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Original Research Article

Improvement of ovarian function and fertility in patients with endometriosis after combination therapy with levonorgestrel-releasing intrauterine system plus gonadotropin-releasing hormone agonist

Aijun Ti[1], Ting Zhang[2], Lili Jia[3], Mingli Chen[1*]
[1]Department of Gynecology, Laizhou People's Hospital, Laizhou, Shandong, 261400, China, [2]Department of Central Pharmacy, Laizhou People's Hospital, Laizhou, Shandong, 261400, China, [3]Qingdao West Coast New District Maternal and Child Health and Family Planning Service Center, Qingdao, Shandong, 266500, China

For correspondence:* ***Email:* *zhaozunyi2009@163.com*

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Abstract

Purpose: *To evaluate the therapeutic efficacy of gonadotropin-releasing hormone agonist (GnRHa) plus levonorgestrel-releasing intrauterine system (LNG-IUS) for moderate and severe endometriosis (EMS).*
Methods: *94 patients with EMS were randomized into experimental and control groups. Patients in the experimental group (EG) received LNG-IUS + GnRHa while those in the control group (CG) received GnRHa. Pre- and post-treatment estradiol (E_2), follicle-stimulating hormone (FSH), luteinizing hormone (LH), interleukin (IL)-2, IL-4, and interferon-γ (INF-γ) levels in serum were measured, and alterations in antral follicle count (AFC), ovarian stroma (OS) and peak systolic velocity (PSV) were observed using an ultrasonic tester. Patients were followed up for one-year during which the severity of dysmenorrhea was determined with the Visual Analogue Scale (VAS), and the clinical efficacy was evaluated at the last follow-up while the recurrence of EMS was counted.*
Results: *EG patients had lower FSH, E_2, LH and IL-2 levels than CG patients after treatment, with higher AFC, PSV, IL-4 and INF-γ ($p<0.05$). The prognostic follow-up showed a continuous decrease in VAS in both groups, with a lower score among EG patients ($p<0.05$). No statistical difference was identified in the recurrence rate of EMS between EG and CG patients ($p>0.05$) even though the overall response rate was higher among EG patients ($p<0.05$).* ***Conclusion:*** *LNG-IUS + GnRHa has better, stable and long-term efficacy against moderate and severe EMS, and higher efficacy in improving patients' ovarian function.*

Keywords: *Endometriosis; gonadotropin-releasing hormone agonist; dysmenorrhea degree; levonorgestrel-releasing intrauterine system*

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INTRODUCTION

Endometriosis (EMS) is a clinically common gynecologic disorder that occurs frequently in women of childbearing age (25 to 45 years) and occasionally in postmenopausal women receiving hormone replacement therapy [1]. According to the statistics of the World Health Organization, the incidence of EMS is about 10 to 15 percent worldwide, and 20 to 90 percent among women with chronic pelvic pain and dysmenorrhea [2]. Approximately 25 to 30 percent of female infertility has been clinically associated with EMS [3]. Dysmenorrhea, the hallmark symptom of EMS, makes most patients ignore or mishandle the disease, directly leading to disease exacerbation in over 60% of EMS patients when they are diagnosed, and eventually evolving into malignant infectious diseases and even gynecological tumors [4]. Except radical hysterectomy, there is currently no complete clinical cure for EMS, which also results in a high likelihood of EMS recurrence, seriously affecting the normal life of patients [5].

Gonadotropin-releasing hormone agonist (GnRHa) has been shown to effectively lower hormone levels in women, promote lesion atrophy after EMS surgery, and reduce the possibility of EMS recurrence [6,7]. The levonorgestrel-releasing intrauterine system (LNG-IUS), a drug-containing T-shaped intrauterine contraceptive device that was initially used for long-term contraception in clinical practice, has been found to be effective in assisting the treatment of EMS and improving the prognosis of patients [8,9]. However, the value of LNG-IUS + GnRHa in EMS is rarely reported. LNG-IUS + GnRHa may provide a more reliable prognostic guarantee for EMS, reducing the recurrence risk of EMS and effectively improving the quality of medical services. Therefore, this study was designed to evaluate the therapeutic efficacy of gonadotropin-releasing hormone agonist (GnRHa) plus levonorgestrel-releasing intrauterine system (LNG-IUS) for moderate and severe endometriosis (EMS).

