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ORIGINAL ARTICLE

Fractional CO_2 laser therapy: a new challenge for vulvovaginal atrophy in postmenopausal women

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ABSTRACT

Objective: To evaluate the effects of CO_2 laser in the treatment of vulvovaginal atrophy (VVA) in postmenopausal women.

Methods: VVA was assessed in 87 postmenopausal women (mean age 58.6 ± 6.9 years) before and after the treatment. The protocol consisted of three monthly treatments and included the treatment of vulva. Subjective measures included VAS (Visual Analog Scale) both for vaginal dryness and dyspareunia; DIVA (Day-by-day Impact of Vaginal Aging); a questionnaire on treatment satisfaction and one about the degree of pain during the procedure. Objective measures included VHI (Vaginal Health Index) and VVHI (Vulvo-Vaginal Health Index). Time points of the study were at the screening visit (T0), at baseline (T1), at week 4 (T2), at week 8 (T3), after 3 months since the last laser application (T4), after 6 months (T5), after 9 months (T6), after 12 months (T7) and after 15 months (T8).

Results: Treatment induced significant improvement in the VAS score. After treatment, VHI and VVHI indicated no VVA and this improvement was long lasting. Multivariate analysis showed that the time of follow-up was correlated with better VHI and VVHI ($p < 0.001$). DIVA improved over time ($p < 0.001$).

Conclusions: This study shows that CO_2 laser treatment induces a significant and long-lasting improvement of symptoms.

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Introduction

Fifty per cent of menopausal women experience symptoms of vulvovaginal atrophy (VVA), recently included in the definition of genitourinary syndrome of menopause (GSM)[1], which includes genital symptoms such as vaginal dryness, burning and irritation; sexual symptoms such as dyspareunia and lack of lubrication; and urinary symptoms of urgency, dysuria and recurrent urinary tract infection[1]. Estrogen deprivation results in an adverse transformation of the vaginal epithelium from a thick, well-wrinkled appearance to one that is thin and pale[2]. The anatomical changes in GSM include connective tissue proliferation, elastin fragmentation and collagen hyalinization with progressive loss of vascularization in the vaginal submucosa. Collagen turnover is increased and vaginal pH becomes less acid in postmenopausal women[2].

Unlike hot flushes and night sweats which resolve spontaneously over time, atrophic symptoms affecting the vagina and lower urinary tract are often progressive and frequently require treatment[3]. The symptoms associated with GSM are often under-reported by women, under-recognized by health-care providers and, therefore, under-treated[4].

All these symptoms may interfere with quality of life and with sexual function[4–9]. Guidelines recommend non-hormonal moisturizers and lubricants as first-line treatment, but they provide only a temporary relief[3]. Local vaginal therapy is the treatment of choice for women non-responding to non-hormonal therapy and for isolated VVA symptoms[3]. However, local estrogen therapy is prescribed with reluctance in patients with hormone-dependent cancer for the fear of potential absorption of estrogens that can stimulate cancer cell growth[10,11]. Even if new formulations with low and ultra-low doses of vaginal estrogens are available[12–19] and can be an option to be discussed with symptomatic women with previous hormone-dependent cancer not responding to non-hormonal therapies[20], reluctance still persist among health-care providers.

In the last few years, laser therapy has been introduced also in gynecology as a new non-hormonal option for the treatment of VVA. For this reason, non-pharmacological tools for GSM treatment, such as the vaginal laser, play an important role when estrogen therapy fails or it is contraindicated. Laser treatment, initially only used in dermatology, was introduced in the gynecological field after *the ex-vivo* study by Salvatore and colleagues[21] who first analyzed the effect of the CO_2 laser on atrophic vaginal tissues. Actually, there are two main types of laser that have been tested in VVA: fractional CO_2[21] and the erbium laser[22]. In the literature, there are not so many data reporting follow-up after laser therapy so the aim of the present study was to evaluate the feasibility and the effectiveness of the fractional CO_2 laser in a cohort of postmenopausal women with GSM.

