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TRANSGENDER HEALTH

Anti-Androgenic Effects Comparison Between Cyproterone Acetate and Spironolactone in Transgender Women: A Randomized Controlled Trial

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ABSTRACT

Background: Spironolactone and cyproterone acetate are commonly used in feminizing hormone therapy to achieve the goal of female range testosterone level; however, the data on the efficacy comparing between these two anti-androgens are scarce.

Aim: To compare the anti-androgenic effects between spironolactone and cyproterone acetate as the component of feminizing hormone therapy among transgender women population.

Methods: The study was single-blinded randomized controlled trial involved 52 transgender women from two transgender health clinics. Each participant received oral estradiol valerate 4 mg/day combined with anti-androgen, spironolactone 100 mg/day or cyproterone acetate 25 mg/day, depending on which group they were randomized to. Clinical and biochemical variables were obtained at baseline and at 12 weeks of feminizing hormone therapy.

Main Outcome Measures: The change of testosterone level from baseline. Other changes including free testosterone, estradiol, prolactin and lipid profile after the therapy.

Results: After a 12 weeks of feminizing hormone therapy, the change of testosterone level in the cyproterone acetate group [558.0 ng/dL (IQR 352.0 to 783.3)] was significantly higher than the spironolactone group [226.2 ng/dL (IQR,-4.3 to 480.1)](p value <0.001). Testosterone and calculated free testosterone in the cyproterone acetate group were significantly lower than the spironolactone group. Consequently, a proportion of the participants who achieved the female range testosterone (<50 ng/dL) was significantly higher in cyproterone acetate group (90%) compared to the spironolactone group (19%). Serious adverse effects observed in cyproterone acetate users were drug-induced liver injury and asymptomatic hyperprolactinemia.

Clinical Implications: The data on the differences between the two anti-androgen could be benefit for the transgender health-care providers in medication selection and adverse-effects counseling.

Strengths & Limitations: The study design was randomized controlled trial and controlled the estrogen component by prescribed the same type and dose for each participant. However, the study was suffered from the confound feminizing effects from previous hormone therapy and the high drop-out rate.

Conclusion: For feminizing hormone therapy, cyproterone acetate had a higher testosterone suppression efficacy than spironolactone. **Burinkul S, Panyakhamlerd K, Suwan A, et al. Anti-Andorgenic Effects Comparison Between Cyproterone Acetate and Spironolactone in Transgender Women: A Randomized Controlled Trial. J Sex Med 2021;18:1299–1307.**

Key Words: Gender dysphoria; Androgen antagonists; Transgender persons; Spironolactone; Cyproterone acetate; Feminizing hormone therapy

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INTRODUCTION

Transgender people define as individuals whose gender identity and/or expression differs from their birth-assigned gender.[1] Despite the improvement in social acceptance, many transgender people still have experienced prejudice and discrimination which can hinder the accessibility of health care services.[2] To deliver a comprehensive transgender health care, a multidisciplinary team approach should be implemented involving primary care provider, psychiatrist, reproductive endocrinologist and plastic surgeon.[3,4] Gender-affirming hormone therapy is one of the cornerstone therapies for gender affirmation. In transgender women, assigned male at birth (AMAB) transgender people, estrogen is typically used as feminizing hormone therapy. Estrogen has an anti-androgenic property by reducing the endogenous testosterone via negative feed-back on hypothalamus-pituitary levels.[5] After estrogen monotherapy, most of the transgender women still have the level of testosterone above the physiologic female ranges.[6] Anti-androgens are usually needed to achieve testosterone levels in the female range, if full de-virilization is required.[7,8,9]

According to World Professional Association for Transgender Health and Endocrine Society guidelines, anti-androgens are recommended as an adjunctive medication in transgender women who had not undergone gender-affirming surgery.[1,8] The commonly used anti-androgens are gonadotropin-releasing hormone agonist, spironolactone and cyproterone acetate. While gonadotropin-releasing hormone agonist is highly effective in testosterone suppression and particularly used for pubertal suppression in adolescents with gender dysphoria,[10,11] administration route and high cost of the medication limits its use in many countries including Thailand.