METHODS

Patients

Following approval for this study by the Laizhou People's Hospital's Ethics Committee, 94 cases of EMS who presented at the Laizhou People's Hospital, Shandong, China between December 2020 and October 2021 were recruited. All subjects signed informed consent forms.

Included in this study were all patients (25 to 45 years) that presented with clinical manifestations of EMS (including abdominal pain, dysmenorrhea). Confirmation of EMS was based on laparoscopy or surgical exploration that included evaluation of the site, number, size and depth of endometriotic infiltration, and degree of adhesions. Patients that scored 1-5 was classified as stage I, 6-15 as stage II, 16-40 as stage III (moderate), and >40 I as stage IV (severe). EMS classification was according to the EMS staging standards [10]. All patients included in the study completed their treatment. Patients that presented with coagulation dysfunction, severe cardio-cerebrovascular disease, organ dysfunction/failure, drug allergy, or surgical contraindications were excluded from the study. In addition, all patients lost to follow up were also excluded.

Study design

The patients were randomized into an experimental group (EG, 47 patients) for LNG-IUS + GnRHa therapy and a control group (CG, 47 patients) for GnRHa therapy. Conservative surgical treatment [11], which was completed by the same surgical team of in the hospital, was performed on all patients. Under the premise of fertility preservation, the visible lesions and neoplasms in the patient's ovary were removed as far as possible, and the pelvic adhesion was separated. Postoperatively, both groups were given Triptorelin (H20054645) injection, one injection of 0.5mg (dissolved in 5 mL saline) once a day for five weeks. After five weeks it was changed to 0.1 mg/dose (dissolved in 1 mL saline). In EG patients, LNG-IUS (J20140088, contains 52 mg of LNG - a slow release system that continued to release LNG at a rate of 20 μg/24h for the first 5 years and roughly 11 μg/24h after 5 years) was placed into the uterine cavity five weeks after Triptorelin injection.

Follow-up for patient prognosis

All patients received one-year prognostic follow-up after the completion of treatment, which was conducted in the form of regular hospital review every 3 months, with a total of 4 reviews. The termination event was EMS recurrence.

Endpoints

All basic information about all study patients (e.g. age, fertility, and course of disease) were collected. Fasting venous blood samples were collected before and after treatment. Radioimmunoassay was performed to quantify serum estradiol (E_2), follicle-stimulating hormone

(FSH) and luteinizing hormone (LH) levels, and ELISA was carried out to determine Th1/Th2 cytokines interleukin (IL)-2, IL-4, and interferon-γ (INF-γ) with kits all supplied by Beijing TransGen Biotech. In addition, the antral follicle count (AFC), and peak systolic velocity of ovarian stroma (PSV) were detected by vaginal color Doppler ultrasonography. The severity of dysmenorrhea was assessed at each follow-up visit using the Visual Analogue Scale (VAS; 0-10 points), with greater scores suggesting more obvious pain. At the last follow-up, the clinical efficacy of patients was evaluated using the treatment guidelines for EMS [12] as given in Table 1.

Table 1: Clinical efficacy rating of patients

Rating	Condition
Cure	All symptoms and pelvic mass disappeared
Marked response	Symptoms basically disappeared and the pelvic mass shrank
Response	Symptoms were relieved, and the pelvic mass did not increase or decrease
Non-response	Symptoms were not improved or even worsened, and the local lesions aggravated

The overall response rate (ORR) was calculated as:

ORR = (cure+marked response+response) cases/total number of people ×100%.

EMS recurrence during the one-year prognostic follow-up was also counted in both groups.

Statistical methods

SPSS22.0 software was used for statistical analysis. χ^2 tests were performed for inter-group comparisons in terms of ORR, incidence of adverse reactions and other categorical variables represented by (%). Independent samples t-test (comparison between two groups), paired t-test (comparison of pre- and post-treatment within the group), variance analysis and LSD post-hoc test (comparison between multiple groups or multiple time points) were carried out to identify differences in patients' age, CA125 and other continuous variables represented by ($\bar{\chi}$±s). The difference between groups was considered statistically significant when $p<0.05$.

RESULTS

Clinical baseline data

Patients' baseline data were first compared to ensure the reliability of experimental results, and found no statistical inter-group differences ($p>0.05$, Table 2), confirming the comparability between EG and CG.

