ORIGINAL RESEARCH

Effect of Low-Frequency Electro-Acupuncture in Unmarried Women With Polycystic Ovary Syndrome: A Randomized Controlled Study

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ABSTRACT

Objectives • We aimed to evaluate the effect of low-frequency electro-acupuncture (EA) in unmarried women with polycystic ovary syndrome (PCOS).

Design • A total of 54 women with PCOS were randomly assigned to either the acupuncture group (n=27) or the sham acupuncture group (control, n=27) for a total of 32 treatments over 16 weeks. In the acupuncture group 26 patients and in the control group 20 patients completed the trial.

Outcome Measures • Main measures were androgen levels including 17-α-hydroxyprogesterone (17-α-OHP), androstenedione (A2), testosterone (T) and dehydroepiandrosterone (DHEA) at 0, 24 and 48 hours after stimulation with a dose of human chorionic gonadotropin (HCG). Other measures included body mass index, waist-to-hip ratio, sex hormone levels, etc.

Results • After treatment, there was no significant difference in the main measures between the 2 groups ($P > .05$), except for the DHEA level at 0 h of HCG stimulation ($P = .024$) and acne score ($P < .05$). Comparison within the acupuncture group found that 17-α-OHP and A2 levels at 0 h and DHEA levels at 24 h of HCG stimulation after treatment were significantly decreased ($P < .05$), whereas T levels at 24 h were significantly increased ($P < .05$). Comparison within the control group showed 17-α-OHP level at 0 h and 17-α-OHP and A2 and DHEA levels at 24 h after treatment were significantly lower ($P < .05$). In addition, weight, BMI, HCG and Ferriman-Gallwey score in the acupuncture group and LH/FSH ratio was significantly reduced in the control group.

Conclusion • Traditional EA is slightly more effective than sham acupuncture in reducing DHEA secretion and the acne score. Meanwhile, sham acupuncture is not completely ineffective. The specific mechanism of the two may be different. (*Altern Ther Health Med.* 2022;28(4):24-33)

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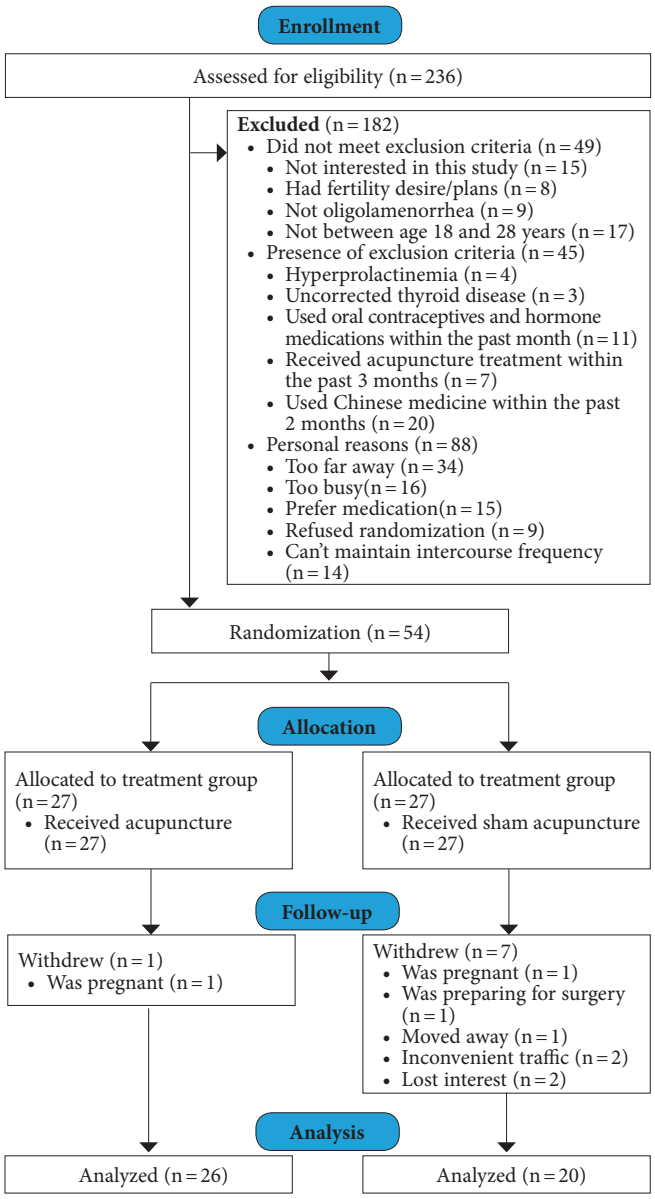
INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common gynecologic endocrine diseases; according to recent epidemiologic surveys, PCOS incidence is 2% to 8.25% in various countries and populations.[1-3] Currently, the treatment for PCOS is only symptomatic. In married women with PCOS, ovulation induction is often used to solve infertility problems, but it is not suitable in the cohort of unmarried women with PCOS who have no immediate wish for fertility. Although there are no detailed statistics, according to clinical experience the number of women in this unmarried cohort is huge. For them, menstrual disturbance, hirsutism and acne, overweight, obesity or metabolic syndrome are often considered significant problems. Contraceptive pills are commonly used to solve the

Figure 1. Study flow chart



problems of hyperandrogenism and irregular menstruation. However, they have many adverse events such as increased risks for thrombosis, abnormal glucose metabolism and insulin resistance, headache and migraine etc. Therefore, supplemental alternatives are worth trying in unmarried women with PCOS.

