



Microablative fractional CO₂-laser therapy and the genitourinary syndrome of menopause: An observational study



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ABSTRACT

Objectives: This study aimed to assess the effect of the Microablative Fractional CO₂ Laser (CO₂-laser) therapy on vaginal pathophysiology and the symptoms of the Genitourinary Syndrome of Menopause (GSM).

Methods: Postmenopausal women with moderate to severe symptoms of GSM underwent three sessions of CO₂-laser therapy at monthly intervals. Participants were evaluated at baseline and 4 weeks after the last treatment.

Main outcome measures: The primary outcomes were Vaginal Maturation Value (VMV) and Vaginal Health Index Score (VHIS). Secondary outcomes included symptoms of GSM, Female Sexual Function Index (FSFI), International Consultation on Incontinence Questionnaire of Female Urinary Tract Symptoms (ICIQ-FLUTS) and Urinary Incontinence Short Form (ICIQ-UI SF), Urogenital Distress Inventory (UDI-6) and King's Health Questionnaire (KHQ).

Results: Fifty-three postmenopausal women completed this study. VMV, VHIS and FSFI increased significantly. Dyspareunia, dryness, burning, itching, dysuria, frequency, urgency, urgency incontinence, stress incontinence and scores on the ICIQ-FLUTS, ICIQ-UI SF, UDI-6 and KHQ decreased significantly. Factors predicting for which women the CO₂-laser therapy was more effective were not identified.

Conclusion: This study suggests that intravaginal CO₂-laser therapy for postmenopausal women with clinical signs and symptoms of GSM may be effective in improving both vaginal pathophysiology and reported symptoms.

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1. Introduction

The Genitourinary Syndrome of Menopause (GSM) affects menopausal women due to the decline mainly of estrogens involving clinical signs and symptoms from both the genital and the lower urinary tract (LUTS) [1]. Women may present with some or all of the clinical signs and symptoms [1]. The GSM definition was introduced to describe more accurately the urogenital changes and the local symptoms appearing at menopause in comparison to the terms of vulvovaginal atrophy (VVA)/atrophic vaginitis [1].

The principles of treatment of VVA are the restoration of urogenital physiology and the alleviation of symptoms [2]. The therapeutic options for VVA can include non-hormonal local therapies (i.e. lubricants, moisturizers) or hormonal ones (i.e. low-dose vaginal estrogens). However, the 2 principles of treatment usually are not achieved. Lubricants offer a temporary relief of vaginal symptoms, without restoration of urogenital physiology. Moisturizers improve lubrication but have no effect on the overall vaginal maturation index/value (VMI/VMV) [2,3]. Lubricants and moisturizers are less effective than the local hormonal therapy [2]. Low-dose vaginal estrogens fulfill both treatment principles for symptoms of VVA [2,4] and LUTS [5] and may provide substantial alleviation of symptoms in patients with more severe GSM [5].

Intravaginal Microablative Fractional CO₂ Laser (CO₂-Laser) is a new non-hormonal proposal for the management of the VVA [6–9].

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Current data are promising regarding the effectiveness of CO₂-Laser in postmenopausal women [6–9]. However, there are limitations in the available data; there are pilot studies focusing mainly in the symptoms of VVA and not the GSM as a whole (VVA-symptoms, sexual function and LUTS). Furthermore, sample size and effect size of the analyzed finding have not yet been calculated and there is lack of data regarding the impact of the CO₂-Laser therapy in the LUTS and the changes of the VMI/VMV.

The aim of the current study was to assess the effectiveness of the CO₂-Laser in postmenopausal women with clinical signs and symptoms of GSM focusing in both the genital and the lower urinary tract and both principles of the treatment. Additionally, we aimed to address possible factors of predicting the improvement of symptoms of CO₂-laser therapy.

2. Methods

This prospective observational study was performed at the outpatient clinic of the Urogynecologic Unit of a tertiary Hospital. The study protocol was approved by the Ethics Committee. All participants signed an informed consent form.

