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Original Article

Effects of letrozole combined with ethinylestradiol and cyproterone acetate tablets on serum sex hormones and lipid metabolism in patients with polycystic ovary syndrome

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Abstract: Objective: To investigate the effects of letrozole combined with ethinylestradiol and cyproterone acetate tablets on serum sex hormones and lipid metabolism in patients with polycystic ovary syndrome (PCOS). Methods: Clinical data of 152 PCOS patients in the First Affiliated Hospital of Guangxi University of Chinese Medicine from May 2019 to June 2021 were collected for a retrospective analysis. Among the patients, 73 treated with ethinylestradiol and cyproterone acetate tablets alone were seen as control group (CG), and the rest 79 with letrozole combined with ethinylestradiol and cyproterone acetate tablets were seen as observation group (OG). The treatment efficacy was observed, and the adverse reactions in the course of treatment were counted. The levels of luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone (T), estrogen (E_2), total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were compared before and after treatment. The number of mature follicles, ovulation rate and pregnancy rate were assessed. Multivariate logistic regression analysis was used to detect the independent risk factors of ineffective efficacy. Results: After the treatment, the total efficacy rate of the OG was higher than that of the CG ($P<0.05$); moreover, the levels of TC, TG, LDL, FSH, LH and T in OG were lower while HDL and E_2 were higher (all $P<0.05$) than those of the CG. Also, the number of mature follicles, ovulation rate and pregnancy rate were higher in OG than those in the CG (all $P<0.05$). There was no obvious difference in the incidence of adverse reactions between the groups ($P>0.05$). Higher fasting glucose, higher Ferriman-Gallway hair score, single drug treatment regimen, higher systolic blood pressure, and lower E_2 before treatment were independent risk factors for ineffective treatment efficacy. Conclusion: Letrozole combined with ethinylestradiol and cyproterone acetate tablets can enhance the treatment efficiency of PCOS and improve serum sex hormones and lipid metabolism in PCOS patients.

Keywords: Letrozole, ethinylestradiol and cyproterone acetate tablets, polycystic ovary syndrome, sex hormone, lipid metabolism

Introduction

Polycystic ovary syndrome (PCOS), a common gynecological endocrine disorder, mainly affects women of reproductive age and adolescence and is one of the main causes of infertility [1, 2]. Some data suggest that PCOS affects 10% of women of reproductive age, resulting in over 75% of ovulatory infertility [3]. PCOS patients may develop comorbidities such as obesity, excessive body hair, acne, menstrual cycle disorders, infertility, hormonal imbalance and depression, posing a health risk to women of reproductive age [4, 5]. The pathogenesis of PCOS is still vague, but it is generally accepted that it is inextricably linked to endocrine changes [6].

For ovulation disorders due to PCOS, ovulation promotion therapy is currently the most impor-

tant assisted reproduction technique [7]. Ethinylestradiol and cyproterone acetate tablets are frequently used in clinical practice and have antagonistic effects on androgen receptors, as well as anti-gonadotropic effects, which inhibit the effects of androgens on the corresponding target organs and enhance the anti-gonadotropic effects. They have a definite effect on reducing androgen secretion, but the effect of individual treatment is poor and needs to be combined with drug therapy to improve the efficacy [8, 9]. Letrozole, a commonly used ovulation-promoting drug in clinical practice and a third-generation nonsteroidal aromatase inhibitor, lowers estrogen levels in patients by inhibiting the conversion of androgens to estrogens, causing the hypothalamus-pituitary gland to secrete more gonadotropins for ovulation-promoting treatment [10, 11]. However, few studies have investigated the effects of letrozole combined with ethinylestradiol and cyproterone acetate tablets on serum sex hormones and lipid metabolism in PCOS patients. As an endocrine metabolic disease, PCOS is characterized by disorders of sex hormone secretion. Studies have also found that most PCOS patients also have abnormal lipid metabolism, so not only should sex hormone metabolism be corrected during treatment, but also lipid metabolism disorders should be regulated [12, 13].

