Int J Clin Exp Med 2021;14(1):51-61
www.ijcem.com /ISSN:1940-5901/IJCEM0121356

Original Article

Effects of Biejiajian pills on T cell subset distribution and surface PD-1/PD-L1 expression in the treatment of endometriosis

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Received August 29, 2020; Accepted September 27, 2020; Epub January 15, 2021; Published January 30, 2021

Abstract: Objective: To investigate the therapeutic effect of Biejiajian pills on endometriosis and its impact on T cell subset distribution and surface PD-1/PD-L1 expression. Methods: A total of 98 patients with endometriosis who were admitted our hospital were divided into two groups by a random number table method, with 49 cases in each group. Patients in the control group was treated with leuprorelin acetate. Patients in the observation group was treated with Biejiajian pills in addition to the treatment of the control group. Before and after treatment, the clinical efficacy, sex hormone levels (follicle stimulating hormone, luteinizing hormone, and estradiol), tumor-related marker levels (anti-endometrial antibody and carbohydrate antigen 125), T cell subset levels (cluster of differentiation 4-positive ($CD4^+$), cluster of differentiation 8-positive ($CD8^+$), and the ratio of $CD4^+$ to $CD8^+$ T cells), expression levels of programmed death 1 (PD-1) and programmed death ligand 1 (PD-L1) on the surface of $CD4^+$ and $CD8^+$ T cells, and the incidence of adverse reactions were compared between the two groups. Results: The total effective rate in the observation group was 95.92%, which was significantly higher than that in the control group 81.63% (P=0.025). After treatment, the $CD4^+$ T cell count and $CD4^+/CD8^+$ ratio in the observation group were lower than those in the control group, while the $CD8^+$ T cell count in the observation group was higher than that in the control group (P<0.05). PD-1/PD-L1 level on the surfaces of $CD4^+$ and $CD8^+$ T cells, and the levels of follicle stimulating hormone, luteinizing hormone, and estradiol in the observation group were lower than those in the control group (P<0.05). The carbohydrate antigen 125 and anti-endometrial antibody levels in the observation group were lower than those in the control group (P<0.05). The incidences of adverse reactions in both groups were not statistically significant (P=1.000). Conclusion: Biejiajian pills as an adjunct are effective in the treatment of patients with endometriosis. They are safe, and can significantly improve the sex hormone levels, reduce tumor-related marker levels and $CD4^+$ T cell count, increase $CD8^+$ T cell count, and inhibit the expression of PD-1/PD-L1 on the surface of T lymphocytes.

Keywords: Endometriosis, leuprorelin acetate, Biejiajian pills, T cell subsets, adverse reactions

Introduction

Endometriosis refers to a gynecological disease in which active endometrial cells implant outside of the uterus. It manifests as dysmenorrhea, abnormal menstruation, and chronic pelvic pain, and infertility may be induced in severe cases [1]. According to research data, the prevalence of endometriosis-associated infertility is as high as 40%, and about one-third of infertilities are associated with endometriosis [2]. At present, the pathogenesis of the disease has not been fully clarified in clinical practice. Current study has focused on endometrial implantation theory or immunological theory, especially the immune mechanism [3, 4]. Immunologically, it is believed that immune system dysfunction will lead to failed removal of ectopic endometrial fragments from the uterus and rejection of ectopic endometrial implantation [5]. Studies have pointed out that most patients with endometriosis suffer from systemic or local cellular and humoral immune dysfunction, which mainly manifests as immune imbalance and immunosuppression [6, 7]. Immune imbalance will lead to changes in the activity and levels of immune molecules and factors, cause complement deposition and

autoantibody production, alter the peritoneal environment, and increase the risk of ectopic endometrial growth and implantation [8, 9]. Programmed death 1 (PD-1) is a negative costimulatory signaling molecule. After binding to programmed death ligand 1 (PD-L1), it will inhibit T cell proliferation and play a role in negatively regulating immune responses.

Currently, laparoscopic surgery and drugs are the mainstay of treatment for endometriosis. However, since most patients want to preserve ovarian function, such treatment cannot completely eradicate the lesions, and there is still a risk of recurrence after surgery. Relevant research data showed that the recurrence rate of endometriosis is as high as 19% within 1 year after treatment, so adjuvant medical therapy is often used after surgery in clinical practice [10]. For example, leuprorelin acetate, a highly potent drug that is currently used to inhibit gonadotropin secretion, and is often applied in the treatment of endometriosis [11]. Since the World Health Organization (WHO) first included Traditional Chinese Medicine (TCM) in the global medical compendium in 2018, the theory and practice of TCM syndrome differentiation have suddenly come to the fore front and offer a new direction in clinical diagnosis and treatment. Biejiajian pills have the effect of promoting blood circulation and dissipating masses, and they are applied in the clinical treatment of various diseases such as liver cancer and hyperplasia of mammary glands [12]. However, there is no systematically controlled study on the application of Biejiajian pills in endometriosis. Therefore, we observed the changes of T cell subsets and surface PD-1/PD-L1 expression in patients with endometriosis treated with Biejiajian pills, in order to explore the therapeutic value and potential mechanism of this drug in the treatment of endometriosis, and provide a reference for clinical drug treatment of endometriosis. The results are reported as follows.

