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ORIGINAL STUDY

CO_2 laser, radiofrequency, and promestriene in the treatment of genitourinary syndrome of menopause in breast cancer survivors: a histomorphometric evaluation of the vulvar vestibule

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Abstract

Objective: This study aimed to compare the efficacy of CO_2 laser, radiofrequency, and promestriene in treating genitourinary syndrome of menopause in women with breast cancer receiving adjuvant therapy and to analyze the clinical and histological findings of the vulvar vestibule.

Methods: Women with moderate-to-severe symptoms of vulvar atrophy were enrolled. The participants were evaluated according to pretreatment and posttreatment protocols using the visual analog scale and clinical assessments, which included a gynecological examination and vestibular biopsy. Participants were randomly assigned into the laser, radiofrequency, or promestriene groups. Participants in the energy treatment groups underwent three consecutive monthly outpatient vulvovaginal treatment sessions, whereas those in the control group were administered promestriene for 4 months. During a follow-up visit 30 days posttreatment, the participant global posttreatment impression of improvement was evaluated using a Likert scale.

Results: Seventy women completed treatment. Histological vulvar atrophy was identified in four (5.7%) of the pretreatment vulvar samples. Postintervention, all histological parameters were normalized. Significant improvements in symptoms were observed, as all three groups showed a reduction in the visual analog scale score, with no statistically significant differences among them. A high level of satisfaction was reported posttreatment in all groups. No damage to the histological structure of the vulvar vestibule or relevant clinical adverse events were identified posttreatment.

Conclusions: Laser, radiofrequency, and promestriene delivered comparable, significant symptom improvements among women with breast cancer receiving adjuvant therapy. These treatments did not cause structural tissue damage or other clinical complications.

Key Words: Breast cancer – CO_2 laser – Genitourinary syndrome of menopause – Promestriene – Radiofrequency – Vulvar biopsy.

Complex alterations in both cutaneous and mucosal functions attributed to postmenopausal hypoestrogenism give rise to urogenital manifestations and associated symptoms. These changes have a noteworthy impact on the perception of sexual identity, self-image, and well-being among women. This influence extends to their life quality and experiences related to sexuality. Consequently, interventions aimed at alleviating these effects are of paramount importance.[1,2]

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The climacteric phase is characterized by substantial transformations in the epithelial lining of the genital tract owing to declining estrogen levels, resulting in the emergence of symptoms and indicators that define genitourinary syndrome of menopause (GSM).[3] This syndrome encompasses an array of symptoms related to the genital, urinary, and sexual complaints, such as burning, dryness, fissures, insufficient vaginal lubrication, dyspareunia, lower urinary tract infection, dysuria, and urinary incontinence. Such symptoms are notably aggravated in women taking medications that induce hypoestrogenism, including adjuvant hormone therapy for the treatment of breast cancer.[4-6]

Breast cancer ranks as the most prevalent malignancy among women and afflicts millions of individuals globally on an annual basis.[7] Its incidence escalates with advancing age, with approximately 75% of cases arising in postmenopausal women.[6] A considerable proportion of breast cancer instances are marked by estrogen receptor positivity,[8] prompting treatment strategies that typically involve the utilization of antiestrogen medications such as selective estrogen receptor modulators and aromatase inhibitors. Nonetheless, aromatase inhibitors lead to profound suppression of estrogen biosynthesis, potentially exacerbating the severity of GSM symptoms and further compromising patient quality of life. Consequently, approximately 74% of women undergoing breast cancer treatment experience urogenital symptoms.[9] However, scant information exists regarding the histological effects of these medications on the urogenital tract.

Currently, the gold standard treatment for GSM is topical estrogen therapy[10]; however, this cannot be used in women with a medical history of breast cancer.[11] Promestriene, a diether of estradiol, is effective in treating atrophic urogenital manifestations and is considered safe owing to its negligible systemic absorption due to low dose. Despite being a suitable option for women with breast cancer, some patients and their oncologists are hesitant about its long-term usage.[12,13] Nonpharmacological alternatives have been developed, particularly for women with contraindications to hormone-based therapy. Physical methods, also known as energy methods, are employed in medicine to promote tissue renewal. Among these methods, microablative fractional CO_2 laser (CO_2L), erbium laser, and microablative fractional radiofrequency (RF) stand out, as they facilitate tissue recovery (skin and mucosa) through controlled thermal effects. Generally, these techniques utilize diverse forms of energy with analogous impacts on tissues, offering the advantage of not carrying risks associated with hormonal treatments.[14,15]

CO_2L and RF appear promising for GSM treatment and are being explored as alternatives for women ineligible for topical hormonal therapy. These technologies are expected to improve local vascularization, stimulate collagen production, and increase vaginal epithelial thickness. Certain researchers have already demonstrated the clinical improvement of GSM after CO_2L treatment compared with topical promestriene.[16] The effectiveness of RF treatment in alleviating GSM has been noted, although numerous studies did not analyze vulvar vestibule tissue samples and lacked a control group.[14,17] Moreover, in 2018, the US Food and Drug Administration issued a warning statement regarding safety concerns and the lack of evidence supporting the use of lasers and other energy-based devices for GSM treatment.

