Effect of Tiaojingzhixue Fang on the expression of sex hormone and endometrial tissue mRNA in perimenopausal patients with abnormal uterine bleeding

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ABSTRACT

This study aimed to investigate the effect of Tiaojingzhixue decoction on the expression of sex hormone and endometrial mRNA in perimenopausal patients with abnormal uterine bleeding. For this purpose, 84 patients with perimenopausal abnormal uterine bleeding who were treated in our hospital from January 2018 to January 2019 were divided into study group and control group, the control group was treated with mifepristone for six months, the clinical efficacy, time of symptom relief and disappearance, endometrial thickness, menstrual volume, adverse events, expression of sex hormone (ER), Progesterone receptor (PR), Lutropin (LH), Follicle-stimulating hormone (FSH), Estradiol (e2) and Vascular endothelial growth factor in endometrial tissue were compared between the two groups. The results showed that the clinical efficacy of the study group (97.61%) was significantly higher than the control group (80.95%) (P<0.05). There was no significant difference in endometrial thickness between the two groups before treatment, but after treatment for 1 month, 3 months and 6 months, endometrial thickness was thinner in the study group (P<0.05). There was no significant difference in menstrual volume between the two groups before treatment (P <0.05), but it was lower in the study group (P <0.05). The incidence of adverse reactions in the study group was 11.90% lower than in the control group (26.19%). The expression levels of ER, PR, LH, FSH and E2 were almost the same before treatment and there was no significant difference between the two groups (P>0.05), but they were lower in the study group after treatment (P<0.05). Before treatment, there was no significant difference in the level of VEGF between the two groups (P>0.05); after treatment, the level of Vegf in the study group was lower than the control group (P<0.05). After treatment, the level of Vegf in both groups was significantly higher than before treatment (P<0.05). In general, Tiaojingzhixue decoction can decrease the level of sex hormone and increase the expression of VEGF in patients with perimenopausal abnormal uterine bleeding.

Introduction

Abnormal uterine bleeding (AUB) is a common disease in gynecology. Patients present with irregular menstrual cycles, including frequency, regularity, duration, and flow outside pregnancy. Studies have shown that as many as one-third of women will experience abnormal uterine bleeding during their lifetime, most often during menarche and perimenopause (1). The normal menstrual cycle is 24 to 38 days, lasts 7 to 9 days, and blood loss is 5 to 80 ml. AUB may be a manifestation of the hormonal environment or a clinical manifestation of benign and malignant lesions of the reproductive tract in perimenopausal women. Postmenopausal bleeding needs special attention because it is a clinical indication of endometrial cancer in developed countries and cervical cancer in China (2). Abnormal uterine bleeding can also be classified as acute or chronic. Acute AUB is excessive bleeding and requires immediate intervention to prevent further bleeding. Acute AUB can occur alone or in combination with chronic AUB, which is irregular menstrual bleeding for most of the past 6 months.

Perimenopause includes the last two to eight years before menopause and one year after the onset. Menorrhagia represents large amounts of regular bleeding for a long time and may be associated with fibroma, adenomyosis, or endometrial polyps.

Menorrhagia and uterine bleeding represent other types of AUB, whose root cause is other endometrial changes (3).

Abnormal uterine bleeding is related to cytokines that regulate cell proliferation in vivo, such as the Basic fibroblast Factor (bGGF) vascular endothelial growth factor, etc. (4, 5). The reduced expression of bGGF will slow down the repair of the endometrium and aggravate the symptoms of abnormal uterine bleeding (6). These effects on the uterus are also related to the expression of progesterone receptors and estrogen receptors in the body (7, 8), and the level of expression is closely related to the severity of the disease. Low levels of estrogen can have negative feedback to the hypothalamus, and the endometrium is disturbed by a single estrogen, leading to polyp hyperplasia, retreat bleeding, and perimenopausal abnormal uterine bleeding.