Eligibility criteria for participating in the current study were: menopause, ≥ 1 symptom of GSM with moderate-severe intensity and clinical signs of GSM [1].

Exclusion criteria were: use of any form of hormone therapy (systemic or local) within the previous 6 months, use of lubricants or vaginal moisturizers within the last month, active genital infections, prolapse stage $\geq II$ according to the pelvic organ prolapse quantification (POP-Q) system [10], and any disease that would interfere with compliance to the protocol.

Postmenopausal women with symptom/s of GSM, were asked to complete a questionnaire reporting their symptom/s (dyspareunia, genital dryness, burning, itching, dysuria, urinary frequency, urgency), followed by a 10-cm Visual Analogue Scale (VAS) of the severity of each symptom, in which zero applied to “absence of symptom” and ten to “symptom as bad as could be”. A score of 4–7 was considered moderate and a score of 8–10 severe. Women with moderate/severe symptom/s were further evaluated by clinical examination assessing clinical signs of GSM and POP-Q. The clinical signs assessed were pallor, erythema, loss of vaginal rugae, epithelial integrity, petechiae and tissue fragility [1].

Eligible participants were allocated at a randomly generated number that was different in each visit and entered in a database. The cytologists and the pH-evaluator were blind to all clinical information. The cytological samples were evaluated by 2 independent cytologists and any discrepancies were resolved by reporting the mean value of findings. A vaginal smear was obtained from the lateral vaginal wall using a spatula and stained according to the Papanicolaou technique. The VMI was evaluated by defining the percentage of superficial, intermediate and parabasal epithelial cells on the smear, followed by the calculation of the VMV $[(1 \times \% \text{superficial}) + (0.5 \times \% \text{intermediate}) + (0 \times \% \text{parabasal})]$ [11]. VMV is considered to be an indicator of the estrogenic stimulation, whereas values of 0–49%, 50–64% and 65–100% indicate absent/low, moderate and high estrogenic effect on the vaginal epithelium, respectively [11].

The evaluation of the clinical signs was performed by 2 independent gynecologists blind to all clinical information. Any discrepancies were resolved by reporting the mean value of findings. During the clinical examinations the evaluators could not guess the identity of the participant and thus the treatment status, using a curtain at the median abdominal site of the participant. The clinical signs evaluated according to Vaginal Health Index Score (VHIS), which includes elasticity, fluid volume, pH and epithelial integrity as components [8,9,11].

Participants of the study protocol received intravaginal therapy once a month for 3 months, with CO₂-Laser system (SmartXide² V²LR, Monalisa Touch, DEKA, Florence, Italy). The settings and procedures were performed as previously described [9,12].

The efficacy of the CO₂-Laser on VVA-symptoms was measured using the following questionnaires: frequency of sexual intercourse/month, 10cm-VAS questionnaire for the intensity of dyspareunia, genital dryness, burning, itching and dysuria, 10cm-VAS questionnaire for the overall sexual satisfaction, Female Sexual Function Index (FSFI) [13] and 10cm-VAS questionnaire for the pain during the insertion of the vaginal speculum. The efficacy of the CO₂-Laser on the LUTS was measured using the International Consultation on Incontinence Questionnaires (ICIQ) modules [14–16]: ICIQ-Female Urinary Tract Symptoms (ICIQ-FLUTS Filling Domain) and ICIQ-Urinary Incontinence Short Form (ICIQ-UI SF). Patients also completed the Urogenital Distress Inventory (UDI-6), and the King's Health Questionnaire (KHQ). The participants completed all the questionnaires at the baseline and 4-weeks after the last laser therapy (12-weeks follow-up). Satisfaction of the treatment was assessed with the Patients Global Impression of Improvement (PGI-I) at 12-weeks' follow-up. The participants completed the questionnaires without any interference from the researchers involved in the current study. For the KHQ the minimal important clinical difference (MICD) (the smallest change in score that patients perceive as beneficial) was set at –5 points for each KHQ domain [17].

