

Clinical Evaluation on Treatment of Oral Lichen Planus with Yiqiyangyin Mixture and Its Mechanism

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Abstract

To observe the clinical effects of Chinese herbal medicine Yiqiyangyin Mixture on oral lichen planus (OLP) and to explore its mechanism, we randomly selected two groups of newly diagnosed patients, they were treated with Yiqiyangyin Mixture and total glucosides of paeony (TGP), respectively, for three months, and the clinical effects of the two treatment system were compared by using semi-quantitative REU scoring system; and the changes of NF- κ B-dependent inflammatory medium TNF- α , IL-1 β , IL-6, IL-8 and TH1/TH2 representative cytokines IFN- γ , IL-10 levels were also observed before and after treatment. The overall effective rates on OLP were no significant difference between trial group and control group, but the therapeutic effect of Yiqiyangyin Mixture on the erosive OLP was more significant, its subjective symptoms and objective signs were all evidently improved, and compared with the TGP group, $P < 0.05$; the changes of NF- κ B-dependent inflammatory mediators TNF- α , IL-8 and TH1/TH2 representative cytokine IL-10 were significantly before and after treatment ($P < 0.05$). It shows that Chinese herbal medicine Yiqiyangyin Mixture is effective for treatment of OLP, especially the effect on erosive OLP is more significant, and the prescription may plays a role through regulating Th1/Th2 immune balance and blocking activation of NF- κ B information pathway.

Keywords: oral lichen planus; Chinese herbal medicine Yiqiyangyin Mixture; NF- κ B dependent cytokines; TH1/TH2 cytokines.

1. Introduction

Oral lichen planus (OLP) is a chronic non-specific skin and mucous inflammatory disease related to autoimmunity, one of the common oral mucosa diseases. Its prevalence is 0.1% -4%, the lesion morphologies are diversified, they are divided into the common type and the erosive type according to the status of basal mucosa, and the erosive type has the most severely affection on the patients in clinical. OLP is generally considered to have a

cancerous tendency and is a precancerous condition [1].

The etiology and pathogenic mechanism of OLP is not yet clear. The current studies suggest that mainly it relates to T lymphocyte-mediated immunological abnormalities, the antigenic nature of the oral epithelial cells changes under the effect of various pathogenic factors and the oral epithelial cells are ingested by Langerhans cells and macrophages, and presents to T lymphocytes, activating T lymphocytes, releasing various cytokines, and initiating a series of immune responses [2]. Studied found a wide variety of cytokines, in which, the changes of interferon, various interleukin, and tumor necrosis factor level were associated with the development of oral lichen planus [3].

Currently the treatment of OLP is ineffective, mainly the lesion is difficult to completely disappear and the recurrence rate is higher. The western medicine and physical therapy are always used abroad [4], while the treatments of Traditional Chinese Medicine and combination of Chinese medicine with the western medicine are paid more attention at the domestic. Yiqiyangyin Mixture is one of Chinese patent medicine made by Beijing Hospital of Chinese medicine, with the effects of invigorating qi and nourishing yin as well as activating blood to remove stagnation. The clinical practices of three generations old TCM in Beijing Hospital of Chinese Medicine have proved that the therapeutic effects on skin disease patients with yin deficiency, blood stasis and qi deficiency are effective by using Yiqiyangyin Mixture for evidence-based treatment, with small side effects, easy to take and good stability. For clinical use and observation over a long period found that the Mixture has a very good effect on the regulation of human immunity, and improvement of microcirculation.

This trial uses a clinical design method of randomized, single blind positive drugs parallel to the control to evaluate the therapeutic effects of traditional Chinese herbal medicine Yiqiyangyin Mixture on OLP via observation on the clinical effects of both Chinese herbal medicine Yiqiyangyin

Mixture and total glucosides of paeony (TGP) for OLP, and select NF- κ B-dependent inflammatory mediators TNF- α , IL-1 β , IL-6, IL-8 and the TH1/TH2 representative cytokines IFN- γ , IL-10 as the observation indicators. The affection of the prescription on OLP patients' peripheral blood cytokines is further explored via detecting the changes of peripheral blood cytokine levels in OLP patients before and after treatment of Yiqiyangyin Mixture.