This study mainly studied the effects of Tiaojing Zhixue Prescription on the expression of sex hormones and endometrial tissue mRNA in perimenopausal patients with abnormal uterine bleeding. The report is as follows:

Materials and methods
General Information

A total of 84 patients with perimenopausal abnormal uterine bleeding treated in our hospital from January 2018 to January 2019 were selected as the research objects, and they were divided into study groups and control groups, with 42 patients in each group. The study group was 45-54 years old, with an average age of (49.11±4.01) years. The course of the disease was 2-7 months, with an average course of (4.95±1.23) months. Pregnancy 1-4 times, there were 19 cases of mild anemia, 18 cases of moderate anemia and 5 cases of severe anemia. The control group was 43-53 years old, with an average age of (47.32±4.98) years. The course of the disease was 1-7 months, with an average course of (4.22±1.45) months. Pregnancy 1-4 times, there were 20 cases of mild anemia, 17 cases of moderate anemia and 5 cases of severe anemia. There was no statistically significant difference in age and course of disease between the two groups (P<0.05), indicating comparability. This study has been approved by the Ethics Association of our hospital.

Inclusion Criteria

(i) All patients were diagnosed with abnormal perimenopausal uterine bleeding; (ii) Patients have no contraindication to the drugs used; (iii) Patients were not treated with other hormone drugs before treatment; (iv) None of the patients had fertility requirements; (v) Patients can cooperate with researchers; (vi) Patients and their families were informed of the study and signed informed consent.

Exclusion Criteria

(i) Patients with malignant tumors; (ii) patients with cervical lesions; (iii) patients with diseases of the endocrine system; (iv) patients with severe dysfunction of liver, kidney and other organs; (v) patients with combined mental diseases.

Research Methods

Immunohistochemistry was used to detect the expression of the estrogen-progesterone receptor in tissue samples from 84 patients. Dewaxing and hydration 4μm thick paraffin tissue slices were washed with buffer solution 3 times, each time for 3min, and then put into the microwave oven for continuous heating at 92°C ~ 98°C for 12min. After that, goat serum was dropped to seal the non-specific antigen, and then mouse anti-human ER monoclonal antibody was added (Company name: Abcam, Type: Rabbit anti-human polyclonal antibody) was placed in a refrigerator at 4°C overnight and rewarmed for 30min. The indirect method of Streptomyces antibiotin protein with horseradish peroxidase was added to detect the receptor expression, and chromogenic agent was used for chromogenic process (9). After chromogenic process, hemoxylin was used for redyeing and neutral adhesive was used for sealing. According to the improved Sinicrope method, PR and ER staining intensity ratings of uterine tissue cells were as follows: 0 for colorless or almost no staining, 1 for light brown-yellow staining, 2 for light brown-yellow staining and 3 for dark brown-yellow staining; Scores of PR and ER staining cells in sections: 0 scores for positive cells less than 5%, 1 score for 5%-25%, 2 scores for 25%-50%, 3 scores for 50%-75% and 4 scores for 75%-100%. The final PR ER staining score was obtained by adding the staining intensity to the staining cell number score. After adding staining intensity score and staining cell

number score, 0 ~ 2 was divided into grade 1, 3 ~ 4 was divided into grade 2, and 5 ~ 6 was divided into grade 3. All stained sections were examined by 2 physicians. If the results were consistent, the results would be final.

Intravenous plasma was collected on fasting in the morning before and after treatment in all patients and centrifuged in a centrifuge at 1700r/min at low temperature. The supernatant was removed and stored in a refrigerator at 4℃ for later detection.

Patients in the study group were treated with meridian regulating hemostasis formula, which was prescribed as follows: Astragalus membranaceus 18 g 12 g, dangshen 30 g, Fried atractylodes, motherwort 30 g, cattail pollen carbon 12 g (Fried), 15 g, 12 g field thistle madder, 30 g purslane, herba schizonepetae, sanguisorba 30 g, 6 g ink dry lotus 30 g, 6 g cohosh, notoginseng powder (a blunt), glue 9 g, 3 g anemarrhena asphodeloides bge 9 g, phellodendri 6 g, burnt hawthorn, burnt divine qu, burnt malt 9 g, decocted in water, one dose daily, morning and evening. The drug was administered for 5 to 10 days, starting on the fifth day of rebleeding, and was observed for 2 consecutive cycles of three menstrual cycles.