Acupuncture, as a distinctive treatment in traditional Chinese medicine (TCM), has a more than 3000-year history in treating gynecologic diseases such as delayed menstruation, amenorrhea and irregular menses, which have similar symptoms to PCOS. According to TCM theory, acupuncture can regulate menstruation by reconciling Yin and Yang, regulating meridians of the thoroughfare vessel and the conception vessel, tonifying the kidneys, and promoting the circulation of Qi and blood with almost no adverse events. In addition, several studies[4-6] have reported that electro-acupuncture (EA) may decrease circulating serum testosterone (T) and luteinizing hormone (LH) to follicle-stimulating hormone (FSH) ratio in patients with PCOS, increase ovulation rate and improve menstrual frequency, which indicates that EA may be an optional effective choice in treating PCOS.

In this study, we sought to evaluate the efficacy of low-frequency EA on androgen secretion with and without human chorionic gonadotrophin (HCG) stimulation in unmarried women with PCOS.

MATERIALS AND METHODS

Participants

This trial was approved by the Chinese Ethics Committee of Registering Clinical Trials (ChiECRCT-2012030) and registered on the Chinese Clinical Trial Registration site (ChiCTR-TRC-12002529). All participants provided written consent for their participation in the study.

Unmarried women with PCOS were recruited through advertisements on bulletin boards and physician referrals from November 2012 to March 2016 in the outpatient department of Integrated Traditional Chinese and Western Medicine of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, and the department of obstetrics and gynecology of Hubei Province Hospital of Traditional Chinese Medicine, Wuhan, China. After signing the informed consent, 54 eligible patients were randomly assigned to either the acupuncture group (low-frequency EA) or sham acupuncture group (control), with 27 women in each group. Baseline measurements were performed before receiving the first treatment (Figure 1).

Inclusion Criteria. (1) unmarried women age between 18 and 28 years, (2) no fertility wish within 4 months, (3) confirmed diagnosis of PCOS according to the Rotterdam criteria: oligomenorrhea (menstrual cycle >35 days, and less than 8 cycles per year), or amenorrhea (menstrual cycle >90 days) and either clinical or biochemical hyperandrogenism and/or polycystic ovarian morphology.

Exclusion Criteria. (1) with hyperprolactinemia, (2) with abnormal increased androgen caused by adrenal or ovarian tumors, (3) with uncorrected thyroid disease (thyoid-stimulating hormone [TSH] <0.2 uIU/mL or > 5.5 uIU/mL) except patients with normal TSH in the past year, (4) with suspected Cushing syndrome, (5) who had received estrogen, progesterone or oral contraceptives and hormone medications within the past month (takes at least 1 month to eliminate these medicines from the body), (6) who received other medications (such as anti-obesity medications, anti-diabetic medications, TCM, etc) that influence reproductive function or metabolism within the past 2 months, (7) who had received acupuncture treatment within the past 3 months.

Random Assignment

This was a prospective randomized single-blinded controlled study with 2 parallel groups. Random assignment was undertaken via a computerized randomization program

by an independent statistician. Patients were randomly assigned to either the acupuncture or control group according to computer-generated random numbers. Acupuncture was performed by acupuncturists who knew the different acupuncture protocols but were blinded to the baseline datum collection. Patients in both groups were blinded to the stochastic scheme and outcomes, and received different acupuncture protocols at different sites or at different times to make sure they were totally blind to the randomization process. The baseline datum collection, outcome assessment and statistical analyses were performed by clinical research assistants and statisticians who were blinded to the random allocation.

Treatment Protocol

The acupuncture protocols were fixed and per the project design of Kuang H, et al.[7] Treatment started after a spontaneous period or a withdrawal bleeding with progestin. All patients received either treatment with active EA or Sham acupuncture for 30 minutes twice a week with a maximum of 32 treatments.

Protocol in the Acupuncture Group. In the acupuncture group, 2 sets of acupuncture points were used in an alternating fashion. The first set of acupoints consisted of conception vessel (CV) 3, CV 6, governor vessel (GV) 20 and hibateral stomach (ST) 29, spleen (SP) 6, SP 9 and large intestine (LI) 4. Disposable sterilized stainless steel needles (size 0.25 × 40 mm, lot 251226; Wuxi Jiajian Medical Instrument, Wuxi, China) were inserted to a depth of 15 to 35 mm and stimulated manually to evoke needle sensation ("Deqi" in TCM), which includes soreness, numbness or distension around the puncture site and sometimes along the corresponding meridians. CV 3 and CV 6, hibateral ST29, and hibateral SP6 and SP9 were then connected to electrical stimulators (Export Abteilung, Schwa-Medico GmbH, Ehringshausen, Germany) and stimulated with 2Hz, 0.3 ms pulse length low-frequency EA. The intensity was adjusted to the maximum patient-tolerated level before reaching the point of pain or discomfort. The needles that were not connected to the electrical stimulator were manually stimulated to evoke the needle sensation every 10 minutes a total of 4 times.

The second set of acupoints consisted of CV 3, CV 6, GV 20 and bilateral ST 25 and 29, bilateral SP 6, liver (LR) 3 and pericardium (PC) 6. Bilateral LR3, SP6, ST 25 and 29 were stimulated with low-frequency EA in the same fashion as the first set.

Control Group Protocol. In the control group, 4 pseudoacupoints with 2 points on each shoulder and 2 on each upper arm, which were not located on any meridian, were used to exclude the acupuncture effect. Disposable sterilized needles (size 0.16 × 25 mm; lot 491226; Wuxi Jiajian Medical Instrument, Wuxi, China) were inserted superficially to a depth of <5 mm without any manual stimulus to evoke needle sensation. Electrodes were connected to the needles, but the stimulators were turned on at 0 intensity just to mimic EA in the acupuncture protocol. The acupuncturist explained that this sham protocol was a new acupuncture therapy and informed the patients that "the therapy is not ineffective and the purpose of our study is to compare the differences between the 2 acupuncture therapies."