DISCUSSION

Progressive neuronal cell death is a characteristic feature of neurodegenerative diseases that result in dementia, cognitive impairment, loss of motor control, impairment of functional abilities and ultimately, death. Owing to the lack of effective treatments, neurodegenerative diseases are becoming an increasing economic burden on society [12]. Therefore, there is an urgent to identify compounds that prevent neuronal cell death in order to facilitate the development of therapeutic strategies to combat this global health problem. Neuroinflammation and oxidative stress have been reported to be hallmarks of neurodegenerative diseases, which contribute towards disease progression [1,2]. Currently, researchers are investigating the therapeutic potential of natural antioxidants as treatments for neuroinflammation and oxidative stress and/or as preventatives of the neurodegenerative disorders that are present with these two conditions [13].

Comparison of pre- and post-treatment sex hormone levels

EG and CG did not differ significantly in ovarian function test results before treatment ($p>0.05$). Reductions were observed in FSH, E_2 and LH levels in both groups after treatment, with even lower levels in EG ($p<0.05$, Figure 1).

Table 2: Clinical baseline data

Variable	EG (n=47)	CG (n=47)	t or χ2	P
Age	35.34±3.10	36.17±3.96	1.13	0.26
Course of disease	3.11±1.36	3.06±1.01	0.17	0.86
Fertility (childbearing/ non-childbearing)	40/7	37/10	0.65	0.42
Staging of EMS (III/IV)	26/21	22/25	0.68	0.41
Family history of disease (Yes/No)	6/41	8/39	0.34	0.56

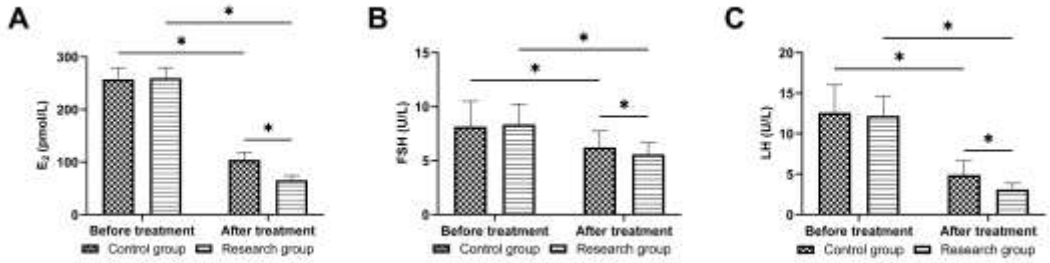

Figure 1: Sex hormone levels of E2 (A), FSH (B), LH (C)
**p<0.05. E_2: estradiol, FSH: follicle-stimulating hormone, LH: luteinizing hormone*

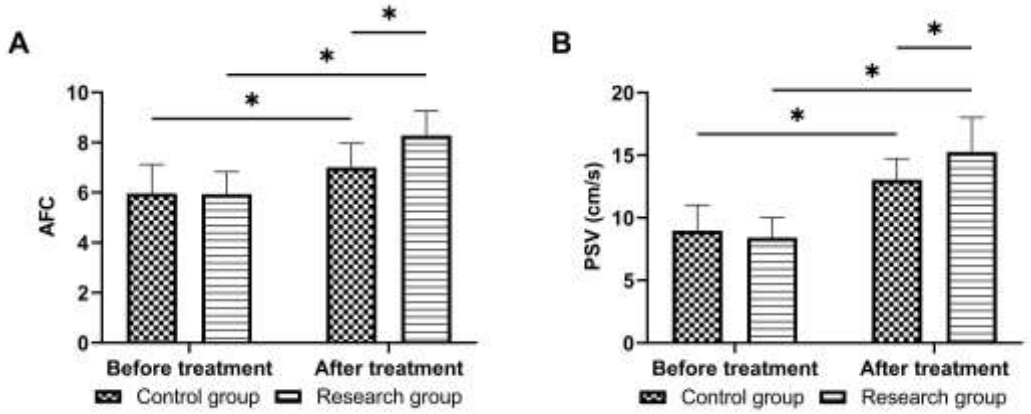

Figure 2: Ovarian reserve function. Antral follicle count (A), Peak systolic velocity (B)
**p<0.05. AFC: antral follicle count, PSV: peak systolic velocity of ovarian stroma*