The control group was given didrogesterone tablets (Chinese drug approval Batch No. H20050395, drug specification: 5mg) once a day, 12.5mg/ time

Observation Indicators

(i) Compare the clinical efficacy of the two groups after treatment. Significant effect: Abnormal uterine bleeding disappeared effectively: abnormal uterine bleeding was significantly reduced than before; Ineffective: Abnormal uterine bleeding symptoms, menstrual time, cycle and so on have no significant difference with before treatment, or even more serious; (ii) The symptom relief time and complete disappearance time were compared between the two groups after treatment. (iii) Color vaginal ultrasound was used to examine the endometrial thickness of the two groups before and after treatment for six months. (iv) The menstrual volume of the two groups was detected and compared by menorrhagia. According to the area of blood-stained with tampons in the two groups of patients, the scoring rules are as follows: 1 point for blood-stained area less than 1/3; The area between 1/3 and 2/3 is 5 points; More than 2/3 of the

area is 20 points, the higher the score means more menstruation.(5) The incidence of adverse reactions between the two groups was compared; (6) The expression levels of ER, PR, LH, FSH and E2 were compared between the two groups. Serum LH, FSH and E2 levels were analyzed by ELISA. The contents of ER and PR were determined by adding staining intensity score and staining cell number score. (7) The expression level of VEGF in endometrial tissue was compared between the two groups. The expression of VEGF was detected by immunohistochemistry.

Statistical methods

SPSS 22.0 software was used to analyze the obtained data. Mean ± standard deviation was used to represent the counting data. T-test was used for comparison between the two groups. [N (%)] was used to represent the count data, and χ^2 was used to test the difference between groups. Rank sum test was used for grade data and $P<0.05$ was considered statistically significant.

Results and discussion
Comparison of clinical efficacy between the two groups after treatment

The experimental results showed that the clinical efficacy of the study group (97.61%) was higher than that of the control group (80.95%), and the comparison between the two groups was statistically significant ($P<0.05$), as shown in Table 1.

Table 1. Comparison of clinical effect between the two groups after treatment [n(%)]

	Study group (n=42)	Control group (n=42)	χ^2	P
Markedly effective	32 (76.19)	18 (42.85)		
Effective	9 (21.43)	16 (38.10)		
Invalid	1 (2.38)	8 (19.05)		
Total	41 (97.61)	34 (80.95)	5.697	0.007

Comparison of symptom relief time and complete disappearance time between the two groups after treatment

After the experiment can be obtained, the symptom relief time and symptom disappear time of the study group are less than the control group. There was significant statistical significance between the two groups ($P<0.05$), as shown in Table 2.

Table 2. Comparison of Symptom Relief Time and complete disappearance time between the two groups (x̄±s)

	Study group (n=42)	Control group (n=42)	t	P
Symptom relief time (D)	37.11±8.17	43.15±8.77	5.897	0.005
Symptom resolution time (D)	90.45±10.01	110.21±12.76	6.012	0.003

Comparison of endometrial thickness between the two groups before and after treatment

After the experiment, the endometrial thickness of the two groups before treatment was almost the same, and there was no significant statistical significance between the two groups (P<0.05), which was comparable. The endometrial thickness of the study group was thinner than that of the control group after treatment for 1 month, 3 months and 6 months, and the comparison between the two groups was statistically significant (P<0.05). The endometrial thickness of the two groups after treatment was thinner than that before treatment, and there was statistically significant difference between the two groups before and after treatment (P<0.05), as shown in Table 3.

Table 3. Comparison of endometrial thickness between two groups before and after treatment (x̄±s)

		Study group (n=42)	Control group (n=42)	t	P
Endometrial thickness (mm)	Before the treatment	13.67±2.49	13.63±2.50	0.414	0.947
	1 months of treatment	12.31±2.31a	12.91±2.73a	2.214	0.049
	3 months of treatment	9.68±1.67ab	10.82±1.84abc	3.012	0.031
	6 months of treatment	6.24±1.26ab	8.11±1.64abc	3.947	0.024

Note: Compared with the same group before treatment, aP<0.05, abP<0.05; Compared with the control group after treatment, cP<0.05.

Comparison of menstrual volume between the two groups before and after treatment

After the test, the menstrual volume of the two groups was almost the same before treatment, and there was no significant statistical significance before treatment (P<0.05), which was comparable. After treatment, the menstrual volume of the study group was lower than that of the control group, and there was significant statistical significance between the two groups (P<0.05). After treatment, menstrual volume in both groups was lower than before, and the

comparison between the two groups before and after treatment was statistically significant (P<0.05), as shown in Table 4.

