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Original Article

Study of peripheral blood inflammatory factor levels and their clinical value in patients with lupus nephritis

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Abstract: Objective: To explore the effect of traditional Chinese and western medicine on the levels of inflammatory cytokines in the peripheral blood of patients with lupus nephritis (LN). Methods: A total of 80 patients with LN admitted to the hospital from August 2016 to August 2017 were retrospectively analyzed. They were equally separated into an experimental group and a control group by the different types of medications. The control group was treated with western medicine, and the experimental group was treated with the combination of traditional Chinese and western medicines. The therapeutic effects were compared. Results: The levels of IL-6, IL-18 and TNF-α in the experimental group after treatment were (5.47±1.66) pg/ml, (31.66±3.87) pg/ml, and (9.28±3.06) pg/ml, respectively, which were significantly lower than (13.71±3.86) pg/ml, (68.47±4.26) pg/ml, and (22.17±6.54) pg/ml before treatment. The difference was statistically significant (t1 = 12.403, t2 = 40.450, t3 = 11.291, all P<0.001). In the control group after treatment, the levels of IL-6, IL-18 and TNF-α were (12.68±1.32) pg/ml, (68.22±3.42) pg/ml, and (19.78±5.57) pg/ml, respectively. The difference in control and experimental groups was statistically significant (t1 = 21.501, t2 = 44.771, t3 = 10.449, P<0.001). The total effective rate was 95.00% (38/40) in the experimental group and 80.00% (32/40) in control group, (X^2 = 4.114, P<0.001). There SLEDAI scores of the experimental group were much lower than control after 8 and 12 weeks of treatment (t1 = 8.186, t2 = 20.776, P<0.001). Moreover, the liver and kidney Yin deficiency symptoms in both groups were significantly improved after treatment (P<0.01). Conclusion: The combined treatment of traditional Chinese and western medicine can successfully prevent the secretion of serum IL-6, IL-18 and TNF-α, control the development of disease, boost the therapeutic outcome, and alleviate the immune injury of the body.

Keywords: Lupus nephritis, peripheral blood inflammatory factors, clinical value

Introduction

Systemic lupus erythematosus (SLE) often causes renal damage and results in lupus nephritis (LN). Immune cells, cytokines and other immune abnormalities play a key role in SLE. Patients with LN often exhibit asymptomatic hematuria and proteinuria [1, 2], and over 50% of SLE patients present with kidney damage [3]. Therefore, LN is an important cause of renal failure, and LN patients are classified into I-VI types according to the severity, and this classification is of great significance for accurately determining prognosis and treatment [4]. The cause of SLE remains unclear, and its current purpose is to effectively control the activity of LN, protect renal function, and delay the progression of renal fibrosis. Although biological agents or hormone intervention have been applied for LN disease in Western medicine, it has proven to be not ideal, with more adverse reactions [5, 6]. Traditional Chinese medicine believes that SLE is characterized by dampness-evil, blood stasis etc., and it has made significant breakthroughs in SLE treatment in recent years. It is reported that the combination of Chinese and western medicine treatment has become an important measure to improve therapeutic outcome and decrease toxicity [7-9]. Based on this, in order to further investigate the influence of traditional Chinese and western medicine on the changes of inflamma-

tory factors in the peripheral blood of LN patients, the clinical data of 80 patients with LN admitted to our hospital from August 2016 to August 2017 were retrospectively analyzed in the present study.

Materials and methods

General information

This retrospective trial reviewed the clinical data of 80 patients with LN who were equally divided into an experimental and a control group based on the different types of medications. The experimental group was aged between 25-44 years, and the course of the disease ranged from 2.5-4.0 years; the control group was aged between 26-45 years, and the course of the disease ranged from 2.6-4.1 years. The research was conducted according to the principles of the World Medical Association Declaration of Helsinki. This study was approved by the Ethical Medical Committee of the First Affiliated Hospital of Hainan Medical University. All subjects submitted written informed consent.

Inclusion criteria

(1) Patients who were diagnosed with LN by clinical diagnostic criteria; (2) Patients who matched the standards of traditional Chinese medicine syndrome differentiation of liver and kidney Yin deficiency; (3) Patients who were aged between 18 and 60 years old.

Exclusion criteria

(1) Patients with other lupus diseases (lupus myocarditis, lupus pneumonia, lupus encephalopathy, etc.); (2) Patients with diabetes mellitus or severe renal failure; (3) Women who were pregnant or lactating; (4) Patients who are allergic to the drugs used in the study.