Spironolactone is categorized as a potassium-sparing diuretic due to aldosterone antagonism, and used primarily to treat hypertension.[12] Mechanisms underlying the anti-androgenic effects of spironolactone are androgen receptor antagonism and inhibition of the enzyme in the testosterone biosynthesis pathway, cytochrome P450 content, which results in testosterone reduction.[5,13,14] Adverse effects associated with spironolactone are hypotension, hyperkalemia and increased urinary frequency. However, the adverse effects are uncommon in transgender women population.[15-17]

Cyproterone acetate, an anti-androgenic progestin compound, is used in the treatment of androgen-dependent condition such as prostate cancer, hirsutism and also for feminizing hormone therapy.[8,18] Cyproterone acetate exerts anti-androgenic effect by inhibiting luteinizing hormone (LH) secretion along with androgen receptor antagonism which suppresses both testosterone action and production. Adverse effects of cyproterone acetate are venous thromboembolism, hyperprolactinemia, elevated liver enzymes and rarely, the risk of developing meningioma.[19,20] Despite its high potency, the United States FDA has not approved the medication due to reported cases of severe hepatotoxicity.[21]

Many factors influence the selection of anti-androgens: efficacy, side effects, availability of medication, patient and care provider preferences. To date, there are scarce data on the efficacy comparing between these 2 anti-androgens and most of the data are based on reports from previous retrospective studies.[22-25] In this present study, we aimed to compare the anti-androgenic effects including testosterone suppression efficacy and decrease of clinical masculinization between spironolactone and cyproterone acetate as the component in feminizing hormone therapy among transgender women population.

METHODS

The study protocol had been registered in Thai Clinical Trial Registry and was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

Study Populations

Transgender women who attended to the "Climacteric and Gender health clinic," King Chulalongkorn Memorial Hospital and "Tangerine community health center," Thai Red Cross AIDS Research Center, were approached. Information pertaining to the study were provided to transgender women who desired to receive gender-affirming hormone therapy. Written informed consent was obtained from the individuals who interested to participate in the study.

Transgender women aged 18−40 years, had not undergone orchidectomy or gender-affirming surgery and had no underlying psychiatric illness or other neurological condition, were enrolled into the study. Participants were excluded if they had anti-androgen washout period less than 3 months, had underlying disease associated with higher risk of adverse effects from hormone therapy included venous thromboembolism, stroke, uncontrolled hypertension, diabetes, liver and renal disease. We also excluded participants who had abnormal laboratory results at enrollment included female range testosterone level at enrollment (< 50 ng/dL), serum transaminitis (2-fold rising of liver enzymes), glomerular filtration rate < 30 mL/min, hyperkalemia (serum potassium >5.0 nmol/L) and hyperprolactinemia (prolactin > 25ng/ml).

During the study period, the participants were withdrawn from the study if they had poor medication adherence (less than 80%), developed serious adverse effects, or had abnormal safety laboratory parameters as mentioned above.

Randomization and Blinding

The study was single-blinded randomized controlled trial. Computer generated blocked-of-four randomization was used to allocate participants into 2 study groups (1:1 ratio). Medication and allocation number were prepared and contained in an opaque envelop by the research nurse and stored in the hospital pharmacy. After enrollment, each participant received a study identification number which was used for prescription by the research pharmacist. Both research nurse and pharmacist were not involved in the evaluation and outcome measurements. The

investigators, laboratory staffs and statisticians were blinded to the treatment arm of the participants. However, the participants were aware of the allocated arm because of the differences in the feminizing hormone regimens.

Interventions

Screening and Baseline Visit. All transgender women who visited either of the two transgender health clinics were invited to participate in the study. The study protocol, effect of feminizing hormone and possible adverse effects were informed and written inform consents were obtained from interested individuals. Participants who met the inclusion criteria were sent to confirm gender dysphoria diagnosis and assess possible psychiatric co-morbidities by the psychiatrist. Serum samples were collected and measured for baseline blood chemistry and hormones. The eligible participants were then scheduled for their next visit to receive the study identification number and feminizing hormone therapy.

