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CLINICAL RESEARCH

Effects of Wenshen Nuangong formula on pain and PGE_2, $PGF_{2\alpha}$ and VEGF levels in patients with cold congelation and blood stasis type of endometriosis dysmenorrhea

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Keywords

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Abstract

Objective To explore the effects of Wenshen Nuangong formula on pain and levels of prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and vascular endothelial growth factor (VEGF) in patients with cold coagulation and blood stasis type of endometriosis (EM) dysmenorrhea. **Methods** From June 2019 to June 2021, 100 patients with cold coagulation and blood stasis type of EM dysmenorrhea admitted to our hospital were selected as research objects, and were divided into a control group and an observation group using the random number table method, with 50 cases in each group. The control group was treated with oral mifepristone tablets, and the observation group was treated with Wenshen Nuangong formula on the basis of the treatment used in the control group. The pain, PGE_2 level, $PGF_{2\alpha}$ level, VEGF level, traditional Chinese medicine (TCM) syndrome scores and adverse reactions before and after treatment were compared between the two groups. **Results** After treatment, COX dysmenorrhea symptom scale scores, visual analogue scale (VAS) scores, PGE_2 level, $PGF_{2\alpha}$ level, VEGF level, and TCM symptom scores were significantly lower than those before treatment (P<0.05), and the levels in the observation group were significantly lower than those in the control group (P<0.05). After treatment, there was no significant difference in the incidence of adverse reactions between the two groups (P>0.05). **Conclusion** Wenshen Nuangong formula can effectively alleviate pain, relieve clinical symptoms, and reduce serum PGE_2, $PGF2_{2\alpha}$, and VEGF levels of patients with cold coagulation and blood stasis type EM dysmenorrhea, with significant efficacy and safety.

Introduction

Endometriosis (EM) is a common gynecological disease, which occurs in the endometrial glands or stroma outside the covered mucosa of the uterine cavity. Patients with EM often have secondary dysmenorrhea, chronic pelvic pain, sexual intercourse pain and other symptoms [1]. Dysmenorrhea is the most typical clinical symptom of EM, and statistical data show that the proportion of secondary dysmenorrhea in EM patients is as high as 80% [2]. Western medicine mostly uses non-steroidal anti-inflammatory drugs, contraceptives, gonadotropin releasing hormone agonists and other drugs to treat EM dysmenorrhea. These drugs mainly affect the level of adrenaline or sex hormones in patients, promote ectopic tissue atrophy, and achieve the purpose of relieving patients' pain. Mifepristone is a progesterone receptor antagonist, which is often used to treat EM dysmenorrhea. However, long-term use of hormone drugs is easy to cause EM dysmenorrhea patients to form hormone dependence, and has the disadvantages of decreased sexual desire, menopause, vaginal bleeding and other adverse reactions, which should not be used alone for a long time [3]. Wenshen Nuangong formula is a self-made prescription formulated from clinical experience by Professor Quansheng Wu, a famous traditional Chinese medicine (TCM) professor in Gansu Province. It can treat both manifestation and root cause of disease, and has the effects of dispersing cold and removing stasis, and warming kidney and enhancing Yang, which is widely used to treat cold coagulation and blood stasis type of dysmenorrhea [4]. At present, there are few studies on the combination of Western medicine and Wenshen Nuangong formula in the treatment of cold coagulation and blood stasis type of EM dysmenorrhea. The purpose of this study is to explore the clinical efficacy of Wenshen Nuangong formula combined with mifepristone tablets in the treatment of cold coagulation and blood stasis type of EM dysmenorrhea, as well as its effects on the levels of prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and vascular endothelial growth factor (VEGF) in serum, to provide a reference for clinical treatment of EM patients with cold coagulation and blood stasis. The research results are reported as follows.

Materials and methods

General data

A total of 100 patients with cold coagulation and blood stasis type of EM dysmenorrhea who were diagnosed and treated in our hospital from June 2019 to June 2021 were selected as the research objects, and they were randomly divided into an observation group (n=50) and a control group (n=50) by the random number table method. There was no significant difference in age, course of disease and menstrual period between the two groups (P>0.05), as shown in Table 1. This study has been approved by the medical ethics committee, and the patients were informed and consented.