Limited research has scrutinized the clinical and histological effects of these novel techniques. Furthermore, no study has conducted a comparative analysis of CO_2L, RF, and promestriene regarding the vulvar vestibule—a region within the urogenital area most affected by GSM. Therefore, this study aimed to describe and compare the effects of CO_2L, RF, and promestriene treatments on both the clinical and histological dimensions of GSM in women with breast cancer receiving adjuvant therapy.

METHODS

This pilot study focused on evaluating the clinical and histological outcomes of energy-based treatments for GSM in breast cancer survivors who had undergone adjuvant therapy. The investigation served as the secondary outcome of an ongoing, multiarm, randomized controlled trial. Using a promestriene clinical success rate of 75% and accounting for a potential 25% loss to follow-up, the sample size was determined to achieve 80% statistical power in detecting a 15% difference among the groups.

This study received approval from the Hospital São Paulo-UNIFESP institutional review board and was carried out at the Department of Gynecology, a tertiary referral academic center within the Federal University of São Paulo. Data collection occurred between September 2019 and September 2021, as part of the larger LARF study (NCT04081805) under the designation "LASER and Radiofrequency Alternatives for the Treatment of GSM in Women with Breast Cancer (EPMLARF-arm2)."

Female breast cancer survivors with moderate-to-severe GSM symptoms and currently receiving adjuvant endocrine therapy were eligible for participation. The severity of each GSM manifestation and symptom, such as burning, itching, dryness, general discomfort, dyspareunia, lack of lubrication during sexual intercourse, fissures, thinning of the vaginal rugae, and trophism reduction (reported by the participant), was assessed using a 10-point visual analog scale (VAS). Inclusion criteria encompassed women presenting at least one moderate-to-severe sign or symptom (≥4) of GSM on the VAS.

The exclusion criteria comprised the use of estrogen therapy in the previous 6 months, pelvic organ prolapse beyond the vaginal introitus, ongoing vulvovaginal infections, active urinary tract infection, and abnormal vaginal bleeding.

All participants provided written informed consent before proceeding. Women were subjected to pretreatment and posttreatment protocols, which involved completing the VAS for GSM, as well as clinical evaluations, including standardized gynecological examinations and vestibular biopsies. Biopsies were acquired from the right side of the vulvar vestibule before treatment initiation and from the left side after treatment using a Medina clamp with a maximum diameter of 4 mm. The obtained vestibular specimens were promptly fixed using formalin. After the necessary processing, these specimens were embedded in paraffin, and appropriately sized 5-μm sections were stained with hematoxylin and eosin.

Upon enrollment, the women were randomly allocated into one of three groups: CO_2L, RF, or promestriene groups,

maintaining an allocation ratio 1:1:1. Block randomization was performed utilizing a computerized random number generator in Microsoft Excel version 16.75.2 (Microsoft Corporation, USA), and the resulting list was maintained by the nurse overseeing the protocol. Women who disclosed a prior history of genital herpes were administered 125 mg famciclovir twice daily for a span of 5 days, serving as prophylaxis before each treatment session.

Participants in the CO_2L and RF groups underwent a series of three consecutive monthly outpatient energy treatment sessions. Initially, a 4% lidocaine gel was administered to the vulva and vaginal introitus. After a 30-minute interval, the participants were positioned in a lithotomy position and the gel removed to perform the energy application. For CO_2L administration, the vaginal epithelium was dried using gauze. Although for RF application, the vaginal epithelium was moistened with saline solution. Following vaginal application, both groups underwent vulvar application using a vulvar probe, covering the introitus, vulva vestibule, as well as the labia majora and minora.

In the CO_2L group, the equipment employed was the SmartXide Touch V^2LR (MonaLisa Touch; Deka, Calenzano, Italy), which has received clinical approval in Brazil, the European Union, and the United States, albeit with warnings and restrictions. For vaginal applications, this equipment was configured in D-pulse mode with a power of 40 W, a dwell time of 1,000 μs, dot spacing of 1,000 μm, and a stack of 2. The procedure involved utilizing a 360° vaginal probe. Vulvar applications were executed using the vulvar probe, with generator settings comprising D-pulse mode, a power output of 24 W, a dwell time of 800 μs, dot spacing of 800 μm, and a stack of 1.