Study Measurements

Assessment of Androgen Levels With and Without HCG Stimulation. The primary outcome measurements were androgen levels including 17-α-hydroxyprogesterone (17-α-OHP), androstenedione (A2), testosterone (T) and dehydroepiandrosterone (DHEA) with and without HCG stimulation. Both at baseline and after treatment, patients received an intramuscular injection of 5000 IU HCG (Livzon Pharmaceutical Co., Ltd, Zhuhai 519020, China) immediately after a spontaneous period or withdrawal bleeding by progestin, and then blood samples were collected at 0, 24 and 48 hours.

Measurements of Biometric and Biochemical Features. The secondary outcome measurements included anthropometric measurements, sex hormone levels and adverse events. Anthropometric measurements included vital signs, height, weight, waistline, hipline, Ferriman-Gallwey hirsutism scores[8] and standard acne scores. Sex hormone levels were collected on an empty stomach on the morning of the 2nd to 3rd day of menstruation. Deviation events and adverse events were recorded, classified and described in a list. Serious adverse events included any events that were immediately life-threatening, severely or permanently disabling, required prolonged hospitalization or was thought to be serious by the investigators.[9]

Statistical Analysis

In the data description, categorical variables were summarized as frequencies and percentages and continuous variables were summarized as mean and standard deviation. The androgen levels at 0, 24 and 48 hours after HCG stimulation were compared by paired *t* test. The main outcome measurements were analyzed as the difference in efficacy between the 2 groups using covariance model analysis with baseline as covariate considered the central effect. Secondary outcome measurements were compared using analysis of variance or the Mann-Whitney U test. The chi-square test was used for the classification indexes between the 2 groups. The dropout rate in each group was compared using the CMH-x2 test.

Sample size was determined based on primary objective of comparing change rate in androgen levels after HCG stimulation between EA and control group. Assuming PCOS patients treated with EA may have a change rate of 10% (SD = 0.1) in androgen levels and control treatment did not work. With an alpha of 0.05, beta of 0.1 and EA group to control group ratio of 1:1, 23 subjects were required in each group. A sample size of 54 was sufficient, taking into account a 15% drop rate.

The IBM SPSS® 22.0 (IBM Corp., Armonk NY USA) was used for statistical analysis. The two-tailed value of $P < .05$ was considered statistically significant.

Table 1. Baseline Patient Characteristics

Parameter	Acupuncture Group (n=27)	Control Group (n=27)	*P* value
Biometric Feature			
Age (years)	23.3 ± 2.7	22.3 ± 2.4	.174
Height (cm)	161.6 ± 2.7	159.8 ± 4.8	.141
Weight (kg)	60.1 ± 15.6	55.7 ± 12.8	.263
BMI (kg/m^2)[a]	22.9 ± 5.5	21.9 ± 5.3	.431
Waistline (cm)	78.3 ± 14.2	76.1 ± 12.0	.640
Hipline (cm)	94.9 ± 10.0	92.0 ± 9.1	.271
WHR	0.82 ± 0.08	0.62 ± 0.08	.914
Pulse (per min)	78.2 ± 7.2	76.3 ±7.3	.896
Respiration (per min)	17.7 ± 1.1	15.5 ± 0.7	.341
Systolic pressure (mmHg)	105.3 ± 8.7	104.2 ± 8.6	.803
Diastolic pressure(mmHg)	67.3 ± 6.0	66.6 ± 11.0	.446
Ferriman-Gallwey score[b]	2.6 ± 3.3	2.3 ± 2.6	.957
Acne score	0.26 ± 0.59	0.59 ± 0.80	.054
Acanthosis nigricans score	0.04 ± 0.19	0.00 ± 0.19	.317
History			
Time with PCOS diagnosis (months)	10.1 ± 6.6	9.6 ± 6.8	.778
Diet control	6/27 (22.2%)	5/27 (18.5%)	.759
Regular exercise habit	4/27 (14.8%)	5/27 (18.5%)	.759
Had acupuncture before	2/27 (7.4%)	3/27 (11.1%)	.671
Previous menstrual cycle (days)	105 ± 93	95 ± 86	.624
Ultrasonography Findings			
Polycystic ovaries per Rotterdam criteria[c]	23/27 (85.2%)	25/27 (92.6%)	.834
Fasting Serum Levels			
LH (mIU/ml)	10.3 ± 6.6 (n = 27)	13.2 ± 9.6 (n = 25)	.327
FSH (mIU/ml)	5.60 ± 1.8 (n = 26)	6.1 ± 2.6 (n = 26)	.461
LH/FSH ratio	1.82 ± 1.23 (n = 27)	2.34 ± 1.61(n = 24)	.206
P (ng/ml)	0.73 ± 0.55 (n = 25)	0.90 ± 0.58(n = 23)	.201
Total T (ng/dl)	0.59 ± 0.29(n = 27)	0.65 ± 0.28(n = 27)	.450
E_2 (ng/ml)	46.50 ±28.8(n = 27)	77.8 ± 89.8(n = 27)	.159
PRL (ng/dl)	15.1 ± 7.6 (n = 26)	15.6±7.2 (n = 26)	.644

Note: Each value represents mean ± standard deviation or absolute number (percentage).

[a]Body mass index is the weight in kilograms divided by the square of the height in meters.
[b]Scores on the modified Ferriman-Gallwey scale for hirsutism range from 0 to 44, with higher scores I ndicating a greater degree of hirsutism.
[c]Polycystic ovaries were defined as an antral follicle count of 12 or more or a volume >10 cm3 in at least 1 ovary.

