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Curative effect of kangfuyan capsule combined with antibiotic treatment on pelvic inflammatory disease

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Abstract: This study aims to investigate the curative effect of Kangfuyan capsule in the treatment of damp-heat and blood stasis type of pelvic inflammatory disease (PID), and its influence on serum inflammatory factors IL-6, CRP and TNF-α. A total of 83 patients with PID were randomly divided into two groups: Western medicine group (control group, n=41) received oral antibiotics (azithromycin + metronidazole) alone and the traditional Chinese medicine combined with Western medicine group (experimental group, n=42) received Kangfuyan capsule based on Western medicine therapy. Clinical efficacy between these two groups and the influence of drugs in serum inflammatory factors (IL-6, CRP and TNF-α) were compared. The total effective rate was 78.05% in the control group and 97.62% in the experimental group and difference between these two groups was statistically significant (P<0.01). The symptoms and signs in the two groups significantly improved after treatment (P<0.05) and improvement rate was significantly better in the experimental group than in the control group (P<0.05). After treatment, serum inflammatory factor levels in the two groups were significantly lower than levels before treatment (P<0.05) and improvement rate was significantly better in the experimental group than in the control group (P<0.05). Kangfuyan capsule combined with antibiotics can effectively relieve the symptoms and signs of patients, improve the efficiency of treatment, provide high safety, and does not increase adverse reactions.

Keywords: Kangfuyan capsule; Pelvic inflammatory disease; damp-heat and blood stasis type; Curative effect

INTRODUCTION

Pelvic inflammatory disease (PID) is a group of diseases caused by inflammation of the upper genital tract in women, including endometritis, salpingitis, tubal ovarian abscess and pelvic peritonitis (Soper, 2010). Literature reported that there are approximately 750,000 patients diagnosed with PID in the United States each year (Brunham *et al*., 2015; Workowski and Bolan, 2015) and the age of these patients ranges between 15-29 years old. Furthermore, there is high incidence of PID, in which most of these diseases are caused by chronic asymptomatic infections (Ziklo *et al*., 2016). These diseases are characterized by prolonged courses and recurrent inflammation. If patients do not receive timely and correct treatments, they may develop ectopic pregnancy, infertility, chronic pelvic pain and other sequelae (Ford and Decker, 2016; Goller *et al*., 2017), which increase family and social burdens. Therefore, it is very necessary to seek an economic, effective and safe treatment method. At present, pelvic inflammatory diseases are mostly treated with anti-infection therapy using antibiotics. However, single antibiotic therapies can easily result in resistance and toxic side effects and their curative effect is unsatisfactory. At present, many doctors advocate combined treatment of traditional Chinese medicine (TCM) and Western medicine. Kangfuyan capsule has been verified to have a valid curative effect on inflammation in the female reproductive system through a large number of basic experiments and clinical trials. This drug is produced from 11 medicinal herbs including dandelion, coix seed, red peony root, dahurian patrinia herb, atractylodes rhizome, Chinese angelica, chuanxiong rhizome, nutgrass galingale rhizome, rhizoma corydalis, oriental water plantain rhizome and spreading hedyotis herb. A number of records have been found in ancient literatures regarding these key medicinal herbs. The main effects of these herbs are clearing heat, detoxification, dissolving clots, removing blood stasis, eliminating dampness and arresting leucorrhea. Therefore, through a random case-control study, the clinical efficacy of the Fuyankang capsule combined with antibiotics on PID and its impact on serum inflammatory factors (IL-6, CRP and TNF-α) were investigated. Details are reported as follows.

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MATERIALS AND METHODS

General information

According to the following diagnostic criteria and inclusion and exclusion criteria, 83 patients were diagnosed with damp-heat and blood stasis type of PID in the Outpatient Department of the Affiliated Obstetrics and Gynecology Hospital of Zhejiang University and the Third Affiliated Hospital of Zhejiang Chinese Medicine University from June 2014 to November 2015. This study

was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of our hospital, with the Institutional Ethics Committee approval number 20140053.

