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Randomized, double-blind, placebo-controlled clinical trial for evaluating the efficacy of fractional CO_2 laser compared with topical estriol in the treatment of vaginal atrophy in postmenopausal women

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Abstract

Objective: The aim of the study was to evaluate efficacy of fractional CO_2 vaginal laser treatment (Laser, L) and compare it to local estrogen therapy (Estriol, E) and the combination of both treatments (Laser + Estriol, LE) in the treatment of vulvovaginal atrophy (VVA).

Methods: A total of 45 postmenopausal women meeting inclusion criteria were randomized in L, E, or LE groups. Assessments at baseline, 8 and 20 weeks, were conducted using Vaginal Health Index (VHI), Visual Analog Scale for VVA symptoms (dyspareunia, dryness, and burning), Female Sexual Function Index, and maturation value (MV) of Meisels.

Results: Forty-five women were included and 3 women were lost to follow-up. VHI average score was significantly higher at weeks 8 and 20 in all study arms. At week 20, the LE arm also showed incremental improvement of VHI score ($P = 0.01$). L and LE groups showed a significant improvement of dyspareunia, burning, and dryness, and the E arm only of dryness ($P < 0.001$). LE group presented significant improvement of total Female Sex Function Index (FSFI) score ($P = 0.02$) and individual domains of pain, desire, and lubrication. In contrast, the L group showed significant worsening of pain domain in FSFI ($P = 0.04$), but FSFI total scores were comparable in all treatment arms at week 20.

Conclusions: CO_2 vaginal laser alone or in combination with topical estriol is a good treatment option for VVA symptoms. Sexual-related pain with vaginal laser treatment might be of concern.

Key Words: CO_2 laser – Dyspareunia – Estriol – Female urogenital disease – Postmenopause – Vulvovaginal atrophy.

Vulvovaginal atrophy (VVA) is a common disorder among postmenopausal women as a result of declining estrogen levels with menopause. It affects up to 50% of postmenopausal women, causing great impact in both quality of life and sexual function.[1-5]

Postmenopausal estrogen deficiency promotes morphological and secretory changes in the vulva and vagina. Reduced vascularization and blood flow leading to altered lubrication, loss of tissue elasticity, thinning of the vaginal epithelium, and tissue friability are some of the local changes that contribute to sexual-related symptoms.[6] A substantial decline in glycogen production due to thinning of the vaginal epithelium promotes changes in the vaginal pH and flora with decreased lactobacilli (which normally dominates the vaginal flora), high bacterial diversity, and increase susceptibility to inflammation.[7]

Signs and symptoms of VVA including dyspareunia, dryness, mucosal irritation, itching, and dysuria tend to worsen within 4 to 5 years after menopause.[8] An online survey investigated 56,000 women's perception of VVA symptoms, and found dryness (55% of participants), dyspareunia (44%), and irritation (37%) to be the most commonly reported symptoms.[6,9,10]

Topical hormonal treatment is considered the gold standard therapy for postmenopausal vaginal symptoms, promoting restoration of epithelial integrity, vaginal flora, and improving VVA symptoms.[5] Low-dose vaginal estrogen has also been shown to be superior to systemic therapy for vulvovaginal symptomatic improvement.[11,12] This treatment is, however, associated with poor compliance due to multiple and inconvenient self-applications and increased vaginal discharge. The

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prescription of topical estrogens should also be avoided in women with history of breast cancer, estrogen-sensitive tumors, and thromboembolism, emphasizing the necessity for treatment alternatives.[6,13] Lubricants and moisturizers are available options to help improve dryness, but not enough data addressing efficacy have been published.[13] Ospemifene is another alternative and has been shown to decrease symptoms related to hypoestrogenism.[14]

In the context of individualizing management of VVA symptoms, fractional CO_2 laser treatment has emerged as an alternative treatment option for the management of vulvovaginal symptoms.[6,15,16,17] Fractional CO_2 laser collagen remodeling and increased vascularization effects have been described ex vivo.[17] Microablative fractional CO_2 laser therapy has also improved vaginal health by restoring vaginal flora to premenopause status with predominant lactobacilli.[18]

A significant improvement of VVA symptoms has been described after laser treatment in observational case series.[3,16,19] Sokol and Karram also described lasting effects of microablative fractional CO_2 laser at a 1-year follow-up.[20]

Currently, there are no clinical trials demonstrating the efficiency and safety of fractional CO_2 laser or comparing it to other well-established therapies. This study focused on the evaluation of fractional CO_2 laser treatment safety in comparison to topical estriol and the combination of both treatments in postmenopausal women presenting with VVA symptoms.