Materials and methods

General data

A total of 98 patients with endometriosis who were admitted to the Zibo Hospital of Traditional Chinese Medicine from December 2017 to December 2019 were selected and divided into two groups by a random number table, with 49 cases in each group. This study was approved by the Medical Ethics Committee of Zibo Hospital of Traditional Chinese Medicine.

Inclusion criteria

Inclusion criteria: Patients who met the diagnostic criteria for endometriosis according to the Norms on the Diagnosis and Treatment of Endometriosis [13]; patients who met the criteria for qi stagnation and blood stasis syndrome according to Guidelines for Clinical Research of New Chinese Medicines [14]; patients with primary symptoms of lower abdominal pain before or during menstruation, and distending pain in the breast and chest; patients with secondary symptoms of impeded discharge of menses or scant menstruation; patients with clots in the menstrual blood, dark purple menstruation, and relief of pain with the discharge of clots; patients who felt irritable; patients with chest tightness, dry mouth and constipation; patients with purple or petechiae tongue, and string-like and unsmooth pulse; patients who signed an informed consent.

Exclusion criteria: Patients with congenital defects of the immune system; patients with uterine malformations; patients with hysteromyoma; patients with ovarian diseases; patients with pelvic inflammatory disease; patients with an allergy to drug used in this study; patients with confirmed infertility; patients with a history of depression, anxiety, schizophrenia, cognitive dysfunction and other mental illness before diagnosis; patients with incomplete clinical data.

Methods

Control group: The control group was treated with leuprorelin acetate (Takeda Pharmaceutical Company Limited), 3.75 mg subcutaneously within 4 days of menstruation, once a month, for a total of 3 months.

Observation group: The observation group was treated with Biejiajian pills on the basis of the treatment in the control group, with a prescription including: stir-fried Carapax Trionycis 90 g, red niter 90 g, Catharsius 45 g, Radix Paeoniae Alba 37 g, Eupolyphaga seu Steleophaga 37 g, cortex moutan 37 g, Nidus Vespae 30 g, Radix Bupleuri 22.5 g, stir-fried Rhizoma Belamcandae 22.5 g, pillbug 22.5 g, Radix Scutellariae 22.5 g, Rhizoma Zingiberis 22.5 g, Ramulus Cinnamomi 22.5 g, Mangnolia offici-

nalis 22.5 g, Folium Pyrrosiae 22.5 g, stir-fried Colla Corii Asini 22.5 g, Radix et Rhizoma Rhei 22.5 g, Flos Campsis 22.5 g, Semen Persicae 15 g, Dianthus Superbu 15 g, Rhizoma Pinelliae 7.5 g, Radix Ginseng 7.5 g, and Semen Lepidii 7.5 g. These medicines were ground into powder, processed into honey pills with each pill weighing 3 g. The pills were given to the patients 1 pill/time, 3 times/d, for 3 months. All the herbal medicines were purchased from Sinopharm Zhonglian Pharmaceutical Co., Ltd., and the pills were prepared by the Preparation Room of Zibo Hospital of Traditional Chinese Medicine.

Outcome measures

Primary outcome measures: (1) Clinical efficacy: Markedly effective: the patients' clinical symptoms such as abdominal pain and dysmenorrhea disappeared, and B ultrasound revealed no pelvic mass. Effective: the patients' clinical symptoms such as abdominal pain and dysmenorrhea were significantly improved, and B ultrasound revealed a shrinkage of pelvic mass by ≥30%. Ineffective: the patients' clinical symptoms such as abdominal pain and dysmenorrhea did not improve, and B ultrasound revealed a shrinkage of pelvic mass by <30%, or no shrinkage, and even signs of enlargement. Total effective rate (%) = (Markedly effective cases + effective cases)/total number of cases * 100.

(2) Immune function: Before and after treatment, 5 mL of venous blood was drawn from the patient with a vacuum blood collection tube containing heparin. Then the samples were mixed evenly with an equal volume of phosphate-buffered saline (PBS), slowly added to 5 mL of lymphocyte separation solution, and centrifuged at 600 g for 30 min at room temperature (slowly heating up and cooling down). After centrifugation, buffy coat cells were aspirated, washed once in PBS, centrifuged at 500 g for 10 min, and resuspended to a concentration of 5 * 10^6/mL. Then 100 μL of suspension was added with Fc block, and incubated at 4 degrees for 15 min. Subsequently, the suspension was added with antibodies (CD3-APC-H7, CD4-PE, CD8-Percp-Cy5.5, PD1-BV421, and PD-L1-PE-Cy7), stained at 4 degrees for 30 min, washed once in PBS, and finally resuspended in 300 uL of 1% paraformaldehyde in PBS for analysis of immune functions using an analyzer (BD LSR Fortessa). After that, FlowJo software was used to analyze the results. The above antibodies and isotype controls were purchased from Becton, Dickinson and Company (BD, USA).

Secondary outcome measures: (1) Sex hormones levels: Before and after treatment, 4 mL of fasting venous blood was taken from the patients and centrifuged at 2500 r/min for 5 min. Then the levels of follicle stimulating hormone (FSH), estradiol (E_2), and luteinizing hormone (LH) were measured by radioimmunoassay. OTA-400 automatic biochemical analyzer was provided by Shenyang Wantai Medical Equipment Co., Ltd. The test kits were provided by Shanghai Chemtron Biotech Co., Ltd (FSH, E_2 and LH test kits; cat. nos. 4781-50, 1533237375 and 1532989770, respectively). Specific steps were performed in strict accordance with the instruction manuals.

