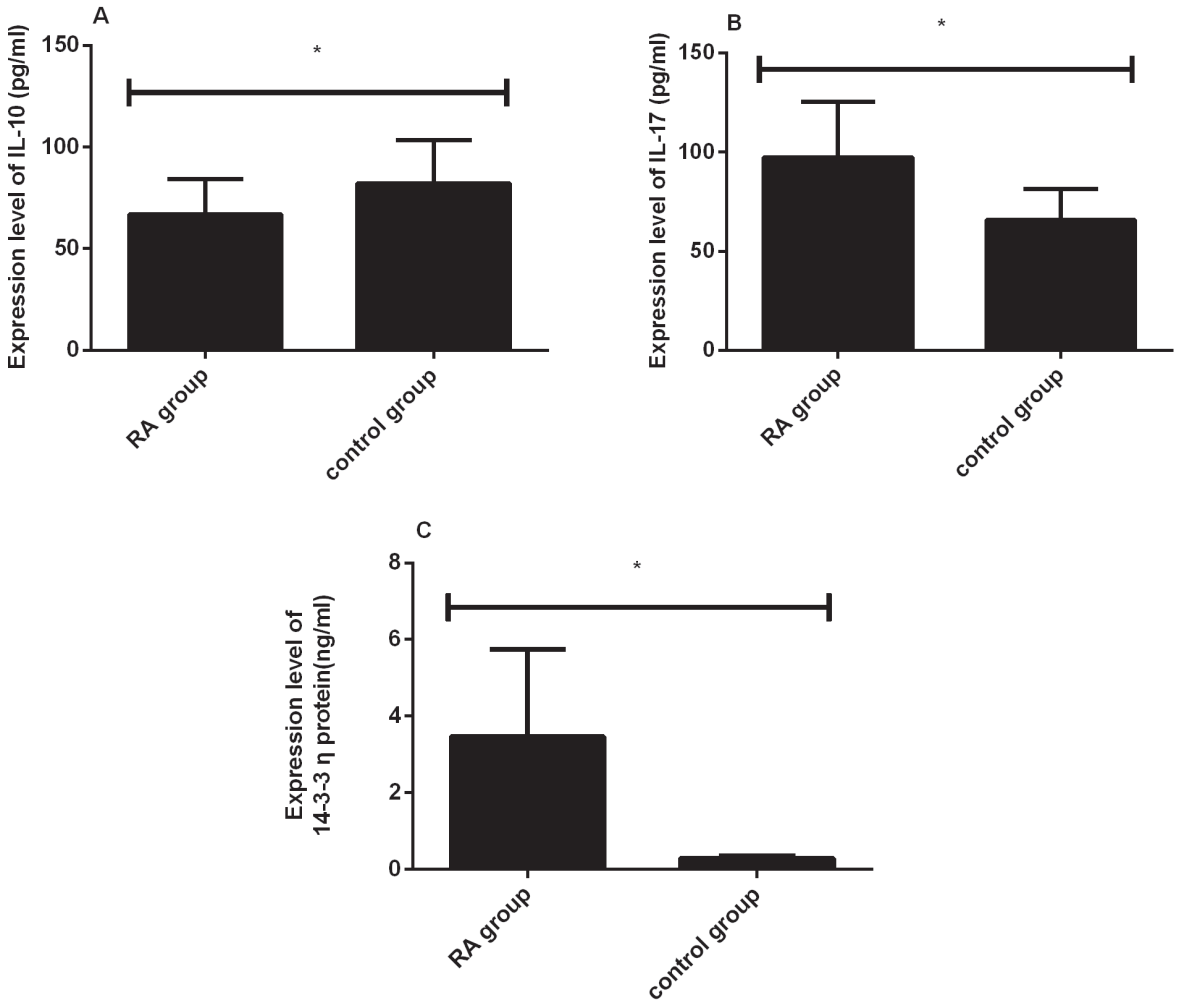

Figure 1. Expression levels of serum IL-10, IL-17 and 14-3-3η protein in two groups of patients. The results of ELISA showed that: ***A,*** Patients in the RA group had markedly lower serum IL-10 level than those in the control group, with a statistically significant difference (p<0.001). ***B,*** Patients in the RA group had remarkably higher IL-17 level than those in the control group, with a statistically significant difference (p<0.001). ***C,*** Patients in the RA group had higher 14-3-3η protein level than those in the control group, with a statistically significant difference (p<0.001). *p<0.001.

