Horsetail mixture on rheumatoid arthritis and its regulation on TNF-α and IL-10

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Abstract: Taking autoimmune inflammation of rheumatoid arthritis as entry point, this paper discussed the clinical effect of horsetail mixture on rheumatoid arthritis (RA) and its mechanism. A total of 60 cases of patients with RA were randomly divided into experimental group and control group using randomized controlled trial. We observed its biochemistry, TNF-α and IL-10 before and after treatment, and then systematically assessed the clinical effect of horsetail on RA. Results showed that the total effective rate of experimental group was 80%, while that of control group was 16.67%. After statistical treatment, the differences between two groups were significant ($p<0.01$). Comparison of the difference value of TNF-α ($p<0.05$) and IL-0.05 in serum between groups before and after treatment, there were significant differences. Comparison of CRP within group before and after treatment was significantly different ($p<0.05$), while comparison of CRP between groups was not significantly different ($p>0.05$). Comparison of ESR and RF within group before and after treatment was significantly different ($p<0.01$), and comparison of them between groups was also significantly different ($p<0.05$). Comparison of difference values within group before and after treatment were also significantly different ($p<0.01$). It was concluded that horsetail mixture has remarkable curative effect on rheumatoid arthritis, and its clinical application is safe and reliable. It has obvious down regulatory effect on cell factor TNF-α related to RA, that is, it can down regulate the level of pre-inflammatory factor TNF-α as well as the level of anti-inflammatory factor IL-10. Therefore, it is considered that the regulating effect of horsetail mixture on TNF-α and IL-10 is one of the mechanisms of its treatment on RA.

Keywords: Rheumatoid arthritis, TNF-α, IL, horsetail mixture.

INTRODUCTION

The evolution of the cognition of modern medicine on rheumatoid arthritis is from the initial rheumatoid factor (RF) and immune complex theory and T cell in rheumatoid arthritis to cytokine network theory (Duanyong *et al*., 2010). The brief retrospect of this evolutionary process makes us clearly realize the problem that we should face at present. Waaler firstly discovered RF in 1939 (Xiuyuan *et al*., 2011). Then Rose discovered that rheumatoid factor (RF) gathers sheep red blood cells in 1948. Moreover, Kunnel proved that RF was the antibody that combined in the segment of immunoglobulin FC (Yali *et al*., 2014). Then Zvaifler revealed that RF was the promoter of RA mediated by immune complex in 1973 (Jinghui, 2013). After that, immune complex theory can explain many expressions of RA during acute stage, but this hypothesis is still insufficient to explain the clinical manifestation of specificity of RA. Although immune complex theory can explain many expressions of RA during acute stage, the apparent intra-articular infiltration proved that T cell is one of key factors. Researches found (HLA)-DR, which exists in MHC, participates in antigen presentation and closely related to RA. It is firmly believed that the participation of T cell in pathogenesis and pathological process is ground-breaking idea. Some animal models with arthritis are used to study the mechanism of arthromeningitis. For example, arthritis induced by collagen or adjuvant is dependent from by T cell, which has provided contact for clinic and clinical research (Yue *et al*., 2014). In the late 1980s, with the new progress of molecular technique, people can detect various cell factors in synovium and joint fluid, and then we have the chance to verify the driving effect of T cell in RA. TNF-α is mainly generated from synovium macrophage in RA synovium and stimulates the proliferation of firoblast and the activity of lymphocyte. The generation of TNF and other inflammatory cytokines also collect other inflammatory cells, and successively release cell factors so as to magnify immune reaction. The appearance of TNF inhibitor showed the success of RA transcription. Based on the characteristics of cytokine network, as auto-stimulating factor, TNF-α in synovium can affect other cytokines. TNF-α inhibitor can slow down or prevent the progressive damage of RA bone and cartilage (Ronghua and Jianjun, 2013), which may be related to its function of inhibiting osteoclast (Kurt *et al*., 2002). Based on the modern pathological cognition of RA, and through the observation and comparison of proinflammatory cytokine change in RA pathology before and after treatment of patients with RA, this paper verified that horsetail medicine might intervene and down-regulate the level of proinflammatory factor TNF-α, up-regulate inflammatory cytokines IL-10 level and regulate the immune

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inflammatory of RA (Yinshan, 2001). Therefore, this paper conducted research on the regulating effect of horsetail mixture on TNF-α and IL-10 in RA.

