International Journal of Reproduction, Contraception, Obstetrics and Gynecology
Khatun MS et al. Int J Reprod Contracept Obstet Gynecol. 2025 Sep;14(9):2853-2858
www.ijrcog.org
pISSN 2320-1770 | eISSN 2320-1789

DOI: https://dx.doi.org/10.18203/2320-1770.ijrcog20252709

Original Research Article

Efficacy of melatonin and letrozole combination therapy compared to letrozole alone in the treatment of symptomatic endometrioma in infertile women

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Received: 15 July 2025
Accepted: 08 August 2025

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ABSTRACT

Background: Endometriosis is an estrogen-driven inflammatory condition involving endometrial tissue growth outside the uterus. Letrozole, an aromatase inhibitor, lowers estrogen levels to reduce pain and lesion size. Melatonin offers additional benefits through its antioxidant, anti-inflammatory, and anti-proliferative effects on endometrial tissue. The present study was conducted to compare the effects of a combination of melatonin and letrozole with letrozole alone in infertile women with symptomatic endometrioma.
Methods: This randomized controlled trial was conducted in the Department of Reproductive Endocrinology and Infertility of Bangabandhu Sheikh Mujib Medical University from July, 2023 to June, 2024. Total 40 infertile women with symptomatic endometrioma were included in this study. They were randomly allocated to receive either tab. letrozole 2.5 mg, twice daily plus tab. melatonin 3mg, 3 tablets at night daily or only tab. letrozole 2.5 mg, twice daily for 3 months.
Results: Mean VAS score (6.88±2.39 vs 2.64±1.96) and mean size of endometrioma (3.77±1.22 vs 3.05±1.17 cm) were significantly decreased after 3 months of treatment with melatonin and letrozole combination therapy and mean VAS score (7.11±1.81 vs 3.22±1.89) and mean size of endometrioma (3.81±1.00 cm vs 3.27±0.85) were also significantly decreased after 3 months of treatment with letrozole alone group .But there were no statistically significant difference in terms of reducing pain score and size of endometrioma when compared between two groups.
Conclusions: Melatonin and letrozole combination therapy compared to letrozole alone provide similar efficacy on symptomatic endometrioma in terms of reduction of pain score and size of endometrioma.

Keywords: Endometrioma, Endometriosis, Letrozole, Melatonin, and infertile women

INTRODUCTION

Endometriosis is a disease characterized by the presence of tissue resembling endometrium (the lining of the uterus) outside the uterus (WHO). Ten percent of women who are of reproductive age have endometriosis.[1] Endometriosis prevalence in infertile women is thought to be between 25% and 40%.[2] Endometriosis is a chronic estrogen-

dependent inflammatory illness that can cause a range of symptoms, such as subfertility, dysmenorrhea, dyspareunia, chronic pelvic pain, and the formation of endometriomas. Ovaries, uterosacral ligaments, and the posterior cul-de-sac are the most common affected areas by endometriosis. The growth of endometrial tissue in the ovaries can result in the formation of a cyst, which is called endometrioma. It is among the most typical endometriosis symptoms. 17-44% of people with endometriosis had endometriomas.[3] The disease's precise mechanism is still unknown. Despite the existence of various other proposals, Sampson's theory of retrograde menstruation is the most well recognized explanation. Other factors such as impaired immune function, abnormal endocrine profile, heredity, role of proinflammatory environment, effects of free radicals, proangiogenic pathways, and environmental toxins have been implicated. One factor that has been definitively established as having a causative role in the development of endometriosis is estrogen.[4] It has been demonstrated that aromatase and 17B-hydroxysteroid-dehydrogenase type 1, which convert androstenedione to estrone and estrone to estradiol, respectively, are expressed in endometriotic implants. However, the enzyme 17B-hydroxysteroid dehydrogenase type 2, which deactivates estrogen, is lacking in implants.[5] As association or sequential to above mentioned factors, endometrial lesions have high estradiol biosynthesis and low estradiol inactivation. Abundant steroidogenic acute regulatory protein (STAR) activity and aromatase enzyme activity in stromal cells isolated and cultured from endometriomas. The above findings provide the basis for clinical trials with aromatase inhibitors in women with endometriosis. Third-generation non-steroidal aromatase inhibitor letrozole inhibits aromatase selectively, thereby effectively suppress estrogen production both locally and systematically. Medical therapies other than aromatase inhibitors only inhibit estrogen action or its production from the ovaries, do not inhibit local estrogen production by endometriotic implants.[6] Blocking estrogen production by inhibiting aromatization, letrozole will cause regression of endometrioma and reduction of pain associated with endometriosis. Free radicals are recognized as essential signaling molecules in endometriosis. Free radicals use a range of proinflammatory cytokines to mediate their effects. Melatonin, or N-acetyl-5-methoxytryptamine, is an indole that is primarily produced in the pineal gland of mammals during the dark phase. Melatonin is a widely recognized potent antioxidant and free radical scavenger.[7] Several studies suggest that melatonin may have therapeutic potential as an anti-endometriotic drug. Melatonin has been shown to regulate the growth and invasion of endometriotic cells via a variety of mechanisms, including increased antioxidant activity, an anti-inflammatory effect, regulation of matrix remodeling, suppression of the epithelial to mesenchymal transition (EMT), induction of apoptosis, decreased angiogenesis, inhibition of cell proliferation, inhibition of cell adhesion, inhibition of invasion, regulation of immune modulation, and neurotrophic effects. Both melatonin and letrozole are effective in the treatment of endometriosis and have beneficial effects on the treatment of infertility with acceptable side effects. Letrozole causes a regression in the size of endometriotic implants but has no effect on adhesion formation and free radicals, but melatonin reduces adhesion formation. So, with this background, the present study was conducted to evaluate the effect of melatonin and letrozole in the treatment of symptomatic endometrioma. This study aimed to compare the effects of a combination of melatonin and letrozole with letrozole alone in infertile women with symptomatic endometrioma.