Within the RF group, the equipment employed was the Wavetronic6000 Touch System with Megapulse HF FRAXX (Loktal Medical Electronics, São Paulo, Brazil), which has been approved for clinical application in Brazil and Europe. The vaginal and vulvar probes were equipped with 64 microneedles distributed in an 8 × 8 matrix, with a needle separation of 1.27 mm. The 4 MHz generator was configured to the Fraxx mode with low intensity, boasting an activation time of 40 ms and a power of 45 W. For vaginal applications, the probe was activated throughout the vaginal walls, creating matrix lines under direct visualization with the assistance of a speculum. Vulvar applications involved the same equipment settings: Fraxx mode, low intensity, an activation time of 40 ms, and a power of 45 W, but using a vulvar probe.

The promestriene group received guidance for the domiciliary application of vaginal promestriene at a dosage of 10 mg daily for a period of 21 days, followed by 10 mg on 2 nonconsecutive days per week for a duration of 4 months. The weight of the vaginal promestriene tubes was recorded before dispensing and subsequently at each monthly visit to ascertain the actual medication usage.

A period of 5 days of sexual abstinence was recommended following each energy application sessions. Furthermore, participants across all three groups were instructed to abstain from using vaginal lubricants or moisturizers for the duration of their participation in this study.

A follow-up visit was performed 120 days after the commencement of each treatment, adhering to the pretreatment evaluation protocol. During the visits, the participant overall posttreatment impression of improvement was gauged using a five-point Likert scale (1 = much worse, 2 = worse, 3 = neutral, 4 = better, 5 = much better). The occurrence of complications was assessed between the treatment sessions and follow-up visits through clinical evaluation, which included physical examinations.

Histomorphological evaluation evolved with sample suitability defined by adequate tissue quantity, devoid of any artifact features, such as diminished fixation quality or clamping artifacts, and featuring accurate representation of both the epithelium and stroma (chorion or submucosa). The selection process was predicated on various features or parameters for histological evaluation, including the presence of hyperkeratosis (excessive keratin on superficial epithelial layer, >50% of the average keratin thickness), epithelial thickness (measured using a Breslow ruler, considering the thickest section of tissue at 10× optical enhancement), the number of epithelial cell layers (5-10 considered reduced, whereas 10-20 was deemed normal), epithelial cell maturation status (presence of basal, intermediate, and superficial cells considered normal; reduction of superficial and intermediated cells indicating maturation reduction), and presence of dermal papillae (epithelial tissue invagination within the stroma, with more than one third of its thickness possessing the normal lining of the corresponding basal membrane). A count of at least five papillae in a minimum of two distinct regions was regarded as normal. Identifying >10 papillae per area was classified as papillae enhancement, whereas <2 papillae per area was denoted papillae reduction. Vascularization was assessed by enumerating the number of capillaries within two distinct stromal areas (reduced, <2 capillaries; normal, 2-5 capillaries; enhanced, >10 capillaries). A diffuse capillary distribution within the stroma indicated a normal pattern, whereas the presence of subepithelial vessels in the chorion or submucosa was characterized as an altered configuration.

Histological analyses were performed by two pathologists who were blinded to treatment time and type. In cases where a discrepancy emerged during the assessment of qualitative data, a reevaluation of the slide was carried out to establish a consensus between the two evaluators. For numerical measurements, the average of two estimates was employed.

As per a prior definition, histological atrophy was characterized by a notable reduction in the number of epithelial layers. Moreover, in this context, basal cells should comprise over half of the epithelial thickness, whereas the intermediate and superficial layers of the squamous epithelium should exhibit reduced or negligible presence. Furthermore, the epithelial papillae should be absent, and vascularization should be diminished.

Statistical analysis

Statistical analysis was performed using Minitab Statistical Software (V19 Minitab, LLC, State College, PA). The normality of the data was assessed. Continuous variables were analyzed utilizing analysis of variance (ANOVA), whereas categorical variables were assessed through chi-square tests. Generalized

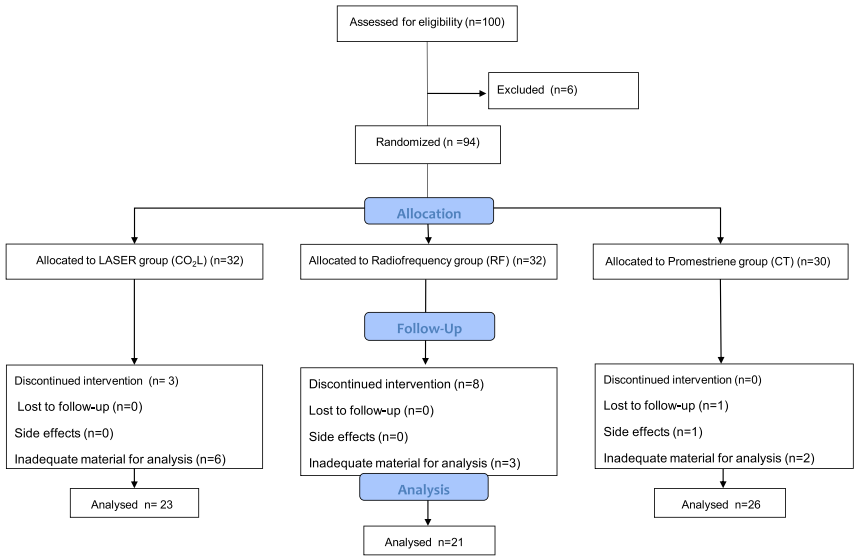

FIG. 1. Flowchart.