Abbreviations: BMI, body mass index; WHR, waist-hip-ratio; FSH, follicle stimulating hormone; LH, luteinizing hormone; P, progesterone; T, testosterone; E, estradiol; PRL, prolactin.

RESULTS

Patient Baseline Demographics

A total of 236 patients were screened initially; 142 unmarried women were eligible for the study and 54 finally participated. In the end, 1 patient in the acupuncture group and 7 in the control group withdrew from treatment. A summary of patient flow can be found in Figure 1.

There were no significant differences between patients in the acupuncture and control groups at baseline with regard to biometric features (including age, height, weight, BMI, waistline, hipline, WHR, pulse rate, respiration rate, systolic pressure, diastolic pressure, Ferriman-Gallwey score, acne score and acanthosis nigricans score), past history (including time with PCOS diagnosis, ratio of diet control, ratio of regular exercise habits, ratio having received acupuncture previously and previous menstrual cycle), the ratio of polycystic ovaries or serum levels of sex hormones (including LH, FSH, LH/FSH ratio, progesterone, total testosterone, DHEAs, estradiol and prolactin) (see Table 1).There were no significant differences between the two groups at baseline in biometric features, past history and serum levels of sex hormones (see Table 1).

Table 2. Comparison of Androgen Levels With HCG Stimulation at 0, 24 and 48 Hours

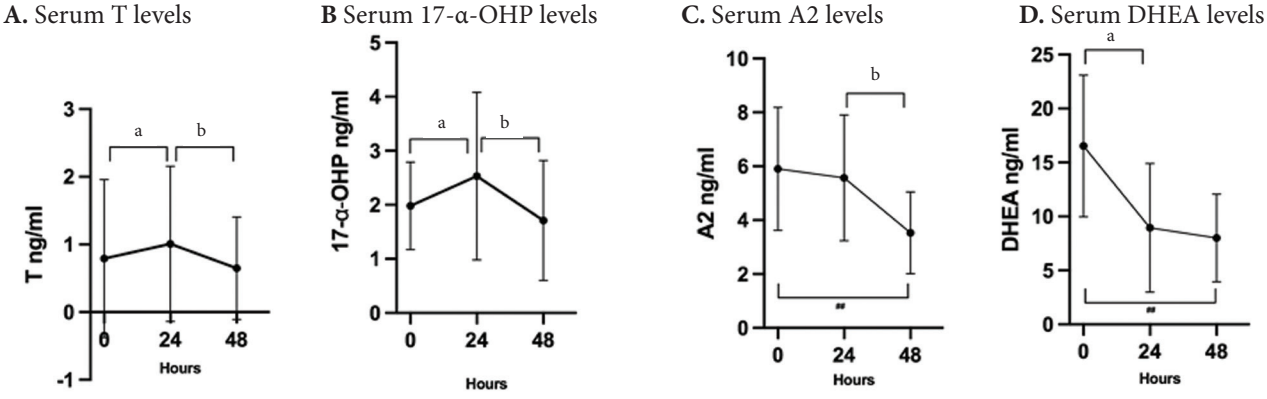
Outcome	HCG Stimulation 0 Hours	HCG Stimulation 24 Hours	HCG Stimulation 48 Hours	0 and 24 hours with HCG Stimulation *P* value	0 and 48h with HCG Stimulation *P* value	24 and 48h with HCG Stimulation *P* value
T (ng/ml)	0.79 ± 1.17 (44)	1.01 ± 1.15 (44)	0.65 ± 0.76 (44)	.000[a]	.086	.000[b]
17-α-OHP (ng/ml)	1.98 ± 0.81 (43)	2.53 ± 1.55 (43)	1.71 ± 1.11 (43)	.009[a]	.140	.001[b]
A2 (ng/ml)	5.90 ± 2.29 (44)	5.57 ± .33(44)	3.53 ± 1.52 (44)	.165	.000[c]	.000[b]
DHEA (ng/ml)	16.54 ± 6.57 (43)	8.95 ± 5.69 (43)	8.02 ± 4.07 (43)	.000[a]	.000[c]	.320

Note: *t* test of paired samples was used for analysis.

[a]*P* <.01
[b]*P* <.01
[c]*P* <.01

Abbreviations: T, testosterone; 17-α-OHP, 17-α-hydoxy progesterone; A2, androstenedione; DHEA, dehydroepiandrosterone.

Figure 2. Comparison of androgen levels at 0, 24 and 48 hours with HCG stimulation at baseline



[a]the comparison between 0 and 24 hours with HCG stimulation, *P* < .01
[b]the comparison between 24 and 48h with HCG stimulation, *P* < .01

Abbreviations: T, testosterone; 17-α-OHP, 17-α-hydoxy progesterone; A2, androstenedione; DHEA, dehydroepiandrosterone.