As primary outcomes were regarded the changes of the objective assessment tools of VMV and VHIS. As secondary outcomes were regarded the changes of the subjective assessment tools as evaluated by the above mentioned questionnaires and the evaluation of possible predicting factors of the CO₂-Laser efficacy.

2.1. Statistical analysis

Statistical analysis was performed comparing baseline data and 12-weeks' follow-up data. A priori calculation of the sample size required for the primary efficacy outcomes to achieve a power of 90%, level of significance 5% and effect size derived from the previously published pilot study [12]. Due to the large size effect of the VHIS in the pilot study and the small sample size required for the current study, we calculated a sample size based on the VMV. The changes of the VMV after the CO₂-Laser have not been reported in previous studies, thus we used a level of significance 5%, power of study 90% and a hypothetical effect size of 0.5 (medium effect). The required sample would be 47 participants. Considering a 10% drop-out rate, 52 participants would be necessary to be included. Post-hoc computing of the power of study and effect size of all the outcomes was performed using the G-power statistical software. The distribution of data was assessed using the Shapiro-Wilk test. For abnormally distributed variables Wilcoxon signed-rank test for related paired samples and Spearman correlation coefficient was used. For categorical variables chi-square test was used. Logistic regression analyses were performed using as dependent variables the presence or absence of symptom/s and thresholds of 15 and 49 for VHIS and VMV, respectively at the 12-weeks' follow-up. All tests were based on a significance level of 5% (p -value < 0.05). Data were presented as mean \pm SD and as percentages (%). SPSS statistical software was used for the analyses.

3. Results

Fifty-eight women were eligible to be included in the study. Three women declined the protocol, as it was difficult for them to follow the protocol schedule and 2 hesitated to participate due to the novelty of the therapy. The protocol was possible in all eli-

Table 1
Baseline characteristics of the participants in the study.

Number (N)	53
Age	57.2 ± 5.4 ^a
BMI	26.0 ± 4.8 ^a
Years since last menstrual period	8.9 ± 6.6 ^a
Level of Education	
University	42/53 (79)
Secondary	11/53 (21)
Smokers	
Yes	16/53 (31)
No	37/53 (70)
Number of cigarettes/day	13.9 ± 6.8 ^a
Married	
Yes	45/53 (85)
No	8/53 (15)
Sexual activity	
Yes	25/53 (47)
No	28/53 (53)
Causes for no sexual activity	
Vulvovaginal symptoms	16/28 (57)
No sexual partner	7/28 (25)
Other	5/28 (18)

^a Data are displayed as mean ± SD. All other data are displayed as percentages.

gible women despite the severity of the vaginal atrophy. Thus, fifty-three participants (mean age 57.2 ± 5.4 years) were enrolled in this study, their baseline characteristics are presented in Table 1. All participants completed the study protocol without any serious side effects. Only a temporary mild irritation of the introitus was noted that started immediately after the laser treatment, lasted up to 2 h and resolved spontaneously.

The VMV and the VHIS increased significantly after the completion of the study protocol (Table 2). At the baseline none of the participants had VMV > 49% and VHIS > 15. At the 12-weeks' follow-up 57% (30/53) and 89% (47/53) of the participants had VMV > 49% and VHIS > 15, respectively. The participants that could not overpass the thresholds of VMV > 49% and VHIS > 15, had an improvement of 10–40% and 57–180%, respectively. At the baseline VMV was correlated with the years since last period ($r=0.4$, $p=0.003$), but at the 12-weeks' follow-up this correlation was not present.