Table 4. Comparison of menses between the two groups before and after treatment (x̄±s)

		Study group (n=42)	Control group (n=42)	t	P
Menstrual volume (ml)	After treatment	156.12±26.47	155.84±28.14	0.048	0.987
	After treatment	41.35±11.89d	56.14±17.11d	4.015	0.001

Note: dP of the two groups before treatment was <0.05

Comparison of adverse reactions between the two groups after treatment

The results showed that the incidence of adverse reactions in the study group was 11.90% lower than that in the control group (26.19%), and the comparison between the two groups was statistically significant (P<0.05), as shown in Table 5.

Table 5. Comparison of adverse reactions between the two groups after treatment [n(%)]

	Study group	Control group	χ2	P
Breast pain	2 (4.76)	4 (9.52)		
Nausea	1 (2.38)	3 (7.14)		
Vomiting	1 (2.38)	2 (4.76)		
Dizzy	1 (2.38)	2 (4.76)		
Total adverse reaction	5 (11.90)	11 (26.19)	5.473	0.006

Comparison of ER, PR, LH, FSH and E2 expression levels between the two groups before and after treatment

The results showed that the expression levels of ER, PR, LH, FSH and E2 were similar before treatment, and there was no significant statistical significance between the two groups (P<0.05), indicating comparability. After treatment, the expression levels of ER, PR, LH, FSH and E2 in the study group were lower than those in the control group. After treatment, the expression levels of ER, PR, LH, FSH and E2 in the two groups were lower than before treatment, with significant statistical significance (P<0.05), as shown in Table 6.

Comparison of VEGF expression levels between the two groups before and after treatment

After the test, VEGF levels in the two groups were almost the same before treatment, and there was no

significant statistical significance before treatment (P>0.05). After treatment, the level of VEGF in the study group was lower than that in the control group, and there was statistical significance between the two groups after treatment (P<0.05). VEGF levels in both groups were higher after treatment than before, with significant statistical significance (P<0.05), as shown in Table 7.

Table 6. Expression of ER、PR、LH、FSH and E2 before and after treatment in two groups (x̄±s)

		Study group (n=42)	Control group (n=42)	t	P
ER	Before the treatment	4.03±0.17	4.10±0.18	0.654	0.865
	After the treatment	0.91±0.21e	1.34±0.32e	3.451	0.031
PR	Before the treatment	3.97±0.19	3.96±0.18	0.945	0.812
	After the treatment	0.83±0.13e	1.26±0.11e	5.141	0.006
LH	Before the treatment	9.31±0.98	9.51±0.91	0.742	0.764
	After the treatment	5.16±0.51e	7.99±0.78e	3.014	0.036
FSH	Before the treatment	12.55±1.20	12.69±1.16	0.847	0.798
	After the treatment	6.78±0.69e	10.97±1.06e	4.125	0.026
E2	Before the treatment	390.01±36.45	389.12±38.01	0.947	0.813
	After the treatment	164.12±16.98e	196.75±20.36e	5.012	0.009

Note: Compared with before treatment, eP<0.05.

Table 7. Comparison of VEGF expression between two groups before and after treatment (x̄±s)

		Study group (n=42)	Control group (n=42)	t	P
VEGF (ng/L)	Before the treatment	706.41±77.14	709.45±75.47	0.954	0.725
	After the treatment	751.94±84.67f	869.84±68.27f	6.482	0.001

Note: Compared with before treatment, fP<0.05.

ABU is a very common female disease, accounting for 1/3 of gynecological diseases, more than 70% of which are perimenopausal, and its main clinical manifestations are frequent and regular cycles, blood loss and abnormal period time (10). The principle of its pathogenesis is that with the increase of women's age (9), ovarian function declines and the mechanism of regulating sex hormones in the body is disturbed, which eventually leads to breakthrough or withdrawal bleeding (11). The endometrial estrogen receptor volume increases, resulting in changes in the endometrium (12-14). The most common cause of abnormal uterine bleeding before and after menopause is endometrial polyps, which are more common in postmenopausal women with atypia and malignant tumor proliferation. Endometrial polyps may occur in women of childbearing age and postmenopause, and they are one of the most common causes of abnormal