estimating equations were employed for comparing groups and tracking changes in categorical variables over the follow-up period. To compare groups and their changes over time in quantitative variables, a mixed-model ANOVA was utilized, with G representing group, T denoting time, and G × T representing the interaction factor. In cases where a significant interaction factor difference was identified, a post hoc Tukey's test was applied. The level of statistical significance was defined as $P < 0.05$. This pilot study aimed to scrutinize the clinical and histological aspects of the vulvar vestibule among adjuvant therapy users; however, the power of this study was not assessed beforehand.

RESULTS

A total of 70 women were enrolled in the multiarm randomized controlled trial, with 23 in the CO_2L group, 21 in the RF group, and 26 in the promestriene group. All participants successfully completed the treatment, and appropriate pretreatment and posttreatment materials were gathered for analysis (Fig. 1).

Table 1 presents the demographic and clinical pretreatment characteristics of the participants. The mean age of the participants was 53.57 (±8.08) years. The majority of participants (67/70 [91.4%]) were in the postmenopausal stage. The mean duration since menopause was 7.65 (±7.78) years. All participants were in current use of adjuvant endocrine therapy, with 25 women (35.71%) receiving tamoxifen, and 45 women (64.29%) receiving anastrozole. The mean duration of medication usage was 2.78 (±1.34) years. A significant number of participants were sexually active (48/70 [68.5%]), with the majority being nonsmokers (66/70 [94.29%]).

Regarding the evaluation of GSM symptoms, the collected data demonstrated noteworthy improvements in all reported clinical parameters across all groups. These improvements were consistent without any variations among the groups, and they were associated with a reduction in the VAS score for each evaluated symptom (Table 2). Furthermore, there was a reduction in the number of women experiencing moderate-to-severe symptoms (VAS ≥ 4) (Table 3).

Moreover, significant levels of satisfaction were reported following the treatment protocol, gauged through a subjective perception of improvement using the Likert scale across all evaluated groups (CO_2L, 4.783 [±0.518]; RF, 4.150 [±0.813]; promestriene, 4.280 [±1.13]; $P = 0.055$).

Regarding the histological outcomes (Fig. 2), pretreatment histological atrophy was detected in only four participants (4/70 [5.71%]). Specifically, three cases, exhibiting reduced maturation (two in the RF group and one in the promestriene group), and one case characterized by a reduction in the epithelial

TABLE 1. *Clinical and demographic parameters before treatment*

Parameter	CO_2L ($n = 23$)	Radiofrequency ($n = 21$)	Promestriene ($n = 26$)	P value
Age, mean (SD), y	52.43 (±8.14)^{a,b}	51.00 (±5.18)^{a}	56.65 (±9.17)^{b}	0.039
BMI, mean (SD), kg/m^2	26.44 (±3.32)	28.85 (±5.37)	27.25 (±5.73)	0.361
Adjuvant therapy in use				0.930
Tamoxifen, *n* (%)	8 (34.78%)	7 (33.33%)	10 (38.46%)	
Anastrozole, *n* (%)	15 (65.22%)	14 (66.67%)	16 (61.54%)	
Sexual active, *n* (%)	17 (73.91%)	12 (57.14%)	19 (73.08%)	0.402
Smokers, *n* (%)	1 (3.84%)	1 (4.34%)	2 (9.52%)	0.967

Mean (standard deviation); chi-square test; Tukey's test: similar letters (a, b) denote similar results, whereas different letters indicate different results.
BMI, body mass index; CO_2L, CO_2 laser.

TABLE 2. *Clinical response of the participants when CO_2L, RF, and promestriene were used for the treatments of GSM (VAS score)*