The severity and the presence of VVA-symptoms decreased significantly, while the sexual function (as assessed by the FSFI) improved significantly (Table 3). At the baseline the presence of severe and moderate intensity of the VVA-symptom/s was 49% (26/53) and 51% (27/53), respectively. At the 12-weeks' follow-up the presence of severe, moderate, mild and zero intensity of the VVA-symptoms was 2% (1/53), 13% (7/53), 51% (27/53) and 34% (18/53), respectively. The 8 participants presenting with moderate to severe symptoms at the 12-weeks' follow-up had severe symptoms at the baseline. The improvement of symptoms in these participants varied from 20 to 60%.

All but one of the participants (15/16) who did not have sexual activity at baseline, due to GSM symptoms, resumed their sexual activity. The participant who did not resume sexual activity was due to personal reasons. The frequency of sexual intercourse/month increased significantly from a mean 1.6 ± 2.1 to 4.1 ± 2.1 ($p < 0.001$, effect size 1.5).

LUTS were improved significantly as reflected by the significant reduction of the scores of ICIQ-FLUTS, UDI-6, ICIQ-UI SF and KHQ (Table 4). At the baseline 40% (21/53), 34% (18/53), 23% (12/53) and 4% (2/53) of the participants passed urine during the day 1–6, 7–8, 9–10 and ≥ 13 times, respectively. At the 12-weeks' follow-up 47%

(25/53), 47% (25/53), 4% (2/53) and 2% (1/53) passed urine during the day 1–6, 7–8, 9–10, and 11–12 times, respectively. The change of KHQ questionnaire was > 5 points in all participants. A history of recurrent urinary tract infections was present in 4% (2/53) of the participants. However, relevant symptoms were not reported at the baseline or during the study protocol.

At the PGI-I 30% (16/53), 60% (32/53) and 9% (5/53) of the participants reported very much better, much better and a little better, respectively.

At the logistic regression analyses the results were as follows: 1) VMV and VHIS increased independently of the participants' baseline characteristics (i.e years since last period), 2) The presence or absence of VVA symptoms at the 12-weeks' follow-up was independent of the participants' baseline characteristics and 3) No predictor of the intensity of the VVA symptoms at the 12-weeks' follow-up was identified.

4. Discussion

This study assesses the efficacy of CO₂-Laser therapy in postmenopausal women with clinical signs and symptoms of GSM as a whole, focusing not only in the genital tract but also in the lower urinary tract. This efficacy was assessed in both principles of treatment (improvement of local pathophysiology and alleviation of symptoms) and it was independent to the participants' baseline characteristics (i.e years since last period). In particular, improvement following the CO₂-Laser was observed on VMV, VHIS, VVA symptoms, LUTS, sexual female function and general health perception of the women. This improvement was not only statistical significant but also clinically meaningful. This finding was ratified by the PGI-I. The majority of the participants expressed they felt “much better” or “very much better” after the end of the therapeutic protocol.

The VMI and the VMV are considered an inexpensive measurement of evaluating the hormonal status of women [11]. A negative correlation between the years since last period and VMV exists [18]. Hence, VMV is recommended in the evaluation of the VVA [11,18] and it is used often as a measurement of the local vaginal therapy [3,11,18–21]. The vaginal estrogens have been proved to induce the vaginal mucosal maturation with a significant rise of the VMV [18–21]. In our study the significant increase of the VMV by the use of CO₂-Laser indicates the estrogen-like effect of the therapy. At the baseline VMV was correlated with the years since last period indicating the hormonal status. However, at the 12-weeks' follow-up such correlation was absent, confirming further the estrogen-like effect of the CO₂-Laser on the vaginal mucosal.

Moreover, the VHIS increased significantly indicating the positive effect of the CO₂-Laser on vaginal health. This effect was in accordance to the findings of previous studies [7–10]. The improvement of the VHIS was observed as soon as 1 month after the first therapy [10]. However, the percentage of the participants that reached non-atrophic values of VHIS after the completion of the CO₂-Laser therapy, have not been previously reported. In our study, at the 12 weeks' follow-up, non-atrophic values of the VHIS were observed in 89% of the participants, independently of their baseline characteristics.