(2) Tumor markers levels: Before and after treatment, 4 mL of fasting venous blood was taken from the patients. Then the serum was collected through centrifugation and detected for the levels of tumor markers including carbohydrate antigen 125 (CA-125) and anti-endometrial antibody (EMAb) were measured by enzyme-linked immunosorbent assay. The test kits were provided by Shanghai Jianglai Biotechnology Co., Ltd (CA-125, and EMAb test kits; cat. nos. 1534962010 and 1533777884, respectively). Specific steps were performed in strict accordance with the instruction manuals.

(3) Adverse reactions: The patients' adverse reactions during treatment were recorded, including interstitial pneumonia, liver damage, endometrorrhagia, dyspareunia, hot flashes, and decreased libido. The total incidence of adverse reactions was calculated as follows: total incidence = (cases of adverse reactions/total number of cases) * 100%.

Statistical methods

All data were processed using SPSS 18.0 statistical software. Measurement data were expressed as mean ± standard deviation ($\bar{x}$ ± sd), and were examined by independent t test. Enumeration data were described as percentage, and were examined using χ^2 test. The difference was statistically significant at $P<0.05$.

Table 1. Comparison of general data between the two groups ($\bar{x} \pm sd$)

Group	Age (years)	Disease course (years)	Body mass index (kg/m^2)
Control group (n=49)	35.2±4.7	1.22±0.48	22.01±1.96
Observation group (n=49)	35.7±4.7	1.21±0.44	22.51±2.21
t	0.527	0.108	1.185
P	0.599	0.915	0.239

Table 2. Comparison of clinical efficacy between the two groups (n (%))

Group	Markedly effective	Effective	Ineffective	Total effective rate
Control group (n=49)	20 (40.82)	20 (40.82)	9 (18.37)	40 (81.63)
Observation group (n=49)	30 (61.22)	17 (34.69)	2 (4.08)	47 (95.92)
χ^2				5.018
P				0.025

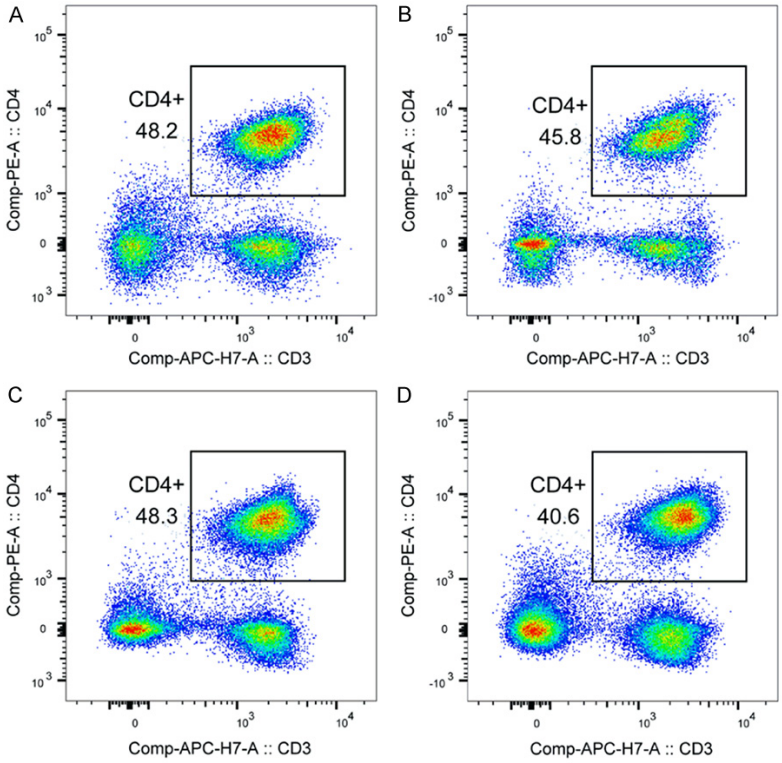

Figure 1. Comparison of CD4$^+$ T cell count between the two groups. A. CD4$^+$ T cell count in the control group before treatment; B. CD4$^+$ T cell count in the control group after treatment; C. CD4$^+$ T cell count in the observation group before treatment; D. CD4$^+$ T cell count in the observation group after treatment. CD4$^+$: cluster of differentiation 4-positive.

Results

General data

There was no significant difference in the general data between the two groups (P>0.05), and they were comparable. See **Table 1.**

Clinical efficacy

The total effective rate in the observation group was 95.92%, which was significantly higher than that in the control group 81.63% (P= 0.025), suggesting that Biejiajian pills can effectively improve the clinical efficacy of patients with endometriosis. See **Table 2**.

Immune function

Before treatment, there was no significant difference in the cluster of differentiation 4-positive (CD4$^+$) T cell count, cluster of differentiation 8-positive (CD8$^+$) T cell count, and the ratio of CD4$^+$ to CD8$^+$ T cells (CD4$^+$/CD8$^+$ ratio) between the two groups (P> 0.05). After treatment, the CD4$^+$ T cell count and CD4$^+$/CD8$^+$ ratio in the observation group were lower than those in the control group, while CD8$^+$ level in the observation group was higher than that in the control group (P<0.001). Both results indicated that Biejiajian pills can effectively improve the immune function of patients with endometriosis. See **Figures 1-3**.