METHODS

Study design

Forty-five women were randomized in a double-blind, placebo-controlled clinical trial to compare the therapeutic responses to CO_2 laser, topical estriol, and the combination of both in the treatment of postmenopausal VVA. Participants were block-randomized into three groups of 15 participants consisting of CO_2 vaginal laser + placebo vaginal cream (laser, L), vaginal estriol cream + sham laser (estriol, E), or vaginal estriol cream + CO_2 vaginal laser (laser/estriol, LE) in an outpatient menopause clinic, located at Centro Atencao Integral Saude da Mulher (CAISM) in Sao Bernardo do Campo, Brazil, between January 2015 and May 2015. The study protocol was approved by ABC School of Medicine IRB and informed consent was obtained from all participants. Study was registered at the US National Institutes of Health (ClinicalTrials.gov) #NTC39495014.0.0000.0082.

Study population

Study population included women between the ages of 45 and 70 who presented with amenorrhea for 24 months or longer and at least one moderate symptom of VVA (dyspareunia, dryness, or burning). Participants rated each of three VVA symptoms from 0 (no symptom) to 10 (very severe symptom) using the Visual Analog Scale (VAS) and symptoms were considered moderate if reported to be equal to or greater than 4 in VAS. Participants were excluded in the presence of BMI $\geq 35\,kg/m^2$; chronic kidney or liver disease; drug-induced menopause; history of any form of cancer; previous vaginal radiotherapy; pap smear consistent with atypical squamous cells of undetermined significance, low-grade intraepithelial lesion or high-grade intraepithelial lesion in the previous 12 months; current use of vaginal lubricants or moisturizers; use of anabolic steroids, ospemifene or systemic estrogen therapy in the past 6 months or diagnosis of vulvovaginitis within 30 days prior to the study.

Study interventions

Participants were randomized into one of three treatment arms: combined laser and estriol arm (LE), laser arm (L), and estriol arm (E). LE arm underwent two sessions of fractional CO_2 laser treatment at weeks 0 and 4 using the SmartXide2 system (Monalisa Touch, DEKA Laser, Florence, Italy) combined with 1 mg vaginal estriol therapy (Stele, Biolab, Brazil) 3 times a week for 20 consecutive weeks; L arm underwent two sessions of fractional CO_2 laser treatment at weeks 0 and 4 using the SmartXide2 system, combined with 1 mg estriol-substitute placebo 3 times a week for 20 consecutive weeks; and the E arm was assigned to two sessions of sham laser treatment at weeks 0 and 4 combined with 1 mg vaginal estriol (Stele, Biolab, Brazil) 3 times a week for 20 consecutive weeks.

Laser

The fractional microablative CO_2 laser SmartXide2 system (Monalisa Touch) was used in this study. A vaginal probe was gently inserted and manually rotated to provide a 360^o treatment of the vaginal mucosa. Laser settings were based on previous studies and set to a power of 300 W, dwell time of 1,000 ms, dot spacing of 1,000 μm, and smart stack of 2.0.[16] Participants were advised to avoid sexual activity for at least 3 days after each laser application as a mild inflammatory reaction previously reported could last up to 48 hours.[17]

Placebo

Adequate placebo of fractional CO_2 laser was provided by adjusting power to 0.0 W while maintaining dwell time of 1,000 ms, point spacing of 1,000 μm, and smart stack of 2.0. This procedure is identical to real treatment; even the noise involved with firing the laser was maintained. The 1 mg estriol-substitute placebo topical cream was similar in appearance, odor, consistency, and packaging to the estriol cream.

Blinding

An unblinded nurse was responsible for programming laser parameters before the doctor and participant entered the room. Once programmed, a cover was placed over the screen to ensure proper blinding. The same nurse was responsible for the selection of the appropriate topical cream of either placebo or estriol for each participant. The same physician conducted all appointments and laser sessions.

