The Effect of Vaginal Microablative Fractional CO_2 Laser Treatment on Vaginal Cytology

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Background and Objectives: Most recently vaginal laser treatment was introduced as a new option for women with genitourinary syndrome of menopause, vaginal dryness. Our objective was to assess the effects of intravaginal CO_2 laser treatment on vaginal cytology.

Study Design/Materials and Methods: Fifty-two women with symptoms of vaginal dryness were enrolled and underwent vaginal laser treatment using a fractional CO_2 laser. Patients received three vaginal laser treatments 4 weeks apart. Vaginal cytology was obtained before the first treatment and 4 weeks after each additional treatment. Vaginal dryness was assessed by using a Visual Analog Scale (VAS).

Results: Out of the 52 women enrolled, 34 were in menopause. Postmenopausal women had significantly lower vaginal maturation values (VMV) compared with premenopausal women at the baseline visit (mean ± standard deviation [SD], 42 ± 23 vs. 68 ± 13, $P < 0.01$). The vaginal dryness VAS was higher (worse) in postmenopausal women compared with premenopausal cases (mean ± SD, 5.7 ± 4 vs. 2.4 ± 3, $P < 0.01$). The VMV did not change significantly over time after vaginal laser treatment. However vaginal dryness VAS improved significantly after each treatment. Both in the premenopausal and postmenopausal groups, vaginal dryness scores improved significantly from baseline after the three treatments (postmenopausal 5.7 ± 4 vs. 1.6 ± 2.5, $P < 0.01$ and premenopausal 2.4 ± 3 vs. 0.2 ± 0.5, $P < 0.01$). Those patients who had improvement in VMV had significantly better (lower) dryness VAS compared with those women without an improvement in VMV after the three treatments (mean ± SD, 0.3 ± 0.8 vs. 1.6 ± 2.6, $P = 0.04$).

Conclusions: Vaginal dryness VAS improved significantly in a cohort of premenopausal and postmenopausal women undergoing vaginal CO_2 laser treatment despite no significant change in vaginal cytology. Lasers Surg. Med. © 2020 Wiley Periodicals, Inc.

Key words: CO_2 laser; vagina; vaginal cytology; fractional laser

INTRODUCTION

Vaginal dryness is a common bothersome condition affecting many premenopausal, perimenopausal, and postmenopausal women. The etiology of vaginal dryness is frequently related to decreased estrogen production. In postmenopausal women, a persistent low estrogenic environment is responsible for the development of the genitourinary syndrome of menopause (GSM), which is the new term for vulvovaginal atrophy (VVA) according to the International Society for the Study of Women's Sexual Health and the North American Menopause Society [1,2]. The syndrome together with its bothersome symptoms affects 40–54% of postmenopausal women and negatively influences their everyday life [3–5]. Traditionally. the treatment option for GSM/VVA was vaginal estrogen supplementation, but many women are either unable to or are afraid to use hormones. There are several nonhormonal treatment modalities for the management of GSM-related symptoms, especially for the most bothersome symptoms of vaginal dryness. The 2013 position statement of The North American Menopause Society indicates that the first-line therapies to alleviate symptoms of GSM should include nonhormonal vaginal lubricants and moisturizers as well as regular sexual activity [2]. If nonhormonal therapy does not provide satisfactory symptomatic relief and there are no contraindications, locally applied estrogen may be offered [2].

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However, many women are reluctant to use hormonal products and desire a more durable treatment.

Most recently vaginal laser treatment was introduced as a new option for women with GSM and vaginal dryness. Several clinical studies have investigated the efficacy and revealed the beneficial effects of vaginal laser treatment [6–9]. Currently, there are promising data regarding improvement in subjective symptoms of GSM, vaginal health and flora, sexual function and dyspareunia, and urinary incontinence [7–17]. Prior publications revealed that vaginal cytology had improved significantly, reflected by an increase in the vaginal maturation values (VMV) after vaginal laser treatment in postmenopausal women with GSM [14,18,19]. Vaginal maturation values less than 49 are considered to represent a low estrogenic environment [20]. Vaginal laser treatment has been shown to significantly increase VMV in women with cytological evidence of atrophy, but there is no data on the effects of laser treatment on women with symptoms of GSM without cytological atrophy [14,18,19]. One of the proposed mechanisms of action of vaginal laser treatment for the alleviation of vaginal dryness is the improvement in the vaginal epithelium and thickening of the atrophic vaginal mucosa. In addition, laser treatment results in the production of new extracellular matrix elements such as collagen, glycosaminoglycanes, proteoglycans, and multiadhesive glycoproteins contributing to the beneficial effects of vaginal laser treatment [21].