Table 1 Comparison of general data between the two groups

Group	Case	Age (years)	Course of disease (years)	Menstrual period (d)
Observation group	50	36.24±4.22	3.85±0.46	32.28±1.57
Control group	50	36.92±4.19	3.71±0.48	32.36±1.23
t		-0.809	1.489	-0.284
P		0.421	0.140	0.778

Diagnostic criteria

Western medicine diagnosis referred to the diagnostic criteria for dysmenorrhea caused by EM in the *Diagnosis and treatment of endometriosis* [5]. TCM diagnosis referred to the description of cold coagulation and blood stasis type of dysmenorrhea in the *Guiding principles for clinical research of new traditional Chinese medicine* [6]: the menstrual

volume is small, the menstrual blood is dark and stagnant, the body and limbs are cold, the tongue coating is white and greasy, and the pulse is heavy and astringent.

Inclusion criteria

(1) Those who meet the diagnostic criteria of TCM and Western medicine for EM dysmenorrhea; (2) Those with cold coagulation and blood stasis type of EM dysmenorrhea; (3) Those with no relevant hormonal medication recently; (4) Those with complete clinical data and no history of drug allergy.

Exclusion criteria

(1) Patients with serious heart, liver, kidney and other systemic diseases and immune dysfunction; (2) Patients with malignant tumor or mental abnormality; (3) Patients with hysteromyoma, primary dysmenorrhea, pelvic infection and other gynecological diseases.

Treatment methods

The control group was given mifepristone tablets (Hubei Gedian Renfu Pharmaceutical Co., Ltd., approval number of NMPA H20033551, specification: 25mg), 25 mg each time, once a tablet, and stopped taking drugs during menstruation, with a total treatment for 3 menstrual cycles. On the basis of the control group, the observation group was additionally treated with Wenshen Nuangong formula, and the main ingredients of the formula were: 15g of Lujiao Shuang (*Cornu Cervi Degelatinatum*), Yin Yang Huo (epimedium herb) and Danggui (Chinese angelica), 12g of Baishao (debark peony root), 18g of stir-fried Baizhu (largehead atractylodes rhizome), 15g of stir-fried Shanyao (common yam rhizome), 15g of Fulin (Indian bread), 30g of roasted Huangqi (milkvetch root), 10g of Ganjiang (zingiber), 9g of Qianghuo (notopterygium root), 15g of Shu Dihuang (prepared rehmannia root), 10g of Jiu Yurou (*Fructus Corni*), 9g of San Leng (*Rhizoma sparganii*), 15g of E Zhu (*Curcuma phaeocaulis Valeton*), 10g of Tu Biechong (ground beetle), 6g of Gancao (liquorice root). The above drugs were boiled with water to take 400ml of juice, and the decoction was taken with lukewarm water in the morning and before dinner, one dose a day for 3 menstrual cycles [4].

Detection indicators

(1) Pain: before and after treatment, the symptoms of dysmenorrhea were evaluated with the COX dysmenorrhea symptom rating scale, which uses a 5-level scoring method and covers 18 sub-items (e.g. abdominal pain, vomiting, headache and diarrhea) as well as includes two dimensions of severity and duration. The higher the final score, the more serious the symptoms of dysmenorrhea [7]. Visual analogue scale (VAS) was used to assess the degree of pain. The total score was 10 points, and the higher score represents the higher degree of pain. Scoring standard: 0 point: no dysmenorrhea; 1-3 points: mild dysmenorrhea, and no impact on daily work and life; 4-6 points: moderate dysmenorrhea, limited daily activities, and effective with painkillers; 7-10 points: severe dysmenorrhea, painkillers were suboptimal and patients required bed rest [8]. (2) Serum PGE_2, $PGF_{2\alpha}$ and VEGF levels: before and after treatment, 3 ml fasting venous blood was drawn from patients in the two groups in the morning for testing, and PGE_2, $PGF_{2\alpha}$ and VEGF levels were measured by enzyme-linked immunosorbent assay (ELISA). The ELISA kits were purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd. (3) TCM syndrome scoring: before and after treatment, a total of eight TCM syndromes were scored, and three main symptoms including the lower abdomen pain and chills, pain reduction in the heat, and feeling pain while pressing were classified into four grades: none, mild, moderate, and severe, which were scored as 0, 2, 4, and 6 points, respectively; Five accompanied symptoms including chilly sensation and the cold limbs, inhibited menstruation, pale complexion, menstruation with light color and blood clot, and delayed menstruation were classified as none, mild, moderate, and severe, which were scored as 0, 1, 2, and 3 points, respectively [9]. (4) Adverse events such as irregular vaginal bleeding and abnormal liver function were recorded in both groups.