Study outcomes

The study consisted of five clinical visits (participant selection, weeks 0, 4, 8, and 20). Primary outcomes included

improvement in Vaginal Health Index (VHI) and in VVA symptoms using the VAS. Both VHI and VVA were assessed at weeks 0, 8 and 20.

VHI analyzes the following five components on a scale of 1 to 5: elasticity, fluid volume, pH, epithelial integrity, and moisture. A minimal total score of 5 points indicates severe VVA, and a maximal total score of 25 points indicates no clinical signs of VVA.

Participants reported intensity of VVA symptoms using a 10-cm VAS. The scale's left extremity indicates the complete absence of symptoms (0) and the right extremity indicates the worst possible symptom (10). Participants rated VVA symptoms (dyspareunia, dryness, or burning) from 0 to 10.

Secondary outcome measures were assessed at weeks 0, 8, and 20 and consisted of the analysis of vaginal smear samples and the assessment of quality of sexual function using the validated Portuguese version of the Female Sexual Function Index (FSFI).[21]

Vaginal smear samples were collected in a single scraping of the middle third of the lateral vaginal wall with an Ayre spatula; 100 cells were analyzed per specimen by the Pathology Department. Parabasal (P), intermediary (I), and superficial (S) cell counts were performed and multiplied by 0.0, 0.5, and 1.0, respectively. The sum of all three values comprises the maturation value (MV) of Meisels, and an increased percentage of P cells and I cells suggests a decrease in estrogen levels.[22] MV values ranging from 0 to 49 indicate low estrogen effect, 50 to 64 indicate moderate estrogen effect, and 65 to 100 indicate high estrogen effect on the vaginal epithelium.[23]

The FSFI questionnaire addresses six different domains (desire, arousal, lubrication, orgasm, satisfaction, and pain/discomfort) ranging from 0 (no sexual activity in the past 4 wk) or 1 (very dissatisfied) to 5 (very satisfied). The sum of questions related to each individual domain was multiplied by its unique predetermined factor and the sum of the 6 domain final scores was reported as the full-scale score. Full scale scores ranging from 2.0 (severe dysfunction) to 36.0 (absence of dysfunction) were used to evaluate sexual function throughout the study, with increased FSFI scores correlating to an improvement of symptoms.[19] An optimal cut score of 26, reported by Wiegel et al,[24] is currently used to differentiate between women with and without sexual dysfunction.

Sample size

A standard deviation of 2.5 was adopted from an observational study on CO_2 laser therapy for VVA symptoms.[16] To detect a three-point difference in the VHI with a $P < 0.05$ and a power ≥ 0.8, 12 women per arm were required. Taking into consideration a potential loss of 20% of participants throughout the study, 15 women were included in each treatment arm for a total study enrollment of 45 women.

Randomization

An independent nurse included women in either the combined, laser, or estriol treatment group according to a computer-generated randomization list. Randomization was carried out in blocks (block randomization) of 15 individuals with the help of a computer program.

Statistical analysis

Average and standard deviation were performed in quantitative continuous data analysis, medians, and interquartile range in quantitative discrete data and qualitative variables were summarized using absolute numbers and percentages. Intention-to-treat (ITT) analysis and per-protocol analysis were used for analysis of primary outcomes. ANOVA was used in the comparison among study arms and multiple significant comparisons among arms were obtained using the least significant difference test. Comparison of treatment groups was performed using the Kruskal–Wallis test when data presented nonnormal distribution. A repeated measures ANOVA was used for data analysis in multiple time points and the Friedman test was used when data presented nonnormal distribution. Paired t test and Wilcoxon signed-rank test was used when data presented nonnormal distribution for comparison of baseline and week 20 within groups. Comparison of categorical data was performed using the χ^2 test. Cohen d test was used to calculate effect sizes for primary outcomes and effect sizes were considered small if 0.2, medium if 0.5, and large if 0.8. Multivariate analysis was used for analysis of outcomes when baseline characteristics were statistically different among groups. Differences were considered significant with a P value of 0.05. Data were analyzed using the statistical analysis tool WinStat for Microsoft Excel version 2009.

RESULTS

A total of 45 participants from a total of 50 preselected women were randomized into three treatment groups: 2 participants from the L group and 1 from the E group were lost to follow-up (Fig. 1). As a result, primary outcomes were analyzed per protocol and by ITT analysis. No adverse effects of the fractional CO_2 laser treatment or pain during laser application were observed during the study.