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Methods

Study design

This study was conducted between April 2014 and April 2016 at the Sedes Sapientiae Institute of Turin and included postmenopausal women with symptoms related to VVA. All women gave written informed consent and an Independent National Advisory Board reviewed this protocol approved by the Division Ethics Committee.

Study population

A total of 91 patients were enrolled in the study. The inclusion criteria consisted of menopausal status (amenorrhea for at least 12 months or for 6 months with serum levels of estradiol $\leq$30 pg/ml and follicle stimulating hormone $\geq$50 IU/l); negative cervical smear in the 12 months before the study; symptoms of VVA (vaginal dryness and/or dyspareunia and/or absence of sexual intercourse). Exclusion criteria were: use of hormone replacement therapy up to 6 months before the study recruitment, active genital infection (e.g. herpes genitalis, candida), obesity (body mass index $>$40 kg/m^2), use of photosensitizing drugs, inability to sign the informed written consent, use of drugs that may interfere with the efficacy and tolerability of the laser therapy.

Study data

Eight relevant time points were considered for the evaluation of treatment results: baseline (T0), before the first laser application (T1), before the second laser application (T2), before the third laser application (T3), 3 months after the last laser application (T4), 6 months after the last laser application (T5), 9 months after the last laser application (T6), 12 months after the last laser application (T7), and 15 months after the last laser application (T8). Relevant demographic characteristics, pretreatment clinical data and inclusion/exclusion criteria were recorded at T0.

Women were evaluated by using objective and subjective parameters. Objective parameters included the Vaginal Health Index (VHI) and the Vulvo-Vaginal Health Index (VVHI) evaluated at each time point of the study (T0, T1, T2, T3, T4, T5, T6, T7, T8). The VHI consists of five measures: elasticity, fluid volume, pH, epithelial integrity and moisture. Each parameter is graded from 1 to 5. If the total score is $<$15, the vagina is considered atrophic[22]. The VVHI consists of eight measures: labia majora, labia minora, clitoris, urethra, vaginal introitus and elasticity, color, bother and pain, other elements (e.g. ulcerations, petechiae). Each parameter is graded from 0 to 3. If the total score is $>$8 or if the total is 3 for any of the categories, the vulva is considered atrophic[23].

Subjective parameters included: Visual Analogic Scale (VAS) for pain both for dyspareunia and vaginal dryness, evaluated at each time point of the study (T0, T1, T2, T3, T4, T5, T6, T7, T8), the Day-by-day Impact of Vaginal Aging (DIVA) questionnaire, completed at T0 and T4, a questionnaire about the degree of pain encountered in the procedure (insertion of the probe, vaginal treatment and vulvar treatment), completed after each laser treatment, a questionnaire on treatment satisfaction (very satisfied, satisfied, uncertain, dissatisfied and very dissatisfied) completed at T4.

The VAS is a 10-cm scale, where the left extreme indicates 'absence of symptom' and the right indicated 'symptom as bad as it could be'[24]. DIVA is a four multi-item scale addressing vaginal symptom impact on activities of daily living, emotional well-being, sexual functioning, self-concept and body image[25].

Study protocol

Patients were treated with the fractional microablative CO_2 laser system (SmartXide^2 V^{2}LR, Monalisa Touch, DEKA, Florence, Italy) using the following setting: for endovaginal treatment dot power 40 watt and smart stack parameter from 1 to 3.

A vaginal probe was inserted up to the top of the vaginal channel, withdrawn and rotated in order to provide a complete treatment of the vaginal walls. Then the vaginal introitus and the vulvar zone (labia majora, labia minora, clitoris and interlabial sulci) were treated with a lower power: for the treatment of introitus dot power ranging from 15 to 30 watt and smart stack parameter 1 or 2; for the vulvar treatment dot power from 15 to 30 and smart stack parameter 1 or 2. The treatment of the introitus and vulvar zone was performed with a special probe, similar to the one used for skin. The external limits of the treated vulvar area were: anterior labial commissure (superior), labia majora (lateral) and posterior labial commissure (inferior). The protocol included three laser treatments, one every 4 weeks. The procedure was performed in the outpatient clinic with a prior application, in some cases, of topical anesthetic cream. The use of lubricants or moisturizers was not allowed during the study period.