2. Materials and Methods

Case inclusion criteria

- (1) Age 18-65 years;
- (2) Meet the diagnostic criteria of oral lichen planus (skin changes can be combined) and both Qi and Yin injuries with stasis syndrome (yin deficiency blood stasis syndrome) the criteria of syndrome differentiation of TCM;
- (3) Signed informed consent form.

Syndrome differentiation of TCM

(1) Qi deficiency syndrome: Main symptoms: shortness of breath, fatigue, lassitude, weak pulse. Secondary symptoms: spontaneous sweating, lazy words, pale tongue.

(2) Yin deficiency syndrome: Main symptoms: mouth and throat dryness, thirst but not want to drink, red tongue and few saliva. Secondary symptoms: fatigue, upset, irritability, thready pulse.

(3) Blood stasis syndrome: dark purple tongue or ecchymoses, astringent or sink pulse, string late. Anyone with two items of the main symptoms or one item of the main symptoms and 2 items of the secondary symptoms can be diagnosed as Yin deficiency and blood stasis syndrome type of OLP.

Exclusion criteria

- (1) Patients with other established oral mucosal disease;
- (2) Patients with more serious systemic disease, and cancer;
- (3) Patients used antibiotics within 1 month or immune agents within 3 months;
- (4) Patients whom may be caused lichenoid reaction with certain drugs or silver amalgam fillings;
- (5) Smokers and alcoholics within 3 months;
- (6) Pregnancy, breast-feeding women;
- (7) Anyone can not be prescribed medication or whose trial process records are incomplete, affecting judgment of the efficacy.

Treatment protocol

80 patients with OLP were randomly divided into treatment group and control group, the treatment group was oral administrated with Yiqiyangyin Mixture, 10ml/time, 3 times/d; the control group was oral administrated with TGP capsules, 2 granule s/time, 3 times/d; continuous administration for 3 months in both groups, 1 re-examination per month,

and the observation indicators were recorded at patients' initial and final re-examination by the same examiner.

Evaluation of therapeutic effect

By using semi-quantitative REU scoring system [5] (Table 1), the mouth is divided into 10 regions (lip, left cheek, right cheek, dorsal tongue, ventral tongue, floor of mouth, hard palate, soft palate, maxillary gingival and mandibular gingival), The signs and symptoms were scored, respectively, for each area and then a comprehensive evaluation of therapeutic effect is carried out.

Table 1. Scores of REU signs for oral lichen planus.

Types of clinic	Scores of signs
Reticulate / plaque-type (R)	0 = none, 1 = white streaks or patches
Congestive / atrophic (E)	0 = none, 1 = lesions <100mm ² , 2 = lesions 100mm ² to 300mm ² , 3 = lesions > 300mm ²
Ulceration (U)	0 = none, 1 = lesions <100mm ² , 2 = lesions 100mm ² to 300mm ² , 3 = lesions > 300mm ²
Total	$\Sigma R + \Sigma E \times 1.5 + \Sigma U \times 2$

Scores of OLP symptoms

Degrees of pains are divided into: Severe pain, Moderate pain, Mild pain, and No pain

Evaluation Criteria of therapeutic effects

Recovery: signs score and symptoms score as 0;

Effective: Signs score and symptoms score decrease;

Ineffective: signs score and symptoms score unchange or increase.

Trial method

Method of sampling

5ml of fasting quiet venous blood was sampled for OLP patients before treatment, and 5ml of fasting venous blood was sampled on the next day after end of treatment; 5ml of fasting quiet venous blood was sampled for healthy volunteers in the control group. After separation of serum, the samples were stored in -20 °C ready for use.

Detection of cytokines

The levels of cytokines in the serums were detected by double antibody sandwich ABC-ELISA method, and the operation was carried out according to the instructions of ELISA kit, programming on the HTS7000 Plus. After the standard curve was read out correctly using the standard material, the formal experiment was carried out. 100ul of re-thawed supernatant was taken for each sample and was added to the corresponding hole plate, and then

adding biotin, concentrated enzyme-linked material and chromogenic liquid in sequence. Finally, OD values were detected by the analyzer and the contents of samples were automatically calculated. All samples are detected using multiple holes, and the average value was taken as the final result.