uterine bleeding (15). According to studies, endometrial polyps were detected in about 26% of AUB patients (16-18). In most cases, polyps are benign, but between 0.5% and 13% can grow or become malignant. Postmenopausal women with hypertension and obesity are more likely to develop cancer (19-21). In perimenopausal patients with abnormal uterine bleeding, mRNA expression is also abnormal, in which the level of VEFG changes greatly, which is the most specific and effective factor in regulating abnormal uterine bleeding. VEGF expression can regulate itself or other secretory mechanisms to stimulate endothelial vascular proliferation and structure formation, which can promote vascular growth and improve vascular permeability. Repair the vascular endothelium in the bleeding uterus, accelerate the formation of vascular formation factors in the uterus, endothelial cells to form new blood vessels. And the endometrium after repair of capillaries, reduce uterine bleeding. The level of VEGF in perimenopausal patients with abnormal uterine bleeding is lower than that of normal people, so when bleeding, it cannot be regulated, forming a vicious cycle.

In this study, many of the herbs of tiaojing Hemostasis prescription have the function of supplementing qi, nourishing blood and stopping bleeding. For example, Sanqi powder has the effect of stopping bleeding, dispersing stasis and calming pain. Ejiao has the effect of nourishing blood (22). Tuckahoe has the effect of improving water permeability, invigorating the spleen and calming heart. Scutellaria charcoal has the effects of clearing heat, dampness, relieving fire and detoxifying (23). Perimenopausal AUB patients are often accompanied by varying degrees of anemia, so this prescription can repair the patient's uterus and replenish blood and qi.

The clinical treatment effect of the study group was better than that of the control group, and the adverse reactions were less than that of the control group, indicating that tiaojing Zhixue Prescription had a good effect on the repair of patients' uterus, and patients almost had no adverse reactions. Among them, the Traditional Chinese medicine of supplementing qi and nourishing blood plays a great role, possibly because it can reduce the generation of sex hormones in the uterus, which can reduce the stimulation of sex hormones to the uterus so that the patient's uterine

bleeding volume can be significantly reduced, and the expression of VEGF in the uterus can be increased to repair it. The decrease in adverse reactions is due to the fact that drugs regulate the body during treatment. When the disease occurs, the patient's blood loss increases, and the patient's qi and blood will be lost, and the drugs in the prescription will coordinate with each other to regulate the qi and blood in the body. The level of sex hormone in the study group was also significantly lower than that before the treatment and lower than that in the control group, indicating that the regulation of jingjing hemostasis was obvious in the regulation of sex hormone in the patients, which may be to regulate and protect the secretion of sex hormone through the regulation and protection of the decline of the ovary to restore the normal level of sex hormone in the body. The expression level of VEGF in uterine tissues was rise less than the control group, because the experiment formula of patients with uterine bleeding after, to regulate the expression of VEGF, which makes the body of the expression of VEGF levels increase, and then to repair of the uterus, menstrual quantity gradually returned to normal, the thickness of the lining of the uterus to normal levels. The excessive increase of VEGF may lead to endometrial hyperplasia in patients, resulting in aggravation of the disease, so the treatment effect of the study group is better.

In the study of Martins et al. (24), it was detailed that abnormal uterine bleeding has many causes. Uterine polyps, adenomyosis, uterine malignant tumors and hyperplasia, ovulation dysfunction, are related to the disorder of sex hormones and abnormal mRNA expression in patients. Therefore, after understanding it, we can prevent the disease or carry out the symptomatic treatment. In the study of Jewson et al. (25), progesterone was involved in the regulation of abnormal uterine bleeding, and the regulation of PRA and PRB receptors played A role, and their increase in the body would lead to endometrial hyperplasia, which may deteriorate into endometrial cancer. Hormones and sex issues are very important in married life (26, 27). All of these are consistent with the study of this paper and have theoretical help for the treatment of abnormal uterine bleeding in the perimenopausal period.

In conclusion, Tiaojing Zhixue Prescription can regulate the expression of sex hormones and mRNA in perimenopausal patients with abnormal uterine bleeding, and play a therapeutic effect.

Acknowledgments

Interest conflict
The authors declare no conflict of interest.

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