No significant differences were found at baseline among groups. Participants characteristics, VHI, and FSFI were similar with the exception of burning, significantly milder in the E group (Table 1).

ITT analysis showed no differences in VHI average score among groups at baseline ($P = 0.8$) and week 8 ($P = 0.5$), although a significant difference among groups at week 20 was observed ($P < 0.01$). Per protocol analysis showed that VHI average score was significantly higher at week 8 ($P < 0.05$) and week 20 ($P < 0.01$) in comparison to baseline in all study arms. LE group also showed incremental improvement from week 8 to week 20 in the VHI score ($P = 0.01$) and the L group had a lower VHI score at week 20 compared with other study arms ($P < 0.05$) (Fig. 2). Mean difference between groups L and E, L and LE, and E and LE at week 20 were −2.87 (95% CI: −5.99 to 0.26), 4.73 (95% CI: 2.42-7.07), and 1.87 (95% CI: −0.59 to 4.31), respectively.

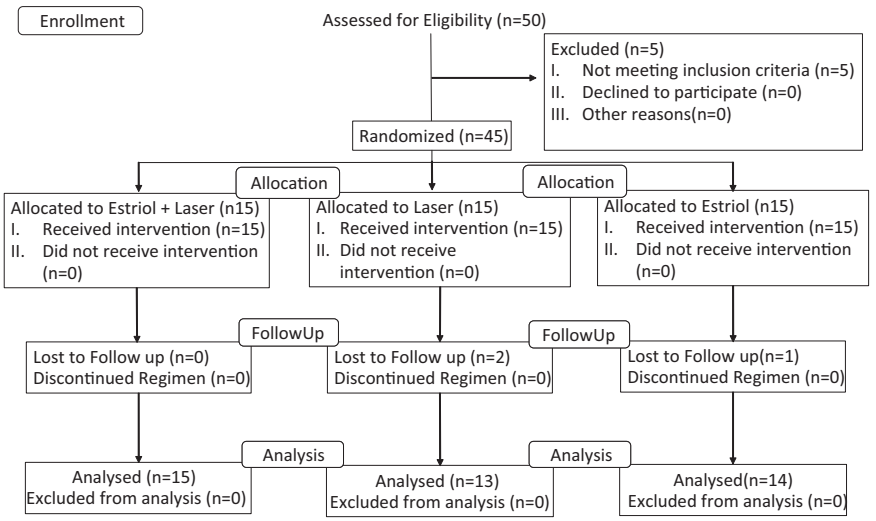

FIG. 1. Study flowchart.

L and LE groups showed a significant improvement of dyspareunia, burning, and dryness, and the E arm presented improvement of reported dryness ($P < 0.001$) in ITT analysis (Table 2). VAS symptoms were comparatively milder in the E group at baseline, and burning was shown to be significantly lower compared with the same symptom in the other treatment arms. Therefore, burning assessment at week 20 in the E group was directly compromised by baseline findings ($P = 0.014$; $R^2 = 0.119$). As ITT, per-protocol analysis, analysis also showed a significant difference at baseline among groups for burning ($P = 0.02$) and no significant improvement of burning symptoms in the E group ($P = 0.5$). Mean difference between groups L and E, L and LE, and E and LE at week 20 were 0.05 (95% CI: −1.27 to 1.36), 0.35 (95% CI: −0.83 to 1.53), and 0.40 (95% CI: −0.98 to 1.78) for dyspareunia; 0.13 (95% CI: −1.49 to 1.75), −0.93 (95% CI: −1.96 to 0.09), and −0.80 (95% CI: −1.49 to 1.75) for dryness; and 0.13 (95% CI: −0.73 to 1.0), −0.07 (95% CI: −0.96 to 0.82), and 0.07 (95% CI: −0.71 to 0.84) for burning, respectively.