Laboratory Assessment. Blood chemistry and hormones levels were collected at baseline and at the end of the study: serum creatinine, serum potassium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), high-density lipoprotein (HDL), total cholesterol, albumin, sex-hormone binding globulin (SHBG), testosterone, estradiol and prolactin. Serum samples were immediately processed after collection to prevent hemolysis and carried out in a research central laboratory. Serum testosterone and estradiol were measured using electrochemiluminescence immunoassay (Elecys; Roche Diagnostics Thailand). The inter-assay coefficient of variation (CV) for testosterone was 3.2% and estradiol was 4.7%. Prolactin was measured using chemiluminescent microparticle immunoassay (Architect; Abbott Thailand) with inter-assay CV of 3.3%. Laboratory serum limit of quantitation was 2.5 ng/dL for testosterone, 5.0 pg/mL for estradiol and 0.6 ng/ml for prolactin.

Feminizing Hormone Therapy. Combined estrogen and anti-androgen were adopted in our study. Each participant received equal dose of oral estrogen, 4 mg of estradiol valerate (Progynova; Bayer, Germany). For anti-androgen, spironolactone 100 mg/day (Aldactone; Pfizer, UK) or cyproterone acetate 25 mg/day (Androcur; Bayer, Germany) was given to the participants according to their assigned arm. After allocation, the participants were scheduled for the return-visit at week 4 and week 12 to assess for adverse effects and outcome measurements. Serum potassium level and liver enzymes were measured at every follow-up visit to detect hyperkalemia and hepatotoxicity.[21,26] The adherence to the medications was performed by the pharmacist using the pill count method.

Outcomes Measurement

The primary outcome of the study was the change in testosterone level after 12 weeks of feminizing hormone therapy between two study groups. Change of testosterone level was calculated; the pre-treatment level was subtracted by the post-treatment level. Secondary outcomes were calculated free testosterone,[27] proportion of participants that achieved female range testosterone level, estradiol, prolactin and HDL level.[22,28,29] Decreased penile erection was reported to occur shortly after feminizing hormone therapy was initiated.[8] Thus, frequency of morning erection (times/week) was also included as one of the secondary outcomes. If the measured hormones were less than the limit of quantitation, the value of limit was input for statistical analysis.

Sample Size Calculation and Statistical Analysis

Sample size was calculated by differences of means formulation using changes of testosterone level from previous study as references.[17,24] In total, for 90% power and 5% type 1 error, 23 participants were required for each group to detect the difference of serum testosterone change between spironolactone and cyproterone acetate users. After adjusting for 10% dropout rate, total sample size needed for the study was 52 participants.

The descriptive statistics were expressed as frequency, percentage, mean with standard deviation (SD) or median with inter-quartiles range (IQR). Chi-square, independent *t*-test or Mann-Whitney *U* test was used to compare the outcomes between the 2 study groups. The kolmogorov-smirnoff test was also applied to verify the normality of the data. Both per-protocol and intention-to-treat analysis were performed for primary outcome comparison. In the intention-to-treat analysis, last observation carried forward method was used to impute the missing data. The statistical significance was presented as *P* value, 2-sided and considered significant at less than .05. All of the data analysis was performed using IBM SPSS statistic version 22.0 software.

RESULTS

Participants

From June 2019 – February 2020, a total of 167 transgender women were invited to participate in the study. Eighty-three participants met the inclusion criteria. Fifty-two participants were enrolled into the study and underwent randomization into two study groups (26 participants for each group). The number of participants who completed the study was 21 and 20 for spironolactone group and cyproterone acetate group, respectively, which were included in the per-protocol analysis. (Figure 1) Most of the participants, 46 out of 52, had a history of prior hormone use, all of which were self-medicated. The previous hormone therapies were classified into 4 categories included estrogen alone, estrogen plus anti-androgen, combined oral contraceptive pills and custom compound hormone. While there were various types and routes of estrogen (oral estradiol, transdermal estradiol patch/gel, parenteral estradiol), cyproterone acetate was the only anti-androgen used by the participants. All of the baseline clinical