Thus, this research aimed to investigate the efficacy of letrozole combined with ethinylestradiol and cyproterone acetate tablets in PCOS patients, and to observe the changes in serum sex hormones and lipid metabolism before and after treatment, so as to provide better guidance for clinical diagnosis and treatment.

Data and methods

Patient data

Altogether 152 PCOS patients in the First Affiliated Hospital of Guangxi University of Chinese Medicine from May 2019 to June 2021 were retrospectively enrolled in this study. Thereinto, 73 patients treated with ethinylestradiol and cyproterone acetate tablets alone were assigned to the control group (CG), with a mean age of 26.37 years and disease duration of 41.96 days (or months?). The rest 79 cases with letrozole combined with ethinylestradiol and cyproterone acetate tablets were seen as the observation group (OG), with a mean age of 27.05 years and a disease duration of 38.53 days (or months?). The study was approved by institutional review board (IRB20190408) and inform consent form was obtained from all patients.

Inclusion and exclusion criteria

Inclusion criteria: All patients were diagnosed with PCOS and all met the diagnostic criteria from the Rotterdam Conference [14], i.e. the presence of 2 of the following 3 items: (1) Sporadic ovulation or anovulation; (2) Clinical manifestations of hyperandrogenemia or hyperandrogenism; (3) Ultrasonography performed at 3-5 d of the menstrual cycle or bleeding after progesterone withdrawal, showing small follicles of ≥12 and 2-9 mm in diameter in both ovaries, i.e. ovarian polycystic-like changes or increased ovarian volume; Patients not taking hormones and drugs with effects on glucose and lipid metabolism for the previous 3 months prior to participation in this research; Patients with complete clinical data.

Exclusion criteria: Patients with an allergic reaction to the therapeutic drug; Patients with poor adherence to treatment; Patients with other endocrine disorders; Patients with ovulation disorders due to other causes.

Treatment options

In the CG, one tablet of ethinylestradiol and cyproterone acetate (Bayer Weimar GmbH und Co. KG, each tablet contains 2 mg of cyproterone acetate and 0.035 mg of ethinylestradiol) was administered orally once a day for 21 d from the 5th day after menstruation. While in the OG, ethinylestradiol and cyproterone acetate were used in the same manner as CG, and additional letrozole was prescribed. letrozole (Jiangsu Hengrui Pharmaceutical Co., Ltd., 2.5 mg) was given orally 2.5 mg once a day from the 3rd to 7th day of menstruation. Treatment was continued for 3 months, and both groups were followed up for 1 year.

Efficacy assessment criteria

The efficacy assessment criteria were as follows: Markedly effective: successful pregnancy or normal menstrual cycle with expulsion of mature follicles and normal metabolic and

Table 1. Baseline data

	OG (n = 79)	CG (n = 73)	t/X^2	P
Age (year)	27.05±5.85	26.37±5.45	0.740	0.461
BMI (kg/m^2)	21.65±2.11	22.18±2.21	1.512	0.133
Course of disease (month)	38.53±14.79	41.96±16.96	1.331	0.185
Systolic blood pressure (mmHg)	102.34±12.63	105.49±12.95	1.518	0.131
Diastolic blood pressure (mmHg)	78.29±4.4	78.17±4.83	0.160	0.873
Fasting insulin (μa/ml)	7.88±1.94	7.83±2.23	0.148	0.883
Fasting blood glucose (mmol/L)	7.08±0.64	6.93±0.63	1.455	0.148
Ferriman-Gallway hair score	6.47±1.07	6.6±1.14	0.725	0.469
Years of infertility (year)	3.15±1.3	3.38±1.25	1.110	0.269
Type of infertility			0.817	0.366
Primary infertility	49 (62.03)	40 (54.79)		
Secondary infertility	30 (37.97)	33 (45.21)		
Place of residence			1.463	0.226
Cities and towns	61 (77.22)	62 (84.93)		
Countryside	18 (22.78)	11 (15.07)		

BMI, Body Mass Index.

endocrine indicators; Effective: improvement of the menstrual cycle, with one to two mature follicles expelled and clinical symptoms, endocrine and metabolic indicators approaching normal; Ineffective: no normal ovulation manifested, and clinical symptoms, endocrine and metabolic indices did not improve. Total effective rate = (effective + effective)/total number of cases × 100%.