Study arm sizes were considered adequate for detecting differences in VHI scores, and the LE, L, and E groups presented large effect sizes of 0.85, 0.72, and 0.81, respectively. Effect size estimates were also calculated for VVA symptoms, and the LE, L, and E groups presented effect sizes of 0.63, 0.45, and 0.33 for burning, 0.87, 0.82, and 0.75

TABLE 1. *Demographic and clinical characteristics of study participants at baseline*

Characteristic	Laser ($n = 15$)	Estriol ($n = 15$)	Laser + estriol ($n = 15$)	P
Demographic				
Age	55.9 ± 5.2	56.9 ± 6.0	55.7 ± 4.4	0.83
BMI	26.2 ± 3.7	27.8 ± 2.7	24.9 ± 3.9	0.10
Pregnancies	2.9 ± 1.6	2.5 ± 1.1	2.5 ± 0.9	0.62
Years since menopause	8.2 ± 7.0	8.7 ± 6.4	8.3 ± 6.7	0.98
VHI	11.7 ± 2.9	12.6 ± 4.2	12.6 ± 4.2	0.76[a]
FSFI	19.60 ± 7.55	21.80 ± 8.95	16.19 ± 8.78	0.21
VAS				
Dyspareunia	4.2 ± 3.8	3.2 ± 3.4	6.5 ± 3.9	0.065
Dryness	6.9 ± 3.7	5.6 ± 2.9	7.9 ± 3.0	0.153
Burning	3.4 ± 4.4	0.9 ± 1.6	4.9 ± 3.8	**0.01**[b]
		No. (%)		
Previous HT	1 (7.7)	5 (35.7)	5 (33.3)	0.18[c]
Smoking	2 (15.5)	2 (14.3)	2 (13.3)	0.98[c]
Hot flushes	7 (53.8)	6 (42.9)	10 (66.7)	0.43[c]
Orgasm	7 (53.8)	9 (64.3)	9 (60.0)	0.85[c]
Libido	7 (53.8)	11 (78.6)	11 (73.3)	0.34[c]

Items listed as means ± SD. P values of 0.05 were considered statistically significant.
BMI, body Mass Index; FSFI, Female Sexual Function Index; HT, hormone therapy; VAS, Visual Analog Scale (0-10, where 0 = no symptom and 10 = severe symptom); VHI, Vaginal Health Index.
[a]Kruskal-Wallis test.
[b]Least significant difference analysis showed group E vs LE and E vs L: $P < 0.05$.
[c]Chi-square test.
All others: ANOVA.

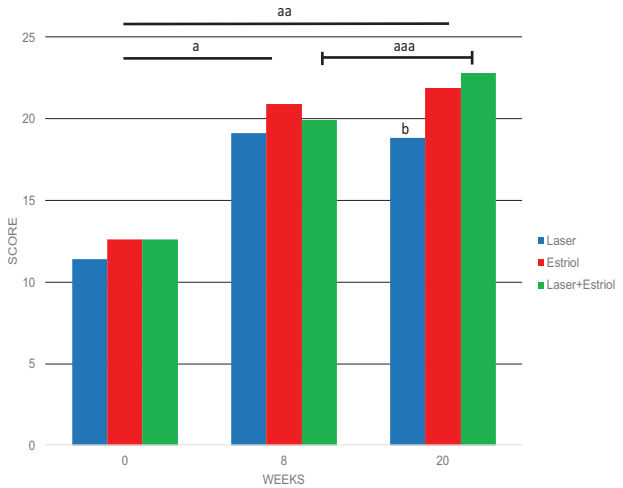

FIG. 2. VHI score of different treatment arms at multiple time-points. [a]Wilcoxon test $P < 0.05$, all groups; [aa]Wilcoxon test $P < 0.001$, all groups; [aaa]Wilcoxon test, LE week 8 vs week 20, $P = 0.01$; [b]Kruskal-Wallis test, L vs E and LE, $P < 0.05$; Friedman test for multiple time-points, $P < 0.001$, all groups.

for dryness, and 0.68, 0.60, and 0.52 for dyspareunia, respectively.

Table 3 summarizes FSFI full-scale scores and individual domain analysis. The LE group presented significant improvement of the total FSFI score ($P = 0.02$) and in individual domains of pain ($P = 0.02$), desire ($P = 0.005$), and lubrication ($P = 0.02$) compared with baseline. A significant difference in pain at baseline among all arms was found ($P = 0.04$), particularly between the LE and E groups ($P < 0.05$), but these findings did not persist at subsequent assessments where an overall symptomatic improvement was observed. A difference among groups ($P = 0.006$) in pain domain score was also observed at week 20.