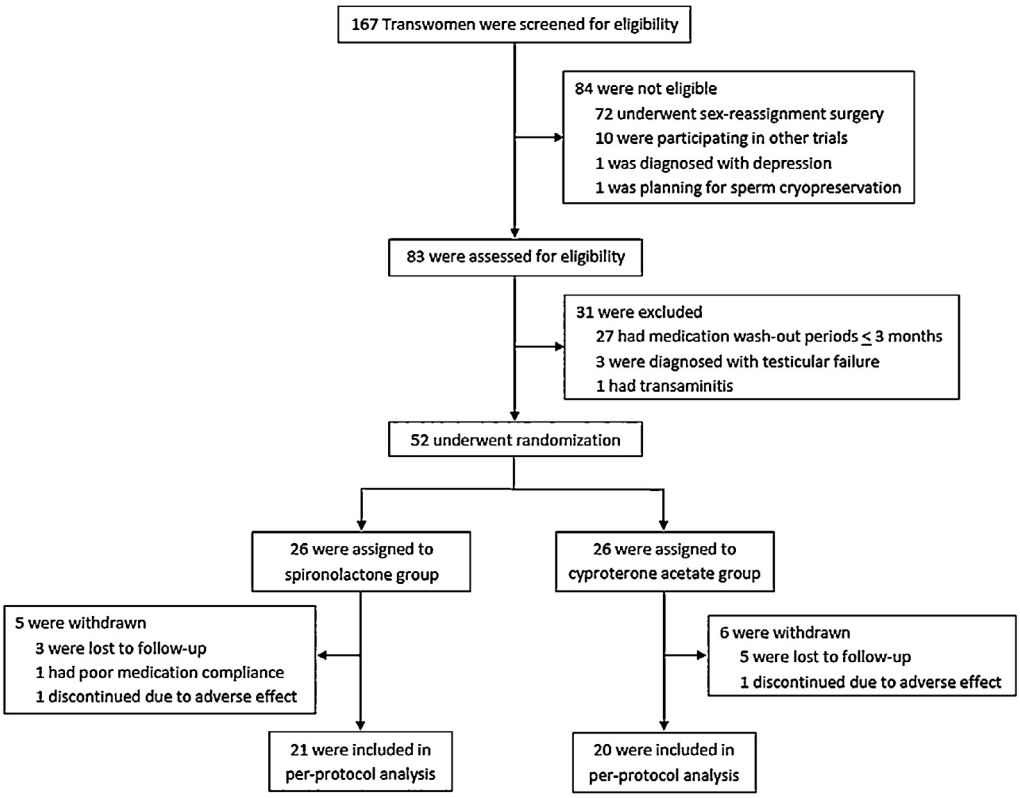

Figure 1. Study flow.

and biochemical characteristics were similar between the 2 study groups. (Table 1)

Change of Testosterone, Clinical and Biochemical Variables After Feminizing Hormone Therapy

Baseline median testosterone level was 645.0 ng/dL (IQR, 466.7−1027.7) in the spironolactone group and 655.5 ng/dL (IQR, 402.6−872.7) in the cyproterone acetate group. Both were within the male range[30] and similar between the 2 study groups. After 12 weeks of feminizing hormone therapy, change of testosterone level in the cyproterone acetate group [558.0 ng/dL (IQR, 352.0−783.3)] was significantly higher than the spironolactone group [226.2 ng/dL (IQR, -4.3 to 480.1)] (P value < .001). The difference remained statistically significant even after intention-to-treat analysis (P value = .04). Additionally, after 12 weeks of treatment, testosterone and calculated free testosterone in the cyproterone acetate group were significantly lower than the spironolactone group. Consequently, the proportion of the participants who achieved the female range testosterone level (< 50 ng/dL) was significantly higher in the cyproterone acetate group (90%) compared to the spironolactone group (19%). Baseline and post-treatment testosterone levels for both study groups are shown in Figures 2 and 3.

After 12 weeks of therapy, there were no significant differences in the mean arterial pressure, frequency of morning erection and estradiol level between the two study groups. Spironolactone users showed significantly higher HDL level than the cyproterone acetate users while prolactin level was significantly lower compared to the cyproterone acetate users.

Adverse Effects

Loss of libido was the most common adverse effects detected in both study groups. The other observed adverse effects were breast tenderness, myalgia and increased urinary frequency, all of which were well-tolerated by the participants. (Table 3) Hyperprolactinemia was diagnosed in one participant in the cyproterone acetate group at the end of the study. Even though her prolactin level was 135.4 ng/mL, she had no symptoms and refused further evaluation.