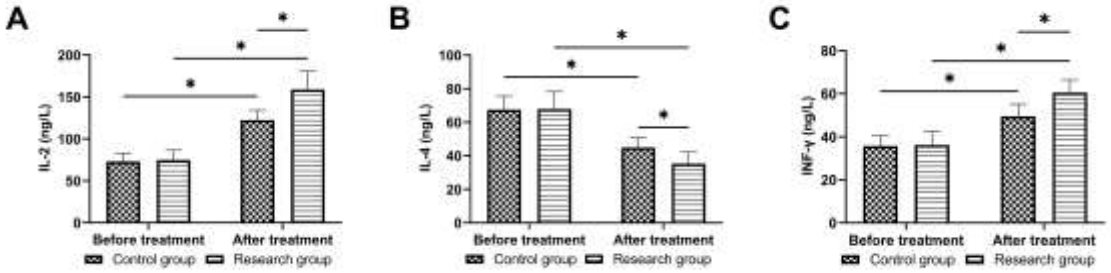

Figure 3: Levels of Th1/Th2 cytokines. IL-2 (A), IL-4 (B), INF-γ (C).
*p<0.05. IL-2/4: interleukin 2/4, INF-γ: interferon-γ

Comparison of pre- and post-treatment ovarian reserve function

AFC and PSV were not statistically different between groups prior to treatment (p>0.05), while the two indexes elevated markedly after treatment in both patient cohorts (p<0.05), especially in EG (p<0.05, Figure 2).

Comparison of pre- and post-treatment levels of Th1/Th2 cytokines

The results of pre- and post-treatment level of IL-2, IL-4 and INF-γ showed that IL-4 were significantly decreased in both patient cohorts after treatment, with lower levels in EG as compared to CG (p<0.05). In contrast, IL-2 and INF-γ levels were elevated after treatment compared with before treatment and were higher in the study group than in the control group (p<0.05, Figure 3).

Improvement of dysmenorrhea

No significant inter-group difference was identified in pre-treatment VAS scores (p>0.05). During the follow-up period, the VAS of both groups decreased gradually with the increase of the number of visits, reaching the lowest value at the fourth follow-up (p<0.05); moreover, significant lower VAS scores were identified in EG compared to CG at each follow-up (p<0.05, Figure 4).

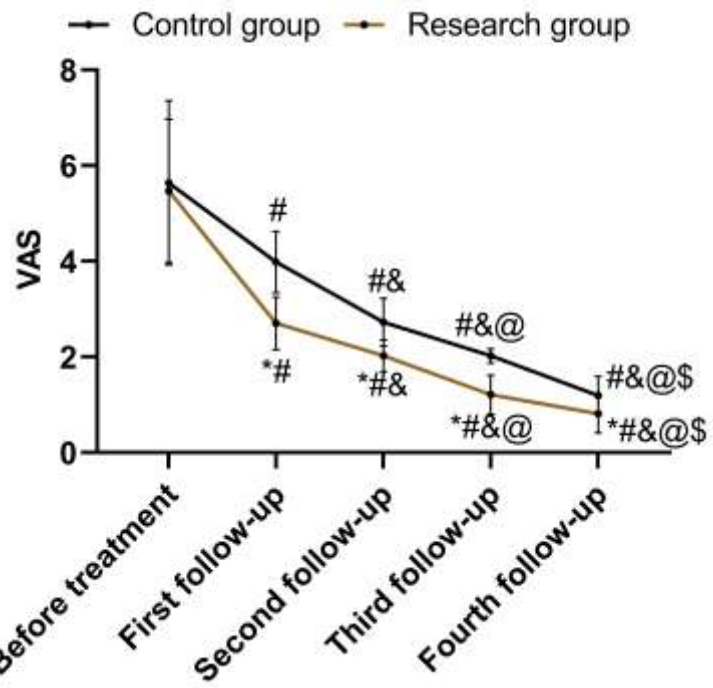

Figure 4: Changes in VAS scores**.** *, #, @ and $ mean statistically significant differences between and control group, before treatment, first follow-up, second follow-up and third follow-up, respectively ($p<0.05$)

Table 3: Clinical efficacy and recurrence after 1 year of prognostic follow-up

	n	Cure	Marked response	Response	Non-response	ORR	EMS recurrence rates
EG	47	11 (23.40)	22 (46.81)	6 (12.77)	8 (17.02)	82.98%	7 (14.89)
CG	47	18 (38.30)	25 (53.19)	3 (6.38)	1 (2.13)	97.87%	3 (6.38)
t (or χ^2)						6.02	1.79
P						0.01	0.18

Clinical efficacy and recurrence rate

After the one-year prognostic follow-up, the ORR was calculated to be 97.87% in EG patients and 82.98% in CG patients, demonstrating higher clinical efficacy of LNG-IUS + GnRHa therapy ($p<0.05$). The EMS recurrence rates of EG and CG were 6.38% and 14.89%, respectively, showing no statistical difference ($p>0.05$, Table 3).