Although the Food and Drug Administration approved CO_2 laser and YAG laser therapy for several dermatological and plastic surgical indications, GSM or vaginal dryness is not among those indications. As previous studies have revealed the beneficial effects of vaginal microablative fractional laser treatment in GSM, our aim was to investigate the effects of laser treatment on vaginal cytology in pre and postmenopausal women. Vaginal dryness is very common in menopausal and perimenopausal women. Prior studies have not evaluated the effects of laser treatment on a mixed population of premenopausal and postmenopausal women with complaints of vaginal dryness. We hypothesized that vaginal cytology would improve this, VMV scores will be higher after women undergoing vaginal laser treatment and they would report improvement in vaginal dryness (lower vaginal dryness Visual Analog Scale [VAS]). Our goal was to assess the effects of vaginal CO_2 laser treatment on vaginal cytology.

MATERIAL AND METHODS

A prospective cohort study was carried out after informed consent was obtained at the urogynecology outpatient clinic of the Department of Obstetrics and Gynecology, University of Debrecen, between 3/2017 and 9/2018. Enrollment criteria were the presence of vaginal dryness (VAS for vaginal dryness >1). Exclusion criteria were pregnancy, hormone therapy (local or systemic) in the past 6-month, recent use of any over-the-counter vaginal product (within 6 months), vaginal infection at presentation, cytological atypia, dysmenorrhea, pelvic organ prolapse (POP) > stage 2 prolapse according to the pelvic organ prolapse quantification system (POP-Q) or severe urinary or fecal incontinence.

Women enrolled into the study underwent three sessions of intravaginal microablative CO_2 laser therapy 4 weeks apart, and vaginal cytology was obtained in a standardized fashion at the baseline before the first treatment, after the first and the second sessions and 4–6 weeks after the final, third treatment of the CO_2-Laser system (SmartXide2V2LR, Monalisa Touch; DEKA, Florence, Italy).

Demographic and pertinent clinical information was recorded prospectively and stored in a dedicated database. Our study was approved by the Hungarian National Institutional Review Medical Research Council. All women signed written informed consent before participating in our research.

VAS for Vaginal Dryness

Participants reported the intensity of the vaginal dryness symptom using a 10-cm VAS. The scale's left extremity indicates the complete absence of symptoms (0) and the right extremity indicates the worst possible symptom (10) [12].

Vaginal Cytology

Vaginal smear samples were collected in a single scraping of the middle third of the lateral vaginal wall with a spatula; 200 cells were analyzed per specimen by an independent board-certified cytopathologist blinded to the clinical information. Parabasal (P), intermediary (I), and superficial (S) cell counts were performed and multiplied by 0, 0.5, and 1.0, respectively. The sum of all three values comprises the VMV of Meisels, and an increased percentage of P cells and I cells suggests a decrease in estrogen levels [20]. VMV values ranging from 0 to 49 indicate low estrogen effect, 50–64 indicate moderate estrogen effect, and 65–100 indicate high estrogen effect on the vaginal epithelium [22].

Laser Therapy

For laser treatment, a microablative, fractional CO_2 laser system (SmartXide2V_2LR; Deka) was applied, with a specific 360° probe for intravaginal procedures. Laser beams were fractionally emitted in small points (DOT's) around the vaginal mucosa during treatment. To achieve the required effect, the laser was used in D-Pulse mode, depth was set, and laser power, dwell time and spacing was adjusted. According to literature data for treatment, we used laser parameters as the following SmartStak 1 (SmartXide2V2LR; Monalisa Touch, DEKA, Florence, Italy), 30 W power, 1,000 µs dwell time and 1,000 µm spacing [23].