Statistical analysis

Statistical analyses was performed using SPSS 20.0. And enumeration data were compared with χ^2 test, while the measurement data were expressed as mean ± standard deviation（ $\bar{x}$±s）. Independent samples t-test was used for comparison between the two groups, and paired samples t-test was used for comparison before and after treatment. $P < 0.05$ was taken as statistically significant.

Results

Comparison of pain between the two groups

Before treatment, there was no significant difference in COX and VAS scores between the two groups (P>0.05). After treatment, the COX and VAS scores of the two groups were significantly lower than those before treatment (P<0.05), and the scores in the observation group were significantly lower than those in the control group (P<0.05), see Table 2.

Comparison of PGE_2, $PGF_{2\alpha}$ and VEGF levels between the two groups

Before treatment, there was no significant difference in PGE_2, $PGF_{2\alpha}$ and VEGF levels between the two groups (P>0.05). After treatment, the PGE_2, $PGF_{2\alpha}$ and VEGF levels in the two groups were significantly lower than those before treatment (P<0.05), and these levels in the observation group were significantly lower than those in the control group (P<0.05), see Table 3.

Table 2 Comparison of pain between the two groups （ $\bar{x}$±s, points)

Group	Case	COX score		VAS score	
		Before treatment	After treatment	Before treatment	After treatment
Observation group	50	28.43±3.16	10.51±2.13*	6.90±0.72	3.34±0.36*
Control group	50	28.62±3.85	14.48±2.27*	6.63±0.88	3.62±0.49*
t		-0.270	-9.018	1.679	-3.256
P		0.788	0.000	0.096	0.002

Note: compared with that before treatment: *P<0.05.

Table 3 Comparison of PGE_2, $PGF_{2\alpha}$ and VEGF levels between the two groups （ $\bar{x}$±s）

Group	Cases	PGE_2 (pg/mL)		$PGF_{2\alpha}$ (pg/mL)		VEGF (ng/L)	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Observation group	50	478.65±32.16	231.17±26.78*	325.07±42.27	108.57±27.14*	224.48±31.06	117.36±20.51*
Control group	50	476.50±33.85	308.35±26.24*	322.25±44.16	161.89±28.55*	223.12±32.11	165.43±25.72*
t		0.326	-14.556	0.326	-9.571	0.215	-10.333
P		0.745	0.000	0.745	0.000	0.830	0.000

Note: compared with that before treatment: *P<0.05.

Comparison of TCM symptom scores between the two groups

Before treatment, there was no significant difference between the two groups in the scores of 8 items of TCM symptoms (lower abdomen pain and chills, pain reduction in the heat, feeling pain while pressing, delayed menstruation, chilly sensation and the cold limbs, low volume or inhibited menstruation, pale

complexion and menstruation with light color and blood clot) (P>0.05). After treatment, the score levels of these TCM symptoms in the two groups were significantly lower than that before treatment (P<0.05), and the levels in the observation group were significantly lower than those in the control group (P<0.05), see Table 4.