Traditional Chinese medicine syndrome differentiation standard

Based on the *Principles of Clinical Research on the Treatment of Lupus with Traditional Chinese Medicine* [10], we established the standard of liver-kidney Yin deficiency syndrome, with the symptoms including tinnitus, soreness of waist, dry throat and mouth, hair loss, dry eyes, vexation.

Methods

Health education was conducted for all patients upon admission to inform patients of disease related knowledge and precautions.

Patients in the control group were treated with western medicine. Prednisone [SFDA approval number: H12020201; manufacturer: Tianjin Tianyao Pharmaceutical Co., LTD; Specification: 5 mg*100 s), 1 mg/(kg*d)], was taken with warm water in the morning every day. The drug dosage was increased or decreased after 5-7 weeks according to the condition, and the drug dose was maintained at 7-10 mg/d. In severe cases, methylprednisolone (0.5-1 g/d) was injected intravenously, and prednisolone was taken orally after remission. Patients were given cyclophosphamide [SFDA approval number: H32024654; manufacturer: Jiangsu Shengdi Pharmaceutical Co., LTD; Specification: 50 mg), each dose 600-900 mg/m^2]. Intravenous injection was performed after dilution with 20-40 ml normal saline, once a week, and the treatment lasted for 3 months.

On this basis, the experimental group was treated with traditional Chinese medicine according to the syndrome differentiation and classification of patients. The prescription was as follows: raw astragalus 50 g, Duhua 15 g, Chuanqi 15 g, Astragalus membranaceus, Salvia miltiorrhiza and Chinese yam 20 g each, 1 dose per day. The decocted dose was 500 ML, taken in the morning and evening, 250 ML each time, and the course of treatment was 3 months.

Detection method

Three ml of each patients' fasting venous blood was drawn in the morning before and after treatment and was collected, the serum was centrifuged to separate and stored in a -30°C freezer for use. The values of TNF-α, IL-6 and IL-18 in the serum of the two groups of patients were measured by ELISA kit (Xiamen Lunchang Biotechnology Co., LTD). The operation was carried out strictly according to the instructions, and the expression levels of inflammatory cytokines IL-6, IL-18 and TNF-α in peripheral blood of patients before and after treatment were observed.

Table 1. Comparison of the general data

Group	n	Gender		Age (years, x±s)	Course of disease (years, x±s)
		Female	Male		
Experimental group	40	33	7	34.57±4.52	3.27±0.65
Control group	40	34	6	34.62±4.46	3.24±0.62
x^2/t		0.092		0.050	0.211
P		0.762		0.960	0.833

Evaluation index

According to the criteria formulated by *International Society of Nephrology* [11], a reduction of ≥80% in systemic lupus erythematosus disease activity index (SLEDAI) score after treatment as compared with that before treatment was defined as markedly effective; if the (SLEDAI) index score decreased by ≥60% but <80% it was deemed effective; if it failed to meet the above standards it was determined as ineffective.

SLEDAI was used [12] to evaluate at 0, 8 and 12 weeks of treatment. The full score was 20, and 0-4 was basically inactive; 5-9 was light activity; 10-14 was moderate activity; ≥15 was strong activity.

Traditional Chinese medicine syndrome score includes tinnitus, pharynx dry mouth dryness, hair loss and vexation, each with 5 points in full. Higher score indicates the more serious symptoms.

Statistics analysis

The experimental data were analyzed by SPSS 20.0 software, and the counting data were analyzed by χ^2 test and represented by [n (%)]. Measurement data were given as (x±s) and were analyzed by t-test. P<0.05 indicated the difference was statistically significant.

Results

General information comparison

No statistical difference was found in the general data such as gender, age and disease course between these two groups (P>0.05), as shown in **Table 1**.

Comparison of levels of TNF-α, IL-6 and IL-18 in peripheral blood

The levels of TNF-α, IL-6 and IL-18 in the peripheral blood of the experimental group after treatment decreased, and the outcome was significantly better than the control group (P<0.05), as shown in **Figure 1A-C**.

Comparison of clinical efficacy

The total effective rate of treatment in the experimental group was higher than that in the control group (P<0.05), as shown in **Table 2**.

Comparison of SLEDAI score before and after treatment between the two groups

Before treatment, the SLEDAI scores of these two groups were similar (P>0.05). After 8 weeks and 12 weeks of treatment, SLEDAI score of the experimental group was significantly lower than the control group (P<0.05), as shown in **Table 3**.