Comparison of Androgen Levels at 0, 24 and 48 Hours After HCG Stimulation at Baseline

As for T, it increased by 27.8% 24 hours after HCG stimulation (0 hours: 0.79 ± 1.17 vs 24 hours: 1.01 ± 1.15; *P* < .01) and decreased to the level prior to HCG stimulation 48 hours later (24 hours: 1.01 ± 1.15 vs 48 hours: 0.65 ± 0.76; *P* < .01; 0 hours: 0.79 ± 1.17 vs 48 hours: 0.65 ± 0.76; *P* > .05). As for 17-α-OHP, it increased by 27.8% 24 hours after HCG stimulation (0 hours: 1.98 ± 0.81 vs 24 hours: 2.53 ± 1.55; *P* < .01) and decreased to the level prior to HCG stimulation 48 hours later (24 hours: 2.53 ± 1.55 vs 48 hours: 1.71 ± 1.11; *P* < .01; 0 hours: 1.98 ± 0.81 vs 48 hours: 1.71 ± 1.11; *P* > .05). As for A2, it maintained the same level as before stimulation after 24 hours of HCG stimulation, but decreased by 40.2% after 48 hours of HCG stimulation (0 hours: 5.90 ± 2.29 vs 48 hours: 3.53 ± 1.52; *P* < .01). As for DHEA, it decreased by 45.9% 24 hours after HCG stimulation (0 hours: 16.54 ± 6.57 vs 24h: 8.95 ± 5.69; *P* < .01) and 51.5% 48 hours after HCG stimulation (0 hours: 16.54 ± 6.57 vs 48 hours 8.02 ± 4.07; *P* < .01), which maintained the same level as at 24 hours (24 h: 8.95 ± 5.69 vs 48 hour: 8.02 ± 4.07; *P* > .05). (see Table 2 and Figure 2)

Main Outcome Measurement: Changes of Androgen Levels With and Without HCG Stimulation

Both before and after treatment, there were no significant differences in serum levels of T, 17-α-OHP, A2 and DHEA between the 2 groups at 0, 24 and 48 hours of HCG stimulation *P* > .05; see Table 3).

In comparison, within the groups after 16-week treatment, T levels 24 hours after HCG stimulation in the active group were significantly higher (*P* < .01; see Table 3); 17-α-OHP level at 0 hours of HCG stimulation in both groups and 24 hours after HCG stimulation in the control group were significantly lower (*P* < .05; Table 3); A2 level at 0 hours of HCG stimulation in the active group and 24 hours after HCG stimulation in control group were significantly lower than that at baseline (*P* < .05;

Table 3. Comparison of Androgen Levels With and Without HCG Stimulation in the Two Groups

Outcome	Acupuncture Group		Control Group		Acupuncture and Sham Acupuncture Groups Before Treatment *P* value	Acupuncture and Sham Acupuncture Groups After 16-Week Treatment *P* value	Intragroup Comparison Acupuncture *P* value	Intragroup Comparison Sham Acupuncture *P* value
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment				
T (ng/ml)								
T-HCG 0h	0.95 ± 1.48 (22)	0.89 ± 1.29 (22)	0.79 ± 1.00 (13)	0.93 ± 1.18 (13)	.742	.919	.277	.196
T-HCG 24h	1.11 ± 1.36 (20)	1.32 ± 1.29 (20)	1.02 ± 1.79 (16)	0.94 ± 0.64 (16)	.841	.294	**.010**[a]	.535
T-HCG 48h	0.70 ± 0.91 (20)	0.70 ± 1.14 (20)	0.67 ± 0.76 (16)	0.57 ± 0.58 (16)	.914	.692	.351	.569
17-α-OHP (ng/ml)								
17-α-OHP-HCG 0h	2.01 ± 0.81 (21)	1.52 ± 0.46 (21)	2.02 ± 0.89 (16)	1. 50 ± 0.50 (16)	.981	.882	**.002**[b]	**.017**[c]
17-α-OHP-HCG 24h	2.21 ± 0.98 (20)	1.87 ± 0.92 (20)	2.21 ± 0.98 (20)	1. 87 ± 0.92 (20)	.121	.537	.050	**.040**[c]
17-α-OHP-HCG 48h	1.47 ± 0.72 (20)	1.62 ± 0.69 (20)	2.12 ± 1.54 (16)	1. 55 ± 0.55 (16)	.105	.751	.515	.352
A2 (ng/ml)								
A2- HCG 0 h	5.66 ± 2.25 (22)	4.73 ± 1.87 (22)	6.57 ± 2.81 (13)	4.84 ± 1.79 (13)	.297	.861	**.019**[a]	.152
A2-HCG 24 h	5.41 ± 2.35 (20)	5.03 ± 1.89 (20)	6.03 ± 2.66 (16)	5.02 ± 2.31 (16)	.460	.986	.357	**.032**[c]
A2-HCG 48 h	3.43 ± 1.65 (20)	3.53 ± 2.06 (20)	3.58 ± 1.65 (16)	3.58 ± 2.00 (16)	.787	.934	.687	.996
DHEA (ng/ml)								
DHEA- HCG 0h	16.59±6.44 (21)	13.91±7.02 (21)	17.55±7.23 (16)	18.81±8.24 (16)	.673	.059	.210	.553
DHEA-HCG 24h	8.19 ± 3.27 (20)	5.82 ± 2.72 (20)	11.05 ±8.20 (16)	5.84 ± 2.18 (16)	.163	.982	**.010**[a]	**.004**[d]
DHEA-HCG 48h	8.00 ± 4.22 (20)	6.43 ± 2.72 (20)	7.23 ± 2.81 (16)	8.27 ± 6.44 (16)	.536	.255	.156	.877

[a]*P* <.05
[b]*P* <.01
[c]*P* <.05
[d]*P* <.01

Abbreviations: T, testosterone; 17-α-OHP, 17-α-hydoxy progesterone; A2, androstenedione; DHEA, dehydroepiandrosterone.