Correlation Between Serum IL-10, IL-17 and 14-3-3η Protein in RA Patients

Serum IL-10 level was negatively correlated with 14-3-3η protein level in RA patients (r=-0.582, p<0.001). Serum IL-17 level was positively correlated with 14-3-3η protein level in RA patients (r=0.482, p<0.001). Serum IL-10 level was negatively correlated with IL-17 level in RA patients (r=-0.468, p<0.001). See Figure 2.

Diagnostic Values of IL-10 and IL-17 in RA

The ROC curve of IL-10 for diagnosing RA was analyzed. The results showed that the AUC of IL-10 for diagnosing RA was 0.671, with a 95% confidence interval of 0.602-0.741 and a cut-off value of 87.315. There were 53 positive cases in the RA group with a sensitivity of 45.70%, and 100 in the control group with a specificity of 86.20%.

The ROC curve of IL-17 for diagnosing RA was analyzed. The results showed that the AUC of IL-17 for diagnosing RA was 0.856, with a 95% confidence interval of 0.807-0.905 and a cut-off value of 87.844. There were 81 positive cases in the RA group with a sensitivity of 69.90%, and 102 in the control group with a specificity of 87.90%.

The ROC curve of IL-10 combined with IL-17 for diagnosing RA was analyzed. The results showed that the AUC of IL-10 combined with IL-

Table I. General data [n(%)].

Factors		RA group (No. =116)	Control group (No. =116)	t/χ^2	*p*
Gender				2.559	0.142
	Male	42 (36.21)	54 (46.55)		
	Female	74 (63.79)	62 (53.45)		
Average age (Years)		50.25±10.83	48.68±12.52	1.021	0.308
BMI (kg/m^2)		22.76±3.17	21.64±4.15	2.310	0.022
Course of disease (Years)				1.399	0.293
	≥ Half year	65 (56.03)	56 (48.28)		
	< Half year	51 (43.97)	60 (51.72)		
Smoking				1.490	0.278
	Yes	39 (33.62)	48 (41.38)		
	No	77 (66.38)	68 (58.62)		
RA family history				2.544	0.144
	Yes	61 (52.59)	73 (62.93)		
	No	55 (47.41)	43 (37.07)		
Work/living environment				1.536	0.271
	Wet	80 (68.97)	71 (61.21)		
	Dry	36 (31.03)	45 (38.79)		