These patients were randomly divided into two groups according to the random number table: For the TCM combined with Western medicine group (experimental group, *n*=42), the age of these patients ranged between 18-49 years (average: 32.84±9.26 years old) and the course of the disease ranged between 2-15 months (average: 7.77±2.96 months); as for the Western medicine group (control group, *n*=41), the age of these patients ranged between 19-48 years (average: 8.21±33.29 years old) and the course of the disease ranged between 3-16 months (average: 8.76±3.84 months). The differences in age, duration of disease and clinical manifestations between these two groups were not statistically significant (P>0.05). Details are shown in table 1.

Diagnostic criteria

Diagnostic criteria for Western medicine: Western medicine diagnostic criteria for this disease were developed based on the 2014 Chin "Diagnosis and Treatment Standard of Pelvic Inflammatory Disease (Revised Edition)" and the 2010 "U.S. Centers for Disease Control and Prevention Sexually Transmitted Disease Treatment Guidelines" (7). Specific criteria are as follows: (1) uterine tenderness; (2) attachment tenderness; (3) cervical pain; (4) transvaginal ultrasound or MRI revealed thickening of the fallopian tube and fallopian tube fluid, with or without pelvic effusion and tubal ovarian mass. PID was diagnosed when any of the above four items was met.

Diagnostic criteria for TCM: The diagnostic standard of damp-heat and blood stasis type of PID was developed based on the "Guidelines of Clinical Research of New Drugs of Traditional Chinese Medicine", "Diagnostics of traditional Chinese medicine", and "Chinese gynecology". Primary manifestations are as follows: (1) abdominal pain or distending pain; (2) lumbosacral distending pain; (3) abnormal vaginal discharge (large amount or viscous and yellow or smelly). Secondary manifestations: (1) mental fatigue and lack of strength; (2) aggravated abdominal pain during menstruation; (3) large amount of menstruation; (4) prolonged menstruation; (5) greasy mouth and anorexia; (6) yellow urine or frequent urination; (7) dry stool or sloppy and upset bowel; (8) dry mouth and no requirement for drinking. Tongue and pulse: red or dark red tongue, petechiae or ecchymoses on the margins or the tip of tongue. The coating on the tongue is yellow and greasy and the pulse or taut pulse was stringy and slippery. This disease can be diagnosed when at least two primary manifestations and two secondary manifestations are met, combined with tongue and pulse.

Inclusion criteria

(1) Women of childbearing age (18-49 years old); (2) women with sexual life; (3) women who met the diagnostic criteria for PID; (4) women who met the diagnostic standard for the syndrome of damp-heat and blood-stasis in TCM; (5) women who provided an informed consent.

Exclusion criteria

(1) Pregnant or lactating women; (2) patients with acute appendicitis and tubal ovarian abscess, with surgical indications; (3) patients with other sexually transmitted diseases, endometriosis, and genital tuberculosis; (4) patients with severe diseases of the heart, liver and kidney or disease in the hematopoietic system; (5) patients with allergy or drug allergy; (6) patients with poor compliance, who changed the treatment method or had incomplete information that affected the efficacy of the evaluation.

Treatment methods

(1) Control group: patients received oral antibiotics (azithromycin (Zithromax)), 0.5g per time, once per day. Two days later, this regimen was converted as follows: 0.25g per time, once per day, for five days. At the same time, 0.4g of metronidazole tablets per time was additionally administered twice per day, for fourteen days. (2) Experimental group: Western medicines were given as above and Kangfuyan capsule was additionally used, three capsules each time, twice per day, for 28 days.

Evaluation criteria for curative effect

Clinical symptoms and signs: Incidences of abdominal bearing-down pain, uterine tenderness, and pelvic effusion revealed by ultrasound examination were observed in the two groups of patients before and after treatment.