However, some serious adverse effects were observed and 2 participants had to withdraw from the study. One participant from the spironolactone group developed urticarial rash, after 2 weeks of feminizing hormone therapy, which recovered after cessation of medication. Severe transaminitis was observed in one participant from the cyproterone acetate group. The participant had 10-fold increase of liver enzymes (AST and ALT) detected from safety laboratory assessment at week 4 return-visit. Further investigation and evaluation by gastroenterologist revealed undiagnosed hepatitis B infection. After being withdrawn from the

Table 1. Baseline characteristics of the participants

Baseline variables	Spironolactone Group (N = 26)	Cyproterone Group (N = 26)
Age — y		
— Mean ± SD	25.38 ± 5.4	26.58 ± 5.4
Education — n (%)		
— High school	11 (42%)	5 (19%)
— Bachelor degree	15 (58%)	20 (77%)
— Master degree	0	1 (4%)
Social transition age — y		
— Median (IQR)	18 (16 – 18)	18 (16 – 18)
Previous feminizing hormone regimen — n (%)		
— Hormone-naïve	4 (15%)	2 (8%)
— Estrogen alone	1 (4%)	1 (4%)
— Estrogen plus anti-androgen	14 (54%)	19 (73%)
— Combined oral contraceptive pills	6 (23%)	4 (15%)
— Custom compound hormone	1 (4%)	0
Frequency of morning erection — times/wk		
— Median (IQR)	3.5 (3.0 – 6.0)	3.5 (2.0 – 7.0)
Body mass index — kg/m^2		
— Median (IQR)	21.0 (19.3 – 22.7)	21.3 (19.8 - 23.1)
Mean arterial pressure — mm Hg		
— Mean ± SD	90.3 ± 7.3	93.6 ± 7.0
Testosterone — ng/dL		
— Median (IQR)	645.0 (466.7 – 1027.7)	655.5 (402.6 – 872.7)
Free testosterone — ng/dL*		
— Median (IQR)	11.6 (8.9 – 13.9)	10.4 (8.1 – 13.6)
Estradiol level — pg/mL		
— Mean ± SD	33.3 ± 12.1	32.0 ± 15.6
Sex-hormone binding globulin — nmol/L		
— Median (IQR)	46.2 (32.5 – 68.7)	47.2 (31.9 – 63.7)
Prolactin — ng/mL		
— Median (IQR)	10.6 (7.4 – 15.0)	11.2 (8.3 – 17.6)
High-density lipoprotein — mg/dL		
— Mean ± SD	53.1 ± 10.8	50.4 ± 10.5

IQR = Interquartile range; SD = Standard deviation.
The body-mass index is the weight in kilograms divided by the square of the height in meters.
There were no significant differences of the baseline hormone and lipid profiles between the 2 study groups.
*Free testosterone level was calculated based on testosterone level, SHBG and albumin level.[26]

study, the participant's liver enzymes spontaneously returned to the baseline level without any treatment which suggested diagnosis of drug-induced liver injury. For further addressing serious adverse effects, all participants who lost to follow-up were contacted and inquired about the health status and possible adverse effects by phone call.

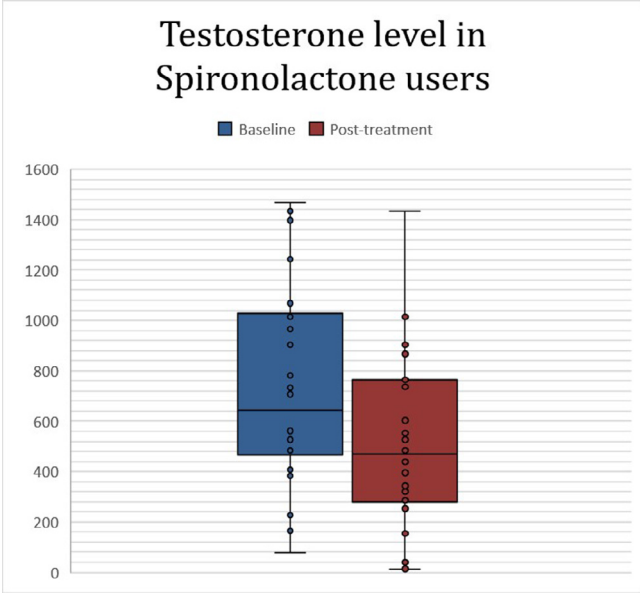

Figure 2. Baseline and post-treatment testosterone levels in the spironolactone group (intention-to-treat analysis).

