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Research

The Effectiveness of Letrozole Adjuvant Therapy - Electro Acupuncture in Reducing the Number of Follicles and Body Weight and Increasing Endometrial Thickness in Women with Polycystic Ovary Syndrome

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age. Acupuncture can improve menstrual cycle recovery and decrease body mass index (BMI) in women with PCOS. The effect of acupuncture can be enhanced by giving letrozol. Researchers are interested in knowing the comparison between letrozol therapy with letrozol + electroacupuncture on body weight, follicle count, and endometrial thickness in women with polycystic ovary syndrome. This study aims to determine the differences in weight loss, follicle number, and endometrial thickness in PCOS patients receiving letrozol treatment compared to letrozol + electroacupuncture.

Subjects and Method: An observational analytic study using a case-control approach and fixed disease sampling technique was carried out at the Gynecology Polyclinic and Sekar Clinic at Dr Moewardi Hospital, Surakarta. Group 1 consisted of 15 infertile SOPK women who received letrozol and group 2 consisted of 15 infertile SOPK women who received electroacupuncture + letrozol. Body weight, follicle count, and endometrial thickness were measured in both groups. Data were analyzed univariate, bivariate and multivariate using the IBM-SPSS version 22 and STATA version 13 statistical applications.

Results: There was a significant difference in the thickness of the endometrium (p= 0.008), the number of follicles on day 6 (p= 0.039), the number of follicles on day 8 (p= 0.033), the number of follicles on day 10 (p= 0.041), the number of follicles on day 12 (p= 0.041)) with letrozol therapy with letrozol + electroacupuncture. There was no significant difference in body weight between groups

Conclusion: There were significant differences between the letrozol group compared to the letrozol + electroacupuncture group in endometrial thickness and on the number of follicles on days 6, 8, 10, 12 in infertile PCOS women. However, there was no significant difference in body weight between groups.

Keywords: number of follicles, endometrial thickness, PCOS, letrozol, electroacupuncture

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BACKGROUND

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age with a prevalence of between 8-13% accompanied by health risks, including infertility, endometrial

hyperplasia, diabetes mellitus (DM), and cardiovascular disease (CVD) (Lee and Rausch, 2013). 2012; Azziz et al., 2016).

One of the therapies used to treat infertility in women with PCOS is to induce ovulation using letrozole. Letrozole is an aroma-tase inhibitor agent that decreases estrogen synthesis. The action of letrozole specifically occurs in inhibiting the change of androgens to estrogens in the ovarian follicles, peripheral tissues, and the brain so that there are two main effects, namely a decrease in circulating estrogen levels and an increase in intra-ovarian androgen levels. This effect has an impact on further follicular growth, more dominant follicle selection, and the occurrence of atresia in smaller follicles (Legro et al., 2014; Franik et al., 2018; Diamond et al., 2015).

Acupuncture is used all over the world to achieve fertility but its benefits are only supported by limited scientific evidence. The effects of acupuncture are partly mediated by a reduction in sympathetic nerve activity (Johansson and Stener-Victorin., 2013). Acupuncture can reduce uterine artery impedance and therefore can increase blood flow to the uterus (Chang, Chung & Rosenwaks, 2002). Acupuncture also raises-endorphin levels. There are suggestions that acupuncture might affect ovulation and fertility (Huang and Chen, 2008).

The effects of acupuncture can be increased by giving letrozole (Li et al., 2016). The use of letrozole combined with herbal medicines and acupuncture is known to smooth the menstrual cycle, lose weight, and increase ovulation and pregnancy rates (Yin et al., 2018). Based on this, the researchers are interested in conducting a study that aims to determine the relationship between body weight, number of follicles, and endometrial thickness with electro-acupuncture in SOPK patients who consume letrozole.

Giving letrozole, especially when combined with electroacupuncture, can have a positive impact, especially in relation to the fertility of people with PCOS (Li et al., 2016). Therefore, researchers are interested in knowing the comparison between letrozole therapy and letrozole plus electroacupuncture on body weight, follicle count, and endometrial thickness in women with polycystic ovary syndrome.

SUBJECTS AND METHOD

1. Study Design

This was an observational analytic study with a case-control study. The study was conducted at the Dr Moewardi Hospital Polyclinic from January to February 2020.