Comparison of liver-kidney Yin deficiency syndrome scores before and after treatment between the two groups

The scores of liver and kidney Yin deficiency symptoms in both groups were significantly lower after treatment than before treatment (P<0.05), and the scores of liver and kidney Yin deficiency symptoms in the experimental group were much lower than in the control group (P<0.05), as shown in **Table 4**.

Discussion

In recent years, with the continuous improvement of medical diagnostic techniques and treatment, the prognosis of SLE has significantly improved. The survival rate of patients with SLE has increased from 50% with 4-year survival rate to 80% with 15-year survival rate, and the 10-year survival rate has also exceeded 90% [13]. Although clinical treatment prolongs the survival of most patients, SLE may cause serious damage to various organs of the body, especially the nervous system and kidneys. LN is a major cause of secondary renal disease and a significant contributor to SLE related

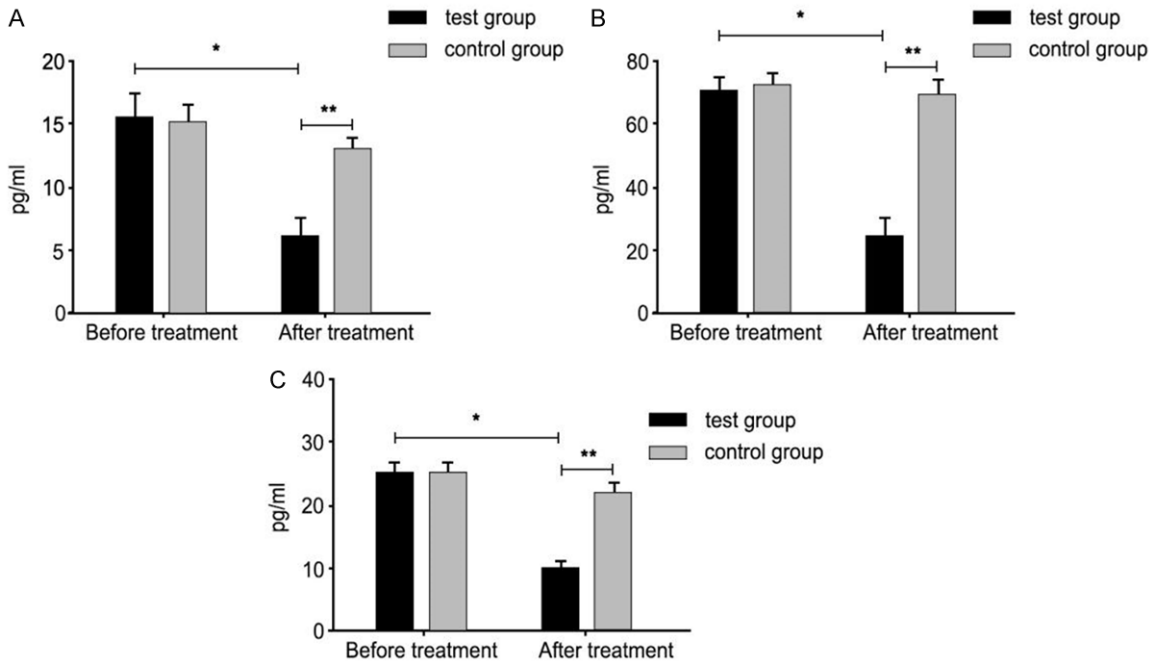

Figure 1. Comparison of IL-6, IL-18 and TNF-α levels. Note: A. The x-coordinate represents pre-treatment and post-treatment, and the y-coordinate represents pg/ml. The IL-6 level of the experimental and control group before treatment was (13.71±3.86) pg/ml and (13.54±3.76) pg/ml, respectively, and the IL-6 level after treatment was (5.47±1.66) pg/ml and (12.68±1.32) pg/ml. Asterisk indicates that the differences were statistically significant. B. The x-coordinate represents pre-treatment and post-treatment, and the y-coordinate represents pg/ml. The IL-18 levels of the experimental group and control group were (68.47±4.26) pg/ml and (69.11±4.58) pg/ml before treatment, and (31.66±3.87) pg/ml and (68.22±3.42) pg/ml after treatment, respectively. Asterisk indicates that the differences were statistically significant. C. The x-coordinate represents pre-treatment and post-treatment, and the y-coordinate represents pg/ml. The TNF-α level in the experimental group and the control group before treatment was (22.17±6.54) pg/ml and (22.24±6.49) pg/ml, respectively; the TNF-α level after treatment was (9.28±3.06) pg/ml and (19.78±5.57) pg/ml. Asterisk indicates that the differences were statistically significant.