No difference was observed in vaginal smear samples among groups (Table 4). The L and E groups had an improvement of MV of Meisels at week 8, but only the L group presented a significant MV increase also at week 20 ($P = 0.01$). In the LE group a nonsignificant increase of MV value was found, although MV increased from 48.4 ± 25.3 to 60.4 ± 8.6 at week 20. A decrease in the percentage of P cells per higher power field occurred in weeks 8 and 20 compared with baseline in the L and E groups, but only the L group also presented an incremental decrease of P cells for both week 8 ($P = 0.03$) and week 20 ($P = 0.02$). Cytological evaluation of vaginal smears was compromised due to inadequate sampling and preparation, resulting in different number of viable samples for analysis between and within groups.

DISCUSSION

Improvement of vulvovaginal symptoms after fractional CO_2 laser has been demonstrated in multiple case series.[3,6,15,16] This study was the first to evaluate the effects of fractional CO_2 laser in comparison to estriol topical therapy, considered the gold standard treatment for local VVA symptoms.[12]

All treatment options used in this study, either fractional CO_2 laser alone, estriol alone, or the combination of both treatments, resulted in improvement of vaginal health and VVA symptoms, and were also seen in vaginal specimen analyses.

The LE, E, and L groups showed statistically significant improvement in the VHI at week 8, suggesting that both fractional CO_2 laser and estrogen therapies promote a fast and significant improvement in the vaginal mucosa. Similar results were reported in studies evaluating the efficacy of fractional CO_2 laser.[16,25]

An incremental improvement of VHI was also noted in the LE arm between week 8 and week 20, suggesting that the

TABLE 2. *Visual Analog Scale scores at 0, 8, and 20 weeks by treatment group*

	Laser (n = 15)	Estriol ($n = 15$)	Laser + estriol ($n = 15$)	P^a
Dyspareunia				
Baseline	4.9 ± 3.7	3.2 ± 3.4	6.5 ± 3.9	0.09
Week 8	2.9 ± 2.9	0.6 ± 1.7	2.5 ± 3.8	0.16
Week 20	0.7 ± 1.5	0.2 ± 0.6	0.9 ± 1.8	0.95
P^b	**0.01**	0.058	**0.009**	
Dryness				
Baseline	8.0 ± 2.6	5.6 ± 2.9	7.9 ± 3.0	0.07
Week 8	3.6 ± 2.6	2.4 ± 2.0	3.3 ± 2.9	0.57
Week 20	1.4 ± 2.0	0.5 ± 1.4	0.3 ± 0.7	0.35
P^b	**<0.001**	**<0.001**	**<0.001**	
Burning				
Baseline	3.9 ± 4.5	0.9 ± 1.6	4.9 ± 3.8	**0.017**[c]
Week 8	1.0 ± 2.0	0.1 ± 0.5	1.2 ± 2.7	0.33
Week 20	0.5 ± 1.5	0.1 ± 0.3	0.4 ± 1.1	0.95
P^a	**0.02**	0.51	**0.002**	

Intention-to-treat analysis. Items listed as mean ± SD. P values of 0.05 were considered statistically significant.
[a]ANOVA.
[b]Friedman test.
[c]Least significant difference analysis showed group E vs LE and E vs L: $P < 0.05$.
All others: Visual Analog Scale (0-10, where 0 = no symptom and 10 = severe symptom).

TABLE 3. *FSFI scores (individual domains and total) at 0, 8, and 20 weeks by treatment group*