Outcome measures

(1) The efficacy of both groups of patients was observed; (2) The adverse reactions that occurred during the treatment in both groups was counted; (3) 20 mL of fasted elbow venous blood was collected from patients before and after the treatment and centrifuged at 4000 rpm for 5 min. The serum was collected and stored at -20°C. Luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone (T), and estrogen (E_2) were measured using a fully automated immunoassay analyzer (Elecsys 2010, Roche) with reagents provided by Roche (26542527, 56215201, 25621058, 56325120). Total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels were measured in both groups using a HITACH 17600-020 fully automated biochemical analyzer; (4) The number of mature follicles, ovulation rate and pregnancy rate were recorded and counted; (5) Independent risk factors for ineffective patient outcomes were explored by conducting univariate and multivariate analysis.

Statistical methods

The collected data were statistically analyzed via SPSS 20.0 (SPSS, Chicago, USA). The counting data were expressed as utilization (%), and then assessed via chi-square test, marked by χ^2. The measurement data were all conformed to normal distribution and expressed as mean ± SD. Independent samples t-test was applied to inter-group comparison, while the intra-group comparison was conducted by using paired t-test. Independent risk factors for ineffective treatment outcomes were explored by multivariate logistic regression analysis. P<0.05 signified statistical difference.

Results

Baseline data

There was no marked difference in age, BMI, course of disease, systolic blood pressure, diastolic blood pressure, fasting insulin, fasting blood glucose, Ferriman-Gallway hair score, years of infertility, type of infertility and place of residence between groups (all P>0.05) (**Table 1**).

Comparison of efficacy between the two groups of patients

The total effective rate of the OG was 92.41%, and that of the CG was 80.82%. It was clear that the effective rate of the OG was higher than that of the CG (P<0.05) (**Table 2**).

Table 2. Comparison of treatment efficacy between patients of the two groups

	OG (n = 79)	CG (n = 73)	X^2	P
Markedly effective	32 (40.51)	23 (31.51)	1.331	0.249
Effective	41 (51.90)	36 (49.32)	0.101	0.750
Ineffective	6 (7.59)	14 (19.18)	4.455	0.035
Total adverse reactions	73 (92.41)	59 (80.82)	4.455	0.035

Table 3. Comparison of adverse reactions between patients of the two groups

	OG (n = 79)	CG (n = 73)	X^2	P
Breast distension	1 (1.27)	1 (1.37)		
Nausea	3 (3.80)	1 (1.37)		
Abdominal pain	2 (2.53)	2 (2.74)		
Headache	2 (2.53)	2 (2.74)		
Total adverse reactions	8 (10.13)	6 (8.22)	0.165	0.685

Comparison of adverse reactions between the groups

The occurrence of adverse reactions in both groups was counted, including experienced breast distension, nausea, abdominal pain and headache. The total adverse reaction rate was 10.13% in the OG and 8.22% in the CG, and there was no statistical difference (P>0.05) (**Table 3**).

Effect of treatment on lipid metabolism in patients

There was no statistical difference in TC, TG, HDL and LDL before treatment (P>0.05). While after treatment, TC, TG and LDL were decreased (P<0.05) and HDL was increased (P<0.05) in both groups, and the levels of TC, TG and LDL were lower (P<0.05) while HDL was higher (P<0.05) in the OG than those in the CG after treatment (**Figure 1**).