Table 4. Comparison of Biochemical Features Between the Two Groups

Outcome	Acupuncture Group		Control Group		Comparison Between Acupuncture And Sham Acupuncture Groups After 16-Week Treatment *P* value	Intragroup Comparison In Acupuncture Group *P* value	Intragroup Comparison In Sham Acupuncture Group *P* value
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment			
LH (mIU/ml)	10.3 ± 6.6 (24)	8.9 ± 5.2 (24)	14.6 ± 10.2(18)	7.9 ± 5.2 (18)	.618	.283	**.035**[a]
FSH (mIU/ml)	5.6 ± 1.8 (18)	6.0 ± 2.2 (18)	5.5± 1.3 (18)	6.2 ± 1.9 (18)	.615	.236	.238
LH/FSH ratio	1.9 ± 1.2 (18)	1.6±1.0 (18)	2.6± 1.6 (18)	1.2±0.7 (18)	.713	.106	**.004**[b]
P (ng/ml)	0.87 ± 0.55 (19)	0.57±0.25 (19)	0.92 ± 0.68 (13)	0.56 ± 0.28 (13)	.929	.08	.060
Total T(ng/dl)	0.60 ± 0.29 (25)	0.55 ± 0.23 (25)	0.64 ± 0.29 (18)	0.59 ± 0.28 (18)	.559	.178	.578
E_2 (pg/ml)	47.0 ± 30.0 (25)	48.4 ± 16.4 (25)	95.4±105.9 (17)	53.0 ± 30.6 (17)	.833	.833	.133
PRL (ng/dl)	14.9 ± 7.5 (26)	12.5 ± 5.1 (26)	15.8 ± 7.6 (18)	14.8 ± 8.1 (18)	.225	.053	.575

[a]*P* < .05
[b]*P* < .01

Abbreviations: P, Progesterone; T, testosterone; E, Estradiol; PRL, Prolactin.

Table 3); DHEA level 24 hours after HCG stimulation in both groups was significantly lower (*P* < .05; Table 3).

Secondary Outcome Measurement: Changes in Sex Hormone Level

After the treatment, there was no significant difference between the 2 groups in sex hormone levels (*P* > .05; Table 4). In within group comparison, there was no significant difference in the acupuncture group (*P* > .05; Table 4). Compared with the in-group comparison, after 16-week treatment, the LH level and LH/FSH ratio in the control group were significantly reduced (*P* < .05; Table 4).

Secondary Outcome Measurement: Changes in Biometric Features

After treatment, there was a significant difference between the 2 groups only in acne scores (*P* < .05) among all the biometric features (Table 5). In comparison, within the

Table 5. Comparison of Biometric Features Between the Two Groups

Outcome	Acupuncture Group		Control Group		Acupuncture and Sham Acupuncture Groups After 16-Week Treatment *P* value	Intragroup Comparison Acupuncture Group *P* value	Intragroup Comparison Sham Acupuncture Group *P* value
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment			
Menses during 4 months (times)	1.70 ± 0.92 (27)	1.96 ± 1.51 (27)	1.85 ± 0.86 (27)	1.98 ± 1.56 (27)	.986	.290	.689
Weight (kg)	59.8 ± 15.9 (26)	58.2 ± 15.6 (26)	54.6± 8.6 (20)	54.1 ± 8.0 (20)	.557	**.001**[a]	.335
BMI (kg/m^2)[b]	22.8 ± 5.5 (26)	22.2 ± 5.4 (26)	21.4± 3.6 (20)	21.2 ± 3.4 (20)	.773	**.001**[a]	.333
Waistline (cm)	78.3 ± 14.5 (26)	76.4 ± 12.1 (26)	73.9 ± 10.0 (20)	73.8 ± 11.7 (20)	.357	.521	.894
Hipline (cm)	95.0 ± 10.1 (26)	94.8 ± 10.2 (26)	91.1 ± 8.6 (20)	91.4 ± 8.8 (20)	.246	**.034**[c]	.720
WHR	0.82 ± 0.08 (26)	0.81 ± 0.07 (26)	0.81 ± 0.07 (20)	0.81 ± 0.07 (20)	1.0	.050	.610
Ferriman-Gallwey score[d]	2.7 ± 3.3 (26)	2.1 ± 2.6 (26)	2.6 ± 2.7 (20)	2.2 ± 2.7 (20)	.747	**.007**[a]	.528
Acne score	0.27 ± 0.60 (26)	0.31 ± 0.55 (26)	0.60 ± 0.82 (20)	0.70 ± 0.73 (20)	.03[e]	.655	.755
Acanthosis nigricans score	0.04 ± 0.20 (26)	0.04 ± 0.20 (26)	0 ± 0 (20)	0 ± 0 (20)	.380	1.00	1.00
Prolactin (ng/dl)	14.9 ± 7.5 (26)	12.5 ± 5.1 (26)	15.8 ± 7.6 (18)	14.8 ± 8.1 (18)	.225	.053	.575

Note: Each value represents mean ± standard deviation (n).

[a]$P < .01$
[b]Body-mass index is the weight in kilograms divided by the square of the height in meters.
[c]$P < .05$
[d]Scores on the modified Ferriman-Gallwey scale for hirsutism range from 0 to 44, with higher scores indicating a greater degree of hirsutism.
[e]$P < .05$

Abbreviations: BMI, body mass index; WHR, waist-hip-ratio.

Table 6. Comparison of Deviation Events Between the Two Groups

Parameter	Acupuncture Group (n = 27)	Control Group (n = 27)	Total	*P* value
Serious Deviation Event				
Being pregnant	1/27 (3.7%)	1/27 (3.7%)	2/54 (3.7%)	1.00
Other Deviation Events				
Termination of treatment >2 weeks	3/27 (11.1%)	4/27 (14.8%)	7/54 (13.0%)	.724
Drug combination	3/27 (11.1%)	5/27 (18.5%)	8/54 (14.8%)	.513
Lack of required laboratory tests	7/26 (26.9%)	6/20 (30.0%)	13/46 (28.3%)	.864
Treatment <32 times (except for follow-up patients)	1/26 (3.8%)	0/20 (0%)	1/46 (2.2%)	.389

Note: Each value represents absolute number (percentage).