Serum levels of inflammatory cytokines: In the morning, 5 ml of fasting venous blood was collected before and after treatment, respectively. Blood samples were immediately placed into a sterile test tube and centrifuged at 3,000 rpm for 10 minutes. The supernatant fluid was obtained and reserved at -80°C for detection. Changes in serum levels of interleukin-6 (IL-6), C-reactive protein (CRP) and human tumor necrosis factor-α (TNF-α) were detected using ELISA. All operation procedures were performed in accordance with specifications.

Incidence of adverse reactions: Patients in these two groups underwent routine blood test, liver and kidney function detection, and pelvic ultrasonography before and after treatment. During the treatment, it could be observed as the occurrence of an adverse reaction if one of the following symptoms appeared once or more than once: headache, dizziness, nausea, vomiting, abdominal discomfort and liver and kidney dysfunction. The types and times of adverse reactions in the treatment were accurately determined and recorded in detail and safety analysis was conducted.

Comparison of clinical efficacy: An evaluation standard was developed with reference to the "Guidelines of Clinical Research of New Drugs of Traditional Chinese Medicine", and the clinical curative effects (TCM syndrome score + sign score + laboratory score) were evaluated according to the sum-score method: Efficacy index = (sum of scores before treatment - sum of scores after treatment) / sum of scores before treatment × 100%. Results were categorized as follows: cured, ≥95% reduction of the sum of scores before and after treatment; excellent, ≥70% but <95% reduction of the sum of scores; effective, ≥30% but <70% reduction of the sum of scores; invalid, <30% reduction of the sum of scores. Total effective rate = (recovery cases + excellent cases + effective cases) / total cases × 100%.

STATISTICAL ANALYSS

Statistical analysis was conducted using SPSS 20.0 software package. Count data were compared using X^2-test. Measurement data were expressed as mean ± standard deviation (x± SD), and were compared using *t*-test. $P<0.05$ was considered statistically significant.

RESULTS

Comparison of clinical symptoms and signs before and after treatment in the two groups

The difference in the incidences of clinical signs and symptoms such as abdominal bearing-down pain, uterine attachment tenderness and pelvic effusion determined by ultrasound examination between the two groups was not statistically significant ($P>0.5$). The above symptoms and signs significantly improved in the two groups compared with pre-treatment ($P<0.05$). In addition, the incidences of abdominal bearing-down pain, uterine attachment tenderness and pelvic effusion were significantly lower in the experimental group than in the control group ($P<0.05$, table 2).

Comparison of serum levels of inflammatory cytokines (IL-6, CRP and TNF-α) before and after treatment in the two groups

Before treatment, the difference in serum inflammatory factor levels between the two groups was not statistically significant ($P>0.05$). Serum inflammatory factor levels in the two groups after treatment were significantly lower than that before treatment ($P<0.05$), and improvement rate in the experimental group was significantly better than in the control group ($P<0.05$, table 3).

Comparison of the incidences of adverse reactions

One case had headache and dizziness, one case had nausea, two cases had vomiting, three cases had abdominal discomfort, and one case had abnormal liver function during the course of treatment in the experimental group; and the overall incidence of adverse reactions was 19.05%. One case had headache and dizziness, one case had nausea, one case had vomiting, two cases had abdominal discomfort and one case had abnormal liver function during the course of treatment in the control group; and the overall incidence of adverse reactions was 14.63%. The difference in the overall incidence of adverse reactions between the two groups was not statistically significant ($P>0.05$, table 4).

Comparison of effective rate

After treatment, results in the experimental group were as follows: 26 patients were cured, 10 patients achieved an excellent curative effect, five patients achieved effective results, and one patient achieved an invalid result; and the overall effective rate was 97.62% (41/42). Results in the control group were as follows: 15 patients were cured, eight patients achieved an excellent effect, nine patients achieved effective results, and nine patients achieved an invalid result; and the overall effective rate was 78.05% (32/41). The overall effective rate was significantly higher in the experimental group than in the control group ($P<0.05$, table 5).