Statistical analysis

The statistical analysis of the data was completed by using SPSS13.0 statistical software. The serologic detection result was expressed as mean \pm standard error ($\bar{x} \pm s$), and the mean numbers between the two groups were compared using t test, and for the enumeration data using χ^2 test.

3. Results

Comparability of data

As to gender, age, course of disease and other basic information for the two groups of patients, the statistical analysis showed no significant difference between the two groups ($P > 0.05$). In treatment

group, there were 12 males and 28 females; in the control group, there were 10 males and 30 females (Table 2).

Comparison of the recoveries of the lesion between the two groups after treatment

The total effective rate was 87.5% in the trial group, and 82.5% in the control group, the statistic data were examined by χ^2 test, $P > 0.05$, no significant differences (Table 3). Comparison of VAS symptom scores for the subjective efficacy showed that VAS scores decreased more significantly after treatment of Yiqiyangyin Mixture group, there was significant statistical difference ($P < 0.05$) (Table 4). Comparison of REU scores in the objective efficacy showed that the score values in both groups were reduced, but no statistical difference (Table 4). Also found that the difference of the therapeutic effects between the treatment group and control group was more significant for erosive OLP, and the comparisons of VAS scores and REU scores were significantly different ($P < 0.05$).

Table 2. Comparison of the basic situations between the two groups.

group	age $\bar{x} \pm s$	course of disease $\bar{x} \pm s$	Type of lesions	
			common	Erosive
Treatment group	47.5 \pm 12.3	16.2 \pm 5.3	16	24
Control group	46.3 \pm 15.4	17.8 \pm 6.1	18	22

Table 3. Comparison of the therapeutic effects between the treatment group and control group.

group	n	recovery	significantly effective	ineffective	effective rate
control	40	4	29	7	82.5%

Table 4. Comparison of VAS symptom scores ($\bar{x} \pm s$) between the treatment group and control group.

group	n	before treatment	after treatment
treatment group ^{a*}	40	7.5 \pm 2.5	2.0 \pm 1.5
common type ^d	16	3.4 \pm 1.2	1.5 \pm 0.3
erosive type ^{c*}	24	10.8 \pm 2.3	3.2 \pm 1.4
control group ^{a*}	40	6.3 \pm 2.2	3.4 \pm 2.4
common type ^d	18	3.3 \pm 1.4	1.8 \pm 0.5
erosive type ^{c*}	22	9.6 \pm 2.2	5.7 \pm 1.3

^a Comparison of treatment group and control group, $P < 0.05$;

^b Comparison of common type between the two groups, $P = 2.94$;

^c Comparison of erosive type between the two groups, $P < 0.05$.

Comparison of NF- κ B-dependent cytokines levels in the OLP patients before and after treatment

The results of trials showed that TNF- α , IL-6, IL-8 levels in common type of OLP patients before treatment were significantly higher than that of the healthy control group ($P < 0.05$); and the TNF- α , IL-

8 levels decreased to normal levels after treatment, compared with before treatment, the difference was significant ($P < 0.05$), although the IL-6 levels decreased but compared with before treatment, no significant difference; while the IL-1 β levels before and after treatment had no significant changes (Table 5).

Table 5. Comparison of REU signs score ($\bar{x} \pm s$) between the treatment group and control group.

group	n	before treatment	after treatment
treatment group a	40	10.1±2.5	2.6±1.5
common type b	16	5.3±1.2	1.4±0.4
erosive type c*	24	14.8±2.3	3.3±1.4
control group a	40	8.7±2.2	3.1±2.4
common type b	18	5.2±1.4	1.8±0.5
erosive type c*	22	12.4±2.2	5.7±1.3

^a Comparison of treatment group and control group, $P = 0.08$;

^b Comparison of common types between the two groups, $P = 1.43$;

^c Comparison of erosive types between the two groups, $P < 0.01$.