METHODS

This randomized controlled trial was conducted in the Department of Reproductive Endocrinology and Infertility of Bangabandhu Sheikh Mujib Medical University from July, 2023 to June, 2024. Total 40 infertile women, aged between 18-40 years, with sonographically diagnosed endometrioma (mean diameter<5cm), associated with dysmenorrhea. Exclusion criteria encompassed contraindications to letrozole and melatonin, existing pulmonary, cardiac, renal, or hepatic diseases, other types of ovarian cysts, history of hormonal treatment within the past three months, previous use of aromatase inhibitors, known case of any psychiatric disturbances. The study was approved by institutional review board. Informed consent was taken from each participant. The patients were randomly allocated into two groups each containing 20 patients: one receiving Tab. Letrozole 2.5mg twice daily orally plus tab. Melatonin 3mg, three tablets at night daily orally for three months and the other arm receiving only Tab. Letrozole 2.5mg twice daily orally for three months. Pre and post-treatment assessment of endometrioma size was performed by transvaginal ultrasonography. All the patients were evaluated for the presence and intensity of pain including dysmenorrhea and pain reduction was recorded by VAS score. The visual analogue scale (VAS) consisted of a straight line of 10 cm with the endpoints defining extreme limits such as' no pain at all=0 and' pain as bad as it could be=10'. Patient was asked to mark his pain level on the line between the two endpoints. The distance (in cm) between 'no pain at all and the 'mark' that defined the subject's pain. Random sequence generation of permuted block was done by computer generated random numbers and allocation concealment was done by sequentially numbered opaque sealed envelopes. Each envelop had a card inside noting the intervention drug. Allocation was never changed after opening the closed envelops. All data were collected by the principal investigator. There was no blinding. Sample size of participants was calculated as 40 in each group using mean formula, for a power of 0.80, a significance level of 0.02. Statistical analyses were carried out by the SPSS program for Windows, version 26.0 (SPSS, Chicago, IL). The qualitative observations were indicated by frequencies and percentages. Chi-Square was used to analyze the categorical variables. Student t-test was used for continuous variables. Paired sample t-test was used for comparing measurements before and after treatment and independent samples t-test (Unpaired t-test) for comparing

between 2 groups of treatment arms. The p value <0.05 was considered as statistically significant.

RESULTS

Total of 35 women completed the treatment course of 3 months and was included in analysis. Table 1 shows sociodemographic characteristics of the study participants. Age distribution among the participants was similar across both groups, with a mean age of approximately 28 years (melatonin plus letrozole: 28.5±4.8; letrozole alone:28.9±6.3). This similarity in age distribution was statistically nonsignificant (p=0.822). Regarding occupational status, most participants in the control group were housewives (85%) compared to 55% in the experimental group, which also included more participants working in service jobs and students, though the difference was not statistically significant (p = 0.117). Residence was evenly distributed between rural and urban areas in both groups, with no significant difference (p = 0.752).

Table 1: Sociodemographic characteristics of study participants (n=40).