The VVA-symptoms at the end of our therapeutic protocol decreased in both prevalence and intensity. All participants experienced improvement in VVA-symptoms. Indeed, even those that reported moderate to severe intensity of VVA symptoms at the end of the protocol, showed a symptomatic improvement up to 60%. Additionally, the sexual function of the participants improved significantly as reflected by the FSFI. It is noteworthy, that all the components of the FSFI (desire, arousal, orgasm, lubrication, satisfaction and pain) changed to clinically meaningful better status as

Table 2
Changes of the primary outcomes (Vaginal Health Index (VHIS), Vaginal Maturation Value (VMV) and their components) of the study.

	Baseline (n = 53) ^b	12 weeks follow-up (n = 53) ^b	Mean of difference ± SD of difference (n = 53) ^b	p-value ^b	Effect size ^b	Power of the study ^b
VMV ^a	11.7 ± 15.6	44.2 ± 13.7	32.4 ± 15.2	<0.001	2.1	100%
Parabasal epithelial cells ^a	74.6 ± 31.6	16.2 ± 23.9	-57.6 ± 29.9	<0.001	1.9	100%
Intermediate epithelial cells ^a	24.6 ± 31.7	80.0 ± 23.4	55.6 ± 30.6	<0.001	1.8	100%
Superficial epithelial cells ^a	0 ± 0	3.3 ± 4.9	3.3 ± 4.9	<0.001	0.7	99%
VHIS ^a	8.4 ± 2.5	20.1 ± 3.0	11.6 ± 2.9	<0.001	4	100%
Elasticity	1.6 ± 0.8	4.2 ± 0.9	2.6 ± 0.9	<0.001	2.9	100%
Fluid Volume	1.0 ± 0	3.1 ± 0.9	2.1 ± 0.9	<0.001	2.3	100%
pH	2.7 ± 1.1	4.2 ± 1.7	1.5 ± 1.0	<0.001	1.5	100%
Epithelial Integrity	1.3 ± 0.9	4.1 ± 1.7	2.8 ± 1.8	<0.001	1.6	100%
Moisture	1.8 ± 1.2	3.1 ± 0.9	2.7 ± 1.2	<0.001	2.3	100%

^a Parabasal epithelial cells, Intermediate epithelial cells and superficial epithelial cells were quantified as percentages in the vaginal smear. VMV was calculated using the formula ((1×%superficial) +(0.5×%intermediate) +(0×%parabasal)) [11]. VHIS is calculated by adding the scores of the 5 components: Elasticity, fluid volume, pH, epithelial integrity and moisture [12–14]. Each component could receive a score from 1 (poorest) to 5 (best). The sum of the 5 components could receive an upper bound score of 25 and lower bound of 5. A Score of ≤15 defined the presence of vaginal atrophy [8,9,11].

^b Mean ± SD values of the outcomes are presented. Wilcoxon signed rank-test for paired samples was used for the statistical analysis. Effect size was calculated based on Cohen's d, derived from the mean of difference ±SD of difference using the G-power statistical program. The thresholds for the interpretation of the effect size were 0.2, 0.5 and 0.8 for small, medium and large respectively. The power of study was calculated post-hoc for the effect size of each outcome at a level of significance 5% and the given number of participants.

Table 3
Changes of the symptoms from the Genital Tract System and the Female Sexual Function Index (FSFI) of participants in the current study.