	Laser ($n = 13$)	Estriol ($n = 14$)	Laser + estriol ($n = 15$)	P
Desire				
Baseline	2.4 [1.5; 3.6]	2.4 [2.1; 3.6]	1.8 [1.2; 3.0]	0.19
Week 8	2.4 [1.8; 3.6]	2.4 [2.2; 3.6]	3.0 [1.2; 3.6]	0.99
Week 20	2.4 [1.8; 3.6]	3.0 [2.4; 3.6]	3.6 [1.8; 3.6]	0.76
P (baseline vs week 20)[a]	0.39	0.63	**0.005**	
Arousal				
Baseline	2.4 [1.4; 4.0]	3.6 [2.1; 4.8]	2.7 [1.5; 4.5]	0.66
Week 8	2.4 [1.4; 3.4]	3.1 [2.0; 4.9]	3.6 [1.8; 4.2]	0.35
Week 20	3.0 [1.5; 3.6]	4.0 [2.2; 4.6]	3.9 [1.8; 4.5]	0.68
P (baseline vs week 20)[a]	1.00	1.00	0.17	
Lubrication				
Baseline	4.2 [2.7; 5.0]	4.2 [2.8; 5.6]	2.7 [1.2; 4.2]	0.15
Week 8	2.7 [1.0; 5.1]	4.5 [2.0; 5,1]	4.2 [2.7; 5.4]	0.49
Week 20	3.0 [0.8; 4.5]	3.9 [2.9; 5,2]	3.6 [2.7; 4.8]	0.29
P (baseline vs week 20)[a]	0.24	0.58	**0.02**	
Orgasm				
Baseline	4.0 [2.0; 4.8]	4.2 [2.9; 4.7]	3.6 [1.2; 4.8]	0.68
Week 8	2.8 [0.6; 4.4]	4.0 [0.0; 6.0]	4.0 [1.6; 5.6]	0.60
Week 20	2.4 [0.0; 4,6]	4.2 [2.3; 6.0]	4.4 [2.8; 5.6]	0.14
P (baseline vs week 20)[a]	0.26	0.95	0.11	
Satisfaction				
Baseline	3.2 [1.6; 4.8]	4.8 [2.1; 5.7]	3.6 [1.2; 4.8]	0.46
Week 8	4.0 [1.4; 4.8]	4.8 [2.3; 5.7]	4.8 [2.8; 5.2]	0.40
Week 20	3.6 [1.4; 4.0]	4.6 [3.0; 5.7]	4.8 [2.8; 4.8]	0.13
P (baseline vs week 20)[a]	0.72	0.60	0.09	
Pain				
Baseline	4.4 [1.6; 5.6]	4.8 [2.2; 5.7]	2.4 [1.2; 3.6]	**0.04**[b]
Week 8	2.0 [0.0; 4.4]	5.2 [0.0; 6.0]	3.6 [1.2; 4.8]	0.39
Week 20	2.0 [0.0; 3.6]	6.0 [3.9; 6.0]	2.8 [1.6; 5.6]	**0.006**[c]
P (baseline vs week 20)[a]	**0.04**	0.16	**0.02**	
Total				
Baseline	18.6 [16.4; 24.6]	23.6 [17.5; 29.8]	18.7 [7.2; 22.6]	0.21
Week 8	18.0 [11.4; 20.7]	22.9 [8.4; 29.7]	22.6 [11.3; 26.3]	0.39
Week 20	14.4 [7.8; 22.4]	25.4 [16.8; 29.3]	23.6 [14.9; 28.6]	0.10
P (baseline vs week 20)[a]	0.26	0.56	**0.02**	

Items listed as median [interquartile range]. P values of 0.05 were considered statistically significant.
FSFI, Female Sexual Function Index.
[a]Paired student t test.
[b]Least significant difference analysis: E vs LE, $P < 0.05$.
[c]Least significant difference analysis: E vs L, $P < 0.05$.
All others: ANOVA.

TABLE 4. *Parabasal cells (percentage per HPF) and Meisels (maturation value) at 0, 8, and 20 weeks by treatment group*

	Laser	Estriol	Laser + estriol	P[a]
Baseline				
n	12	11	10	
P cells	35.4 ± 31.5	45.3 ± 38.1	26.2 ± 36.3	0.47
Meisels	42.4 ± 24.0	36.9 ± 29.7	48.4 ± 25.3	0.61
Week 8				
n	12	14	13	
P cells	14.3 ± 24.4	2.4 ± 2.6	5.2 ± 8.2	0.11
Meisels	64.5 ± 23.1	65.6 ± 6.5	65.0 ± 10.5	0.98
Baseline vs week 8[b]				
P cells	**0.03**	**<0.01**	0.09	
Meisels	**0.01**	**0.01**	0.07	
Week 20				
n	11	9	11	
P cells	16.7 ± 29.2	6.1 ± 11.7	3.9 ± 3.7	0.24
Meisels	58.5 ± 23.7	58.2 ± 8.5	60.4 ± 8.6	0.93
Baseline vs week 20[b]				
P cells	**0.02**	0.11	**0.07**	
Meisels	**0.01**	0.46	**0.07**	