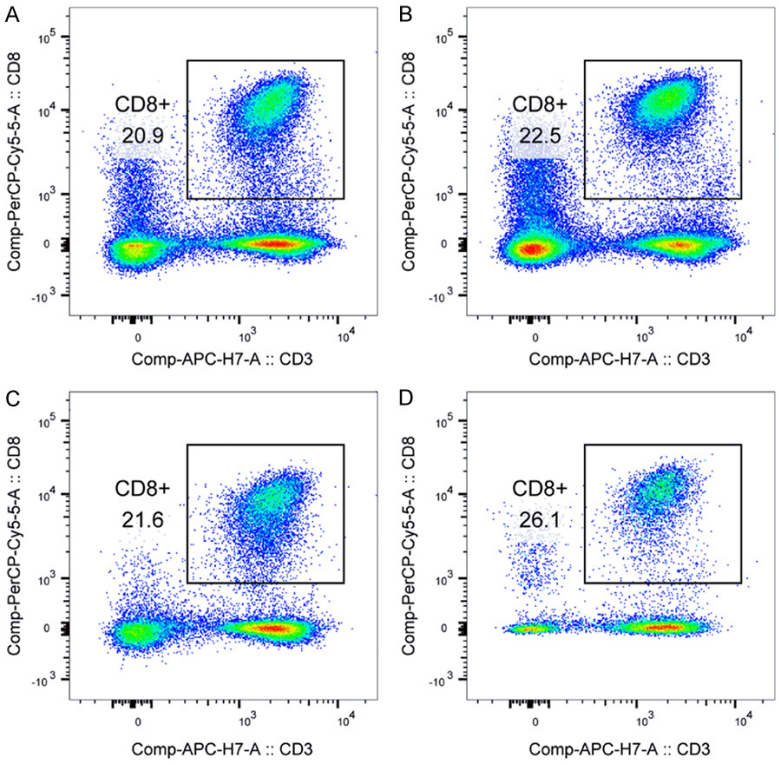

Figure 2. Comparison of CD8⁺ T-cell count between the two groups. A. CD8⁺ T cell count in the control group before treatment; B. CD8⁺ T cell count in the control group after treatment; C. CD8⁺ T cell count in the observation group before treatment; D. CD8⁺ T cell count in the observation group after treatment. CD8⁺: cluster of differentiation 8-positive.

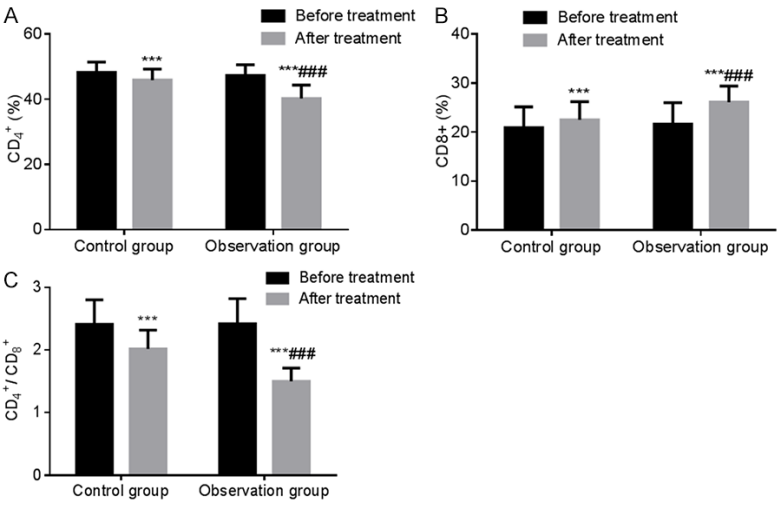

Figure 3. Comparison of immune function between the two groups. A. CD4⁺; B. CD8⁺; C. CD4⁺/CD8⁺. ***P<0.001 as compared with that before treatment; ###P<0.001 as compared with the control group. CD4⁺: cluster of differentiation 4-positive; CD8⁺: cluster of differentiation 8-positive.

PD-1/PD-L1 expression levels

Before treatment, there was no significant difference in the expression levels of PD-1/PD-L1 on the surface of CD4⁺ and CD8⁺ T cells between the two groups (P>0.05). After treatment, the levels of PD-1/PD-L1 on the surfaces of CD4⁺ and CD8⁺ T cells in the observation group were lower than those in the control group (P<0.001). The above results suggested that Biejiajian pills can effectively improve PD-1/PD-L1 expression levels on the surfaces of CD4⁺ and CD8⁺ T cells in patients with endometriosis. See **Figures 4**-**8**.

Sex hormone levels

Before treatment, there was no significant difference in FSH, E_2, and LH levels between the two groups (P>0.05). After treatment, FSH, E_2, and LH levels in the observation group were lower than those in the control group (P<0.01). Both results demonstrated that Biejiajian pills can effectively reduce sex hormone levels in patients with endometriosis. See **Table 3** and **Figure 9**.

Tumor markers

Before treatment, there was no significant difference in CA-125 and EMAb levels between the two groups before treatment (P>0.05). After treatment, CA-125 and EMAb levels in the observation group were lower than those in the control group (P<0.05). The above results showed that Biejiajian pills can effectively lower the level of tumor markers in patients with endometriosis. See **Table 4** and **Figure 10**.

