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# Effect of Smilax China Capsules and azithromycin combined therapy on chronic annexitis

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ABSTRACT

**Objective:** To explore the mechanism of Smilax China (Chinese spelling: Jingangteng) Capsules and Azithromycin combined therapy for chronic annexitis, and offer help to patients with chronic annexitis on relevant clinical therapies. **Methods:** A total of 170 cases of patients with chronic annexitis were selected from the gynecological department in our hospital, and randomly divided to be the combination therapy group and the control group by digital table, 85 cases for each group. Patients in control group were treated with Azithromycin. Patients in combination therapy group were treated by giving Smilax China capsules based on the Azithromycin treatment. Relevant indexes of lymphocyte subsets ($CD3^+$, $CD4^+$, $CD8^+$ and $CD4^+/CD8^+$), cytokines (TNF- α , IL-2, IL-6 and IL-10) and hemorheology (blood viscosity, plasma viscosity, hematocrit, red blood cell aggregation index) in patients of the two groups were detected before and after treatment. **Results:** Before treatment, no statistical significance found on the differences of lymphocyte subsets, cytokines and hemorheology between the two groups of patients ($P>0.05$); After treatment received on the two groups of patients, indexes of $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ were dramatically increased, $CD8^+$, cytokines (TNF- α , IL-2, IL-6 and IL-10) and hemorheology in the combination therapy group were significantly decreased compared with patients in the control group; Statistical significance existed in differences between the two groups ($P<0.05$). **Conclusions:** Patients who received Azithromycin therapy added with Smilax China capsules concurrently could be significantly improved levels of lymphocyte subsets, cytokines and hemorheology index. It is of clinical importance for treatment of patients with chronic annexitis.

## 1. Introduction

Chronic annexitis is a common reproductive system disease in fertile women[1], it manifests as recurrent attacks of chronic inflammation, with the passing of a long time, pelvic cavity hyperaemia could appear, adhesion, even fibrosis of the peripheral tissue could be generated. Since the specificity of the inflammatory site, relevant medicines could not completely arrive at the site of focus to play a role, so that cure rate of chronic annexitis always showed as a low level. Chronic annexitis has a relatively high recurrent rate, and more adverse reactions[2,3]. If no effective and timely therapy received, ectopic pregnancy, infertility and endocrine dyscrasia could be easily caused[4,5]. In our study, the mechanism of Smilax China Capsules and azithromycin combined therapy for chronic annexitis was explored, which could offer assistance to the clnical relevant treatments for patients who had chronic annexitis.

## 2. Materials and methods

### 2.1. General materials

A total of 170 patients with chronic annexitis were selected from

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the gynecological clinic in our hospital from Mar 2014 to Apr 2016. They were randomly divided to two groups by digital table method. There were 85 patients in the combination therapy group, ages were 22-44 years old, the average age was (31.3±3.2) years old, and course of disease was from 4 months to 8 years, the average course was (3.6±2.5) years. There were 85 patients in the control group, ages were 23-46 years old, the average age was (32.7±2.9) years old, and course of disease was from 3 months to 10 years, the average course was (3.8±3.1) years. No statistical significance existed in the differences of age, pregnancy history, and course of disease between the two groups of patients ($P$>0.05).

### 2.2. Inclusive and exclusive standards

Inclusive standards (Refer to the diagnostic criteria in Obstetrics and Gynecology[6]): (1) Medical history: dragging and distending or pain on both sides of the lower abdomen with or without lumbosacral pain, which was aggravated when feeling tired, after sexual activity and around the menstruation, and was also accompanied with irregular menstruation and more leucorrhea. (2) Physical sign: Streaks or flake thickening touched by one side or both sides of uterus, and mild tenderness felt. (3) Fallopian tube thickening or pelvic effusion found on one side or both sides by indication of vaginal ultrasonography. (4) Disease of ectopic pregnancy, endometriosis, pelvic tuberculosis and so forth were excluded.

Exclusive standards: Patients with severe dysfunction on heart, liver or kidney, patients who had other serious gynecological disease, patients who had history of allergic to the above drugs, patients who could not coordinate the treatment or who had bad treatment compliance, patients who were lost to follow up during treatment were excluded.

Our study had been received approval by the ethics committee in our hospital for implementation. All the consent forms were signed.

### 2.3. Therapeutic Methods

Patients in control group: 0.5 g Azithromycin+500 mL 5% GS was given through intravenous drip once a day, 5 days a cycle. Patients in combination therapy group: Smilax China (Chinese spelling: Jingangteng) capsules (Approval number: Z19991031, Hubei Furen Pharmaceutical Co., Ltd.) was added besides azithromycin treatment with 4 capsules a time, 3 /day.