MATERIAL AND METHOD

General Material

A total of 60 patients with rheumatoid arthritis were selected and divided into two groups. In experimental group, 5 cases were men and 25 women, ranging in age from 31 to 76 (mean 53±10 years), and the range of disease course is from 3 months to 10 years (mean 3.5±2.7 years). In control group, 5 cases were men and 25 women, ranging in age from 24 to 67 (mean 51±11 years), and the range of disease course is from 3 months to 20 years (mean 4.1±3.9 years). Patients who accept non-steroid anti-inflammatory drug and hormone therapy should have stable dose for at least 30 days before experiment. However, patients who accept other disease improving drugs must suspend drugs for at least 30 days. Patients and their main family members are sufficiently informed about the therapeutic schedule and risk and they all sign the informed consent. Therapeutic schedule is approved by the Medical Ethics Committee.

The diagnostic criteria of ARA rheumatoid arthritis from American College of Rheumatology in 1987 are (Wenxue, 2008): morning stiffness is at least 1h, with the duration of at least 6 weeks. Three or more joint are swelling; wrist, metacarpophalangeal joints or proximal interphalangeal joint is swelling; symmetry joint is swelling, all of the above swellings are with the duration of at least 6 weeks. Subcutaneous nodule and RF is positive, and titer is more than 1: 32. Of these patients do not include: later period of patients with severe joint malformation; patients with overlap systemic lupus erythematosus, sicca syndrome, severe knee osteoarthritis and other patients with rheumatism; patients with serious disease in heart, brain, liver, kidney, lung and hemopoietic system or with active canker; women in pregnancy or breast feeding stage, and psychopath.

Therapeutic method

Horsetail mixture, specification: 100mL/bottle, produced by Shanghai Guanghua Hospital; methotrexate tablets, specification: 2.5mg/tablet; diclofenac sodium, specification: 25mg/tablet. In experimental group: horsetail mixture 15mL, three times per day; methotrexate 7.5mg, once a week; diclofenac sodium 25mg, three times per day. In control group: methotrexate 7.5mg, once a week; diclofenac sodium 25mg, three times per day. Both treatment courses of two groups are three months. ELISA (Enzyme-Linked Immuno Sorbent Assay) is used to detect index.

Marking criteria of clinical symptoms

Elevation criteria of clinical symptoms are as follows. Conduct grading record on local symptoms of joint: time of joint morning stiffness, index of arthralgia, index of joint swelling, grip. Evaluation criteria: morning stiffness time is calculated by minute; arthralgia index of is calculated by total painful joint number of each patient; joint swelling index is calculated by total swelled joint number of each patient. Integrated function integral of joint: 0 stands for no joint dysfunction; 1 stands for light dysfunction. One or more joints are functionally limited, but it can work normally. 2 stands for moderate joint dysfunction. Joint function is obviously limited and patient can still take care of him self, but he cannot work normally. 3 stands for severe joint dysfunction. Patients lie in bed or in wheelchair, and they cannot take care of themselves. Grip: fold the wristband of blood pressure monitor two times and stuff it in suited cloth bag. Before check, fill it to 30mmHg, then patients hold pressure for three times using both two hands. Record the stated value and draw mean value.

STATISTICAL ANALYSIS

Comparison of measurement data between group and before and after treatment is detected using t. Comparison of rate is detected using χ^2. Results are expressed by x±s.