		CO_2L (n = 23)	Radiofrequency (n = 21)	Promestriene (n = 26)	P value
Fissures	Pre	2.45 (±3.99)	3.0 (±3.93)	3.15 (±3.49)	Group = 0.431
	Post	0.32 (±1.12)	1.68 (±3.48)	0.78 (±1.85)	Time < 0.001; group time = 0.711
Pruritus	Pre	1.54 (±3.26)	3.61 (±3.89)	2.61 (± 3.33)	Group = 0.037
	Post	0.27 (±0.76)	2.21 (±3.34)	0.95 (± 1.96)	Time < 0.001; group time = 0.909
Dyspareunia	Pre	7.27 (±3.65)	7.70 (± 3.78)	6.95 (±3.45)	Group = 0.49
	Post	2.71 (±3.40)	4.07 (±4.08)	2.88 (±3.14)	Time < 0.001; group time = 0.893
Trophism reduction	Pre	5.50 (±2.87)	5.57 (± 3.24)	5.11 (±3.41)	Group = 0.66
	Post	4.00 (±3.05)	3.31 (±2.33)	3.17 (± 3.14)	Time < 0.001; group time = 0.524
Thinning of vulvar rugae	Pre	5.18 (±2.98)	5.52 (±3.28)	5.15 (± 3.40)	Group = 0.92
	Post	3.61 (±2.99)	3.26 (±2.55)	3.17 (±3.14)	Time < 0.001; group time = 0.568

Mixed ANOVA; VAS of GSM symptoms (values are expressed as mean [standard deviation]).
CO_2L, CO_2 laser; RF, radiofrequency; GSM, genitourinary syndrome of menopause; VAS, visual analog scale.

layer (≤5 layers) (promestriene group). Postintervention, all histological parameters returned to normal. However, no instances of the dermal papillae rectification were observed before treatments. Table 4 displays the pretreatment and posttreatment histological parameters of the participants. Regarding hyperkeratosis, a reduction in frequency was observed following energy application but not postpromestriene usage ($P = 0.035$). Moreover, an increase in epithelial thickness was noted in the energy-treated groups, which differed from the observation in the promestriene group. However, this difference did not achieve statistical significance.

After the intervention, a trend toward decreased dermal papillae excess was observed in all groups, with statistical significance ($P = 0.044$).

In the context stromal evaluation, a normal pretreatment density was observed in 68 cases (97.2%), and normal cellularity was noted in 67 cases (95.7%). These two parameters remained unchanged after the treatment. A significant number of vessels were also observed in all groups, and this count decreased following all interventions, predominantly resulting in a normal vessel count after treatment. Furthermore, clinical and histological parameters were comparable between tamoxifen and anastrozole users before and after treatment (Table 5).

Out of the participants, nine received prophylactic treatment for genital herpes: four before CO_2L and five before RF. Subsequent to the intervention, none of the women exhibited herpes lesions. No adverse events related to vestibular introitus, such as scarring or retraction, were either reported or observed during the follow-up visits. In addition, melanosis was not identified either histologically or clinically across all the groups after treatment.

DISCUSSION

In this study, we present the first randomized clinical trial that compares the effectiveness of CO_2L, RF, and promestriene in addressing GSM among breast cancer survivors undergoing adjuvant therapy. The assessment is based on the clinical and histological evaluations of the vulvar vestibule.

Following treatment, all three groups exhibited significant and comparable clinical improvements. This aligns with findings from previous research concerning GSM in individuals not using adjuvant therapy.[15,17,18] The interventions led to a reduction in symptom intensity, with fewer women experiencing moderate-to-severe symptoms. In addition, subjective improvements were evident, as indicated by Likert scale scores.

Although the relationship between GSM symptoms and antiestrogens use is well established,[9] there is limited available information concerning vaginal and vestibular histological characteristics. In addition, women with symptoms of vulvovaginal atrophy are believed to exhibit histological atrophy, which involves a reduction in epithelial layer count, thickness, cell maturation, dermal papillae, and stromal vascularization.[19]

Notably, a significant majority of the study participants were postmenopausal (average 7.65 [±4.95] years, since menopause onset), displayed moderate-to-severe GSM symptoms, and had been using antiestrogens for approximately 2.5 years. However, contrary to expectations, over 90% of cases did not meet the histologic atrophy criteria described earlier.

Instead, a consistent pattern of normal cell maturation in the epithelium was observed both before and after treatment.

Although lacking statistical significance, an observable trend of heightened epithelial thickness emerged in the energy-treated

TABLE 3. *Number of women with GSM (VAS ≥ 4) pretreatment and posttreatment*

		CO_2L (n = 23)	Radiofrequency (n = 21)	Promestriene (n = 26)	P value
Fissures	Pre	6/23 (26.08%)	8/21 (28.57%)	11/26 (42.3%)	Group = 0.284
	Post	0/23 (0%)	3/21 (14.28%)	6/26 (23.07%)	Time = 0.005; group time = 0.416
Pruritus	Pre	4/23 (17.39%)	9/21 (42.85%)	8/26 (30.76%)	Group < 0.001
	Post	0/23 (0%)	2/21 (9.52%)	3/26 (11.53%)	Time < 0.001; group time = 0.756
Dyspareunia	Pre	14/23 (40.86%)	13/21 (61.9%)	15/26 (57.69%)	Group = 0.507
	Post	4/23 (17.39%)	7/21 (33.33%)	7/26 (26.92%)	Time < 0.001; group time = 0.118
Trophism reduction	Pre	19/23 (82.6%)	17/21 (80.95%)	18/26 (69.23%)	Group = 0.687
	Post	12/23 (52.17%)	9/21 (42.85%)	10/26 (38.46%)	Time = 0.001; group time = 0.596
Thinning of vulvar rugae	Pre	18/23 (78.26%)	16/21 (76.19%)	18/26 (69.23%)	Group = 0.878
	Post	10/23 (43.47%)	9/21 (42.85%)	10/26 (38.46%)	Time < 0.001; group time = 0.582

CO_2L, CO_2 laser; GSM, genitourinary syndrome of menopause; GEE, generalized estimating equation.