DISCUSSION

PID often occurs in pluripara, which is a common clinical gynecological disease. This disease has a slow onset, a long disease course, and is easy to relapse. Patients would be prone to sequelae if they receive improper or delayed treatment, which seriously affects the health and quality of life patients. At present, clinical treatment for PID is mainly antibiotics (Workowski *et al*., 2010). For antibiotics selection, France's new guidelines do not recommend fluoroquinolone antibiotics (Brun *et al*., 2016). In outpatient treatments in Australia (Australasian Sexual Health Alliance, 2016), four antibiotic systems are recommended for mild and moderate PID, with a combined use and adequate dose: intramuscular injection of 500mg of ceftriaxone once daily, oral administration of 1 g of azithromycin daily, 400 mg of metronidazole plus 100 mg of doxycycline, with a course of 14 days. Azithromycin is one of the commonly used choices of clinical treatment at present, but its long term use can easily induce adverse effects (Trent *et al*., 2011). Therefore, some scholars recommend the combination of TCM with antibiotics treatment in recent years, which could obtain good results (Chen *et al*, 2008). At present, a number of clinical studies have revealed that Kangfuyan capsule has a good curative effect on PID. However, its specific mechanism remains unknown. Therefore, in order to explore the clinical safety and efficacy and the possible mechanism of action of Kangfuyan capsule in patients with damp-heat and blood stasis type PID, a randomly controlled study was performed in 83 patients with damp-heat and blood stasis type of PID and the effect of Kangfuyan capsule on serum inflammatory factors (IL-6, CRP and TNF-α) was analyzed.

Table 1: General situations of the two group patients

Group	n	Age	Course of disease	Clinical manifestations				
				Abdominal bearing-down pain	Uterine attachment tenderness	Poor uterine mobility	Attachment thickening	Pelvic effusion
received oral antibiotics	42	32.84±9.26	7.77±2.96	41 (97.62%)	1 (97.62%)	14 (33.33%)	13 (33.95%)	39 (92.85%)
Western medicine group	41	33.29±8.21	8.76±3.84	40 (97.56%)	40 (97.56%)	15 (36.58%)	12 (29.27%)	38 (92.68%)
		T= 0.236	T= 1.339	x2=0	x2=0	x2=0.097	x2=0.028	x2=0.001
P		0.814	0.184	0.986	0.986	0.756	0.867	0.976

Note: control group, Western medicine group, azithromycin + metronidazole, received oral antibiotics

Table 2: Comparison of clinical symptoms and signs incidence rates before and after treatment in the two groups (n, %)

Group	abdominal bearing-down pain	uterine attachment tenderness	poor uterine mobility	attachment thickening	pelvic effusion
received oral antibiotics (n=42)					
Before treatment	41(97.62%)★	41(97.62%)★	14(33.33%)★	13(33.95%)★	39(92.85%)★
After treatment	1(2.38%) ◆■	2 (4.76%) ◆■	2(4.76%) ◆■	2 (4.76%) ◆■	1(2.38%) ◆■
Western medicine group (n=41)					
Before treatment	40(97.56%)	40(97.56%)	15(36.58%)	12(29.27%)	38(92.68%)
After treatment	6 (14.63%)◆	7 (17.07%) ◆	8 (19.51%) ◆	6 (14.63%) ◆	10(24.39%)◆

Note: control group, Western medicine group azithromycin + metronidazole, received oral antibiotics
★ before treatment, compared with Western medicine group ($p>0.05$), ◆compared with the same group before treatment ($p<0.05$), ■compared with western medicine group after treatment ($p<0.05$)

Table 3: Comparison of serum levels of inflammatory cytokines (IL-6, CRP and TNF-α) before and after treatment in the two groups

Group	time	IL-6 (pg/mL)	CRP(g/L)	TNF-α(ug/mL)
received oral antibiotics (n=42)	Before treatment	39.19±10.24★	32.50±6.65★	204.10±19.64★
	After treatment	23.24±5.60◆■	8.38±2.99◆■	147.98±21.60◆■
Western medicine group (n=41)	Before treatment	41.24±11.30	33.17±7.03	202.07±16.05
	After treatment	26.93±6.42◆	11.93±4.12◆	160.27±18.14◆