The IL-1 β , IL-6, IL-8, TNF- α levels in the erosive type of OLP patients before treatment were higher than that of the healthy control group ($P < 0.05$); After treatment, IL-1 β , IL-8, TNF- α were recovered to normal levels, and IL-6 level decreased but still higher than that of healthy control group ($P < 0.05$); compared with changes in cytokine levels before and after treatment, only the changes of TNF- α , IL-8 were statistically significant ($P < 0.05$) (Table 6).

Table 6. Comparison of NF- κ B cytokines levels in OLP patients before and after treatment.

group		IL-1 β (pg/ml)	IL-6 (pg/ml)	IL-8 (ng/ml)
Common type	before treatment	0.20±0.03	125.37±36.72 ^a	1.25±0.26 ^a
	after treatment	0.18±0.04	112.69±45.11	0.57±0.34 ^b
Erosive type	before treatment	0.34±0.05 ^a	178.62±36.18 ^a	2.32±0.47 ^a
	after treatment	0.25±0.06	149.45±52.24 ^{ab}	0.64±0.32 ^b
control group		0.19±0.04	108.85±37.25	0.54±0.09

^a $P < 0.05$, compared with the healthy control group; ^b $P < 0.05$, compared with before treatment.

Comparison of TH1/TH2 representative cytokine levels in the OLP patients before and after treatment

The results of trials showed that Th1 representative cytokine IFN- γ and Th2 representative cytokines IL-10, there were no significant changes in the common type of OLP group before and after treatment ($P > 0.05$); in erosive OLP group, IFN-

level in the serum was lower than that of the normal control group before treatment, the difference was statistically significant ($P < 0.05$), and it recovered to the normal level after treatment, while the expression of IL-10 had a down trend after treatment ($P < 0.05$) (Table 7).

Table 7. Comparison of TH1/TH2 cytokines levels in the OLP patients before and after treatment.

group		IL-10	IFN- γ
Common type	before treatment	17.40±1.06	13.97±1.70
	after treatment	17.18±1.54	14.35±1.06
Erosive type	before treatment	18.85±2.20	11.22±2.01 ^a
	after treatment	16.30±2.05 ^b	15.04±2.17
control group		17.92±6.30	14.67±2.65

^a $P < 0.05$, compared with the healthy control group; ^b $P < 0.05$, compared with before treatment.

4. Discussion

The treatment of oral lichen planus with Traditional Chinese Medicine (TCM) has a long history. According to TCM, the oral organ is a part of the whole body, is also the gateway of the digestive tract, it is closely linked with the internal organs, and various meridians and collaterals intersect in the oral cavity, so the disease is closely related with

the meridians organs. According to the epidemiological characteristics of prone incidence in middle-aged women of this disease, in combination with long-term clinical practice, it is considered that the deficiency of both Qi and Yin is the root cause of OLP incidence, in which the Yin deficiency is a main cause that results in the local mucosal dystrophy, while blood stasis occurs in the whole course of lesions, thus the treatment should be based on the

invigorating qi and nourishing yin in combination with activating blood to remove stagnation [6].

This trial uses TCM Yiqiyangyin Mixture to treat the oral lichen planus, and the result shows it is effective, and the total effective rate is 87.5%; compared with TGP, Yiqiyangyin Mixture is significantly more effective for the erosive OLP, and the subjective symptoms and objective signs are improved, $P < 0.05$. Yiqiyangyin Mixture is one of chinese patent medicines homemade by the Beijing Chinese Medicine Hospital, the main ingredients of the formulation are *Glehnia littoralis*, *Rhizoma polygonatum*, *Radix scrophulariae*, *Astragalus*, *Codonopsis tangshen*, *lithospermum*, etc., in which the main effects of *Glehnia littoralis*, *Rhizoma polygonatum*, *Radix scrophulariae*, etc., on nourishing yin and generating body fluid; *Astragalus* and *Codonopsis tangshen* on replenishing qi, focusing on the efficacy of replenishing qi and generating Yin. The modern pharmacological studies suggest that the two ingredients have a role in regulating immune function, inhibiting T cell function, and inhibiting the inflammatory response; *Lithospermum* has effects of cooling and activating blood, as well as detoxification and removal stagnation. The modern pharmacological studies suggest that activating blood and removing stasis can improve the microcirculation and permeability of capillary, regulate metabolism of connective tissue, enhance immune function, anti-inflammatory, anti-allergy, and promote repair of tissue, and they are beneficial to healing of erosion. This formulation not only improve symptoms and shorten the course of the disease, but also restore the local lesions, improve blood circulation, reduce the local inflammatory exudates, promote repair of epithelial, and heal the erosion for patients with oral lichen planus.