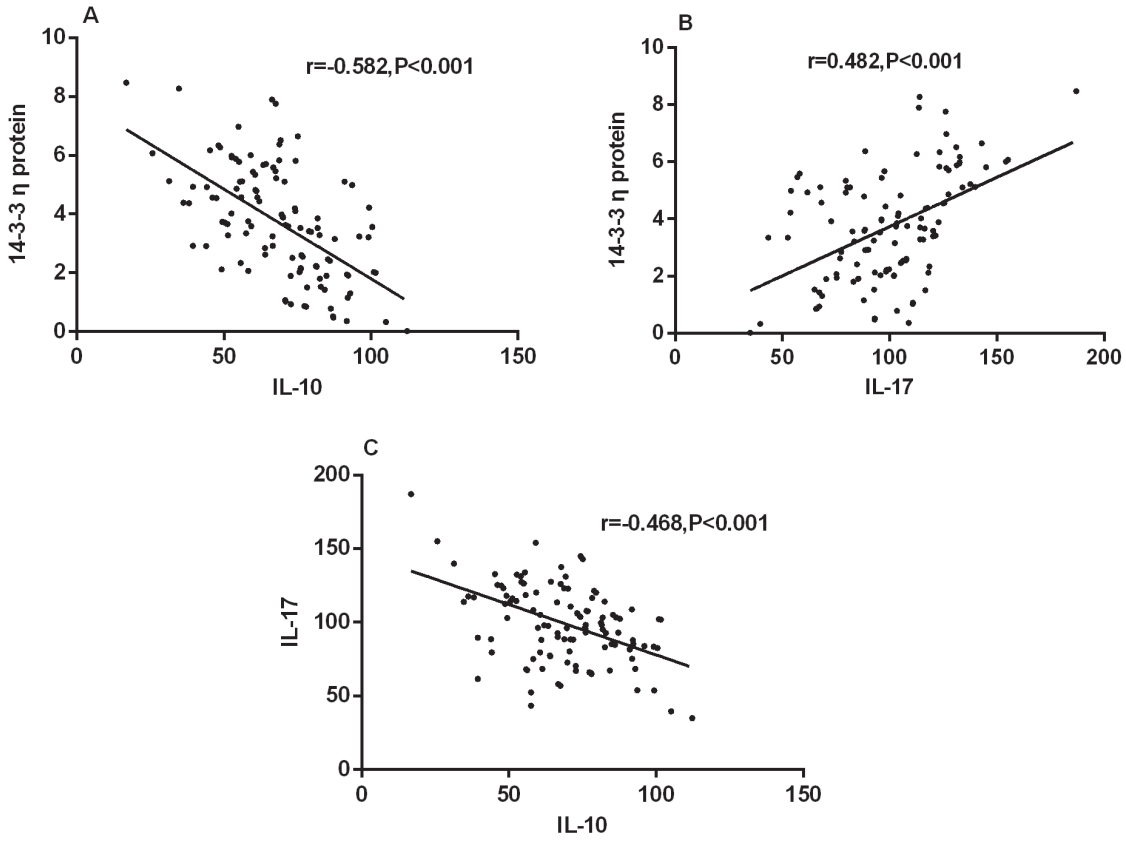

Figure 2. Correlation between serum IL-10, IL-17 and 14-3-3η protein in RA patients. The results of the Pearson analysis showed that: ***A,*** Serum IL-10 level was negatively correlated with 14-3-3η protein level in RA patients (r=-0.582, $p<0.001$). ***B,*** Serum IL-17 level was positively correlated with 14-3-3η protein level in RA patients (r=0.482, $p<0.001$). ***C,*** Serum IL-10 level was negatively correlated with IL-17 level in RA patients (r=-0.468, $p<0.001$).

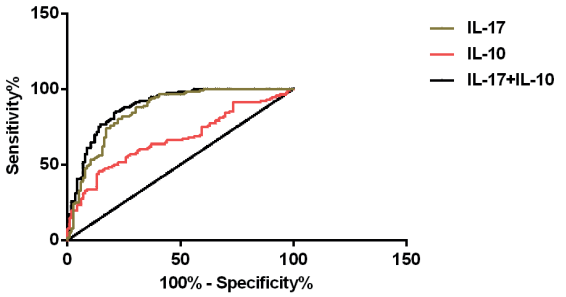

Figure 3. Diagnostic values of IL-10 and IL-17 in RA. The results of the ROC curve showed that the AUC of IL-10 for diagnosing RA was 0.671, with a 95% confidence interval of 0.602-0.741 and a cut-off value of 87.315. There were 53 positive cases in the RA group with the sensitivity of 45.70%, and 100 in the control group with the specificity of 86.20%. The AUC of IL-17 for diagnosing RA was 0.856, with a 95% confidence interval of 0.807-0.905 and a cut-off value of 87.844. There were 81 positive cases in the RA group with the sensitivity of 69.90%, and 102 in the control group with the specificity of 87.90%. The AUC of IL-10 combined with IL-17 for diagnosing RA was 0.887, with a 95% confidence interval of 0.844-0.929 and a cut-off value of 0.482. There were 98 positive cases in the RA group with the sensitivity of 84.50%, and 92 in the control group with the specificity of 79.30%.

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Discussion

Due to the diverse clinical manifestations of RA, the lack of typical symptoms and negative serology in the early stage, patients are often missed and misdiagnosed[18]. Therefore, it is important to improve the serum diagnostic level of RA. 14-3-3η protein is reported to be highly expressed in the serum and synovial fluid of RA patients, which promotes RA-related inflammatory responses[19,20]. Studies[21-23] on experimental animal models have shown that IL-17 has an adverse effect on arthritis. Therefore, Kirkham et al[24] believe that it may play a key role in the pathogenesis of different forms of arthritis by inducing synovial inflammation and promoting bone destruction. IL-10 has been shown to inhibit joint swelling and deformation in RA animal models[25]. Carter et al[26] have shown that B cells which produce IL-10 inhibit arthritis by promoting immune regulation relative to the differentiation of pro-inflammatory T cells. This study aimed at improving the diagnostic level of RA and explore the correlation between IL-10, IL-17, and 14-3-3η protein levels.