Adverse reactions

The incidences of adverse reactions was 8.16% in both groups, and being equal, the difference

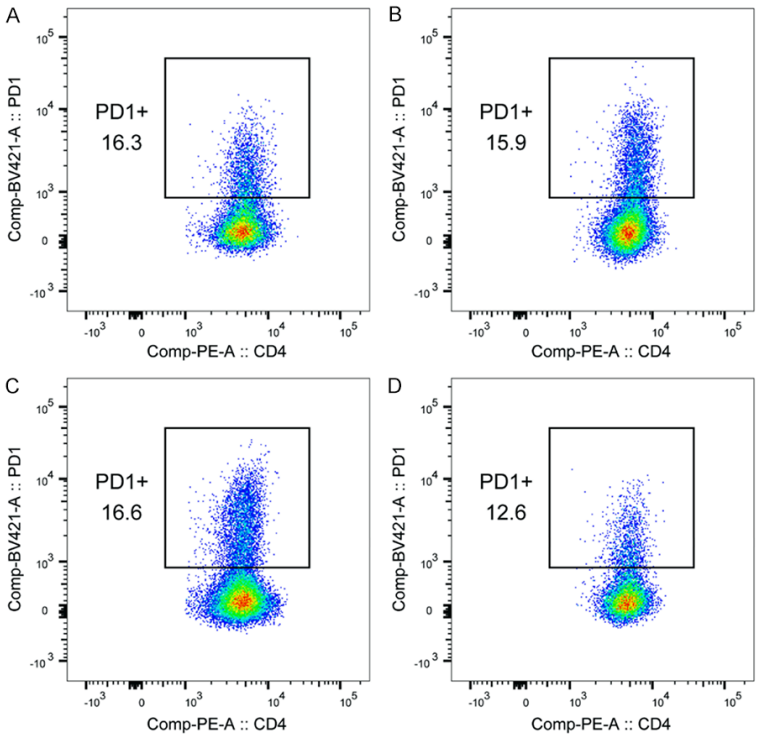

Figure 4. Comparison of flow cytometry of PD-1 expression level on the surface of $CD4^+$ T cell between the two groups. A. PD-1 expression level on the surface of $CD4^+$ T cell in the control group before treatment; B. PD-1 expression level on the surface of $CD4^+$ T cell in the control group after treatment; C. PD-1 expression level on the surface of $CD4^+$ T cell in the observation group before treatment; D. PD-1 expression level on the surface of $CD4^+$ T cell in the control group after treatment. $CD4^+$: cluster of differentiation 4-positive; PD-1: programmed death 1.

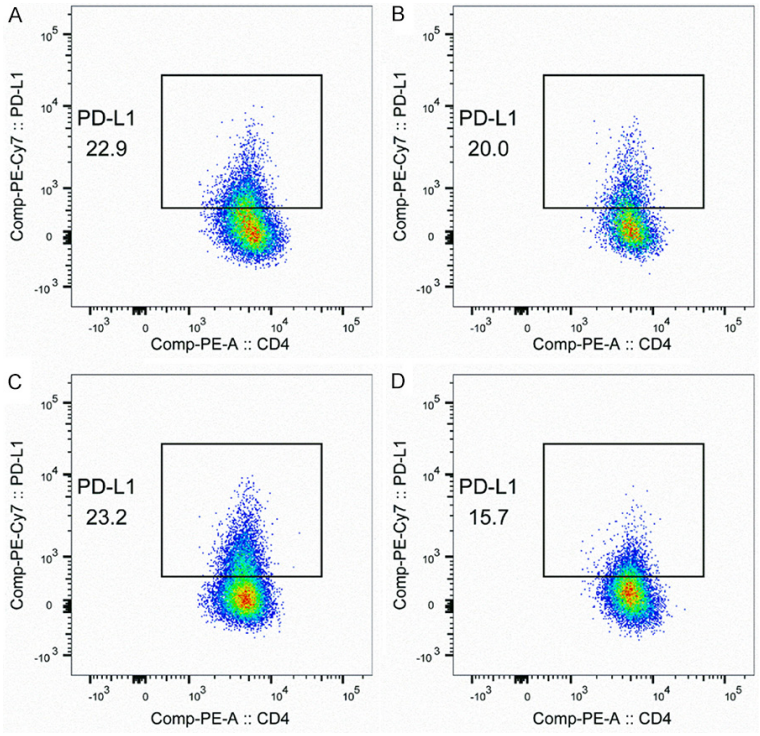

Figure 5. Comparison of flow cytometry of PD-L1 expression level on $CD4^+$ T cell between the two groups. A. PD-L1 expression level on the surface of $CD4^+$ T cell in the control group before treatment; B. PD-L1 expression level on the surface of $CD4^+$ T cell in the control group after treatment; C. PD-L1 expression level on the surface of $CD4^+$ T cell in the observation group before treatment; D. PD-L1 expression level on the surface of $CD4^+$ T cell in the observation group after treatment. PD-L1: programmed death ligand 1; $CD4^+$: cluster of differentiation 4-positive.

between groups was not statistically significant ($P>0.05$), indicating that Biejiajian pills do not increase the incidence of adverse reactions in patients with endometriosis. See **Table 5**.