Items listed as mean ± SD. P values of 0.05 were considered statistically significant.
HPF, higher power field; P cells, parabasal cells.
[a]ANOVA.
[b]Wilcoxon test.
Maturation value of Meisels: repeated measures ANOVA between groups ($P = 0.12$) and within groups ($P < 0.001$).

combined use of local estrogen and fractional CO_2 laser seems to be advantageous. Although maintaining a significantly higher score compared with baseline, the L group VHI score was found to be lower in comparison to other treatment groups at week 20. Sokol and Karram evaluated the efficiency and safety of fractional CO_2 laser for VVA in a 1-year follow-up, and demonstrated that the positive effects on VVA symptoms (burning, dryness, and dyspareunia), VHI, and FSFI full-scale score persisted for at least 1 year after three sessions of fractional CO_2 laser.[20]

It is important to highlight that fractional CO_2 laser effects on the vaginal mucosa persisted for at least 16 more weeks after the last session and that if topical estrogen applications had been interrupted, the efficiency of this treatment would not have been maintained.[11,26]

Fractional CO_2 laser alone and the combined therapy improved reported VVA symptoms of burning, dryness, and dyspareunia throughout the study. The E group presented milder symptoms of burning, dyspareunia, and dryness at baseline; thus dryness was the only symptom that presented a statistically significant improvement at week 20. It is important to observe that symptomatic improvement could have been significant if participants assigned to estriol therapy were more symptomatic.

A significant increase in dyspareunia using FSFI was noted on the L group, although the same symptom improved when assessed by the VAS scale. It is difficult to explain the reason for different results in sexually related pain reported through VAS and FSFI. FSFI is a self-report instrument, whereas the assessment of dyspareunia with the VAS was verbally asked by the physician. There are no studies comparing the accuracy of these assessments in the literature. Salvatore et al used the same laser system and parameters used in this study, but three laser sessions (weeks 0, 4, and 8) were performed instead. Contrasting our findings, FSFI full-scale score and pain individually showed significant improvement at week 12.[3]

Estriol absorption might have had a beneficial effect in the vaginal introitus, decreasing penetration-related pain. Otherwise, it is difficult to explain increasing penetration-related pain in the CO_2 laser arm. Many previous vaginal CO_2 laser studies have reported this side effect.[3,6,8,16,18,20]

Yoruk et al reported a correlation of MV values and vaginal pH to serum estrogen levels in women. The overall improvement of MV indicates higher estrogen effects in the mucosa in all treatment arms.[23] Average percentage of P cells was similar among groups at baseline, but only the L group showed incremental estrogen effect at week 20. Salvatore et al reported similar effects in vaginal mucosa histology after fractional CO_2 laser therapy, suggesting that fractional CO_2 laser promotes morphological changes and mucosal restoration.[17]

The main contribution of this study is the first time comparison of fractional CO_2 laser performance to the use of local estrogen for vulvovaginal symptoms. The study demonstrates vaginal health benefits resulting from fractional CO_2 laser treatment persisting for at least 16 weeks. Fractional CO_2 laser is a convenient alternative to local estriol, in which contraindications, low compliance due to vaginal discharge, and daily self-applications can be of concern.

The inclusion of sham laser treatment in the estriol arm is a strength of this study design. It allowed for a more reliable treatment control group and decreased result bias. Also, a combined estriol plus fractional CO_2 laser treatment arm, the fact that all visits were conducted by the same physician and the small dropout percentage are strong points of this trial. On the contrary, this is a small study, powered to detect difference in VHI and not for all the multiple comparisons made. All findings should be interpreted with extreme caution, mainly VVA symptoms using VAS. Milder VVA symptoms in the estriol group at baseline and the reduced number of viable vaginal smears for vaginal mucosa cytological analysis (MV of Meisels) were also limitations of this study.

CONCLUSIONS

Fractional CO_2 laser is an emerging treatment option for treating VVA symptoms, particularly for women with contraindication for hormone therapy. Fractional CO_2 laser treatment consists of two or three sessions and its effects persisted during our 20-week follow-up. Fractional CO_2 laser effects are similar to topical estriol and the combined treatment. These study findings, either of no differences between groups or differences between CO_2 laser and local estrogen, should all be considered preliminary. Larger placebo-controlled studies evaluating medium- and long-term effects of fractional CO_2 laser treatment are needed. A 1-year follow-up of this study population has been planned to evaluate long-term effects of the three treatments provided.

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