ELISA was performed to detect the levels of IL-10, IL-17, and 14-3-3η protein in the serum of RA patients and healthy people. According to the results, patients in the RA group had markedly lower serum IL-10 level, significantly higher IL-17 and 14-3-3η protein levels than those in the control group, with statistically significant differences ($p<0.001$). Our result is consistent with that of Zhang et al[27], that is, the expression level of IL-17 is remarkably higher in RA patients than that in healthy people. IL-17 increases the migration of synoviocytes and the expression and invasiveness of chemokines, as well as promotes the secretion of metalloproteinases, thereby causing cartilage injury and functioning as an angiogenesis mediator in RA[28]. IL-10 produced by B10 cells improves the occurrence of arthritis by inhibiting the production of Th17 cells[29]. Charbonnier et al[30] have shown that it can prevent the incidence and development of arthritis while inhibiting the secretion of IL-17. It is indicated that destroyed cytokine balance may cause various autoimmune diseases. The study by Maksymowych et al[31] has shown that RA patients have markedly higher serum 14-3-3η protein level than those in the control group, consistent with this study. Extracellular 14-3-3η activates key signaling pathways and induces factors associated with the pathogenesis of RA, which is highly expressed in patients with X-ray lesion and RA progression[31].

Table II. Expression levels of serum IL-10, IL-17 and 14-3-3η protein in two groups of patients.

Group	No. of cases	IL-10 (pg/ml)	IL-17 (pg/ml)	14-3-3η protein (ng/ml)
RA group	116	66.85±17.37	97.43±28.17	3.46±2.29
Control group	116	82.13±21.33	65.85±16.64	0.29±0.07
t		5.983	10.400	14.900
p		0.000	0.000	0.000

Table III. Diagnostic values of IL-10 and IL-17 in RA.

Indicator	AUC	95% Confidence interval	Cut-off value
IL-10	0.671	0.602-0.741	87.315
IL-17	0.856	0.807-0.905	87.844
IL-10+IL-17	0.887	0.844-0.929	0.482

Table IV. Sensitivity and specificity of IL-10 and IL-17 for diagnosing RA.

Indicator	No.	No. of positive cases	Sensitivity	Specificity
IL-10	116	53	45.70	86.20
IL-17	116	81	69.90	87.90
IL-10+IL-17	116	98	84.50	79.30

The results of the Pearson correlation analysis showed that serum IL-10 level was negatively correlated with 14-3-3η protein level in RA patients (r=-0.582, $p<0.001$). Serum IL-17 level was positively correlated with 14-3-3η protein level in RA patients (r=0.482, $p<0.001$). Serum IL-10 level was negatively correlated with IL-17 level in RA patients (r=-0.468, $p<0.001$). The results of the ROC curve showed that the AUC of IL-10 for diagnosing RA was 0.671, with a sensitivity of 45.70% and a specificity of 86.20%. The AUC of IL-17 for diagnosing RA was 0.856, with a sensitivity of 69.90% and a specificity of 87.90%. The AUC of IL-10 combined with IL-17 for diagnosing RA was 0.887, with a sensitivity of 84.50% and a specificity of 79.30%. At present, there are a few studies on the correlation between IL-10, IL-17, and 14-3-3η protein levels and the diagnosis of RA with IL-10 and IL-17. No literature has been found to support our results. However, studies have shown that increasing the secretion of RA vascular endothelial growth factor, IL-17 promotes the secretion of keratinocyte and hepatocyte growth factors in RA and increase the secretion of heparin-binding endothelial growth factor in RA, thereby destroying the joint. It is an *in vitro* stimulating factor released by angiogenesis factors, suggesting that the mechanism of action may be related to the synergistic effect of tumor necrosis factor-α[32]. Bush et al[33] has shown that IL-17 plays an important role in arthritis inflammation and joint destruction, which may be a potential therapeutic target for RA. The study by Heo et al[6] has shown that IL-10 promotes the production of Foxp3 + regulatory T cells through IL-10 receptor signaling, significantly inhibiting the expression of IL-17. It is suggested that IL-10 can be used to treat autoimmune diseases.

Conclusions

We found that RA patients have significantly lower serum IL-10 level and markedly higher IL-17 and 14-3-3η protein levels than healthy people. Serum IL-10 level was negatively correlated with 14-3-3η protein level in RA patients. Serum IL-17 level was positively correlated with 14-3-3η protein level in RA patients. Serum IL-10 level was negatively correlated with IL-17 level in RA patients. IL-10 lacks sensitivity in the diagnosis of RA, while IL-17 has better sensitivity and specificity than IL-10. IL-10 combined with IL-17 is beneficial to improve the diagnostic level of RA which provides reference for the diagnosis, treatment and pathogenesis of RA.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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