Discussion

Triptorelin acetate is a synthetic gonadotropin-releasing hormone (GnRH) analogue that is structurally similar to gonadal hormone-releasing hormone and has an affinity for GnRH receptors. It effectively inhibits the release of FSH, LH, and E_2 mainly by acting on pituitary gland, reduces estrogen levels to menopausal levels, and causes atrophy of estrogen-dependent lesion, ultimately inhibiting or removing lesion growth [15-18]. However, the drug does not have a significant effect on immune function, and can produce more adverse reactions [19, 20]. The results showed that after treatment with triptorelin acetate, 4 patients had adverse reactions such as hot flashes, dyspareunia, and liver damage, which indicated that great importance should be paid to adverse reactions during treatment.

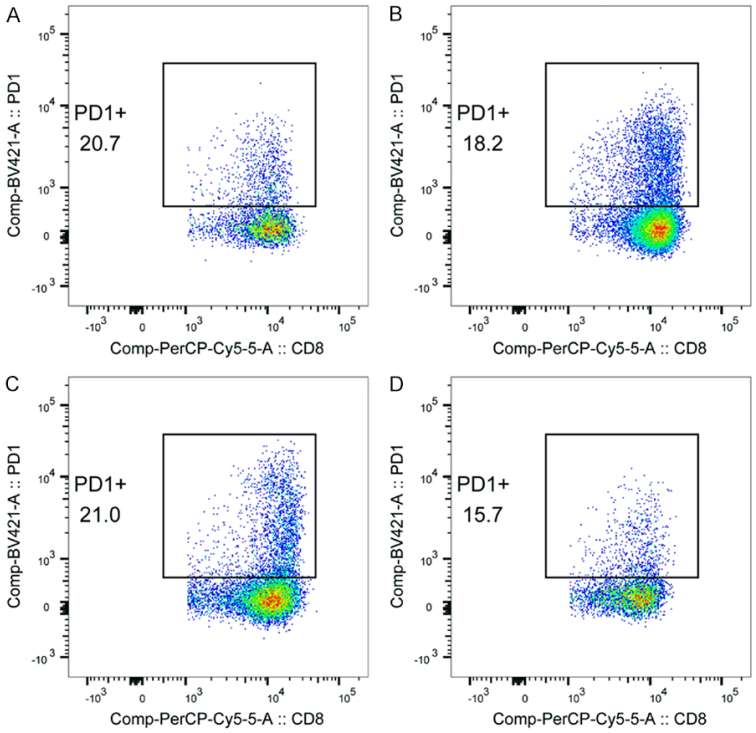

Figure 6. Comparison of flow cytometry of PD-1 expression level on the surface of $CD8^+$ T cell between the two groups. A. PD-1 expression level on the surface of $CD8^+$ T cell in the control group before treatment; B. PD-1 expression level on the surface of $CD8^+$ T cell in the control group after treatment; C. PD-1 expression level on the surface of $CD8^+$ T cell in the observation group before treatment; D. PD-1 expression level on the surface of $CD8^+$ T cell in the observation group after treatment. $CD8^+$: cluster of differentiation 8-positive; PD-1: programmed death 1.

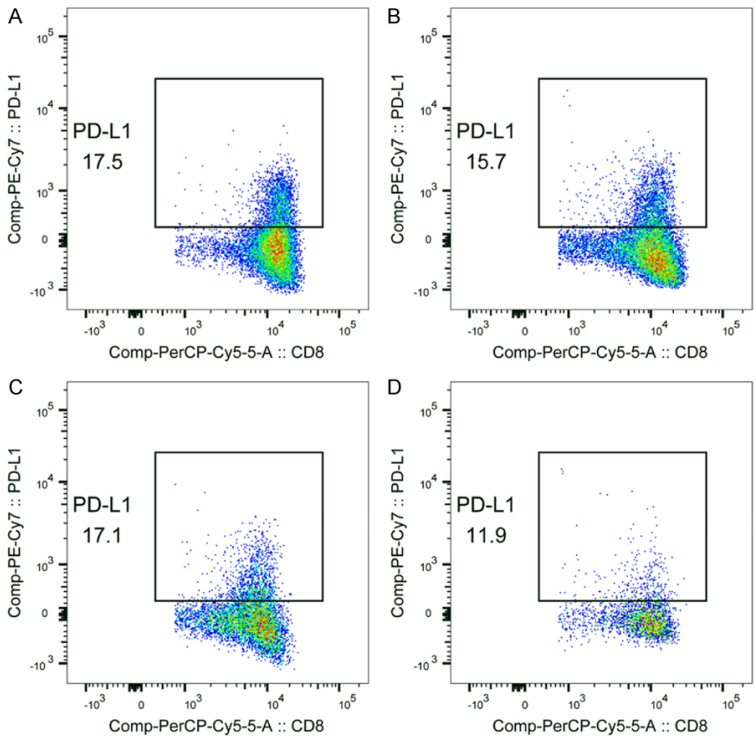

Figure 7. Comparison of flow cytometry of PD-L1 expression level on the surface of $CD8^+$ T cell between the two groups. A. PD-L1 expression level on the surface of $CD8^+$ T cell in the control group before treatment; B. PD-L1 expression level on the surface of $CD8^+$ T cell in the control group after treatment; C. PD-L1 expression level on the surface of $CD8^+$ T cell in the observation group before treatment; D. PD-L1 expression level on the surface of $CD8^+$ T cell in the observation group after treatment. $CD8^+$: cluster of differentiation 8-positive; PD-L1: programmed death ligand 1.