Statistical analysis

All the analysis were performed using the software Stata 13 (Stata Corp., College 2013). Initial descriptive statistics included frequencies and percentages for categorical variables, mean and the standard deviation and median with interquartile range for continuous variables. The same procedure was conducted to describe the average scores of the four main evaluation scales (VAS, VHI, VVHI and DIVA). Potential predictors of dyspareunia, vaginal dryness and vaginal and vulvar atrophy were then investigated through multilevel multivariate linear regression analyses. As the first level, we considered the follow-up time and as second level the patients. Data were then reported as coefficients and *p*-values. A two-tailed *p*-value of 0.05 was considered significant for all analyses.

Each regression coefficient represents the variation of the dependent variable 'y' resulting from a unitary variation of the independent variable 'x'. Therefore each coefficient represents the additional effect of adding that variable to the model if the effects of all other variables in the model are already accounted for.

Results

The main characteristics of this study population are described in Table 1. The mean age was 59 years and most

of the women have a normal body mass index (21.9 kg/m^2, range 20–24 kg/m^2). Only 5% of the women were smokers and 38% of them had at least one vaginal birth. The mean age at menopause was 48 years and 35% of them had previously tried local estrogen therapy.

Among the 91 women enrolled, 87 (95.6%) completed the treatment with fractional CO_2 laser, two dropped out of the study for personal reasons: one left because of concurrent use of a drug interfering with the procedure (amitriptyline) and one because of hyperpyrexia after the first laser application. Out of the 87 patients who completed the treatment, 13 (14.3%) had previous breast cancer. The mean age of the 13 breast cancer survivors was 59 years and all women had a normal body mass index (22.7 kg/m^2, range 22–23 kg/m^2). Eleven women were non-smokers, one was a smoker and one an ex-smoker. Five of them had a vaginal birth and four women had used systemic hormone replacement therapy before the diagnosis of breast cancer. The characteristics of the subgroup are presented in Supplementary Table S1 (see http://dx.doi.org/10.1080/13697137.2017.1319815).

The 3-month follow-up (T4) was completed by 98.8% (86 women), 82.8% (72 women) completed the 6-month follow-up (T5), 56.3% (49 women) completed the 9-month follow-up (T6), 47.1% (41 women) completed the 12-month follow-up (T7) and 12.6% (11 women) completed the 15-month follow-up (T8).

Univariate analysis

Before starting the laser therapy (T1), about 35% of the patients were sexually inactive because of VVA; after two laser treatments (T3) only 13% were still inactive, and this observation persisted up to the 1-year control (T7).

At T1, among sexually active women, the mean VAS score for dyspareunia was indicative of severe symptoms, becoming moderate at T3 and with a further improvement at the 3-month follow-up (T4) visit (mild symptoms, $p < 0.001$).

Vaginal dryness improved from severe at baseline to moderate after only one laser treatment. At T4, vaginal dryness became mild and the benefit was maintained at the follow-up period ($p < 0.001$).

The VHI score improved from baseline (T1), where the score was showing atrophy for all women, to the 12-month follow-up visit (T7) where most of the women (88%) did not report vaginal atrophy anymore ($p < 0.001$) (Table 2).

Also, the VVHI improved from baseline (T1), where scores were indicative of vulvar atrophy in the whole population, to the 12-month follow-up visit (T7) where vulvar atrophy persisted in less than 5% of women ($p < 0.001$).

Table 2 reports the mean (± standard deviation) values of the VAS score both for vaginal dryness and dyspareunia during the study period.