DISCUSSION

In this study, LNG-IUS combined with GnRHa was more effective in treating EMS, which could provide a more reliable safety guarantee for future rehabilitation of EMS patients. FSH, E_2 and LH levels were more significantly improved in EG treated with LNG-IUS + GnRHa compared with CG receiving GnRHa alone, suggesting that LNG-IUS + GnRHa plays a better role in restoring the ovarian function in EMS patients. This is also consistent with the work of Takaesu et al. [13]. LNG-IUS is a key drug for moderate and severe EMS, which can enhance surgical effects and fertility, restore menstrual and ovulation functions, and increase the chance of conception [14]. Moreover, GnRHa can down-regulate pituitary function, resulting in temporary castration of drugs and hypoestrogenic state in vivo, thus playing a role in promoting endometrium degeneration and preventing the development of lesions [15]. However, the disadvantage of GnRHa is that its inhibition of estrogen levels may lead to osteoporosis, and even decreased bone density and bone loss in severe cases [16], requiring strict control over its dosage and duration of use in clinical application. Though GnRHa therapy alone can effectively assist in EMS surgery in the short term, it carries with it a possibility of developing EMS progression or recurrence in patients in the long run. In contrast, levonorgestrel, as a kind of fully synthetic potent progestogen, mainly functions in inhibiting ovulation, preventing the implantation of pregnancy eggs, and increasing the concentration of cervical mucus. This is in addition to preventing sperm from advancing

after female sexual behavior (internal ejaculation) [17]. Therefore, levonorgestrel is a commonly used contraceptive. When applied to moderate and severe EMS treated by therapeutic conservative surgery, it promotes endometrial cell apoptosis, which in turn further atrophies and degenerates the lesions, ultimately reducing the probability of disease recurrence. Meanwhile, LNG-IUS can inhibit the stimulation of lesion tissues by estrogen for a long time and reduce the expression of cells and immune factors in the peritoneal fluid, thus effectively improving the internal environment and exerting a safer and more stable long-term effect [18]. This was also confirmed though the comparison of IL-2, IL-4 and INF-γ between the two groups before and after treatment. In addition, AFC and PSV are key indicators reflecting ovarian fertility function [19]. The higher AFC and PSV in EG after treatment also suggests that LNG-IUS + GnRHa provides a more effective guarantee of prognostic fertility in patients. This may be due to the fact that LNG-IUS therapy only intervenes in eutopic endometrium and down-regulates E_2 and progesterone receptors without affecting estrogen secretion, thereby laying a more reliable foundation for the recovery of patients' reproductive function [20].

During the prognostic follow-up, we found a more obvious reduction in the degree of dysmenorrhea in both groups, with milder pain in EG, further confirming that LNG-IUS + GnRHa has a more stable and long-term therapeutic effect on EMS. Clinical efficacy was investigated through the one-year follow-up, which also showed a markedly higher ORR in EG, speaking volumes for the feasibility of LNG-IUS + GnRHa as a reliable option for future EMS treatment. But despite obviously fewer EMS relapses in EG, no statistical difference was identified between groups in the recurrence rate, which is beyond our expectations and is suspected to be caused by the contingency of the statistical analysis due to the limited number of cases included and the short follow-up time. Therefore, we will increase the number of study cases and extend the follow-up period for validation.

Of course, patient outcomes are also influenced by surgery to a great extent; so we can further study EMS surgical treatment conditions and physician experience to provide more reliable guarantees for patient prognoses. In this study, the GnRHa used was Triptorelin. At present, it is unclear whether there is any difference in the clinical efficacy after the application of other types of GnRHa in clinical practice. Further studies will be needed to ascertain this.

CONCLUSION

LNG-IUS plus GnRHa has demonstrated excellent efficacy against moderate and severe EMS, substantially improving patients' ovarian function and ensuring their fertility, with long-term and stable efficacy. The combination therapy is expected to be a novel option for clinical treatment of EMS, providing a more reliable safety guarantee for patients' prognosis and health.

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DECLARATIONS

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None provided.

Ethical approval

The study protocol was approved by the Ethics Committee of Laizhou People's Hospital, Laizhou, Shangdong, China (Approval No:2021-021-1).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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