There are no records of the name “endometriosis” in traditional Chinese medicine (TCM). According to clinical manifestations of this disease, it is mainly categorized into “dysmenorrhea”, “irregular menstruation”, “infertility”, and “abdominal mass”, which mostly results from stagnation of qi and blood stasis following the deficiency of Chong and Ren meridians. Accumulated blood stasis will block the movement of qi and blood inside the viscera, and generate qi stagnation, phlegm-dampness and turbidity, thus causing obstruction and pain [21]. Therefore, TCM treatment of this disease should be based on syndrome differentiation of blood stasis, emphasize the movement of qi, and follow the principles of promoting blood circulation to remove blood stasis and enhancing the flow of qi to relieve pain. In the formula of Biejiajian pills, the principal drug (monarch) is stir-fried Carapax Trionycis, it can remove abdominal masses and relieve chills and fever; the minister drugs are Rhizoma Belamcandae, Radix et Rhizoma Rhei, cortex moutan, Flos Campsis, Radix Paeoniae Alba, Catharsius, red niter, pillbug, Semen Persicae, and Nidus Vespae, which can eliminate

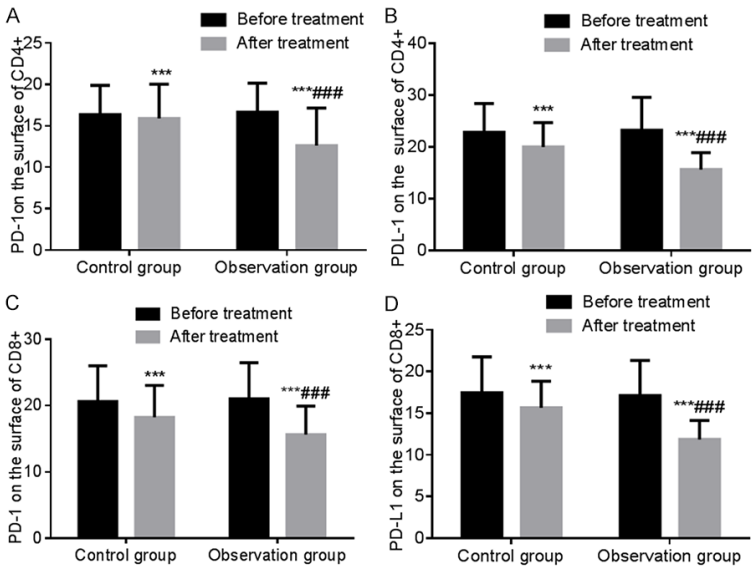

Figure 8. Comparison of PD-1/PD-L1 expression levels on the surfaces of $CD4^+$ and $CD8^+$ T cells between the two groups. A. PD-1 expression level on the surface of $CD4^+$ T cell; B. PD-L1 expression level on the surface of $CD4^+$ T cell; C. PD-1 expression level on the surface of $CD8^+$ T cell; D. PD-L1 expression level on the surface of $CD8^+$ T cell. $^{***}P<0.001$ as compared with that before treatment; $^{\#\#\#}P<0.001$ as compared with the control group. $CD4^+$: cluster of differentiation 4-positive; $CD8^+$: cluster of differentiation 8-positive; PD-1: programmed death 1; PD-L1: programmed death ligand 1.

blood stasis and soften hard masses; the assistant drugs are Folium Pyrrosiae, Semen Lepidii, Rhizoma Pinelliae, Mangnolia officinalis, Radix Bupleuri, Ramulus Cinnamomi, Radix Scutellariae, Dianthus Superbu, and Rhizoma Zingiberis, which can regulate the movement of qi, promote diuresis, and balance cold and heat; the messenger drugs are Colla Corii Asini and Radix Ginseng, which can nourish qi and blood, and enhance immunity. Combined use of these drugs can play a synergistic effect in eliminating abdominal masses and restoring the movement of qi and blood [22]. Also, modern pharmacology has confirmed that Radix Ginseng contains a large number of ginseng polysaccharides, vitamins and amino acids, which are effective in regulating immune system [23].

The results of this study showed that the clinical efficacy of the observation group was higher than that of the control group. After treatment, the improvement of sex hormone levels was greater in the observation group than that in the control group. In the study by Zhan et al., the effective rate of Biejiajian pills in the treatment of hyperplasia of mammary glands was 90.00%, which was significantly higher than 73.68% of Rupixiao tablet [24]. This result was basically consistent with the results of our study, which indicated that Biejiajian pills are beneficial to inhibit the increase of sex hormones, reduce the stimulation of sex hormones to the endometrium, further shrinking or removing lesions and improving clinical efficacy. Carbohydrate antigen 125 (CA-125) is a commonly used marker for the detection of malignant lesions of the uterus, and monitoring of its level has significant reference value for the prevention and treatment of endometriosis and malignant lesions. Anti-endometrial antibody (EMAb) is mostly associated with human immune system disorders, and can be detected in the serum of most patients with endometriosis. The results of our study showed that after treatment, CA-125 and EMAb levels in the observation group were lower than those in the control group. This suggested that Biejiajian pills are conducive to controlling the occurrence and development of the disease, which may be mostly related to the effect of this drug in immune regulation of the body.