Demographic characteristics	Experimental group (melatonin and letrozole) n=20 N (%)	Control group (letrozole) n=20 N (%)	P value
Age (years)			
≤20	1 (5.00)	2 (10.0)	0.822[ns.]
21-30	13 (65.O)	8 (40.0)	
31-40	6 (30.0)	10 (50.0)	
Mean± SD	28.5±4.8	28.9±6.3	
Range (min-max)	20.0-37.0	18.0-38.0	
Occupational status			
House wife	11 (55.0)	17 (85.0)	0.117[ns]
Service	6 (30.0)	2 (10.0)	
Student	3 (15.0)	1 (5.00)	
Residence			
Rural	10 (50.0)	11 (55.0)	0.752[ns]
Urban	10 (50.0)	9 (45.0)	

ns: non-significant

In terms of infertility characteristics (Table 2), primary infertility was slightly more prevalent than secondary infertility in both groups (80% vs. 20% in the experimental group and 75% vs. 25% in the control group), with no significant difference (p=0.500). Additionally, more than half of the participants in both groups had experienced infertility for over five years, again with no significant difference (p=0.749). Overall, the baseline characteristics of the two groups were comparable, supporting the reliability of outcome comparisons between them. The average size of the endometrioma was comparable in both groups, with 3.59±1.26 cm in the melatonin plus letrozole group and 3.82±1.02 cm in the letrozole alone group, showing no significant difference (p=0.526ns). Regarding the pain levels, measured using the VAS showing no significant difference(p=0.413ns). The melatonin plus letrozole group had an average VAS score of 6.65±2.32, while the letrozole alone group had a slightly higher average of 7.20±1.85.

Table 2: Type and duration of infertility of the study participants (n=40).

Variables	Experimental group (melatonin and letrozole) n=20 N (%)	Control group (letrozole) n=20 N (%)	P value
Type of Infertility			
Primary	16 (80.0)	15 (75.0)	0.500[ns]
Secondary	4 (20.0)	5 (25.0)	
Duration of infertility (years)			
≤5.0	9 (45.0)	8 (40.0)	0.749[ns]
>5.0	11 (55.0)	12 (60.0)	

ns: non-significant

Table 3: Pre-treatment and post-treatment comparison of visual analog scale score and size of endometrioma among the study groups (n=40).

Variables	Experimental group (melatonin and letrozole) n=20 Mean±SD	Control group (letrozole) n=20 Mean±SD	P value
Visual analog scale score			
Pretreatment	6.65±2.32	7.20±1.85	0.413^{ns}
Post-treatment	2.64±1.96	3.22±1.89	0.385^{ns}
Size of endometrioma (cm)			
Pretreatment	3.59±1.26	3.82 ±1.02	0.526^{ns}
Post-treatment	3.05±1.17	3.27±0.85	0.541^{ns}

ns: non-significant

Table 3 presents a comparison of pain levels and endometrioma size before and after treatment in both groups. Prior to treatment, the mean visual analog scale (VAS) scores for pain were similar between the groups-6.65±2.32 in the experimental group and 7.20±1.85 in the control group (p=0.413), indicating no significant difference. After treatment, both groups showed a reduction in pain scores, with the experimental group decreasing to 2.64±1.96 and the control group to 3.22±1.89; however, the difference remained statistically insignificant (p=0.385). Similarly, the size of endometriomas was comparable before treatment (3.59±1.26 cm in the experimental group vs. 3.82±1.02 cm in the control group; p=0.526) and after treatment (3.05±1.17 cm vs. 3.27±0.85 cm, respectively; p=0.541), with no significant differences observed.

Table 4 presents the pregnancy rate of study population. In the melatonin plus letrozole group, 15% of the participants (3 out of 20) reported positive pregnancy outcomes. Comparatively, in the melatonin alone group, none of the participants achieved pregnancy. However, this difference in pregnancy rates between the two groups was not statistically significant (p=0.135ns).

Table 4: Pregnancy rate of study population (n=40).

Preg-nancy rate	Experimental group (melatonin and letrozole) (n=20) N (%)	Control group (letrozole) (n=18) N (%)	P value
Yes	3 (15.0)	0 (0.0)	0.135^{ns}
No	17 (85.0)	18 (100.0)	

ns: non-significant

DISCUSSION

This randomized controlled trial was carried out with an aim to evaluate and compare the effects of melatonin and letrozole versus letrozole alone in treating symptomatic endometrioma in infertile women. In this current study at baseline, there were no statistically significant difference between two groups regarding mean size of endometrioma and visual analog scale score. In a study conducted by Schwertner et al reported that the mean pain on visual analogue scale was 6.46±2.6 in melatonin group and 6.89±2.1 in placebo group, the difference was not significant between two groups, which is consistent with my study.[8] This study observed that in experimental group (Melatonin and Letrozole) after 3 months of intervention, VAS score was significantly decreased from the pretreatment value (2.64±1.96 vs 6.88±2.39) with mean difference 4.24. Schwertner et al performed a randomized, double-blind, placebo-controlled trial which investigates the effects of melatonin on women with endometriosis who had 10 mg of melatonin at bed time daily for 8 weeks.[8] There is one study in human which found no role of melatonin in reducing pain associated with endometriosis. Soderman et al conducted a randomized double-blinded, placebo-controlled trial on women with endometriosis who received melatonin 20 mg as a single dose at bed time daily for two consecutive menstrual cycles or months.[9] This study could not show that melatonin had better analgesic effect on pain associated with endometriosis. This was probably due to the research population's overall lower pain scores (2.87). Pain reduction is more pronounced in the greater range of the numeric rating scale than in the lower range. Melatonin's analgesic impact has been demonstrated in prior research to be dose dependent