	Baseline ^a	12 weeks follow-up ^a	Mean of difference ± SD of difference	p-value ^b	Effect size ^b	Post –hoc power of the study ^b
Dyspareunia	7.7 ± 2.5 (98)	2.3 ± 2.2 (71)	5.4 ± 2.6	<0.001(<0.001)	2.1	100%
Genital dryness	6.1 ± 3.1 (87)	1.7 ± 1.9 (53)	4.3 ± 2.9	<0.001(<0.001)	1.5	100%
Burning	1.3 ± 2.9 (22)	0.3 ± 0.9 (12)	1.1 ± 2.4	0.003 (0.04)	0.5	94%
Itching	1.7 ± 3.2 (22)	0.3 ± 1.2 (8)	1.6 ± 3.1	0.002 (0.03)	0.5	94%
Dysuria	0.9 ± 1.7 (34)	0.3 ± 0.7 (13)	0.7 ± 1.1	0.01(<0.001)	0.6	98%
Total	7.4 ± 2.1	1.8 ± 1.8	5.6 ± 1.9	<0.001	3	100%
Pain during the insertion of the speculum	4.9 ± 2.6	1.5 ± 1.9	3.4 ± 2.7	<0.001	1.3	100%
FSFI						
Desire	2.4 ± 0.9	3.8 ± 1.0	1.5 ± 1.0	<0.001	1.5	100%
Arousal	2.1 ± 1.5	3.9 ± 0.9	1.7 ± 1.4	<0.001	1.2	100%
Lubrication	2.5 ± 1.9	4.5 ± 0.9	2.0 ± 1.6	<0.001	1.3	100%
Orgasm	2.2 ± 1.8	4.4 ± 1.0	2.2 ± 1.7	<0.001	1.3	100%
Satisfaction	2.6 ± 1.3	4.6 ± 0.9	1.9 ± 1.4	<0.001	1.3	100%
Pain	1.9 ± 1.6	4.7 ± 1.0	2.8 ± 1.8	<0.001	1.6	100%
Total	13.7 ± 8.1	25.9 ± 4.6	12.1 ± 8.1	<0.001	1.5	100%
Overall satisfaction with sexual life	2.3 ± 2.8	6.5 ± 2.3	3.5 ± 3.6	<0.001	1	100%

^a The presence of each symptom and the calculated p-value of the presence decrease is presented in the parenthesis. The mean values and the presence of all but one symptoms were calculated using the overall number of participants (n = 53). FSFI (mean values) and dyspareunia (mean values and presence) was calculated using the number of participants having sexual intercourse or willing to have sexual intercourse but vulvovaginal symptoms forbade them (n = 40). Mean ± SD values of the outcomes are presented.

^b Wilcoxon signed rank-test for paired samples was used for the statistical analysis. Effect size was calculated based on Cohen's d, derived from the mean of difference ± SD of differences using the G-Power statistical program. The thresholds for the interpretation of the effect size were 0.2, 0.5 and 0.8 for small, medium and large respectively. The power of study was calculated post-hoc for the effect size of each outcome at a level of significance 5% and the given number of participants.

it was implied by the large effect sizes (all ≥ 1.3) of the statistically significant results. Furthermore, the significant increase in the frequency of the sexual intercourse, ratified the findings of the FSFI. The above described findings are in accordance to those of previous studies [6–9].

In addition, in our study LUTS improved significantly (in severity and prevalence), as it was shown by the ICIQ-FLUTS, ICIQ-UI SF and UDI-6 after the CO₂-Laser. Even though in UDI-6 the difficulty of emptying the bladder or the lower abdominal pain, did not reduce significantly after the CO₂-Laser, these are not considered GSM symptoms. Nevertheless, the improvement of the LUTS after the CO₂-Laser was also observed in the KHQ. The MID threshold of 5-point improvement [16], was exceeded in our study in all of its domains, indicating its important effect at the patient level.

In previous studies the efficacy on the Quality of life of the women treated with the CO₂-Laser was assessed by the SF-12, which is a generic questionnaire, not orientated in women with urinary incontinence [7,9,21]. ICIQ-FLUTS, UDI-6, ICIQ-SF and KHQ are considered to be reliable instruments for assessing the LUTS

and their impact in the Quality of Life [16,23–26]. They are usually used for the evaluation of the treatment success of urinary incontinence [22,25]. The findings of the current study indicate that the CO₂-Laser has a positive effect to the lower urinary tract resulting in treatment success of LUTS.