Note: Control group, Western medicine group azithromycin + metronidazole, received oral antibiotics
★before treatment, compared with Western medicine group ($p>0.05$), ◆compared with the same group before treatment ($p<0.05$), ■compared with Western medicine group after treatment ($p<0.05$)

Table 4: Comparation of total incidence of adverse reactions of the two groups (n,%)

Group	n	headache, dizziness	nausea	vomiting	abdominal discomfort	liver and kidney dysfunction	Total incidence of adverse reactions
Received oral antibiotics	42	1(2.38%)	1(2.38%)	2(4.76%)	3(7.14%)	1(2.38%)	8(19.05%)
Western medicine group	41	1(2.44%)	1(2.44%)	1(2.44%)	2(4.88%)	1(2.44%)	6(14.63%)★

Note:control group, Western medicine group azithromycin + metronidazole, received oral antibiotics ★compared with Western medicine group ($x^2=0.288$, $p=0.591>0.05$)

Table 5: Clinical effective rates comparasion of two groups (n,%)

Group	n	Recovery	Excellent	Effective	Without any effect	Total effective rate
received oral antibiotics	42	26	10	5	1	97.62%★
Western medicine group	41	15	8	9	9	78.05%

Note: Control group, Western medicine group, azithromycin + metronidazole, received oral antibiotics ★compared with Western medicine group ($x^2=7.499$, $p=0.006<0.01$)

The occurrence and development of PID include the stages of pathogen microbe infection, host immune system activation and anti-infection factors release. The signaling pathway of Toll-like receptor 4 (TLR-4) plays an important role in the occurrence and development of PID in female patients (Taylor *et al*., 2013; Taylor *et al*., 2014). TLR-4 molecular signaling pathway participates in gynecological chronic inflammation by producing inflammatory cytokines through the myeloid differentiation factor 88 (MyD88) signaling pathway such as IL-6 and TNF-α (Tortorella *et al*., 2014; Xu *et al*., 2004). To participate in the chronic inflammation of gynaecology (Taylor *et al*., 2015).

IL-6 is a multifunctional inflammatory cytokine, which is a key component of the network of inflammatory mediators, playing an important role in inflammatory response. The increase of IL-6 level is related to inflammation (Powell *et al*., 2015; Rier *et al*., 1995), which can mediate activated macrophages and inflammatory cells to travel into the abdominal cavity. This finally adheres to the uterine tube and other local tissues and promotes the growth of granulation and fibrous tissues. Serum IL-6 levels would increase at different degrees. Hence, they can be used to evaluate the severity and prognosis of the disease. TNF-α is a cytokine closely related with infection immunity and reproductive immunity in most recent studies (Siemieniuch *et al*., 2016; Srivastava *et al*., 2008). The decreasing expression of TNF-α in serum can inhibit inflammatory response, which is one of the vital mechanisms for the treatment of chronic PID; and this has become a consensus (Lee *et al*., 2008). Modern pharmacological studies reveal that serum liquid from mice fed with angelica could affect the expression of TNF-α and IL-8 in macrophages in the abdominal cavity in mice, and dandelion could affect the expression of IL-6 (Ling N and Chen, 2013). The dahurian patrinia herb could significantly decrease the secretion of TNF and IL-1 by endotoxin-activated macrophages (Chen *et al*., 2013). Spreading hedyotis herb can enhance humoral immunity and cellular immunity in mice, and enhance the phagocytic function of the reticuloendothelial system and leukocytes. Coix seed contains three major nutrients and lipids, which have antipyretic, sedative and analgesic effects (Dong *et al*, 2010). This study revealed that differences in serum inflammatory factor (IL-6, CRP and TNF-α) levels in patients before treatment between these two groups were not statistically significant (P>0.05). Serum inflammatory factor levels in these two groups were significantly lower after treatment than before treatment (P<0.05) and improvement rate in the experimental group was significantly higher than in the control group (P<0.05). This suggests that Kangfuyan capsule could reduce serum inflammatory factor (IL-6, CRP and TNF-α) levels in PID patients and thus, inhibit chronic PID.