Changes in sex hormones in patients before and after treatment

There was no statistical difference in sex hormones FSH, LH, T and E_2 between the two groups before treatment (all P>0.05). After treatment, FSH, LH and T were decreased and E_2 was increased (all P<0.05) in both groups. Moreover, the levels of FSH, LH, T were lower and E_2 was higher (all P<0.05) in the OG than those in the CG (**Figure 2**).

Effect of treatment on ovulation and pregnancy rate of patients

We discovered that after treatment, the number of mature follicles (P< 0.05) and the ovulation and pregnancy rates (P<0.05) in the OG were higher than those in the CG (**Table 4**).

Univariate analysis of treatment efficacy

Patients were divided into the ineffective group and effective group according to the treatment efficacy. It revealed that the two groups were statistically different (P<0.05) in terms of BMI, disease duration, systolic blood pressure, fasting insulin, fasting glucose, Ferriman-Gallway hair score, treatment regimen, pre-treatment TC, and pre-treatment E_2 (**Table 5**).

Multifactorial analysis of treatment efficacy

We included the factors with statistical differences in the univariate analysis into the multifactorial analysis. The assignment is shown in **Table 6**. Subsequent multifactorial logistic regression analysis manifested that higher fasting glucose, higher Ferriman-Gallway hair score, single drug treatment regimen, higher systolic blood pressure, and lower E_2 before treatment were independent risk factors for ineffective treatment outcomes (**Table 7**).

Discussion

PCOS is a gynecological endocrine disorder with ovulation disorders as its main manifestation. Many female patients do not receive timely treatment because of delayed diagnosis, making PCOS affect the metabolic, reproductive, cardiovascular, and psychological health of many women [15]. PCOS patients present androgen-induced ovarian dysfunction involving the hypothalamic-pituitary-gonadal axis in female patients with various hormonal secretion abnormalities such as hyperandrogenemia, hyperprolactinemia, hyperinsulinemia, and insulin resistance, under the effect of which there are usually a dozen small follicles of varying sizes at different stages of development in their ovaries, but no mature follicles.