2. Population and Sample

The inclusion criteria for study subjects were women aged 20-45 years who experienced infertility. The exclusion criteria in this study were using hormonal therapy for the last three months, having malignant disease, chronic disease, or refusing to be the study sample. The sampling was done using a fixed disease sampling technique.

3. Study Variables

The dependent variables were body weight, number of follicles, and endometrial thickness. The independent variables were electroacupuncture and letrozol.

4. Operational Definition of Variables

The diagnosis of PCOS was confirmed by the Rotterdam 2003 criteria if two of the three symptoms were found, by looking at the patient's medical record data determined by a specialist.

Electroacupuncture is the insertion of a needle into the acupoints selected based on the meridian system to points CV 3 (Zhongji), CV 6 (Qihai), ST 29 (Guilai) bilateral, SP 6 (Sanyinjiao) bilateral, LI 4 (Hegu) bilateral, and ST 36 (Zusanli) bilaterally.

Ovulation induction with letrozol is to stimulate ovulation by administering letrozol on days 2 to 5 of menstruation for 5 days at a dose of 5 mg per day.

Body weight is the measurement of the body in terms of its weight in kilograms which is weighed in a state of minimal clothing without any equipment.

The number of follicles is the number of antral follicles measured in one ovary using transvaginal ultrasound on days 2, 6, 8, 10, and 12 of menstruation.

Endometrial thickness is a measure of the thickness of the endometrium which is measured in milli meters from edge to edge by a transvaginal ultrasound.

5. Data Analysis

The data were analyzed bivariately using the statistical application tools IBM-SPSS version 22 and STATA version 13.

6. Research Ethics

Ethical clearance was obtained from the health research ethics commission of dr. Moewardi Surakarta, Central Java, Indonesia No: 229/II/HREC/2020.

RESULTS

1. Characteristics of study subjects

30 study subjects were divided into 2 groups. Group 1 consisted of 15 infertile SOPK women who received letrozole therapy and group 2 consisted of 15 infertile SOPK women who received electroacupuncture therapy with letrozole.

Table 1. Sample Characteristics

Variable	N	Mean	SD	Min.	Max.	p
Age (year)						
Letrozole + EA	15	30.13	4.44	26	44	0.393
Letrozole	15	31.60	4.81	26	44	
Weight (kg)						
Letrozole + EA	15	65.33	14.93	40	105	0.537
Letrozole	15	64.53	15.85	40	99	
Height (cm)						
Letrozole + EA	15	156.80	4.63	150	163	0.888
Letrozole	15	157.73	3.50	150	167	
Infertile duration (year)						
Letrozole + EA	15	4.73	2.28	1	8	0.784
Letrozole	15	5	2.95	2	10	

The data description of the characteristics of the research subjects is in table 1. It can be seen that the age of the subjects in group I and group II is at least 26 years, and the maximum for each group is 44 years. In group I mean= 31.60; SD= 4.81 and group II mean= 30.13; SD= 4.44. The body weight of the research subjects in group I and group II had a minimum value of 40 kg and a maximum body weight of group I was 99 kg and group II was 105 kg, while the average body weight of group I= 64.53; SD= 14.85 and group II mean= 65.33; SD= 15.93. The height of the subjects in groups I and II is at least 150cm, group I is a maximum of 167 cm and group II is 163 cm with a mean value in group I= 157.73; SD= 14.85 and group II mean= 156.80; SD= 3.63. Group I and group II have a minimum BMI value of 17. The maximum BMI value for group I is 36 and group II is 39, while the mean value is 25.40; SD= 5.08 for group I and for group II mean= 26.00; SD= 5.56. Infertile group I minimum 2 maximum 10 with a mean= 5.00; SD= 2.95, group II minimum value of 1 maximum 8 and mean= 4.73; SD= 2.28. The variables of age, height, weight, BMI and duration of

infertility have a value of P> 0.05 which means there is no difference or characteristic data are homogeneously distributed.