A limitation of the current study is the lack of a control group (i.e pelvic floor physical therapy), as the objective was to evaluate changes before and after the CO₂-Laser therapy in women with GSM as a whole and not to compare its efficacy with other treatment modalities. Thus, a hypothesis of placebo effect of the CO₂-Laser therapy cannot be overruled. However, pathophysiological findings had similar improvement to the decrease of GSM symptoms, implying that probably placebo is not the mechanism of the CO₂-Laser effect. Another potential limitation is the relatively small sample size. Moreover, this study was designed to include women with ≥1 symptom of GSM with moderate/severe intensity regardless of symptoms' origin (genital or lower urinary tract). VVA-symptoms in contrast to LUTS were regarded as the most bothering symptoms by all participants. Moreover, voiding times were calculated

Table 4

Changes of the International Consultation on Incontinence Questionnaire Female Lower Urinary tract systems (ICIQ-FLUTS Filling Domain) and Urinary Incontinence Short-Form (ICIQ-UI SF), Urogenital Distress Inventory (UDI-6) and King's Health Questionnaire (KHQ).

	Baseline ^a	12 weeks follow-up ^a	Mean of difference ± SD of difference	p-value ^b	Effect size ^b	Post-hoc power of the study ^b
ICIQ-FLUTS (n = 53) (Filling Domain)	3.6 ± 2.4	1.6 ± 1.5	2.0 ± 1.6	<0.001	1.3	100%
Day frequency	0.9 ± 0.9	0.6 ± 0.7	0.4 ± 0.5	<0.001	0.8	99%
Nocturia	1.3 ± 1.0	0.6 ± 0.7	0.7 ± 0.6	<0.001	1.2	100%
Urgency	1.2 ± 1.0 (74)	0.3 ± 0.5 (28)	0.9 ± 0.8	<0.001 (<0.001)	1.1	100%
Bladder pain	0.2 ± 0.6	0.08 ± 0.3	0.2 ± 0.5	0.02	0.4	80%
ICIQ-UI SF (n = 35)	8.1 ± 5.6	3.4 ± 4.3	4.7 ± 4.1	<0.001	1.1	100%
UDI-6 (n = 35)	30.2 ± 21.4 (66)	11.9 ± 16.6 (34)	18.3 ± 17.9	<0.001 (<0.001)	1	100%
Frequency	1.8 ± 1.8 (38)	0.7 ± 1.3 (15)	1.1 ± 1.6	0.001 (0.008)	0.7	98%
Urgency incontinence	1.6 ± 1.7 (34)	0.6 ± 1.2 (17)	0.9 ± 1.4	0.001 (0.04)	0.6	92%
Stress incontinence	1.6 ± 1.5 (38)	0.5 ± 0.9 (19)	1.2 ± 1.5	0.001 (0.03)	0.8	99%
Drops	1.8 ± 1.7 (38)	0.5 ± 1.1 (15)	1.3 ± 1.4	<0.001 (0.008)	0.9	99%
Difficulty emptying bladder	0.2 ± 0.8 (6)	0.2 ± 0.9 (6)	0.0 ± 0.6	1 (1)		
Pain discomfort from the lower abdomen or genital region	0.5 ± 0.9 (13)	0.2 ± 0.6 (4)	0.3 ± 0.9	p = 0.1 (p = 0.08)		
KHQ (n = 35)	235.9 ± 226.1	114.1 ± 165.8	121.8 ± 114.9	<0.001	1.1	100%
General Health Perception	35.3 ± 20.6	20.7 ± 14.2	14.6 ± 16.6	<0.001	0.9	99%
Incontinence impact	38.1 ± 37.2	17.6 ± 26.5	20.8 ± 28.0	0.001	0.7	98%
Role limitations	29.5 ± 35.5	12.4 ± 24.4	17.1 ± 23.4	0.001	0.7	98%
Physical limitations	28.1 ± 33.8	11.6 ± 21.7	16.5 ± 22.3	0.001	0.7	98%
Social limitations	17.5 ± 29.4	10.8 ± 23.6	6.7 ± 12.3	0.007	0.5	80%
Personal relationships	18.9 ± 33.3	6.1 ± 20.7	12.8 ± 24.2	0.01	0.5	80%
Emotions	28.9 ± 38.9	10.8 ± 24.9	18.5 ± 28.2	0.001	0.7	98%
Sleep/Energy	20.5 ± 28.9	12.9 ± 23.9	7.9 ± 14.5	0.002	0.5	80%
Severity measures	28.8 ± 29.8	12.8 ± 22.6	15.9 ± 18.7	<0.001	0.9	99%