### 2.4. Indexes detection

A total of 5 mL peripheral blood was extracted separately from the two groups of patients before therapy or 5 days after therapy, and put into -80℃ for test. ELISA method was utilized to measure tumor necrosis factor α (TNF- α ), interleukin IL-2, IL-6 and IL-10. The kits were provided by Wuhan Huamei Biological Engineering Co., Ltd., Shanghai Hengfei Biological Technology Co. Ltd., Nanjing Jinsirui biological science and Technology Co., Ltd. and Shanghai enzyme research Biological Technology Co., Ltd. OD absorbance was determined at 450 nm by Microplate Reader (Model: Infinite 200, company: TECAN, Switzerland), and the corresponding concentration was calculated by standard curve. Flow cytometry (BD Inc., Model: FACSCalibur/ Calibur, USA) was utilized to test the ratio of $CD3^+$, $CD4^+$, $CD8^+$ and $CD4^+/CD8^+$ cells, relevant antibodies were provided by Nanjing Jinsirui biological science and Technology Co., Ltd. Automatic blood rheology detector (Beckman Coulter Inc., Model: DxH 800) was used to detect whole blood viscosity (WBV), plasma viscosity (PV), packed cell volume (PCV) and erythrocyte aggregation index (EAI). All the experimental manipulations were strictly conducted in accordance with the instructions.

### 2.5. Statistical methods

SPSS 20.0 was used for statistical analysis of relevant datas, lymphocyte, cytokine and hemorheology indexes were showed as mode of average number± standard deviation. Comparison of between combination therapy group, control group and intra-groups were conducted by $t$-test, $P$<0.05indicated statistical significant differences.

## 3. Results

### 3.1. Comparison of lymphocyte subsets in two groups of patients before and after therapy

$CD3^+$, $CD4^+$, $CD8^+$ and $CD4^+/CD8^+$ were compared between the two groups of patients before therapy, the differences were not statistical significant ($P$>0.05); $CD8^+$ levels were dramatically decreased in the two groups of patients after therapy, $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ were significantly increased ($P$<0.05), as shown in Table 1.

**Table 1**
Comparison of lymphocyte subsets in two groups of patients before and after therapy.

| Groups | Time | $CD3^+$ (%) | $CD4^+$ (%) | $CD8^+$ (%) | $CD4^+/CD8^+$ |
|---|---|---|---|---|---|
| Combination therapy group | Pre-treatment | 31.18±4.56 | 34.42±3.86 | 53.74±4.02 | 0.65±0.34 |
| | Post-treatment | 63.22±4.15$^{*\#}$ | 37.83±4.11$^{*\#}$ | 24.33±3.72$^{*\#}$ | 1.52±0.38$^{*\#}$ |
| Control group | Pre-treatment | 31.09±5.01 | 33.54±3.56 | 53.55±3.68 | 0.64±0.35 |
| | Post-treatment | 50.48±4.96$^{*}$ | 35.10±4.00$^{*}$ | 32.65±3.38$^{*}$ | 1.08±0.30$^{*}$ |

Note: Compared with the same group before therapy, $^{*}P<0.05$; Compared with control group after therapy, $^{\#}P<0.05$.

### 3.2. Comparison of cytokine in two groups of patients before and after therapy

TNF- α , IL-2, IL-6 and IL-10 were compared between the two groups of patients before therapy, the differences were not statistical significant ($P$>0.05); TNF- α , IL-2, IL-6 and IL-10 levels were significantly decreased in the two groups of patients after therapy ($P$<0.05); TNF- α , IL-2, IL-6 and IL-10 levels in combination therapy group were significantly lower than control group after treatment ($P$<0.05), as shown in Table 2.

### 3.3. Comparison of hemorheology in two groups of patients before and after therapy

Hemorheology indexes were compared between the two groups of patients before therapy, the differences were not statistical significant ($P$>0.05); Hemorheology indexes were significantly decreased in the two groups of patients after therapy ($P$<0.05); Hemorheology indexes in combination therapy group were significantly lower than control group after treatment ($P$<0.05), as shown in Table 3.

## 4. Discussion

Pathogenic microorganism invades to reproductive organs, and causes inflammatory reactions of fallopian tube, ovary and surrounding connective tissue, these reactions are called as annexitis[7]. Situations like Feculent Sexual Intercourse, intrauterine device, gynecologic surgery and multiple miscarries could cause weaken of mucosal barrier function of reproductive organs, and bacterial infections, which could lead to the inflammation[8,9]. Acute annexitis and chronic annexitis could be divided based on course of the disease[10]. Acute annexitis is often appeared as hyperpyrexia, severe abdominal pain and so forth, if treatment could not be received in time or completely, it would often transfer to be chronic annexitis[11]. Common clinical symptoms of chronic annexitis are dragging, distending and pain in lower abdomen, more leucorrhea lumbar and sacral pain, *etc*., tiredness and menstrual period could worsen the condition[12]. In recent years, with the rapid development of economy and society in our country, some factors, like pace of life and life style alteration cause the increased amount of gynecologic diseases. One of the diseases, chronic annexitis, was received with a yearly increased amount of patients. So the therapy of chronic annexitis became a focus of us[13,14]. To find a rapid and effective project for annexitis therapy becomes an urgent affair for a broad of medical workers[15].