death. Previous studies found that in the serum of LN patients, Th1 and Th2 cytokines tended to be increased. These secreted cytokines can promote self-proliferation and inhibit the proliferation of other cell types. Under normal conditions, Th1 and Th2 cells in the body are in a balanced state, while the imbalance results in the occurrence of many diseases. In allergic diseases such as scleroderma and SLE, Th2 is the dominant cell type. Thus, undertaking research on antagonists of Th2 which could effectively reduce cytokine secretion by Th2 cells is an important topic in the treatment of LN [14].

IL-6 is a Th2 type cytokine produced by a variety of cells, and is related to human allergic diseases. It can stimulate the immune response, promote the formation of antibody secreting cells from B lymphocytes, and form antibodies. According to the previous study, B cells do not express IL-6 antibodies in healthy people, but are expressed in LN patients [15, 16]. IL-18 belongs to the Th1 type cytokine, which can increase the expression of natural killer cells (NK) and cause apoptosis of epithelial cells. IL-18 can inhibit the biological activity of peripheral blood and reduce the immune response in LN. Thus, IL-18 plays a key role in the treatment of LN. TNF-α is one of the important causes of immune diseases. TNF-α activates NK cells in the body and promotes B cell chemotaxis, while the increase of TNF-α promotes the increase of IL-6 [17, 18]. Some scholars have speculated that the interaction or abnormal activation of immune active cells is the main cause of SLE, and immune regulation is an effective measure for the treatment of SLE.

In this study, LN was treated with traditional Chinese and western medicine, and the expression levels of IL-6, IL-18 and TNF-α were effectively inhibited, which were significantly different from those in the control group (P<

Table 2. Comparison of treatment effect between the two groups [n (%)]

Group	n	Significant effective	Effective	invalid	Total effective rate
Experimental	40	24 (60.00%)	14 (35.00%)	2 (5.00%)	95.00% (38/40)
Control	40	19 (47.50%)	12 (30.00%)	8 (20.00%)	77.50% (31/40)
x^2					5.165
P					0.023

Table 3. Comparison of SLEDAI score before and after treatment between the two groups (x±s)

Group	n	Before the treatment	8 weeks of treatment	After 12 weeks of treatment
Experimental	40	14.86±3.52	7.06±1.02	4.78±0.42
Control	40	14.79±3.49	9.25±1.35	7.24±0.62
T		0.089	8.186	20.776
P		0.929	<0.001	<0.001

Table 4. Comparison of liver-kidney Yin deficiency syndrome scores before and after treatment between the two groups (x±s)

Group	n	Time	Tinnitus	Dry throat dry mouth	Hair loss	Five upset hot
Experimental	40	Before treatment	3.91±0.28	3.93±0.38	3.83±0.35	4.05±0.36
	40	After treatment	1.13±0.21**	1.06±0.08**	1.17±0.18**	1.01±0.32**
Control	40	Before treatment	3.89±0.32	3.92±0.43	3.86±0.39	4.07±0.32
	40	After treatment	2.13±0.28*	2.28±0.23*	2.21±0.21*	2.03±0.22*

Note: *indicates the comparison between these two groups after treatment, P<0.05; **represents the comparison of the experimental group before and after treatment, P<0.05.

0.01). A study by Goyal et al. indicated that dampness and toxic blood stasis run through the whole disease [19-21]. Therefore, it is advised to promote blood circulation and remove blood stasis as the basic criteria for the treatment of LN. It is believed that traditional Chinese medicine can effectively remove excess antigens from the patient's blood, regulate the immune function of the body, and hinder the production of the immune complex. However, due to the small number of cases selected and the absence of long-term follow-up of patients, the study results may be biased, thus further studies are still needed.

To sum up, integrated traditional Chinese and western medicine treatment can effectively inhibit the secretion of inflammatory factors in peripheral blood of LN patients, so as to control clinical symptoms, improve the therapeutic effect, reduce disease activity, and facilitate the recovery of renal function. Therefore, it is worthy of clinical application.

Disclosure of conflict of interest

None.

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