Table 2. Mean pre- and post-intervention for each outcome

Outcome	No	Pre Intervention		Post Intervention		The difference Post-Pre		p
		Mean	SD	Mean	SD	Mean	SD	
Endometrial thickness at day 12								
Letrozole + EA	15	5.77	1.05	7.08	1.89	1.23	1.19	0.026
Letrozole	15	5.76	1.87	8.37	1.84	2.68	2.06	
Number of follicles on day 2								
Letrozole+ EA	15	17.20	2.42	14.93	2.37	2.27±	2.08	0.011
Letrozole	15	16.87	2.47	16.27	2.71	0.60	1.12	
Number of follicles on day 6								
Letrozole+ EA	15	15.9	2.54	13.27	2.37	2.67±	2.35	0.003
Letrozole	15	15.73	2.68	15.40	2.99	0.33±	1.44	
Number of follicles on day 8								
Letrozole+ EA	15	15.07	2.54	11.60	2.82	3.47	2.41	0.002
Letrozole	15	14.73	2.72	13.73	2.37	1.00	1.25	
Number of follicles on day 10								
Letrozole + EA	15	13.87	2.25	10.07	2.91	3.80	2.21	0.002
Letrozole	15	13.73	2.34	12.20	2.54	1.53	0.83	
Number of follicles on day 12								
Letrozole + EA	15	13.47	2.25	9.00	2.92	4.47	1.99	0.001
Letrozol	15	13.27	2.43	11.13	2.50	2.13	0.83	
Weight								
Letrozole + EA	15	68.87	16.99	66.37	15.95	2.47	1.95	0.156
Letrozole	15	68.20	13.76	66.70	13.34	1.60	1.18	

Table 3. Analysis of groups I and II before treatment

Variable	Before Letrozol treatment		Before treatment Letrozol + EA		p
	Mean	SD	Mean	SD	
Endometrium thickness H 12 (mm)	5.76	1.88	5.78	1.06	0.305
Number of Follicles Day 2	16.87	2.48	17.20	2.43	0.744
Number of Follicles Day 6	15.73	2.69	15.93	2.66	0.838
Number of Follicles Day 8	14.73	2.71	15.07	2.55	0.806
Number of Follicles Day 10	13.73	2.34	13.87	2.26	0.870
Number of Follicles Day 12	13.27	2.43	13.47	2.26	0.870
Weight (kg)	68.20	13.77	68.87	16.99	0.935

2. Bivariate Analysis

In table 3 above the p value> 0.05 on all variables studied, it can be stated that there is no significant difference between group I and group II before treatment, so that the data before the treatment of the two groups is equal (fulfilling the research requirements).

Table 4. Endometrial Thickness Analysis, Number of H2-H12 Follicles and Body Weight for Group I

Variable	Before the Letrozole treatment		After treatment Letrozol		95 CI %	p
	Mean	SD	Mean	SD		
Endometrium thickness H 12 (mm)	5.76	1.88	7.08	1.89	-0.02 – 1.22	0.001
Number of Follicles Day 2	16.87	2.48	16.27	2.71		0.057
Number of Follicles Day 6	15.73	2.69	15.40	2.99	-0.47 – 1.14	0.388
Number of Follicles Day 8	14.73	2.71	13.73	2.37	0.31 – 1.69	0.008*
Number of Follicles Day 10	13.73	2.34	12.20	2.54	1.07 – 1.99	<0.001*
Number of Follicles Day 12	13.27	2.43	11.13	2.50	1.67 – 2.60	<0.001*
Weight (kg)	68.20	13.77	66.70	13.34	0.83 – 2.17	<0.001*

In the mean difference results, it can be seen that the variables that have a significant difference (p <0.05) between before and after treatment in group I are endometium thickness, number of H8 follicles, number of H10 follicles, number of H12 follicles and body weight. There was no significant difference in the number of H2 follicles and the number of H6 follicles (p> 0.05).

Table 5. Endometrial Thickness Analysis, Number of H2-H12 Follicles and Body Weight for Group II

Variable	Before treatment Letrozol + EA		After treatment Letrozol + EA		95% CI	p
	Mean	SD	Mean	SD		
Endometrium thickness H 12 (mm)	5.78	1.06	8.37	1.84		<0.001
Number of Follicles Day 2	17.20	2.43	14.93	2.37		0.003
Number of Follicles Day 6	15.93	2.66	13.27	2.37	1.37 – 3.97	0.001
Number of Follicles Day 8	15.07	2.55	11.60	2.82	2.13 – 4.81	<0.001
Number of Follicles Day 10	13.87	2.26	10.07	2.92	2.58 – 5.02	<0.001
Number of Follicles Day 12	13.47	2.26	9.00	2.93	3.36 – 5.57	<0.001
Weight (kg)	68.87	16.99	66.37	15.96	1.41 – 3.59	<0.001

In group II, it can be seen that the variables that have a significant difference (p <0.05) between before treatment and after treatment are endometium thickness, number of H2 follicles, number of H6 follicles, number of H8 follicles, number of H10 follicles, number of H12 follicles and body weight.