RESULTS

Results analysis of curative effect

Laboratory index: blood sedimentation, C-reactive protein, RF, TNF-α and IL-10. They are all calculated according to conventional criteria. Evaluation criteria of total therapeutic effect are as follows. Obvious effect: the sum of integral of index after treatment is lowered 70% than that before treatment; effectiveness: the sum of integral of index after treatment is lowered 50% to 70% than that before treatment; improvement the sum of integral of index after treatment is lowered 30% to 50% than that before treatment; invalid: the sum of integral of index after treatment is lowered less than 30% than that before treatment as shown in tables 1 and 2.

From table 2, it was clear that serum TNF-α of two groups after treatment obviously decreased, while IL-10 increased. Comparison of the difference value of TNF-α ($p<0.05$) and IL-10 ($p<0.01$) within group before and after treatment was apparently significant. Both two groups had regulating effect on the above two indexes, and that of experimental group was obviously superior to that of control group.

Comparison of the change of CRP, ESR and RF before and after treatment

As shown in table 3, respective comparison within group of CRP before and after treatment was significantly different ($p<0.01$), while respective comparison between groups of that was not significantly different ($P<0.01$). Respective comparison within group of ESR and RF

Table 1: Comparison of clinical effect of horsetail mixture on RA

Group	Obvious effect	Improved	Effective	Invalid	Mixture	Total effective rate
Experimental group	26	22	0	30	80.00	
Control group	0	25	4	1	30.00	16.67%
Sum	2	31	26	1	60.00	

Table 2: Effect of horsetail mixture on serum TNF-α and IL-10 (x±s)

Group	Case		IL-10(ng/mL)	TNF-α (ng/mL)
Experimental group	30	Before treatment	46.06±69.00	44.75±7.06
		After treatment	88.08±83.89	40.80±7.58
Control group	30	Before treatment	52.22±73.81	44.51±8.53
		After treatment	75.54±73.31	42.52±7.90

Table 3: Comparison of the change of CRP, ESR and RF before and after treatment (x±s)

Group	Case	CRP (mg/L)		ESR (mm/h)		RF (mm/h)	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Treatment group	30	33.16±18.16	13.80±9.15	51.37±29.82	20.70±12.73	315.80±194.45	145.78±122.35
Control group	30	33.14±34.30	17.04±20.36	36.15±34.82	25.03±22.39	197.42±192.70	143.60±180.54

Table 4: Changes of morning stiffness, grip, number of arthralgia, number of swelled joint and grading of joint function before and after treatment (x±s)

Group	Case		Morning stiffness (min)	Grip (mm Hg)	Number of arthralgia	Number of swelled joint	Grading of joint function
Treatment group	30	Before treatment	132.67±55.61	75.06±18.61	17.73±3.63	14.06±5.93	1.77±0.68
		After treatment	54.36±32.21	99.88±30.56	7.93±3.46	5.64±3.54	0.77±0.63
Control group	30	Before treatment	113.45±42.49	77.09±20.12	20.00±3.32	16.43±4.82	1.91±0.84
		After treatment	51.82±24.84	85.69±25.48	11.40±4.11	8.67±3.69	1.03±0.68

before and after treatment was significantly different ($p<0.01$), and that between groups was also significantly different ($p<0.01$). It was indicated that the improvement of test rating in experimental group except CRP was superior to that in control group.

Score change of each function before and after treatment

As shown in table 4, respective comparison of morning stiffness time, number of arthralgia hand grip, number of swelled joint and grading of joint function within group was significantly different ($p<0.01$). Comparison of joint function grading between groups was not significantly different ($p>0.05$). Comparisons of difference value of the other were all significantly different ($p<0.01$). It showed that the grading of joint function before and after treatment of two groups was not significantly different, while other indexes were all improved after treatment, and that of experimental group was superior to that of control group.

Adverse reaction

We strictly observed and detailed recorded various adverse reactions during and after the period of medicine. In treatment group, we found two patients with diarrhea in the whole process of treatment. In control group, two patients appeared the decline of white blood cell, three of abnormal liver function and one of dental ulcer. All patients were visited without exception.