Quality of life, as measured by DIVA questionnaire, daily living, emotional well-being, sexual functioning, self-concept and body image significantly improved at T4 in comparison with T1 (activities of daily living: 4.8 ± 4.6 vs. 1.7 ± 3.1, emotional well-being: 8.6 ± 5.2 vs. 4.2 ± 3.6, sexual functioning: 8.5 ± 5.1 vs. 5.1 ± 3.4; and self-concept and body image:7.5 ± 4.2 vs. 3.9 ± 3.8; $p < 0.001$).

As for the effects on mood of vaginal symptoms, many women, in the pre-therapy questionnaire (T0), said that these vaginal symptoms have often caused them sadness, depression, frustration and dissatisfaction with themselves.

As to sexual functioning, many women, before starting the treatment, said they were dissatisfied about their sexual life.

In addition, at baseline, the majority of women had an image of themselves as aged, less seductive, undesirable, as if they had lost something because of their vaginal symptoms. At the 3-month follow-up (T4), women felt more attractive and more desirable.

As regards the grade of satisfaction with the laser treatment at T4, 32 (37.7%) women were very satisfied, 45 (52.9%) satisfied, 6 (7.1%) uncertain and 2 (2.4%) very dissatisfied.

The pain encountered during the laser applications progressively improved over time. At the probe insertion in the first laser treatment, pain was slight or moderate in half of the cases, becoming absent in 60% of cases during the second laser application and in 92% during the last laser application.

The pain during introitus treatment was also slight or moderate in up to 50% of cases at the first laser treatment, becoming absent in 90% of cases during the third laser application. Treatment of vulva was the most painful: at the

Table 1. Baseline characteristics of the study population ($n = 87$). Data are given as mean ± standard deviation (SD), n (%) or median (range).

	Mean ± SD	*Median (range)*	*n*	*%*
Age (years)	58.6 ± 6.9	59 (53–62		
Body mass index (kg/m^2)	22.3 ± 2.7	21.9 (20–24)		
Smokers			5	5.75
Non-smokers			75	86.21
Ex-smokers			7	8.05
Years of smoke exposure	21.8 ± 10.3			
Number of cigarettes/day	10.5 ± 6.7	9.5 (5–14)		
Previous breast cancer			13	14.94
Vaginal birth			33	37.93
Age at menopause (years)	48.23 ± 5.01			
Previous systemic HRT			25	28.74
Duration of previous HRT (months)	51.32 ± 45.90	50 (45–52)		
Previous LET			31	35.63
Duration of previous LET (months)	30.52 ± 40.29			

HRT, hormone replacement therapy; LET, local estrogen therapy.

Table 2. Vaginal Health Index, Vulvo-Vaginal Health Index and vulvovaginal symptoms (vaginal dryness and dyspareunia) during the study period. Data are given as mean ± standard deviation (number of women).

	T1	*T2*	*T3*	*T4*	*T5*	*T6*	*T7*	*T8*
Vaginal Health Index	8.3 ± 2.2 (87)	12.5 ± 2.7 (87)	16.4 ± 3.0 (87)	17.9 ± 3.4 (86)	18.3 ± 3.5 (72)	19.4 ± 3.6 (49)	19.6 ± 3.6 (41)	20.3 ± 3.0 (9)
Vulvo-Vaginal Health Index	16.3 ± 2.6 (87)	12.3 ± 2.5 (87)	5.9 ± 1.9 (87)	5.3 ± 2.7 (84)	4.3 ± 2.6 (72)	3.4 ± 2.5 (49)	3.2 ± 2.6 (41)	2.3 ± 1.8 (9)
VAS of vaginal dryness (cm)	8.8 ± 1.71 (87)	5.7 ± 1.7 (87)	3.7 ± 2.3 (87)	2.8 ± 2.3 (86)	2.8 ± 2.4 (72)	2.8 ± 2.5 (49)	2.9 ± 2.8 (41)	2.5 ± 2.3 (11)
VAS of dyspareunia (cm)	8.7 ± 1.7 (56)	6.24 ± 1.9 (62)	4.63 ± 2.6 (76)	3.7 ± 2.6 (74)	3.6 ± 2.7 (61)	3.5 ± 3.0 (43)	3.6 ± 3.2 (36)	1.9 ± 2.2 (10)

VAS, Visual Analog Scale.