in both human and animal models.[10,11] In our study, in experimental group (Melatonin and Letrozole), mean size of endometrioma was significantly decreased from the pretreatment value (3.05±1.17 cm vs 3.77±1.22 cm) with mean difference 0.72 cm after 3 months of intervention. Yildirim et al investigated the effects of letrozole and melatonin on surgically induced endometriosis in an experimental rat model.[12] Endometrial implants were significantly reduced in treatment group having letrozole and melatonin. Rat endometriotic implants shrink in size when exposed to melatonin, according to animal research.[13-15] Cetinkaya et al also reported a good dose-dependent association with the size reduction.[16] In present study, in control group (Letrozole alone), mean VAS score was significantly decreased from pretreatment value (3.22±1.89 vs 7.11±1.81) with mean difference 3.89 after 3 months of intervention. Madny et al (2014) observed that the mean VAS decreased from 7.65 to 6.1 with mean change 1.55 after administering letrozole 2.5 mg for six months to a total of 20 women with endometriosis.[17] The reduced response may be due to lower dose of letrozole. Letrozole treatment reduced the mean pain score in the Amir et al trial from 6.06 to 4.00 after three months, and to 1.12 after six months.[18] That means the decrease over a half-year was greater. The research conducted by Madny et al and the case studies by Razzi et al and Hefler et al made it clear that letrozole by itself can significantly reduce pain scores.[17,19,20] In this current study, in control group (Letrozol alone), mean size of endometrioma was significantly decreased from pretreatment value (3.27±0.85 cm vs 3.81±1.00) with mean difference 0.54 cm after 3 months of intervention. According to Amir et al, following three and six months of letrozole treatment, the mean size of endometrioma decreased from a baseline of 6.06±2.40 cm to 5.23±1.37 cm and to 4.59±1.25 cm respectively.[18] In our study percentage reduction in size of endometrioma was 14.17%. In this study it was observed that after 3 months treatment of intervention, the difference in mean VAS score and mean size of endometrioma between two groups were not statistically significant. According to Soderman et al, the average pain related to endometriosis was 2.9±1.9 in the group that took melatonin and 3.3±2.0 in the group that took a placebo that was not significant between two groups, which support with my study.[9] More over the present study used letrozole in combination with melatonin. Most of the effect may be due to letrozole with minimal effect of melatonin. In our study, mean percentage reduction of VAS score (61.62% vs 54.71%) and size of endometrioma (19.09% vs 14.17%) were a bit more in experimental group than control group, but the difference was not statistically significant. According to Schwertner et al, there was a mean 39.30% reduction in pain in the melatonin group.[8] Even in women with severe endometriosis, Koninckx et al showed a 30% reduction in pain severity in women having placebo.[21] Therefore, melatonin show superiority over placebo which confirm their effectiveness. In this study the pregnancy rate was 3(15.0%) in experimental group and not found in control group. This may be due to melatonin has direct effect on ovarian function and acts as powerful antioxidants and free radical scavengers. As a result, developmental potential of the oocytes was improved. Letrozole may activate accessible antral follicles and cause pregnancy, according to research by Amir et al.[18] The letrozole arm of the Amir et al research did not result in any pregnancy.[18]

This study has few limitations. The study was conducted with a small sample size and for a short period of time due to restricted time frame, the participants were recruited from one department of a single tertiary level hospital thus challenging the external validity of study findings. Participants and investigators were not blinded to the treatment after randomization. Melatonin plasma concentrations were not assessed, which would have yielded important information.

CONCLUSION

Melatonin & letrozole combination therapy compared to letrozole alone provide similar efficacy on symptomatic endometrioma in terms of reduction of pain score and size of endometrioma. Future studies should include a larger, more diverse sample size and a longer follow-up period. Blinded, multicenter trials with serum melatonin level assessments and alternative delivery methods, such as transdermal applications, may better elucidate melatonin's role and optimize its therapeutic potential in endometriosis management.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Khatun MS, Islam A, Islam T, Islam MA, Ishrat S, Deeba F, et al. Efficacy of melatonin and letrozole combination therapy compared to letrozole alone in the treatment of symptomatic endometrioma in infertile women. Int J Reprod Contracept Obstet Gynecol 2025;14:2853-8.