Table 7. Comparison of Adverse Events Between the Two Groups

Parameter	Acupuncture Group (n = 27)	Control Group (n = 27)	Total	*P* value
Serious adverse events				
Hyperplasia of mammary glands	0/27 (0%)	1/27 (3.7%)	1/54 (1.9%)	.326
Adipoma	1/27 (3.7%)	0/27 (0%)	1/54 (1.9%)	.326
Other adverse events				
Feeling of numbness	2/27 (7.4%)	0/27 (0%)	2/54 (3.7%)	.168
Headache	1/27 (3.7%)	0/27 (0%)	1/54 (1.9%)	.326
Bruise at acupuncture site	1/27 (3.7%)	1/27 (3.7%)	2/54 (3.7%)	1.00
Itch at acupuncture site	1/27 (0%)	0/27 (3.7%)	1/54 (1.9%)	.326
Swelling around the navel during treatment	1/27 (0%)	0/27 (3.7%)	1/54 (1.9%)	.326
Cough	0/27 (0%)	1/27 (3.7%)	1/54 (1.9%)	.326
All adverse events	7/27(25.9%)	3/27(11.1%)	10/54(18.5%)	.759

Note: Each value represents absolute number (percentage).

group, after 16 weeks of treatment, weight, BMI, hipline and Ferriman-Gallwey score in the active group were significantly reduced ($P < .05$; Table 5). In the within-group comparison in the control group there was no significant difference ($P > .05$; Table 5).

Occurrence of Deviation and Adverse Events

The serious deviation event was accidental pregnancy, and there was one in both the acupuncture and control groups. Other deviation events included termination of treatment after more than 2 weeks, drug combinations, lack of required laboratory tests and treatment received less than 32 times. There was no significant difference in either serious deviation event or other deviation events between the 2 groups ($P > .05$; Table 6).

Adverse Events

There were 2 serious adverse events reported. Hyperplasia of the mammary glands was reported in 1 patient in the control group and 1 case of adipoma was reported in the active group. Other adverse events included a feeling of numbness, headache, bruise at the acupuncture site, itch at acupuncture site, swelling around the navel during treatment and cough. There was no significant difference in either serious adverse events or other adverse events between the 2 groups ($P > .05$; Table 7).

DISCUSSION

Since 1992,[10] clinical reports of acupuncture treatment in PCOS have gradually increased year after year. Currently, more than 100 clinical trials have reported the efficacy of acupuncture in treating PCOS, which is reported to manifest by inducing ovulation, weight loss, reducing androgens, adjusting the menstrual cycle and so on. However, recent meta-analyses[11,12] have shown that there were only a limited number of well-designed randomized controlled trials (RCTs) and there was insufficient evidence to support the use of acupuncture in women with PCOS. Furthermore, 2 other studies[5,13] reported that the effect of acupuncture was equal to the effect of sham acupuncture. Different populations, observation indicators, acupuncture design comfort, sample numbers and other factors led to the heterogeneity of these conclusions. Our study is an RCT aimed at evaluating the efficacy of EA in unmarried women with PCOS who had no desire to become pregnant. For them, it would be much more urgent to regulate their abnormal endocrine systems, such as in hyperandrogenism, and to resolve problems with irregular menstruation. Therefore, our research focused on observing the influence of acupuncture on endocrine conditions, especially androgen secretion, in these patients.

Androgens

As we all know, hyperandrogen is an important factor in the pathogenesis of PCOS, and is also one of the important factors required for the diagnosis of PCOS. According to the recognized 2003 Rotterdam standard,[14] any kind of hyperandrogen can be used for the diagnosis of PCOS. However, not every patient with PCOS in the clinic shows hyperandrogenemia. There are 5 main types of androgens in women: DHEAs, DHEA, A2, T, and dihydrotestosterone (DHT).[15] Of these, the first 3 types of androgens must be converted into T or DHT in the target organs and then combined with the androgen receptor (AR) to exert their androgen effects.[15] T is the most commonly used in the clinic. 25% of T comes from the adrenal tract, 25% comes from the direct synthesis of ovarian stroma and 50% comes from the transformation of A2 in the circulation through tissues other than the adrenal glands (such as the ovaries).[16] In addition to T, this study selected 17-α-OHP, A2 and DHEA as the main efficacy indicators. According to the classical theory of sex hormone synthesis, 17-α-OHP, A2, DHEA and T are important steroids; pregnenolone derived from cholesterol can synthesize DHEA and then A2 via the delta 5 pathway; pregnenolone can also synthesize 17-α-OHP and then A2 via the delta 4 pathway; A2 and T can transform each other, and T can further synthesize estrogen. Before ovulation, the ovaries mainly synthesize estrogen through the delta 5 pathway, while after ovulation the ovaries mainly synthesize estrogen through both the delta 5 and delta 4 pathways.