^a Mean ± SD values of the outcomes are presented. The presence and the calculated p-values of the presence decrease is presented in the parenthesis. The presence of each LUTS was calculated using the number of the included participants (n = 53).

^b Wilcoxon signed rank-test for paired samples was used for the statistical analysis. Effect size was calculated based on Cohen's d, derived from the mean of difference ± SD of difference, using the G-Power statistical program. The thresholds for the interpretation of the effect size were 0.2, 0.5 and 0.8 for small, medium and large respectively. The power of study was calculated post-hoc for the effect size of each outcome at a level of significance 5% and the given number of participants.

using the ICIQ-FLUTS and not voiding dairies. In addition, this study was not designed to include women with a history of recurrent UTIs and/or vaginitis, and we cannot derive conclusions of the CO₂-Laser effect in this group of women. Furthermore, there is a relevant short follow-up period and it remains to be seen for how long these results would last.

Despite the above potential limitations, this study has several strengths. This is a prospective study, with a well-defined group of participants. The assessors of all samples, parameters and data were blinded to all details related to the participants, although a possible breach in the blindness of the evaluators cannot be excluded. The assessment of the CO₂-Laser effect was performed with the intention to investigate both principles of the treatment. Furthermore, confirmation of the results from the previously published studies on the CO₂-Laser and its effect on the VVA-symptoms, FSFI and VHIS, is provided. Additional strengths of this study are: the performance of a power calculation (a priori and post-hoc), the estimation of the effect size of the results and the assessment of baseline characteristics as potential predictors of the women who could benefit the best from the CO₂-Laser therapy.

5. Conclusions

The CO₂-Laser therapy could be a valid non-hormonal therapeutic option for the clinical signs and symptoms of the GSM. This finding is of great importance particularly for women that are not allowed to use vaginal estrogens due to a history of estrogen-sensitive cancer or not willing to use them due to personal perceptions. The results of our study indicates that CO₂-Laser may be effective in decreasing both the VVA symptoms and LUTS and improving the VMV, VHIS, sexual function and general health perception of the women with clinical signs and symptoms of GSM. The indication of restoration of the local pathophysiology and the alleviation of GSM symptoms by the use of CO₂-Laser, decreases the possibility of a placebo effect of the therapy. However, larger

studies with a control arm are needed, for our findings to be safely generalized, as well as studies for the cost-effectiveness of the treatment.

Contributors

E.P. was responsible for data extraction and analysis, and drafted the manuscript.

T.G. evaluated the participants' clinical signs and drafted the manuscript.

A.T. was responsible for cytological evaluation.

D.Z. was responsible for the random allocation of participants and data collection and entry.

S.A. conceived the idea of the study, acted as project manager, evaluated the participants' clinical signs, and drafted the manuscript.

All authors contributed to the interpretation of the data or the work, were involved in drafting the work or revising it critically for important intellectual content, and read and approved the final version of the manuscript.

Conflict of interest

S.S. has had financial relations (expert testimonies and lectures) with DEKA Laser.

All other authors declare they have no conflicts of interest.

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Ethical approval

The study protocol was approved by the Ethics Committee of the "Alexandra" Hospital, Athens, Greece.

All participants signed an informed consent form.

Provenance and peer review

This article has undergone peer review.

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