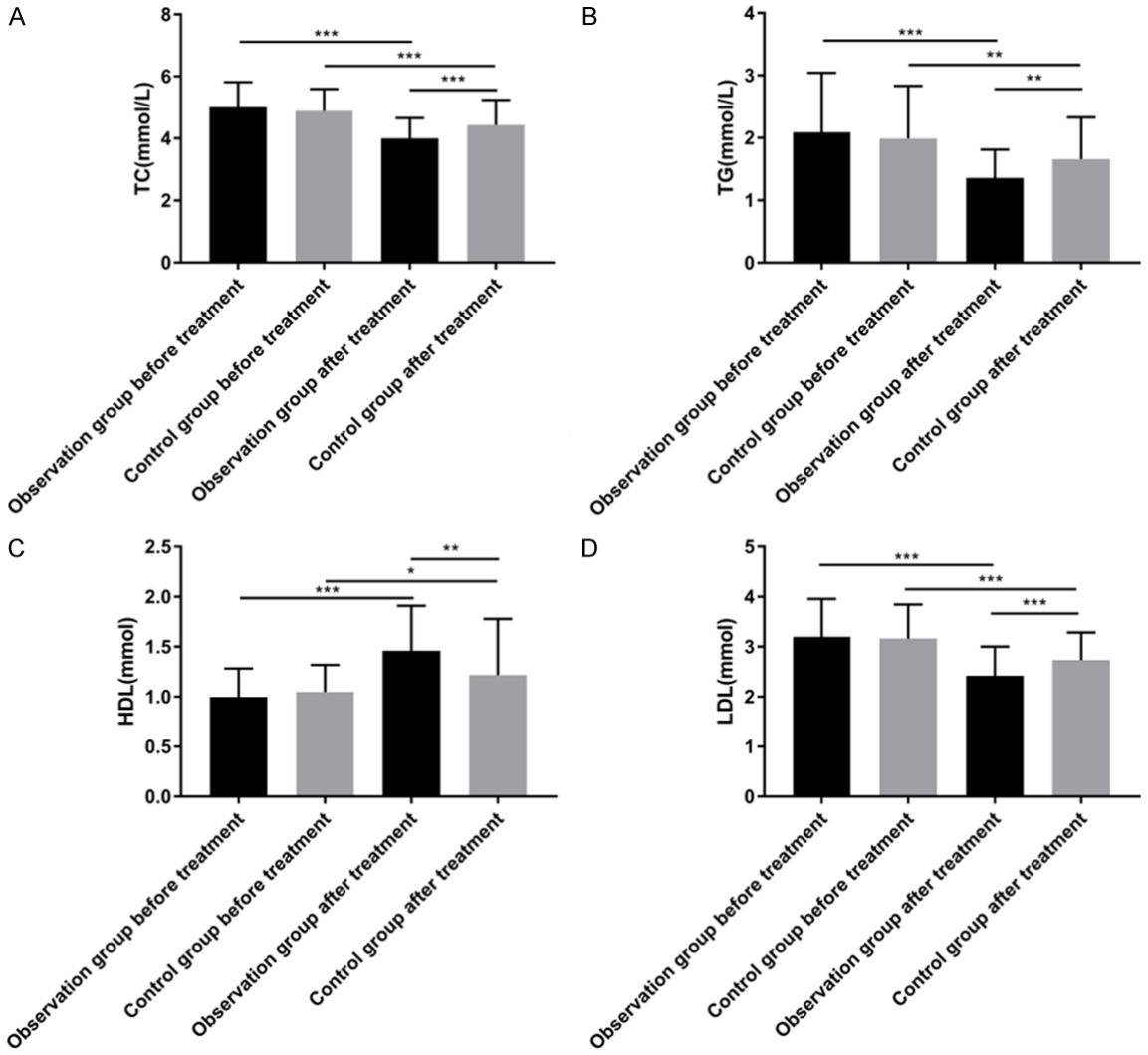

Figure 1. Effect of treatment on lipid metabolism in patients. A. TC was lower in the OG than that in the CG after treatment (P<0.001). B. TG was lower in the OG than that in the CG after treatment (P<0.01). C. HDL was higher in the OG than that in the CG after treatment (P<0.01). D. LDL was lower in the OG than that in the CG after treatment (P<0.001). OG, Observation Group; CG, Control Group; TC, Total Cholesterol; TG, Triglyceride; HDL, High Density Lipoprotein; LDL, Low Density Lipoprotein.

Therefore, patients cannot ovulate normally [16, 17]. Thus, the primary goal of ovulation treatment in PCOS patients is to limit the simultaneous development of multiple follicles and promote the formation of dominant follicles [18]. Ethinylestradiol and cyproterone acetate tablets, as a frequently used drug in the clinical treatment of PCOS infertility, have antagonistic effects on androgen receptors, as well as anti-gonadotropic effects. They inhibit androgen production on the corresponding target organs, enhance anti-gonadotropic effects, and have a definite effect on reducing androgen secretion [19]. Letrozole, as an aromatase inhibitor, causes a hypoestrogenic state through the inhibition of peripheral aromatase. It induces follicular development and maturation and increases ovarian responsiveness through a reflex increase in gonadotropin secretion by the pituitary gland, while stimulating insulin growth [20].

We found that the total effective rate of letrozole combined with ethinylestradiol and cyproterone acetate tablets was higher than that of single drug treatment, indicating that the combined treatment improved the efficacy. There were no obvious adverse reactions, indicating