Unlike in other studies, the HCG stimulation test that is often used in the diagnosis of male infertility was used in this study. HCG is essential and administration of LH can promote the synthesis of androgen in the follicle membrane cells via various pathways such as steroid acute regulatory protein (StAR), cytochrome P450, 17α-hydroxylase (CYP17A1), etc.[17] Previous studies have shown that both basal and HCG-stimulated serum levels of A2 and T were increased by approximately 50% in women with PCOS compared with healthy women.[18] In this study, the HCG stimulation test was considered an auxiliary way to amplify the effect of EA in a limited patient sample. The results also showed that both T and 17-α-OHP increased by 27.8% 24 hours after HCG stimulation, and decreased to the pre-HCG stimulation 48 hours later. However, the levels of A2 did not change significantly after HCG stimulation at 24 hours but decreased 40.2% at 48 hours. In addition, the DHEA level decreased significantly (45.3%) at 24 hours and 51.5% at 48 hours after HCG stimulation. We speculated that the possible reason was that HCG first stimulated the delta 5 pathway to promote T synthesis 0 to 24 hours after stimulation, resulting in an increase in T at 24 hours, and then it mainly stimulated the delta 4 pathway to promote 17-α-OHP synthesis of T and the transformation of T to E_2 during 24 to 48 hours, resulting in a decrease of 17-α-OHP, A2 and T at 48 hours. It is a pity that we did not measure estrogen levels at the same time, or we would have a more comprehensive understanding of the hormonal transition in PCOS.

EA can treat hyperandrogen caused by PCOS, which has been confirmed in many previous studies. In 2010, Stener Victorin, et al reported that after 16 weeks of EA intervention, circulating T decreased by 25%, androsterone glucuronide by 30%, and androstane-3α,17β-diol-3-glucuronide by 28%

compared with physical exercise, but there was no sham acupuncture design. Animal studies have shown that EA could increase the expression of P450arom in ovarian granulosa cell layers and inhibit the expression of p450c17a in follicular membrane cell layer of PCOS rats, so as to promote the normal transformation of androgen into estrogen and improve the local endocrine environment disorder of PCOS ovary.[19] In addition, another study in rats showed that EA could reduce the level of T in peripheral blood, and the mechanism may be through the decrease of opioid receptor in hypothalamus μ and κ.[20]

Main Outcome Measurement

In terms of the main outcome measurements of this study, there was no significant difference between the 2 groups after treatment, even in T and 17-α-OHP levels of HCG stimulation for 24 hours. However, in the intra-group comparison, EA or sham acupuncture showed different improvement effects on androgen, except for the abnormal increase of T at 24 hours in the active group. The 17-α-OHP level at 0 hours of HCG stimulation and DHEA level at 24 hours decreased significantly in both groups. In addition, sham-acupuncture could significantly reduce the 17-α-OHP level 24 hours after HCG stimulation, and EA could also reduce it, but unfortunately there was no statistical significance ($P = .05$). EA could decrease the A2 level at 0 hours of HCG stimulation, while sham-acupuncture could decrease the A2 level 24 hours after HCG stimulation. According to the current literature, we cannot explain the abnormal increase of T 24 hours after HCG stimulation in the EA group. We speculate that the reason may be related to the heterogeneity of androgen. Although there is no difference in the comparison between groups, acupuncture and sham acupuncture can regulate different androgen levels in the intragroup comparison. The results indicate that sham acupuncture may have a certain effect in reducing androgen and it may be different from EA, which may also be one of the important reasons for our negative results in the intergroup comparison. Other reasons may include androgen heterogeneity of patients with PCOS or insufficient sample size.

We must mention our sham acupuncture design. In our trial, we used the same acupuncture and sham acupuncture protocols as Wu Xiaoke's team,[7,21] who carried out a 2 by 2 factorial well-designed randomized clinical trial with a large sample that evaluated the efficacy of acupuncture combined with or without clomiphene citrate in live births in married women with PCOS. The researchers also found that acupuncture and sham acupuncture had a similar effect on androgen secretion. We believe that our sham acupuncture design is a reasonable comfort design. Moreover, in the process of treatment, there is no event of breaking blindness. Therefore, we believe that in some ways this suggests that the specific mechanism of EA and sham acupuncture in treating PCOS may be different. Although EA showed a similar "placebo" comfort effect, it may also reduce the effect of androgen via an unknown mechanism.

In the analysis of biometric and biochemical features of the patient, we can also see the differences between the EA and sham acupuncture groups. Although there was no significant difference between the 2 groups, the effects of EA in reducing body weight, BMI, hipline and Ferriman-Gallwey score was obvious as was sham acupuncture in decreasing the LH level and LH/FSH ratio, which may also indicate different androgen-inhibiting mechanisms. All in all, our study shows that traditional EA is slightly better than sham acupuncture in reducing androgen levels, but sham acupuncture is not completely ineffective, and the specific mechanism of the two may be different.

Safety Analysis

In terms of safety analysis, the EA group and sham acupuncture group both reported 1 serious adverse event, but they had no obvious logical causal relationship with acupuncture. All the adverse events were mild and included a feeling of numbness, headache, bruise at the acupuncture site, itch at the acupuncture site, swelling around the navel during treatment and cough. There was no significant difference in the incidence of adverse events between the 2 groups. The overall incidence of adverse events was 18.5%. In general, acupuncture has a good safety profile.

This study focused on unmarried women with PCOS and also studied the effect of acupuncture on reducing androgens. As a fairly large group, a considerable number of unmarried patients with PCOS did not seek medical attention or were ignored by doctors because of no fertility requirements. This study primarily investigated the effect of acupuncture on reducing androgens in unmarried women with PCOS and we hope that more studies will focus on unmarried women with PCOS.

CONCLUSION

Our study demonstrated that traditional EA is slightly better than sham acupuncture in reducing DHEA secretion and acne score. Sham acupuncture is not completely ineffective. After treatment, the androgen in both groups could be reduced, however, their influences on biometric and biochemical characteristics are totally different. Active EA tends to reduce body weight, BMI, hipline and Ferriman-Gallwey score, whereas control acupuncture tends to decrease LH level and LH/FSH ratio. Therefore, on the one hand, the effect of control/sham acupuncture could not be neglected, but on the other hand, the effect of control/sham acupuncture could not completely take the place of active acupuncture.

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