Azithromycin is an antibiotic which belongs to a kind of macrolide. It has strong sterilizing effects to gram-positive bacterias, gram-negative bacterias, mycoplasmas, chlamydias, *etc*. And its adverse effects are less than erythromycin[16]. Azithromycin has been already widely used for therapies of many inflammations with good therapeutic effects. But varies of adverse reactions, such as gastrointestinal reactions, skin reactions, nervous and cardiovascular systemic effects, could influence on the application of Azithromycin[17]. Multiple active ingredients, like saponin, alkaloid, flavone and organic acid are contained in Smilax China rhizome. Smilax China rhizome plays important roles in activating blood to remove stasis, regulating immune function, diuresis, detoxification and other fields. Smilax China capsule is a Chinese patent medicine using a single Chinese medicine, Smilax China (Jingangteng). It has been widely used in therapies of various inflammations[18]. Therefore, Smilax China capsules and azithromycin combined therapy would be beneficial to chronic annexitis therapy.

In our study, lymphocyte subsets levels, cytokine levels and hemorheology indexes in patients with chronic annexitis who received azithromycin therapy could be dramatically improved when Smilax China capsules added. And the efficacy of combination could be better than single utilization of azithromycin therapy. The possible reason is that azithromycin could effectively kill varies of

**Table 2**
Comparison of cytokine in two groups of patients before and after therapy.

| Group | Time | TNF- α | IL-2 | IL-6 | IL-10 |
|---|---|---|---|---|---|
| Combination therapy group | Pre-treatment | 19.82±5.21 | 13.47±3.32 | 28.58±4.93 | 19.44±3.95 |
| | Post-treatment | 6.56±4.76$^{*\#}$ | 7.14±2.74$^{*\#}$ | 10.76±4.28$^{*\#}$ | 9.54±3.28$^{*\#}$ |
| Control group | Pre-treatment | 19.77±5.03 | 13.42±3.15 | 28.63±4.56 | 19.52±4.01 |
| | Post-treatment | 11.84±4.95$^{*}$ | 10.83±2.99$^{*}$ | 18.01±4.37$^{*}$ | 13.55±3.65$^{*}$ |

Note: Compared with the same group before therapy, $^{*}P<0.05$; Compared with control group after therapy, $^{\#}P<0.05$.

**Table 3**
Comparison of hemorheology in two groups of patients before and after therapy.

| Group | Time | WBV (mPa/s) | | PV(mPa/s) | PCV (%) | EAI |
|---|---|---|---|---|---|---|
| | | BVH | BVL | | | |
| Combination therapy group | Pre-treatment | 6.92±0.53 | 8.32±0.48 | 1.76±0.18 | 48.25±3.48 | 1.41±0.20 |
| | Post-treatment | 5.01±0.57$^{*\#}$ | 6.56±0.51$^{*\#}$ | 1.48±0.20$^{*\#}$ | 36.61±4.11$^{*\#}$ | 1.19±0.17$^{*\#}$ |
| Control group | Pre-treatment | 6.89±0.54 | 8.29±0.43 | 1.75±0.21 | 48.32±4.23 | 1.39±0.17 |
| | Post-treatment | 5.96±0.55$^{*}$ | 7.38±0.46$^{*}$ | 1.62±0.22$^{*}$ | 41.17±3.65$^{*}$ | 1.30±0.18$^{*}$ |

Note: Compared with the same group before therapy, $^{*}P<0.05$; Compared with control group after therapy, $^{\#}P<0.05$.

bacterias in the focal area, such as gram-positive bacterias, gram-negative bacterias, *Staphylococcus aureus,* anaerobions, mycoplasmas and chlamydias. For chronic annexitis treatment, it layed the foundation for improvement of lymphocyte subsets levels, cytokine levels and hemorheology indexes. Smilax China capsule is a Chinese patent medicine using Smilax China rhizome extracts to be a major ingredient, multiple effective ingredients, like saponin, alkaloid, flavone and organic acid are included in it. The function of immune regulation for Smilax China capsule could improve the lymphocyte subsets levels of patients with chronic annexisis, lower the cytokine levels of bodies; The diuresis and detoxify function of Smilax China capsule could lower the side effects of azithromycin to bodies. It is helpful for the disease recovery; Meanwhile, its function of activating blood and dissolving stasis improved the hemorrheology indexes of patients, which was helpful for the blood flow in inflammatory sites, to ensure that the drug could arrive to focal areas[19,20].

Above all, Smilax China capsules and azithromycin combination could significantly improve the lymphocyte subsets levels, cytokine levels and hemorheology indexes in patients with chronic annexitis. It is of quite important clinical value for therapies of patients with chronic annexitis.

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