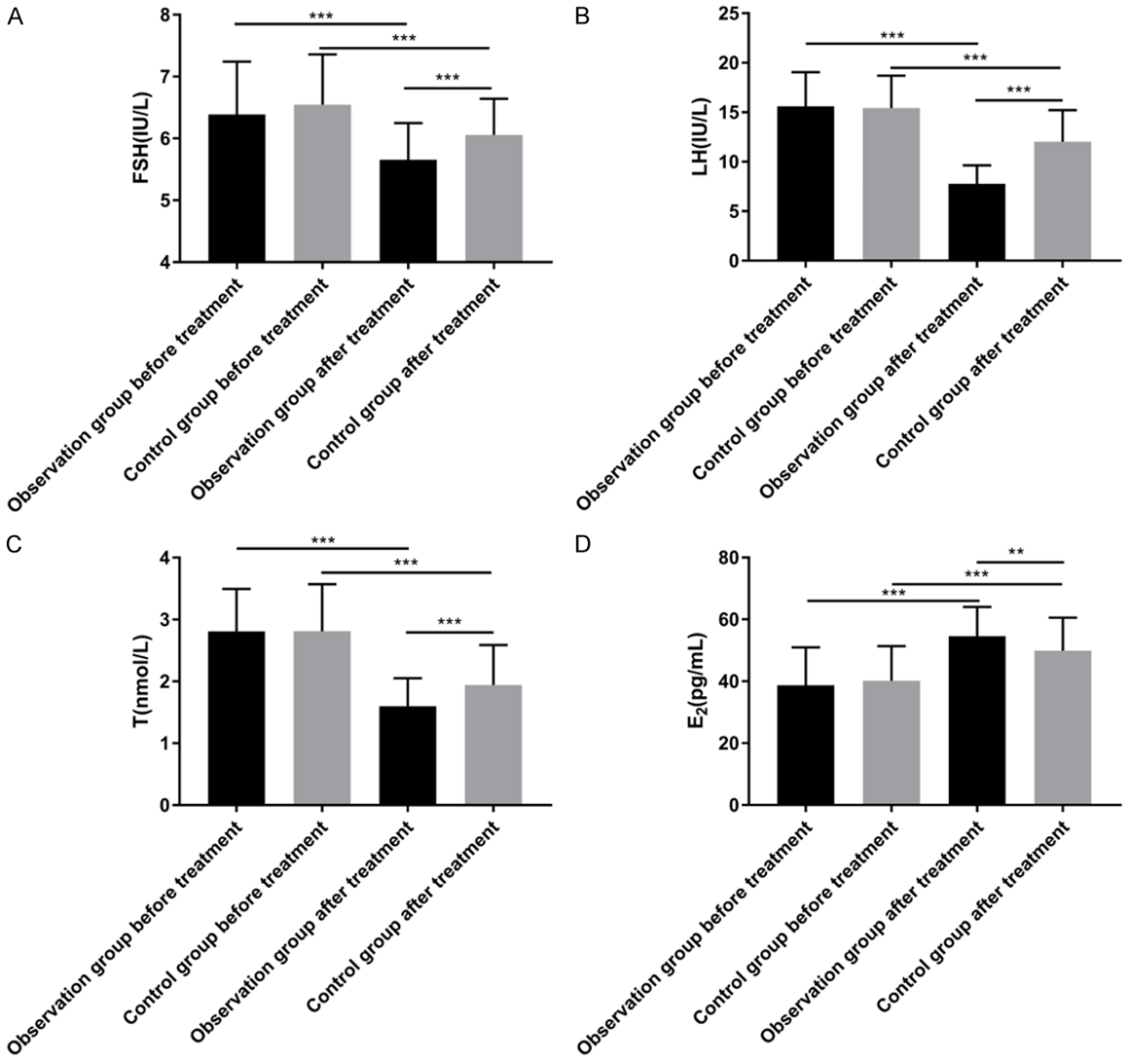

Figure 2. Changes in sex hormones in patients before and after treatment. A. FSH was lower in the OG than that in the CG after treatment (P<0.001). B. LH was lower in the OG than that in the CG after treatment (P<0.001). C. T was lower in the OG than that in the CG after treatment (P<0.001). D. E_2 was higher in the OG than that in the CG after treatment (P<0.01). OG, Observation Group; CG, Control Group; LH, Luteinizing Hormone; FSH, Follicle-Stimulating Hormone; T, Testosterone; E_2, Estrogen.