T lymphocytes are important for cellular and humoral immunity. They are activated under antigen stimulation and can produce various lymphokines to regulate humoral immunity and the balance of $CD4^+/CD8^+$ ratio. Generally, reciprocal restraints and induction of $CD4^+$ and $CD8^+$ will form a T cell network to stabilize immune function. Disruption of this network will lead to abnormal T cell differentiation, ultimately causing immune disorders and pathological damage to the immune system [25]. However, after treatment, the immune function and PD-1/PD-L1 expression levels on the surfaces of $CD4^+$ and $CD8^+$ T cells in the observation group were all improved, but did not improve significantly in the control group. Such results showed that Biejiajian pills can also effectively regulate systemic immunity and enhance the patient's immune function, in

Table 3. Comparison of sex hormone levels between the two groups ($\bar{x}$ ± sd, mmol/L)

Group	FSH		E_2		LH	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Control group (n=49)	5.28±1.58	4.82±1.14***	51.97±10.66	24.68±7.65***	3.96±1.23	1.35±0.65***
Observation group (n=49)	5.32±1.35	4.04±1.04***	52.62±11.25	11.36±4.68***	4.03±1.15	0.67±0.16***
t	0.135	3.538	0.294	10.397	0.291	7.111
P	0.893	0.001	0.769	0.000	0.772	0.000

Note: ***P<0.001 as compared with that before treatment. FSH: follicle stimulating hormone; LH: luteinizing hormone; E_2: estradiol.

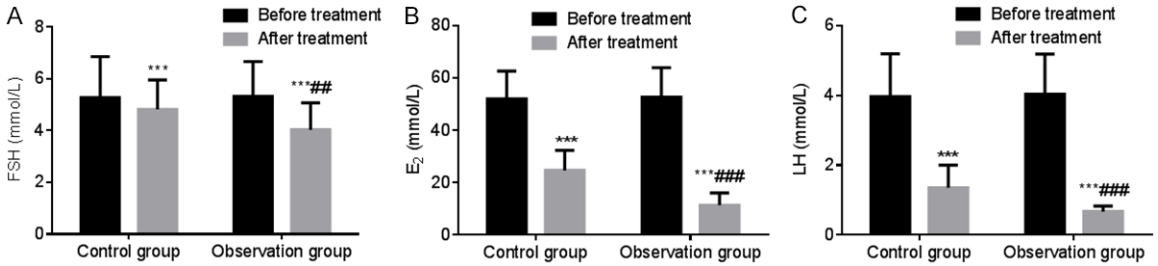

Figure 9. Comparison of sex hormone levels between the two groups. A. FSH; B. E_2; C. LH. ***P<0.001 as compared with that before treatment; ##P<0.01 and ###P<0.001 as compared with the control group. FSH: follicle stimulating hormone; LH: luteinizing hormone; E_2: estradiol.

Table 4. Comparison of tumor marker levels between the two groups ($\bar{x}$ ± sd)

Group	CA-125 (IU/mL)		EMAb (ng/L)	
	Before treatment	After treatment	Before treatment	After treatment
Control group (n=49)	51.28±7.48	45.54±4.74***	0.38±0.08	0.31±0.07***
Observation group (n=49)	50.38±6.54	34.65±4.20***	0.37±0.05	0.19±0.04***
t	0.634	12.037	0.742	10.419
P	0.528	0.000	0.460	0.000

Note: ***P<0.001 as compared with that before treatment. EMAb: anti-endometrial antibody; CA-125: carbohydrate antigen 125.

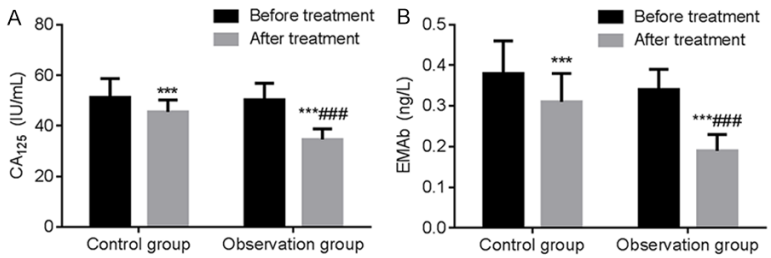

Figure 10. Comparison of tumor marker levels between the two groups. A. CA125; B. EMAb. ***P<0.001 as compared with that before treatment; ###P<0.001 as compared with the control group. EMAb: anti-endometrial antibody; CA-125: carbohydrate antigen 125.

order to promote drug efficacy as well as reduce EMAb level.

In addition, the incidence of adverse reactions in the observation group was similar to that in the control group. This result suggested that traditional Chinese medicine is safe and reliable, which can achieve better therapeutic effect without causing additional damage to the body. However, due to the lack of medical evidence of TCM treatment for endometriosis at present, we only conducted the study with a small sample size. Therefore, a large number of TCM studies are needed to fur-

Table 5. Comparison of the incidence of adverse reactions between the two groups (n (%))

Group	Interstitial pneumonia	Liver damage	Metrorrhagia	Dyspareunia/ decreased libido	Hot flush	Total incidence
Control group (n=49)	0	1 (2.04)	1 (2.04)	1 (2.04)	1 (2.04)	4 (8.16)
Observation group (n=49)	1 (2.04)	0	0	2 (4.08)	1 (2.04)	4 (8.16)
χ^2						0.000
P						1.000

ther confirm our experimental conclusion in the future.

In conclusion, it is confirmed that Biejiajian pills are effective in treating patients with endometriosis. They are safe, and can significantly improve the levels of sex hormones, reduce tumor-related markers, decrease $CD4^+$ T cell count, increase $CD8^+$ T cell count, and inhibit the expression of PD-1 and PD-L1.

Disclosure of conflict of interest

None.

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