DISCUSSION

To our knowledge, this was the first RCT designed to illustrate the differences between 2 antiandrogenic agents, spironolactone and cyproterone acetate, as a part of feminizing hormone therapy in transgender women. The results showed that after a 12-week of therapy, the cyproterone

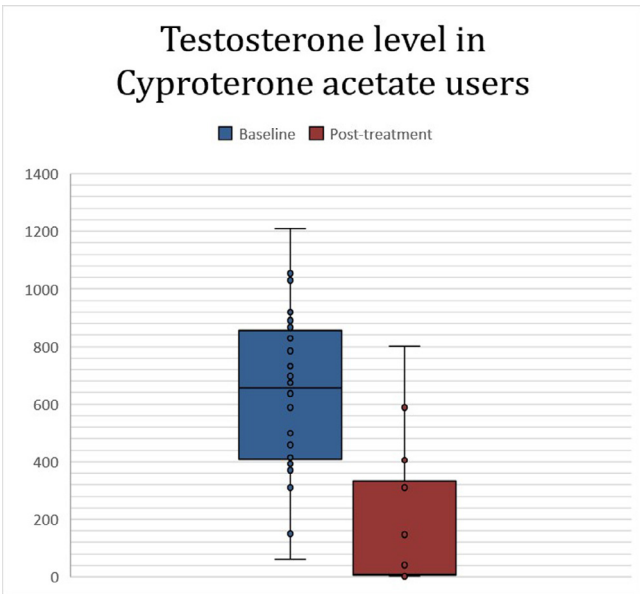

Figure 3. Baseline and post-treatment testosterone levels in the cyproterone acetate group (intention-to-treat analysis).

Table 2. The participants' clinical and biochemical characteristics after 12 weeks of feminizing hormone therapy

Variables	Per protocol analysis			Intention-to-treat analysis*		
	Spironolactone Group (n = 21)	Cyproterone Group (n = 20)	*P* value+	Spironolactone Group (n = 26)	Cyproterone Group (n = 26)	*P* value+
Mean arterial pressure – mm Hg						
– Mean ± SD	85.0 ± 8.6	86.9 ± 5.7	0.40	86.0 + 8.4	87.9 ± 5.6	0.33
Morning erection – times/week						
– Median (IQR)	1 (0 – 1)	0 (0 – 1)	0.43	1 (0 - 2)	1 (0 - 1)	0.37
Testosterone – ng/dL						
– Median (IQR)	410.4 (203.9 – 673.6)	7.4 (5.4 – 13.1)	< 0.001	468.3 (287.0 – 765.4)	9.3 (5.5 – 310.4)	< 0.001
Change from baseline – ng/dL						
– Median (IQR)	226.2 (-4.3 – 480.1)	558.0 (352.0 – 783.3)	< 0.001	48.9 (0 – 468.6)	415.5 (0 – 729.5)	0.04
Achieved female range – n(%)						
(< 50ng/dL)	4 (19%)	18 (90%)	< 0.001[a]	4 (15%)	18 (69%)	< 0.001[a]
Free testosterone – ng/dL†						
– Median (IQR)	6.4 (3.1 – 9.2)	0.14 (0.06 – 0.29)	< 0.001	6.9 (3.7 – 10.1)	0.17 (0.10 – 6.40)	0.002
Estradiol – pg/mL						
– Median (IQR)	72.4 (52.4 – 85.3)	52.7 (36.2 – 72.5)	0.07	64.0 (39.2 – 82.7)	46.8 (35.9 – 71.7)	0.1
SHBG – nmol/L						
– Median (IQR)	48.8 (40.8 – 71.1)	45.9 (35.7 – 73.4)	0.51	55.8 (42.6 – 77.8)	48.0 (38.2 – 74.8)	0.44
Prolactin – ng/mL						
– Median (IQR)	10.4 (9.1 – 13.6)	21.5 (17.2 – 33.2)	< 0.001	10.4 (8.9 – 12.6)	19.4 (11.9 – 28.4)	0.002
HDL – mg/dL						
– Mean ± SD	57.2 ± 12.1	48.5 ± 9.7	0.01	56.4 ± 11.3	49.2 ± 9.9	0.02