Table 4 Comparison of TCM symptom scores between the two groups ($\bar{x}$±s, points)

Observation indicators		Observation group (n=50)	Control group (n=50)	t	P
Lower abdomen pain and chills	Before treatment	4.58±1.35	4.62±1.14	-0.160	0.873
	After treatment	1.95±0.57*	2.80±0.63*	-7.704	0.000
Pain reduction in the heat	Before treatment	4.61±1.38	4.58±1.33	0.111	0.912
	After treatment	1.45±0.49*	2.78±0.66*	-11.441	0.000
Feeling pain while pressing	Before treatment	4.32±1.16	4.25±1.17	0.300	0.764
	After treatment	1.44±0.48*	1.80±0.57*	-3.416	0.001
Delayed menstruation	Before treatment	2.20±0.71	2.13±0.69	0.500	0.618
	After treatment	0.49±0.12*	0.82±0.25*	-8.415	0.000
Chilly sensation and the cold limbs	Before treatment	2.33±0.73	2.25±0.68	0.567	0.572
	After treatment	1.09±0.22*	1.42±0.29*	-6.410	0.000
Low volume or inhibited menstruation	Before treatment	2.02±0.63	1.99±0.65	0.234	0.815
	After treatment	0.79±0.24*	1.11±0.27*	-6.264	0.000
Pale complexion	Before treatment	1.91±0.61	1.87±0.64	0.320	0.750
	After treatment	0.89±0.22*	1.12±0.31*	-4.278	0.000
Menstruation with light color and blood clot	Before treatment	2.22±0.73	2.24±0.68	-0.142	0.888
	After treatment	1.09±0.30*	1.32±0.45*	-3.007	0.003

Note: compared with that before treatment: *P<0.05.

Comparison of adverse reactions between the two groups

The incidence of adverse reactions in the control group was 20.00%, including 6 cases of irregular vaginal bleeding and 4 cases of abnormal liver function. The incidence of adverse reactions in the

treatment group was 16.00%, including 5 cases of irregular vaginal bleeding and 3 cases of abnormal liver function, which were all improved naturally after drug withdrawal. There was no significant difference between the two groups (χ^2=0.271, P=0.603).

Discussion

EM refers to the presence of endometrial tissue outside the uterine cavity, and often occurs in the ovary, fallopian tube and other parts. With the rapid development of society, the incidence rate of EM has increased year by year recently [2]. The typical symptom of EM is dysmenorrhea, accompanied by nausea, vomiting, dizziness and other symptoms. At present, hormone drugs are often used in Western medicine to treat EM dysmenorrhea. Mifepristone tablets are progesterone receptor antagonists, which can promote lesion atrophy and effectively relieve patients' pain. However, hormone drugs are difficult to cure EM dysmenorrhea, and the recurrence rate and adverse reaction rate are high after drug withdrawal so that this drugs are limited in clinical application [10]. TCM has its unique advantages in treating dysmenorrhea on the basis of dialectical treatment and integrity. Wenshen Nuangong formula was founded by Professor Quansheng Wu, which can warm the kidney and enhance Yang, invigorate Qi and nourish blood, and treat both manifestation and root cause of disease, and is widely used to treat cold coagulation and blood stasis type of dysmenorrhea [11]. In this paper, we studied the effects of Wenshen Nuangong formula combined with mifepristone tablets on the pain symptoms, and PGE_2, $PGF_{2\alpha}$ and VEGF levels in patients with cold coagulation and blood stasis type of dysmenorrhea, and the results showed that Wenshen Nuangong formula had a definite effect on EM patients with cold coagulation and blood stasis.