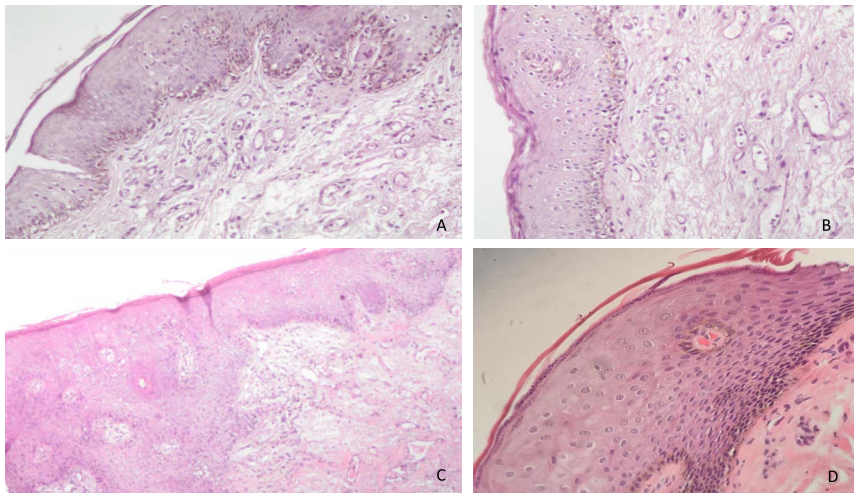

FIG. 2. Histological aspects of vulvar vestibule of women with GSM in use of adjuvant therapy. **(A)** Enhanced number of vessels; pattern presented in 33/70 (47%) women before treatment and in 24/70 (34%) after treatment. **(B)** Hyperkeratosis; pattern presented in 31/70 (44%) women before treatment and in 20/70 (28%) after treatment. **(C)** Enhanced number of dermal papillae; pattern presented in 45/70 (64%) women before treatment and in 33/70 (47%) after treatment. **(D)** Vulvar vestibule: normal keratin layer, normal number of epithelial layers, cell maturation, and number of dermal papillae as well as number of vessels; pattern presented in 6/70 (8.5%) women before treatment and in 17/70 (24%) after treatment.

groups. This observation aligns with the findings of other authors who have investigated the impact of CO_2L on the vaginal mucosa, although not specifically on the vulvar vestibule.[19-21] Furthermore, this enhancement in epithelial thickness was absent in the promestriene group. Nevertheless, the assessment of thickness within a biopsy fragment introduces the potential for low reproducibility, largely owing to the influence of material processing techniques. Given the extremely small fragments, achieving a precise orientation of vulvar specimens during the embedding and sectioning procedures to ensure that the section is perpendicular to the mucosa is challenging. This aspect was not guaranteed in this particular study as well. Therefore, oblique slices might lead to an underestimation or overestimation of the sample thickness measurements. Zerbinati et al[20] highlighted the significance of meticulous cutting of a piece that is perpendicular to the mucosa, which is crucial for accurately assessing epithelial thickness.

Another parameter used to identify cutaneous atrophy is a decrease in the number of dermal papillae, which involves changes in the dermoepidermal junction. Surprisingly, this alteration was not observed in any of the women before treatment, and this pattern has persisted posttreatment as well.