Table 3. Potential predictors of dyspareunia and dryness according to the Visual Analog Scale, multivariate analysis.

	Dyspareunia		*Dryness*	
	Coefficient	*p-value*	*Coefficient*	*p-value*
Age (years)	0.04	0.458	−0.04	0.286
Body mass index (kg/m^2)	0.06	0.482	0.18	0.008
Smoking status				
non-smokers	Ref.		Ref.	
smokers	2.76	0.002	2.38	0.002
ex-smokers	−0.39	0.620	−1.15	0.071
Observation time				
before the 1st laser (T1)	Ref.		Ref.	
before the 2nd laser (T2)	−2.46	<0.001	−3.02	<0.001
before the 3rd laser (T3)	−4.40	<0.001	−4.99	<0.001
after 3 months (T4)	−5.34	<0.001	−5.87	<0.001
after 6 months (T5)	−5.47	<0.001	−5.99	<0.001
after 9 months (T6)	−5.58	<0.001	−6.07	<0.001
after 12 months (T7)	−5.35	<0.001	−5.98	<0.001
after 15 months (T8)	−5.65	<0.001	−5.70	<0.001
Previous breast cancer				
no	Ref.		Ref.	
yes	1.87	0.003	0.24	0.634
Previous vaginal birth				
no	Ref.		Ref.	
yes	0.65	0.174	−0.03	0.786
Years of menopause	−0.47	0.325	0.04	0.210
Previous HRT				
no	Ref.		Ref.	
systemic	−1.18	0.072	0.05	0.957
local	−0.76	0.143	0.46	0.222
both	−1.34	0.101	−0.61	0.326
First therapeutic indication				
absence of sex	Ref.		Ref.	
dyspareunia	−0.77	0.178	0.40	0.409
vaginal dryness	−0.75	0.366	0.19	0.849

first laser treatment, the pain was slight or moderate in 70% of cases, decreased at 53% during the second laser application and at 30% during the last treatment.

Multivariate analysis

In order to define potential predictors of dyspareunia, vaginal dryness, vaginal and vulvar atrophy, we performed a multilevel multivariate analysis adopting the VAS scores, VHI and VVHI scores as continuous variables for two linear regression models (Table 3 and Supplementary Table S2, see http://dx.doi.org/10.1080/13697137.2017.1319815).

Women with a previous breast cancer (coefficient 1.87; $p = 0.003$) showed a higher probability to report dyspareunia; smokers had more severe dyspareunia (coefficient 2.76; $p = 0.002$) and vaginal dryness (coefficient 0.18; $p = 0.008$).

Women with a previous breast cancer (coefficient −1.81; $p = 0.022$) showed a higher probability to report vaginal atrophy, while smokers and women with a previous vaginal birth displayed vulvar atrophy (coefficients 1.81 and 1.04; $p = 0.046$ and 0.024, respectively).

VAS, VHI and VVHI scores improved significantly at the end of laser therapy and during the follow-up period ($p < 0.001$).

Discussion

This study shows that CO_2 laser treatment is effective and easy to perform for VVA symptoms in postmenopausal women. The positive laser effect on the VVA symptoms was already evident after the first laser application, with a further improvement of both objective (increase in VHI score and decrease in VVHI score) and subjective (decrease in VAS score and improvement of DIVA questionnaire) evaluations up to 15 months (T8). The majority of patients were satisfied with laser therapy and no serious adverse events due to fractional CO_2 laser occurred. The results of our study are promising and consistent with the clinical data reported in the literature[22,26–28]. Other studies have evaluated vaginal laser in the literature: four focused on the CO_2 laser[26–28] and one study evaluated the erbium laser[22].