IQR = Interquartile range; SD = Standard deviation.
a; calculated by chi-square test / + *P* value < .05 considered statistical significance.
*Last observation carried forward method was used to impute data for 11 excluded participants.
†Free testosterone level was calculated based on testosterone level, SHBG and albumin level.[26]

Table 3. Adverse effects of the feminizing hormone therapy

Adverse effects, n(%)	Spironolactone Group (N = 21)	Cyproterone Group (N = 20)
Loss of libido	10 (47%)	13 (65%)
Breast tenderness	4 (19%)	1 (5%)
Myalgia	1 (4.3%)	6 (30%)
Urticarial rash*	1 (4.3%)	0
Hyperprolactinemia	0	1 (5%)
Specific to spironolactone		
– Increased urinary frequency	4 (19%)	
– Hyperkalemia / Hypotension	0	
Specific to cyproterone acetate		
– Transaminitis*		1 (5%)

*Participants were withdrawn from the study.

acetate users had a more robust change in the testosterone level compared to the spironolactone users. This result indicated that cyproterone acetate had a higher testosterone suppression efficacy than spironolactone.

Anti-androgen is an essential component of feminizing hormone therapy. Co-administration of estrogen and anti-androgen has shown to be effective in suppressing the testosterone level to a female range, reduced male phenotypes, induced feminization and improved psychological health.[6,8,31] After gender-affirming surgery, antiandrogen will no longer be required and estrogen monotherapy can be used. However, there are many barriers between transgender women and the surgical treatments including lack of trustworthy surgeon, high cost of the surgery and additional post-operative care of the neovagina.[32,33] Effective and safe antiandrogen is still needed for long-term treatment in transgender women who consider living without gender-affirming surgery.

Spironolactone is an anti-androgen widely prescribed in the United States where cyproterone acetate is not available. Evidence regarding testosterone suppression efficacy of spironolactone are conflicting in many literatures.[5,7,17] In this study, the change of testosterone levels were small among spironolactone compared to the cyproterone acetate users and only 19% were able to achieve the female range testosterone level. Interestingly, post-treatment testosterone level increased in some of the spironolactone users (6 out of 21). This can be explained by the mechanism of spironolactone which acts as an androgen receptor antagonist at the hypothalamus. This effect may drive pituitary LH secretion and in turn stimulate testicular testosterone production as a compensation mechanism.[13] Although, we could not prove this hypothesis because LH level was not determined in our study. This phenomenon may be associated with low dose

of spironolactone used in our study, thus it has never been reported before in previous study which used higher dose of spironolactone.

Cyproterone acetate is mostly used in Europe and Asia, including Thailand. The main action of cyproterone acetate is maintaining the negative feedback on the hypothalamus which results in decreasing levels of LH and testicular testosterone. The testosterone suppression efficacy of cyproterone acetate is well-established in many studies, especially when used at high doses (50 mg/day).[24,25,34] Our result was consistent with previous studies[23] which demonstrated higher testosterone suppression efficacy of cyproterone acetate compared to spironolactone. Consequently, a higher number of cyproterone acetate users achieved the female range testosterone level within 12 weeks of therapy.

The anti-androgenic effects are the result of both testosterone suppression and androgen receptor antagonism. Thus, comparing the efficacy between these 2 antiandrogens should be based on clinical of feminization, not testosterone level alone. Most of the feminizing effects typically occur at 3-6 months after consecutive use of feminizing hormone therapy and some characteristics may take years to become noticeable.[35,36] Because of the short study duration, the feminizing parameter that could be assessed was decreased of morning erection which was not significantly different between two study groups. The result suggested the comparable feminizing effects between cyproterone acetate and spironolactone, albeit the higher testosterone level in spironolactone users. We hypothesized that spironolactone may exerts its action directly on the androgen receptor and cause antiandrogenic effects regardless of serum testosterone, thus the testosterone level may not always reflects the anti-androgenic efficacy in spironolactone users.[23] However, the result needed to be cautiously interpreted since the morning erection frequency was a subjective outcome.