The results showed that after treatment, the COX score and VAS score of the two groups were significantly lower than those before treatment, and the scores in the observation group were significantly lower than those in the control group. After treatment, the scores of TCM symptoms in the two groups were significantly lower than those before treatment, and the scores in the observation group were significantly lower than those in the control group. There was no significant difference in adverse reactions between the two groups. The above results suggested that Wenshen Nuangong formula can effectively alleviate the clinical symptoms and pain of patients with cold coagulation and blood stasis type of EM dysmenorrhea, and its effect is safe and reliable. Misoprostone is a 19-nortestosterone derivative, which can competitively bind with progesterone receptor to antagonize progesterone, so as to reduce the disease focus and alleviate pain [12]. TCM has no clear diagnosis and treatment records on EM, and dysmenorrhea is usually classified into the categories of "Zheng Jia" (abdominal mass) and "Yuejing Butiao" (irregular menstruation). Its main pathogenesis is the internal attachment of evil Qi, the deficiency of blood essence, the poor circulation of Qi and blood in the uterus, "obstruction leading to pain" or "pain without nutritional moisturization". Cold coagulation and blood stasis is one of the common types of dysmenorrhea. According to the records in *Gynaecology of traditional Chinese medicine*, the pathogenesis of cold coagulation and blood stasis type of dysmenorrhea is "feeling cold before menstruation and after delivery, or over eating cold food to produce coldness... As a result, Qi and blood are stagnant and blocked, causing pain and dysmenorrhea". Wenshen Nuangong formula takes Lujiao Shuang and Yin Yang Huo as main drugs to promote blood circulation and remove stasis, and takes Danggui, Baishao, Shu Dihuang and Jiu Yurou as adjuvant drugs to nourish Yin and blood. Stir-fried Baizhu, stir-fried Shanyao, roasted Huangqi and Fuling were added to tonify spleen and kidney, while San Leng, E Zhu, Tu Biechong were added to improve blood circulation and disperse stasis. In addition, Gancao was used to regulate the whole prescription, which can not only warm the meridians and disperse the cold, but also remove blood stasis and relieve pain. Modern pharmacological research shows that on the one hand, Wenshen Nuangong formula can improve hemorheology, reduce blood viscosity and relieve pain. On the other hand, it can inhibit pain transmission,

reduce the sensitivity of pain receptors, and thus play an analgesic effect [11].

Modern medicine believes that endocrine disorder is the key to EM dysmenorrhea. Both PGE_2 and $PGF_{2\alpha}$ are prostaglandins secreted by endometrium, in which $PGF_{2\alpha}$ can cause uterine smooth muscle spasm, and PGE_2 can inhibit uterine smooth muscle spasm. Both of them jointly regulate uterine contraction. A study has shown that the levels of PGE_2 and $PGF_{2\alpha}$ in serum of EM dysmenorrhea patients are significantly higher than those of non-dysmenorrhea patients [13]. Neovascularization is one of the key factors to promote the occurrence and development of EM dysmenorrhea. The more sufficient the blood perfusion at the disease focus, the faster the growth of ectopic tissue. VEGF is a vascular endothelial growth factor, whose expression in EM dysmenorrhea patients is significantly higher than that in non-dysmenorrhea patients. And VEGF level is positively correlated with the development of EM dysmenorrhea. The results of this study showed that after treatment, the levels of PGE_2, $PGF_{2\alpha}$ and VEGF in the two groups were significantly lower than those before treatment, and the levels in the observation group were significantly lower than those in the control group, which suggested that Wenshen Nuangong formula can effectively reduce the levels of PGE_2, $PGF_{2\alpha}$ and VEGF. Modern pharmacological research shows that Wenshen Nuangong formula can affect the release of neurotransmitters such as β-endorphins, and it can regulate the secretion of gonadotropin releasing hormone via multiple targets, improve the function of hypothalamus-pituitary-ovary axis, and thus regulate the endocrine function of the body. Cuinan et al. selected Wenshen Nuangong formula to treat cold coagulation and blood stasis type of EM dysmenorrhea, and found that the formula can effectively reduce VEGF level and alleviate pain [4]. Yumei Wang et al. selected Wenshen Nuangong formula, Shaofu Zhuyu decoction and Sanfu Ginger Moxibustion to treat cold coagulation and blood stasis type of EM dysmenorrhea, and they found that Wenshen Nuangong formula can effectively reduce serum PGE_2 and $PGF_{2\alpha}$ levels, which is consistent with the results of this study [11].

In conclusion, Wenshen Nuangong formula can effectively alleviate pain, relieve clinical symptoms, and reduce serum PGE_2, $PGF2_{2\alpha}$, and VEGF levels of patients with cold coagulation and blood stasis type of EM dysmenorrhea, with significant efficacy and safety.

Declaration of conflict-of-interest

The authors declare no conflict-of-interest.

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