In previous studies, the VHI score was employed as an objective parameter while the VAS scale was used for the subjective evaluations. Since, in our study, the laser application was also performed in the vulvar zone, we have also used, as an objective parameter, the VVHI score.

The effectiveness of the treatment on the subjective symptoms experienced by patients is demonstrated both in our study and in the literature. In our study, dyspareunia, evaluated with the VAS scale, greatly improved over time, decreasing from severe to moderate from T1 to T3, with a further improvement at the 3-month follow-up visit (T4). In the 11 cases who completed the 15-month follow-up (T8), a further improvement was observed. Vaginal dryness evaluated through the VAS scale improved from severe at baseline to moderate after only one laser treatment. At T4, vaginal dryness became mild with a long-lasting effect.

In the study of Salvatore and colleagues[26], the VAS scores both for dyspareunia and vaginal dryness improved over time: at baseline, the symptoms were severe while at 4 weeks after the last laser application the symptoms were moderate, becoming mild at 3-month follow-up.

Perino and colleagues[28] observed the same improvement in VAS scores both for dyspareunia and vaginal dryness at the 1-month follow-up. Also in the study of Gambacciani and colleagues[22] with the erbium laser, at baseline VAS scores of dyspareunia and vaginal dryness were indicative of severe symptoms, becoming mild after the third laser application and long-lasting up to the 24-week follow-up.

In our study, baseline VHI values indicated atrophy for all patients in the study. After two laser treatments, VHI scores indicated no atrophy.

Our study is the only one reporting data related to the VVHI score. At baseline, VVHI values indicated atrophy. After two laser treatments, 94% of women had a VVHI indicative of the resolution of the VVA.

In our study, moreover, quality of life was evaluated through the DIVA questionnaire addressing symptom impact on activities of daily living, emotional well-being, sexual functioning, self-concept and body image. All parameters significantly improved after treatment in comparison with baseline. Also in the study by Salvatore and colleagues[27], sexual function, evaluated through the FSFI (Female Sexual Function Index) questionnaire, showed a significant improvement after laser therapy compared to pretreatment evaluation.

Compared to other studies, this study is the one with the greatest number of patients and includes a subgroup of breast cancer survivors. Two studies have been published recently on breast cancer survivors: a retrospective study[29]

included 26 women affected by hormone receptor-positive breast tumors treated for VVA symptoms with the fractional microablative CO_2 laser system and one study[30] included 50 patients who underwent fractional microablative CO_2 laser treatment for dyspareunia in oncological menopause. Treatment resulted in a significant regression of VVA symptoms and procedure-related discomfort versus baseline in both studies.

The follow-up, in our study, was completed to 15 months, although on a small number of patients, while the 12-month follow-up was completed by 47% of patients. In the study by Gambacciani and colleagues[22] on the erbium laser, the follow-up lasted up to 24 weeks, and among studies on the CO_2 laser, this study has the longest follow-up; others are restricted to observation up to 12 weeks after the end of treatment. Moreover, this is the first study in which the laser was also applied to the vulvar zone.

Conclusion

A longer follow-up is needed in order to establish whether laser treatment is effective over years. The major limits of our study are the absence of a control group and the limited number of patients with previous breast cancer. This is a particularly interesting set of women as they cannot be treated with systemic and local estrogens and could benefit from laser treatment. Further studies are therefore needed to explore the effect of laser treatment on a larger population of patients with hormone-dependent cancer.

Conflict of interest N. Biglia had financial relationships (member of advisory boards and/or consultant) with Gedeon Richter, Italfamarco S.p.A and Shionogi Ltd. V. E. Bounous had a financial relationship with Italfarmaco S.p.A. The other authors declare that they have no conflict of interest.

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