that the combined therapy has high safety. This may be because ethinylestradiol and cyproterone acetate tablets inhibit the production of ovarian-derived androgens and promote the metabolism of testosterone to correct hyperandrogenemia. Letrozole promotes the secretion of gonadotropin-releasing hormone and amplifies the follicle-stimulating hormone effect, thus promoting early follicle development, and the combination of the two drugs improves the clinical efficacy. Several studies have shown that improving patients' sex hormone levels is the therapeutic key in PCOS infertility, and the affected function of the hypothalamic-pituitary-ovarian gonadal axis in patients can lead to abnormal LH and FSH levels, and polycystic-like changes in the ovaries can cause abnormal T and E_2 levels [21, 22]. Serum T, LH and FSH levels were lower while E_2 was higher in patients treated with combined drugs than those treated with single drug. It was suggested that letrozole combined with ethinylestradiol and cyproterone acetate tablets helped improve patients' sex hormones. The reason for this may be that letrozole eliminates the feedback inhibition of estrogen on the hypothalamus-pituitary gland and promotes the release of sex hormones [23, 24], while ethinylestradiol and

Table 4. Comparison of ovulation and pregnancy rate between patients of the two groups

	OG (n = 79)	CG (n = 73)	t/X^2	P
Number of mature follicles (number)	2.87±0.49	1.23±0.43	4.403	<0.001
Ovulation	48 (60.76)	29 (39.73)	6.715	0.001
Pregnancy	39 (49.37)	18 (24.66)	9.884	0.002

Table 5. Single factor analysis of factors affecting the treatment efficiency

	Ineffective group (n = 20)	Effective group (n = 132)	X^2/t	P
Age (year)	26.25±5.20	26.80±5.74	0.397	0.692
BMI (kg/m^2)	22.85±2.09	21.76±2.15	2.120	0.036
Course of disease (month)	48.15±15.73	38.97±15.64	2.444	0.016
Systolic blood pressure (mmHg)	111.60±10.36	102.68±12.80	2.97	0.004
Diastolic blood pressure (mmHg)	77.93±5.84	78.27±4.41	0.307	0.759
Fasting insulin (μa/ml)	8.88±1.37	7.70±2.13	2.400	0.018
Fasting glucose (mmol/L)	7.30±0.46	6.96±0.65	2.252	0.026
Ferriman-Gallway Hair Score	7.00±1.21	6.46±1.07	2.067	0.040
Years of infertility (years)	3.15±1.39	3.28±1.27	0.421	0.674
Type of infertility			0.395	0.530
Primary infertility	13 (65.00)	76 (57.58)		
Secondary infertility	7 (35.00)	56 (42.42)		
Place of residence			0.523	0.470
Cities and towns	15 (75.00)	108 (81.82)		
Countryside	5 (25.00)	24 (18.18)		
Treatment options			4.455	0.035
Single treatment	14 (70.00)	59 (44.70)		
Combination therapy	6 (30.00)	73 (55.30)		
Pre-treatment TC (mmol/L)	4.56±0.78	5.01±0.74	2.517	0.013
Pre-treatment TG (mmol/L)	2.09±0.91	2.03±0.90	0.277	0.782
Pre-treatment HDL (mmol/L)	1.01±0.27	1.02±0.28	0.150	0.881
Pre-treatment LDL (mmol/L)	2.97±0.60	3.21±0.73	1.400	0.164
Pre-treatment FSH (IU/L)	6.48±0.66	6.46±0.86	0.100	0.921
Pre-treatment LH (IU/L)	16.23±3.51	15.41±3.33	1.019	0.310
Pre-treatment T (nmol/L)	2.67±0.63	2.83±0.71	0.952	0.343
Pre-treatment E_2 (pg/mL)	32.46±8.37	39.41±11.80	2.536	0.012