TABLE 4. *Histomorphometric parameters pretreatment and posttreatment*

		CO_2L (n = 23)	RF (n = 21)	Promestriene (n = 26)	P value
Epithelium					
Thickness, μm[a]	Pre	2.36 (±0.87)	2.52 (±0.80)	2.55 (±1.10)	Group = 0.736[b]
	Post	2.68 (±0.94)	2.75 (±1.25)	2.32 (±0.76)	Time = 0.460; group × time = 0.195
Hyperkeratosis, n (%)	Pre	13 (56.52%)	11 (52.38%)	7 (26.92%)	Group = 0.151[c]
	Post	9 (39.13%)	4 (19.05%)	7 (26.92%)	Time = 0.035; group × time = 0.263
Dermal papillae, n (%)					
Enhanced, n (%)	Pre	16 (69.57%)	11 (52.38%)	18 (69.23%)	Group = 0.194[c]
	Post	13 (56.52%)	8 (38.10%)	12 (46.15%)	Time = 0.044; group × time = 0.852
Normal, n (%)	Pre	7 (30.43%)	10 (47.61%)	8 (30.76%)	
	Post	10 (43.47%)	13 (61.90%)	14 (53.84%)	
Reduced, n (%)	Pre	0 (0%)	0 (0%)	0 (0%)	
	Post	0 (0%)	0 (0%)	0 (0%)	
Stroma					
Number of vessels					
Enhanced, n (%)	Pre	12 (52.17%)	7 (33.33%)	14 (53.85%)	Group = 0.099[b]
	Post	8 (34.78%)	5 (23.81%)	11 (42.31%)	Time = 0.163; group × time = 0.850
Normal, n (%)	Pre	10 (43.48%)	13 (61.9%)	12 (45.15%)	Group × time = 0.850
	Post	14 (60.87%)	16 (76.19%)	15 (57.69%)	
Reduced, n (%)	Pre	1 (4.35%)	1 (4.76%)	0 (0%)	
	Post	1 (4.35%)	0 (0%)	0 (0%)	

CO_2L, CO_2 laser; RF, radiofrequency.
[a]Mean (standard deviation).
[b]Mixed-model ANOVA.
[c]GEE, generalized estimating equation.

TABLE 5. *GSM (VAS), histological findings, and Likert scale according to the adjuvant therapy used*

	Tamoxifen	Anastrozole	Total	*P* value
GSM (VAS)				
Fissure				
Pre	3.68 (4.05)	2.43 (3.54)	2.88 (3.75)	0.192[a]
Post	0.42 (1.32)	1.18 (2.74)	0.89 (2.32)	0.503[a]
Pruritus				
Pre	3.08 (4.01)	2.3 (3.25)	2.58 (3.54)	0.324[a]
Post	1.5 (2.6)	0.85 (2.12)	1.09 (2.31)	0.215[a]
Dyspareunia				
Pre	6.39 (3.54)	7.94 (3.51)	7.29 (3.57)	0.309[a]
Post	2.63 (2.87)	3.61 (3.89)	3.21 (3.51)	0.426[a]
Trophism reduction				
Pre	4.6 (3.07)	5.82 (3.16)	5.38 (3.16)	0.092[a]
Post	3.21 (3.02)	3.67 (2.79)	3.49 (2.87)	0.494[a]
Thinning of vulvar rugae				
Pre	4.68 (3.08)	5.61 (3.24)	5.28 (3.19)	0.189[a]
Post	3.17 (3.05)	3.46 (2.82)	3.35 (2.89)	0.614[a]
Histological findings				
Epithelium				
Hyperkeratosis, *n* (%)				
Pre	10 (40%)	21 (46.67%)	31 (44.29%)	0.591[b]
Post	7 (28%)	13 (28.89%)	20 (28.57%)	0.937[b]
Epithelium thickness, μm[c]				
Pre	2.6 (0.95)	2.42 (0.94)	2.48 (0.94)	0.390[a]
Post	2.86 (1.02)	2.42 (0.96)	2.57 (0.99)	0.153[a]
Dermal papillae				
Enhanced, *n* (%) Pre	20 (80%)	25 (55.56%)	45 (64.29%)	0.041[b]
Normal, *n* (%) Pre	5 (20%)	20 (44.44%)	25 (35.71%)	
Reduced, *n* (%) Pre	0 (0%)	0 (0%)	0 (0%)	
Enhanced, *n* (%) Post	13 (52%)	20 (44.44%)	33 (47.14%)	0.544[b]
Normal, *n* (%) Post	12 (48%)	25 (55.56%)	37 (52.86%)	
Reduced, *n* (%) Post	0 (0%)	0 (0%)	0 (0%)	
Stroma				
Number of vessels				
Enhanced, *n* (%) Pre	13 (52%)	20 (44.44%)	33 (47.14%)	0.346[b]
Normal, *n* (%)	10 (40%)	25 (55.56%)	35 (50%)	
Reduced, *n* (%)	2 (8%)	0 (0%)	2 (2.86%)	
Enhanced, *n* (%) Post	10 (40%)	14 (31.11%)	24 (34.29%)	0.381[b]
Normal, *n* (%)	14 (56%)	31 (68.89%)	45 (64.29%)	
Reduced, *n* (%)	1 (4%)	0 (0%)	1 (1.42%)	
Likert scale				
Post	4.6 (0.87)	4.3 (0.91)	4.41 (0.9)	0.081[a]

GSM, genitourinary syndrome of menopause; VAS, visual analog scale.
[a]Mann-Whitney *U* test.
[b]Chi-square test.
[c]Mean (standard deviation).