PID belongs to the category of leukorrheal disease and woman celiodynia in TCM and the pattern of syndromes mainly manifests as damp-heat accumulation or dampness combined with blood stasis. The "Synopsis of prescriptions of the Golden Chamber" says: "Seven or eight days after cold in female patients, secondary cold-heat complicated syndrome occurs, with regular episodes, and menstruation stops. This is the hot and humid entering the blood chamber, and blood in patients must form stasis. The symptoms are like malaria, which intermittently and often attack." TCM considers that the etiological factors of PID are cold, damp and heat, which invade the body when the patient is weak. This is stasis with the Qi and blood in the Chong Meridian and Ren Meridian, and is accumulated in the uterus. This repeatedly advances and retreats. Thus, Qi and blood are consumed, and asthenia symptoms and reality diseases tangle together. Thus, the disease persists for a long time and is difficult to be cured. Buchang Kangfuyan capsule prescription uses dandelion and rhizoma atractylodis as the sovereign drugs, in order to remove heat and resolve moisture. Coix seed works to resolve moisture and remove pus, nutgrass galingale rhizome works to clear the liver and stop pain, chuanxiong rhizome invigorates the circulation of blood and stops pain, and the spread of hedyotis herb clears away heat and toxic materials. All of the above herbs act as ministerial drugs. Alisma is a diuretic that can drain dampness, red peony root clears heat and activates blood; dahurian patrinia herb clears away heat and removes pus; Chinese angelica enriches and activates the blood; rhizoma corydalis invigorates the circulation of blood and stops pain; and all the above herbs act as adjuvants. Furthermore, chuanxiong rhizome guides all of the other drugs to reach the lesions, thereby acting as a conduction drug. The combination of all the drugs commonly has effects of clearing away heat and toxic materials, promoting blood circulation, removing blood clots, and eliminating dampness and arresting leucorrhea. This study revealed that differences in the incidences of bearing-down pain in the lower abdomen, tenderness in uterine appendages, and effusion in the pelvic cavity before treatment between these two groups were not statistically significant (P>0.5). After treatment, these two groups of patients were significantly better than before treatment (P<0.05), and the incidence of bearing-down pain in the lower abdomen, tenderness in uterine appendages, range of motion of the uterus, and effusion in the pelvic cavity after treatment weere significantly lower in the experimental group than in the control group (P<0.05). This suggests that Kangfuyan capsule combined with antibiotics therapy is more effective in relieving clinical symptoms and signs. At the same time, according to the curative effect standard of the "Guidelines of Clinical Research of New Drugs of Traditional Chinese Medicine", the total effective rate in the control group was 78.05% and the total effective rate in the experimental group was 97.62%; and the difference between these two

groups was statistically significant (P<0.01). Furthermore, the incidences of adverse reaction in these two groups were compared; in which the total incidence of adverse reactions was 19.05% in the experimental group and 14.63% in the control group. The difference in the incidence of adverse reactions between these two groups was not statistically significant (P>0.05). The above results revealed that the clinical effect of Kangfuyan capsule combined with antibiotics treatment for PID is significantly better than simple antibiotics treatment, and the occurrence of adverse reactions did not increase. These results reveal that the combined use of Chinese and Western medicine has a rapid effect and high safety. Furthermore, this approach can also improve the therapeutic effect to a certain extent. This study adopts the combination of traditional Chinese and Western medicine treatment, in order to fully utilize the superiority of TCM and Western medicine. Clinical symptoms and signs were significantly improved after treatment. The effusion and mass in the pelvic cavity were significantly reduced. Furthermore, the total effective rate was significantly improved.

CONCLUSION

In summary, the combined use of antibiotics and Kangfuyan capsule can effectively relieve the clinical symptoms and signs of patients and improve the efficiency of treatment; which has high safety and does not increase the adverse effects of drugs. The possible mechanism is the decrease serum inflammatory factor (IL-6, CRP and TNF-α) levels; thus, inhibiting PID. However, its specific mechanism requires further studies in the future.

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