BMI, Body Mass Index; LH, Luteinizing Hormone; FSH, Follicle-Stimulating Hormone; T, Testosterone; E_2, Estrogen; TC, Total Cholesterol; TG, Triglyceride; HDL, High Density Lipoprotein; LDL, Low Density Lipoprotein.

cyproterone acetate tablets enhance the efficiency of letrozole by decreasing the level of serum androgens, thus lowering sex hormone levels. Estrogen and progesterone can interfere with lipid metabolism and androgens disrupt lipid metabolism by increasing lipoprotein lipase activity. We found lower TC, TG and LDL and higher HDL in the combination treatment group than those in the treatment alone group. The number of mature follicles, ovulation rate and pregnancy rate were higher in the patients treated with the combined drugs than those in the group with ethinylestradiol cyproterone tablets alone, which again confirmed that letrozole combined with ethinylestradiol and cyproterone acetate tablets could improve the pregnancy rate by improving the follicle count. Kar et al. [25] also indicated that letrozole has better pregnancy promotion and ovulation rates and should be promoted more as a first-line drug. Letrozole inhibits estrogen and blocks the negative feedback regulation of the "hypothalamic-pituitary-ovarian" gonadal axis, so the combination can improve sex hormone levels and reduce the chronic inflammation and insulin resistance in PCOS infertility patients. Hao

Table 6. Assignment table

Factor	Assignment
BMI	Data are continuous variables analyzed using raw data
Course of disease	Data are continuous variables analyzed using raw data
Systolic blood pressure	Data are continuous variables analyzed using raw data
Fasting insulin	Data are continuous variables analyzed using raw data
Fasting glucose	Yes = 1; No = 0
Ferriman-Gallway Hair Score	Data are continuous variables analyzed using raw data
Treatment options	Combination treatment = 1; Single treatment = 0
Pre-treatment TC	Data are continuous variables analyzed using raw data
Pre-treatment E_2	Data are continuous variables analyzed using raw data
Efficacy	Ineffective = 1; Effective = 0

BMI, Body Mass Index; E_2, Estrogen; TC, Total Cholesterol.

Table 7. Multi-factor analysis of factors affecting the treatment efficiency

	B	S.E	Wals	Sig.	Exp (B)	95% CI of EXP (B)	
						lower limit	upper limit
Fasting glucose	0.165	0.067	6.134	0.013	1.179	1.035	1.344
Ferriman-Gallway hair score	0.685	0.303	5.266	0.022	2.003	1.107	3.626
Treatment options	-1.550	0.738	4.407	0.036	0.212	0.050	0.902
Systolic pressure	0.085	0.031	7.666	0.006	1.089	1.025	1.156
Pre-treatment E_2	-0.080	0.034	5.390	0.020	0.923	0.863	0.988

E_2, Estrogen.

et al. [26] also found that better sex hormone improvement by combining letrozole could provide better improvement in follicular development and ovulation. Finally, we found that higher fasting glucose, higher Ferriman-Gallway hair score, single drug treatment regimen, higher systolic blood pressure, and lower E_2 before treatment were independent risk factors for ineffective treatment outcomes by performing a multifactorial analysis. It may be explained by the patients' worse disease severity and hormonal disorders, and therefore require more attention to these risk factors during the treatment.

Nevertheless, there are some shortcomings. The treatment was associated with only mild adverse effects without serious adverse effects such as ovarian hyperstimulation. Because of the limited follow-up time, pregnancy and fetal outcomes could not be measured in longer cycles, so more studies are needed to confirm its efficacy and safety. Meanwhile, pregnancy or not is also affected by their partners, so it is difficult to control the pregnancy outcomes completely and objectively.

To sum up, letrozole combined with ethinylestradiol and cyproterone acetate tablets can enhance the treatment efficiency of POCS and improve serum sex hormones and lipid metabolism of patients.

Disclosure of conflict of interest

None.

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