Hyperkeratosis, frequently found before treatment, may lead to itching and fissures owing to reduced skin elasticity. Hyperkeratosis was less common after energy-based treatments. A decrease in keratin content can enhance skin elasticity and decrease susceptibility to trauma, which may explain the alleviation of symptoms, particularly pruritus, fissures, and penetration dyspareunia.

No major structural changes were observed in the stroma. There was a trend toward vascular normalization following the proposed therapies. In a Doppler study of periurethral vessels in tamoxifen users, Faria[22] observed an increase in vascularization related to it use. This observation may clarify the frequent occurrence of bleeding during physical examinations in patients with GSM and rationalize the observed improvement posttreatment. Stromal density remained consistent across the groups and within the normal range both before and after treatment. Furthermore, no histological melanosis was observed in the reticular dermis following the application of energy-based treatments.[22]

Tamoxifen and anastrozole are the most commonly prescribed agents during adjuvant therapy. Although tamoxifen is categorized as a SERM with supposed agonist estrogen effect in the vagina, this effect was observed only in animals and has not been previously demonstrated in humans. No difference has been reported in the frequency and intensity of symptoms between tamoxifen users and anastrozole (an antiestrogen drug) users. Moreover, the histological vulvar vestibule structure was also similar between the groups, except for the enhanced number of dermal papillae in tamoxifen users before treatment, which witnessed comparable reduction in both groups posttreatment. In addition, the clinical responses to treatment were similar for both adjuvant therapies.

A subset of women (5.71%) included in this study exhibited histological atrophy in vestibular tissue before treatment, notwithstanding their symptomatic condition and concurrent adjuvant hormone therapy. Remarkably, all patients demonstrated substantial improvement in clinical parameters and modified histological parameters postintervention. Consequently, a decoupling of clinical and histological findings emerged. This outcome aligns with the findings of Davila et al,[23] where evaluation involving questionnaires, pH measurements, and the vaginal maturation index among 135 women revealed a lack of correlation between atrophy symptoms and the maturation index.

The majority of the study participants were nonsmokers (94.29%), potentially contributing to the notable clinical response, as tobacco is recognized for its antiestrogenic effect.[24] A substantial proportion of participants were also sexually active (68.5%), likely motivating their involvement. According to Goldstein et al,[25] alterations in vestibular tissue related to GSM are intimately connected to dyspareunia owing to the endodermal origin and extensive nerve innervation of the region. Regular sexual activity is recognized to enhance vaginal trophism and supports therapeutic responses to GSM. This characteristic may be a factor delaying structural changes and might also be associated with improved responses to provided treatments.[25]

One limitation of this study was the histological assessment (hematoxylin and eosin) method used, which may not be optimal for evaluating the vascularization patterns and extracellular matrix composition. Nonetheless, it provided an initial and unprecedented descriptive evaluation of the effects of adjuvant therapy on vulvar vestibule tissue, as well as the response to the proposed treatments among women.

The absence of a sham control group could potentially raise doubts about interpreting clinical outcomes beyond the energy-treated groups. However, employing a sham control group was not deemed ethically viable, given that these women were already dealing with breast cancer and GSM. Therefore, it was imperative to utilize a therapeutic control.

The adverse events reported and observed in this study, such as pain during CO_2L or RF[21,26] application, as well as vaginal bleeding following promestriene use, align with documented instances in the literature. Importantly, no cases exhibited clinical or histological melanosis or any other form of tissue damage after applying both types of energy methods and promestriene, underscoring their safety.

In a significant shift, the findings presented in this study challenge conventional understanding. It becomes evident that many women experiencing symptoms consistent with vulvovaginal atrophy may not necessarily display histological changes. Drawing on these findings, it can be inferred that the issues manifest in the vulvar vestibule tissue could be attributed to dysfunction rather than a structural alteration itself, particularly in the context of previously established histological atrophy parameters. These symptoms might precede actual tissue structural changes. Consequently, it is essential to conduct further investigations that include immunohistochemistry studies and molecular analyses. This approach will yield deeper insights into the functional characteristics of GSM-affected tissues in women, along with the mechanisms underpinning the actions of these therapies.

CONCLUSIONS

In conclusion, among breast cancer survivors undergoing adjuvant therapy, both CO_2L and RF achieved clinical responses comparable with those of promestriene. All participants reported elevated levels of satisfaction after all treatments, regardless of the type of adjuvant therapy used. Although the prevalence of pretreatment atrophic tissue was limited, it consistently normalized postintervention.

Throughout the 4-month follow-up period, notable enhancements in vulvar atrophy were observed, with no indication of tissue damage posttreatment. Furthermore, the absence of serious adverse events underscores the safety of these methodologies when adhering to equipment usage protocols. However, to establish the full scope of efficacy and safety, it is necessary to undertake more extensive and prolonged trials. These studies will provide a more comprehensive understanding of these methods and validate the initial findings presented in this study.

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