Table 6.Analysis of Differences Between Group I and Group II on Endometrial Thickness H12, Number of H2 Follicles, Number of H6 Follicles, Number of H8 Follicles, Number of H10 Follicles, Number of H12 Follicles and Body Weight

Variable	After treatment Letrozol		After treatment Letrozol + EA		95% CI	p
	Mean	SD	Mean	SD		
Endometrium thickness H 12 (mm)	7.08	1.89	8.37	1.84		0.008
Number of Follicles Day 2	16.27	2.71	14.93	2.37	-0.57 – 3.24	0.163
Number of Follicles Day 6	15.40	2.99	13.27	2.37	0.11 – 4.16	0.039
Number of Follicles Day 8	13.73	2.37	11.60	2.82	0.95 – 0.18	0.033
Number of Follicles Day 10	12.20	2.54	10.07	2.92	0.09 – 4.18	0.041
Number of Follicles Day 12	11.13	2.50	9.00	2.93	0.09 – 4.17	0.041
Weight (kg)	66.70	13.34	66.37	15.96	-10.67 – 11.34	0.951

Table 6 shows a significant difference between group I and group II in the number of H8 follicles, the number of H10 follicles and the number of H12 follicles after treatment (p <0.05). The body weight of group I was 66.70 (Mean= 66.70; SD= 13.34) and II was 66.37 (Mean= 66.37; SD= 15.96) with p= 0.951, which means that there was no significant difference between group I and group II in body weight after treatment.

DISCUSSION

Women with PCOS are more likely to be overweight than underweight, and being overweight worsens the condition of PCOS (Lim et al., 2013). Overweight and obesity are also chronic conditions associated with serious morbidity and mortality. Therefore, the importance of weight loss for overweight or obese patients with PCOS is very important (Yang et al., 2018). Acupuncture can aid weight loss according to a number of studies. A systematic review shows that acupuncture has a beneficial effect in overweight patients who have PCOS (Saleem & Rizvi, 2017). In a 2016 trial of 17 overweight women with PCOS, 5 weeks of electro-acupuncture (EA) treatment improved glucose and adipose tissue androgen regulation (Stener-Victorin et al., 2016). The results showed that body weight between letrozol treatment and letrozol + EA did not have a significant difference. This is in line with the findings that show that letrozol tends to increase body weight or has no effect on body weight. This study found that more than half of the research subjects experienced no increase and some even experienced weight loss (Nyrop et al., (2016).

Although the many benefits of acupuncture have been demonstrated, convincing evidence regarding the use of acupuncture to treat obesity is limited due to its low-quality methodology and small sample size. Two-thirds of acupuncture tests on obesity, drawn from a total of 31 studies, were rated as having low Jadad scores (Cho et al., 2009). By conducting a systematic review of acupuncture and Chinese medicine on obesity, studies conducted in China were of lower methodological quality than studies outside of

China. Limitations in trials conducted in China are still lack of information regarding blinding, randomization methods, and reasons for discontinuation (Sui et al., 2012). Additionally, there is little confirmatory evidence regarding the types of acupuncture offered to obese patients. Various types of acupuncture, including manual acupuncture, electro-acupuncture, auricular acupuncture, auricular acupressure, electro acupressure plus auricular acupressure, and electroacupuncture plus lifestyle modification, are used for the management of obesity. Although electroacupuncture is more frequently used in the management of clinical obesity and obesity research, so far there is no convincing evidence to support its effectiveness in treating obesity (Gao et al., 2015).

On days 6 to 12 there was a significant difference between treatment with letrozol and with letrozol + EA, where treatment using letrozol + EA had a lower mean number of follicles than treatment with letrozol alone. A study explains that the use of letrozol increases the number of follicles and allows the growth of existing follicles due to the effect of letrozol work which has no effect on the central nervous system (Chen et al., 2018). Letrozol only works as an aromatase inhibitor agent and can reduce E2 levels. Another study states that in subjects with PCOS, the use of letrozol can induce follicle growth, but in smaller amounts than the use of other agents (Mukherjee et al., 2018). Letrozol has an effect in increasing the number of follicles. However, this effect is less potent than the use of clomiphene (Badawy et al., 2009). This study also states that although the number of induced follicles is less than clomiphene administration, administration of letrozol results in more mature follicles. However, administration of letrozol alone is not recommended as an appropriate method for ovulation induction. Electro-acunpuncture is said to have a better ovulation induction effect than ordinary acupuncture, thereby reducing the rate of infertility (Karim et al., 2019).