About exact etiology and pathogenesis of OLP are still unknown, and presently it is considered from most scholars' studies that OLP is a T cell-mediated inflammatory disease, in recent years with the research progress on the structure and function of the NF- κ B, the "central control" effect of NF- κ B in inflammation and immune response is gradually highlighted, it can bind specifically to the promoter or enhancer sites of multiple genes, start and regulate the gene transcription, and promote the expression and release of inflammatory mediators [7]. In this study, OLP patients were divided into common type and erosive type, NF- κ B-dependent IL-1 β , IL-6, IL-8, TNF- α levels were observed in serum of patients with inflammatory mediators, the changes of TNF- α , IL-8 were found to be significant before and after treatment, they were significantly higher before treatment, and nearly recovered to normal after treatment; IL-6 also has the same change trend, but its sensitivity was not as high as that of TNF- α and IL-8; and IL-1 β has no significant change. This suggests that

TNF- α and IL-8 can be used as sensitive indicators for monitoring OLP activities, guiding treatment and judging prognosis.

TNF- α almost participates in all links of the immune pathogenesis of OLP, including activation of T lymphocyte inducing cytotoxic, mediation of T lymphocytes-infiltrating into the epithelial tissue and inducing apoptosis of keratinocytes, activation of NF- κ B up-regulating IL-6, IL-8 and expression of TNF- α itself, resulting in a chronic persistent process due to alternating occurrence of the lesion recurrence and the healing in the active and quiescent period of OLP [8,9]. IL-6 and IL-8 are all inflammatory mediators with a strong effect, mainly secreted by monocytes / macrophages, can be used as the markers for cascade activation of cytokines to reflect the host's inflammatory response and disease severity, and are the same results from study by Sun et al [10]. This study showed that IL-6 and IL-8 levels in serum of patients with OLP were significantly higher than that of the normal group, and both levels decreased significantly after drug intervention, but IL-6 level in the serum of patients with erosive OLP was still significantly higher than the normal level, speculating that it may be related to IL-6 derived from a variety of cells, and IL-6 as a defense factor for the early release in the immune response and inflammatory responses of host, continues to play a role in the chronic inflammation process. The IL-8 relative to IL-6 is more sensitive for monitoring the condition of patients with OLP.

The polarization state of Th cells in patients with OLP has attracted more and more attention from scholars [11]. The type and signal strength of cytokines are the most important factors affecting Th cell differentiation, not only determine the type of Th cell subgroup, but also regulate the important mediating and regulatory role of the network on Th cell differentiation through a complex cytokine.

IFN- γ is a classic cytokines secreted by Th1 cells, plays an important role on maintaining the stability of Th1 cells, is the main pro-inflammatory cytokines [12]. IL-10 is mainly produced by secretion of Th2 cells, is cytokine inhibiting inflammatory responses, and plays a key role in the differentiation of Th2 cells [13]. This study found through detection that IFN- γ /IL-10 levels have no significant change in the patients with common type of OLP before and after treatment, but the expression of IFN- γ increased relatively in the patients with erosive OLP before and after treatment while the level of IL-10 declined relatively. This suggests that during the development of OLP, there is Th1/Th2 immune imbalance, and IFN- γ /IL-10 ratio can be an auxiliary examination method for diagnosis of OLP and determination of the treatment effects for OLP.

5. Conclusions

Chinese herbal medicine Yiqiyangyin Mixture is effective for treatment of OLP, especially for erosive

OLP the effect is more significant; activation of NF- κ B information pathway and polarization of Th cell may play an important role in the occurrence and development of oral lichen planus, Yiqiyangyin Mixture may play a therapeutic role via adjusting the Th1/Th2 immune balance and blocking activation of NF- κ B information pathway.

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