Estrogen is another major component of the feminizing hormone therapy. Estrogen is required not only for feminizing effect, but also to prevent bone loss especially in transgender women who had undergone gender-affirming surgery or are medically castrated from anti-androgen therapy.[37,38] Estradiol valerate at a dose of 4 mg daily was selected as the estrogen component in our regimen based on previous report that a dose of 2 mg daily may be inadequate.[6,7] Even with good medication compliance, median estradiol levels were still lower than the premenopausal range for both study groups, and only 6 participants (3 from each group) out of 41 could achieve estradiol level greater than 100 pg/mL.

Even though the HDL level was lower in the cyproterone acetate group compared to the spironolactone group, the difference was subtle and questioning the clinical significance in the cardiovascular context. Our result was in line with previous retrospective studies[22,24] that have reported a decreased HDL level in cyproterone acetate users which related to partial androgenic activity of the medication.[39] Oral estradiol used in our study can potentially mask the change of the lipid parameters, thus additional prospective study using transdermal estrogen may be more appropriate in evaluating the alteration of the lipid metabolism between these two anti-androgens.

Hyperprolactinemia has been reported to be associated with estrogen and cyproterone acetate which both can induce lactotroph hyperplasia.[22,28,40] Our study also demonstrated the higher prolactin level in the cyproterone acetate users, and one participant developed asymptomatic hyperprolactinemia. Considering that median prolactin levels of both study groups were remained in the normal female range, it was difficult to draw the conclusion regarding the association between hyperprolactinemia and cyproterone acetate.

Most adverse effects of feminizing hormone were minimal and well-tolerated in our study.

Although, hyperkalemia was one of the highly concerned events, none of the spironolactone users had the serum potassium level exceeding the withdrawal threshold at 5.0 nmol/L. On the contrary, drug-induced liver injury was developed in one of the cyproterone acetate user who had undiagnosed hepatitis B infection, emphasized the crucial of preexisting liver disease evaluation before starting the medications.[41] Venous thromboembolism was not observed in both study groups possible from the Asian ethnicity and used of oral estradiol instead of ethinyl estradiol in our study.

The present study was randomized controlled trial, concealed the allocation, controlled the estrogen component by prescribed the same type and dose for each participant, and assessed the adherence to the medication. However, a number of limitations need to be considered. First, the participants were not blinded which could influence the subjective outcome measurements. Second, confound feminizing effects from previous hormone therapy were unavoidable since the most of participants were not hormone naïve. Third, the dropout rate was higher than expected which can affect the power of per-protocol analysis. Fourth, the hormone level was measured using the immunoassay method which has a lower accuracy compared to high performance liquid chromatography with mass spectrometry. Finally, the follow-up period of 12 weeks may be inadequate to determine the feminizing effect of the therapy, which limits the application of the study in the long-term treatment situation. There are limited prospective data for feminizing hormone therapy, thus further randomized or prospective trial with a larger sample size and longer duration of follow-up is needed to evaluate the efficacy and adverse effects of feminizing hormone therapy.

CONCLUSION

For feminizing hormone therapy, cyproterone acetate had a higher testosterone suppression efficacy than spironolactone. After 12 weeks of therapy, the proportion of transgender women who achieved the female range testosterone level in the cyproterone acetate group and spironolactone group were 90% and 19%, respectively. Cyproterone acetate, even at low doses, could

induce liver injury. Spironolactone induced hyperkalemia was not observed in the study.

TRIAL REGISTRY

The study had been registered and approved by Thai Clinical Trial Registry since 3 April 2019 (TCTR20190404001).

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STATEMENT OF AUTHORSHIP

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Panyakhamlerd, Krasean: Conceptualization, Methodology, Writing - Review and Editing, Supervision.

Suwan, Ammarin: Methodology, Formal analysis, Data Curation, Writing - original Draft, Visualization.

Tuntiviriyapun, Punkavee: Methodology, Validation, Visualization, Funding Acquisition

Wainipitapong, Sorawit: Methodology, Investigation, Project administration

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