DISCUSSION

TNF-α is about 4~5ng/mL or 0 ~2U/mL in normal person and 35pg/mL in synovia, but its level obviously increased in peripheral blood of patient with RA (TNF-α is about 4.3~330pg/m in serum of RA patient and 120~137pg/mL in synovial) (Wei *et al.*, 2009).

Modern pharmacological research has mentioned that the stem of solanum vine contains steroid glucoside, and its extractive steroid saponin has the effect of anti-tumor

(Yanling and Huishan, 2004). The whole herb of dandelion contains taraxasterol, choline, synanthrin, pectin, etc, which has the effect of anti-pathogeny microorganism, anti-gastric ulcer, anti-tumor, etc. In addition, it also has the function of broad-spectrum antibacterial, choleretic and hepatic protective, anti-endotoxin, stomachic and immunological enhancement (Changling *et al*., 2011). Sarsaparilla contains isoengelitin, disogenin, etc, which has the effect of anti-inflammatory, anti-tumor, and antibacterial. Horsetail can reduce blood pressure and lipid, keep clam, anti-convulsion, anti-viral, inhibit platelet aggregation, etc (Dongyi and Ting, 2014). Though the pharmacological research of modern Chinese medicine chemistry and Chinese patent medicine on this prescription do not directly act on report of RA, it can indirectly indicate its therapy mechanism on RA from the perspective of its effects like anti-inflammatory, analgesis, etc.

Clinical effects of horsetail mixture on RA are as follows. This research data showed that the total effective rate of treatment in experimental group that added small dose of western medicine to horsetail mixture was 80%, and that in control group with pure western medicine therapy was 16.67%. Experimental group was apparently superior to control group. It hinted that the drug combination of horsetail mixture and methotrexate was superior to western medicine therapy only TNF-α in serum of two groups after treatment obviously increased, while IL-10 obviously decreased. Comparison of the difference value of two groups was significantly different. It indicated that both of the two groups had regulating effect on the above two cell factors index, and experimental group was superior to control group. Seen from laboratory index of ESR, RF, etc, the respective comparison within two groups before and after treatment was apparently different ($p<0.01$), and the different value comparison between two group was also different ($p<0.01$). It showed that the improvement of the above two indexes in experimental group was superior to that in control group. Respective comparison of morning stiffness time, number of joint pain, hand grip, number of swelled joint and grading of joint function within group before and after treatment was all significantly different ($p<0.01$). It indicated that there was no obvious difference on the grading of joint function of two groups before and after treatment, while the other indexes were all improved before and after treatment of two groups, and experimental group was superior to control group. Mechanism of horsetail mixture on RA was as follows. The clinical observed results showed that horsetail mixture with small dose of western medicine can apparently down regulate the TNF-α level in serum and up regulate IL-10 level. Compared with pure western drug, the difference was significant ($p<0.01$). It hints that the down regulating effect of horsetail with small dose of western drug on TNF-α is superior to that in control group. Therefore, it is considered that through regulating TNF-α, horsetail mixture has got significant therapeutic effect.

CONCLUSION

The above research is under the guidance of traditional Chinese medicine. Taking patients combine with disease and syndrome and animal model as objects, and taking RA autoimmune inflammation theory for entry point, this paper successfully observed the clinical effects of horsetail mixture on humid heat RA and its mechanism. Therefore, we get the following conclusions: horsetail mixture with small dose of MTX has remarkable effect on RA patient, not yet with any adverse reaction. Horsetail mixture has apparent regulating effect on the level of TNF-α and IL-10 in serum of RA. That is to down regulate TNF-α level and up regulate IL-10 level. The regulation of horsetail mixture on the pre-inflammatory factor (TNF-α) and inflammatory cytokines (IL-10) is one of its mechanism of clinical effects. Horsetail mixture is considered as the effective prescription of clinical treatment on RA, which deserves further systematic study and popularization and application.

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