Other studies have shown that electroacupuncture improves follicular development and improves reproductive endocrine dysfunction in the ovaries by regulating hypothalamus, pituitary, and ovarian functions. In a previous study, electroakufastening in CV-3 and CV-4 accupoints, which are located in the same spinal segmental nerve region of the reproductive system, stimulated P450arom expression in follicular granular cells in PCOS mice and increased the conversion of androgens to estrogens, which improved Follicular dysplasia and dysfunctional ovulation, reduce excessive androgen levels and improve ovarian hormone disorders PCOS. Aquaculture has shown neurophysiological effects via accupoint stimulation, which is associated with high density of peripheral nerve endings (Li et al., 2004).

Several studies have shown that electroacupuncture in PCOS reduces high androgen levels to improve menstrual disorders and ovulation. Acupuncture affects the production of beta-endorphins, which can affect the secretion of Gonadotropin Releasing Hormone to regulate the hypothalamic-pituitary-ovarian axis. Acupuncture stimulation can play a role in ovulation induction and fertility (Sato et al., 2015). Low-frequency electroacupuncture has been shown to affect neuroendocrine function in PCOS by regulating the peripheral nerves (ovaries) and central nervous system (hypothalamus). In addition, electroacupuncture improves blood flow, and ovarian tissue morphology, and reduces AMH levels and ovarian volume in women with PCOS. The findings of this study indicate that electroacupuncture has an effect in regula-

ting AMH and hyperandrogenism in PCOS (Shi et al., 2019).

The analysis test on the endometrial thickness showed a significant difference between the letrozol group and the letrozol + EA group, the results showed that the mean endometrial thickness in the letrozol + EA group was higher than the letrozol + EA group. In recent years, a number of studies have demonstrated the effectiveness of electroacupuncture (EA) in the field of artificial reproduction (Jo and Lee, 2017; Huang et al., 2018). Several studies have reported that EA affects uterine microcirculation, increases the expression of estrogen receptors (ER) and progesterone receptors (PR) on the surface of the endometrium, increases serum estrogen levels, promotes endometrial regeneration, and improves pregnancy rates (Li et al., 2011; Ma et al., 2017; So et al., 2010).

Acupuncture is becoming popular in research and clinical practice, and recent studies have shown that acupuncture can improve ovulation, improve endocrine profile by reducing circulating sex steroids, increasing menstrual frequency, and losing weight. Several studies have shown that stimulating acupuncture points can regulate the myoelectric activity of the uterus and neuroendocrine system, as well as increase endometrial blood flow, thereby increasing endometrial receptivity and providing conditions conducive to embryo implantation (Xia et al., 2019). Additionally, electroacupuncture has been shown to improve insulin sensitivity in experimental trials. Electroacupuncture can significantly decrease blood glucose levels after insulin injection, indicating an increase in insulin sensitivity (Li et al., 2016).

A study found that the use of letrozol in combination with acupuncture is known to improve menstrual cycles, lose weight, and increase ovulation rates and pregnancy rates (Yin et al., 2018). This study also states that the combination of letrozol and acupuncture minimizes the side effects that can be caused by letrozol (Li et al., 2016). It is generally believed that an adequate endometrial thickness is necessary to optimize the pregnancy rate. This is because the thickness of the endometrium is affected by the flow of uterine arterial blood. With its main inhibitory effects of sympathoin, acupuncture may contribute to reducing uterine artery impedance and therefore, increasing blood flow to the uterus (Chang et al., 2002).

AUTHOR CONTRIBUTION

Todung Antony Wesliaprilius, Uki Retno Budihastuti, Eriana Melinawati, Sri Sulistyowati, Ida Nurwati, and Cahyono Hadi collected the data, measured the follicles, did acupuncture and electro acupuncture, and wrote the paper.

CONFLICT OF INTEREST

The author states that this research was conducted without any relationship with financial or commercial factors which could be interpreted as a conflict of interest.

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