Statistical Analysis

The normality of continuous variables was examined using graphical plots. Descriptive statistics were reported as mean and standard deviation (SD) for continuous variables and frequencies and percentages for all categorical variables. Sample characteristics were compared between

TABLE 1. Demographic and Clinical Characteristics of Women Undergoing Vaginal Laser Treatment With a CO_2 Microablative Laser

	Premenopausal group ($N = 18$)	Menopausal group ($N = 34$)	P value
Age (years, mean ± SD)	46 ± 6	63 ± 6	<0.01
Gravida (median, range)	2 (1–6)	2 (1–7)	NS
Para (median, range)	2 (0–4)	2 (0–4)	NS
Years in menopause (years, mean ± SD)	NA	14 ± 6	
Body mass index (kg/m^2, mean ± SD)	24.9 ± 5	26.9 ± 4	NS
Previous Cesarean section (N, %)	2 (11)	4 (12)	NS
Previous hysterectomy (N, %)	2 (11)	5 (14)	NS

premenopausal and postmenopausal participants using Student's t test. To examine the change of VMV scores and vaginal dryness under the effect of vaginal laser treatment over time, a mixed model analysis approach was used—given that data was collected repeatedly over three different visits. Mixed model regression analysis accounts for the within-patient variance resulting from the repeated measure design. The reported means, standard errors and P-values have been adjusted based on within-group and between-group interactions as well as the potential for skewed distributions between variables. All statistical analysis was performed using SAS 9.4 statistical software (SAS Institute Inc., Cary, NC, USA), with the risk of Type I error set at $\alpha = 0.05$.

The changes of the VAS after the CO_2 laser treatment have been used to compute sample size in similar studies in the past [16]. Assuming a decrease of three points in mean VAS vaginal dryness from a baseline of 5, we estimated a sample size of 42 patients is needed in order to achieve a power of 80% with a level of significance 5%. Considering a 20% drop-out rate, the final required sample size would be about 52 participants.

RESULTS

Out of the 52 women enrolled, 34 were in menopause. Women in the postmenopausal group were significantly older compared with the premenopausal women (mean ± SD, 63 ± 6 vs. 46 ± 6 years, $P < 0.01$). The rest of the clinical and demographic characteristics are described in Table 1. Postmenopausal women had significantly lower VMV compared with premenopausal women at the baseline visit (mean ± SD, 42 ± 23 vs. 68 ± 13, $P < 0.01$). The vaginal dryness VAS was higher (worse) in postmenopausal women compared with premenopausal (mean ± SD, 5.7 ± 4 vs. 2.4 ± 3, $P < 0.01$).

There was a weak negative significant correlation between VMV and vaginal dryness VAS in the total group ($r = -0.282$, $P < 0.01$).

The VMV did not change significantly over time after vaginal laser treatment (Table 2). However, vaginal dryness VAS improved significantly (lower VAS value) after each treatment (Table 3). Both in the premenopausal and postmenopausal groups, vaginal dryness VAS improved significantly (lower VAS value) from baseline after the three treatments (postmenopausal 5.7 ± 4 vs. 1.6 ± 2.5, $P < 0.01$ and premenopausal 2.4 ± 3 vs. 0.2 ± 0.5, $P < 0.01$).

Those patients who had an improvement in VMV (higher VMV after each treatment) had significantly better (lower) dryness VAS compared with those women without an improvement in VMV after the three treatments (mean ± SD, 0.3 ± 0.8 vs. 1.6 ± 2.6, $P = 0.04$). However, both groups (improvement in VMV vs. no-improvement in VMV) had significantly lower dryness VAS after the three treatments compared with baseline (mean ± SD, 0.3 ± 0.8 vs. 4.0 ± 3.6, $P < 0.01$ and 1.6 ± 2.6 vs .4.6 ± 4.0, $P < 0.01$).

DISCUSSION

In our study, vaginal dryness VAS improved significantly in a cohort of premenopausal and postmenopausal women undergoing vaginal CO_2 laser treatment despite no significant change in vaginal cytology. We were surprised to find no significant changes in vaginal cytology after vaginal CO_2 treatment. Previous trials with vaginal laser treatment revealed significant changes in vaginal cytology as well as a significant improvement in vaginal dryness.

TABLE 2. Vaginal Cytology Values Before and After First Treatment, After Second Treatment and 4 Weeks After the Third Treatment of CO_2 Vaginal Laser Treatment

	Baseline	After first treatment	After second treatment	After third treatment
Maturation value (mean ± SD)	51 ± 24	53 ± 25	50 ± 26	52 ± 26
Superficial cells % (mean ± SD)	20 ± 22	24 ± 25	20 ± 22	22 ± 26
Intermediate cells % (mean ± SD)	63 ± 30	60 ± 31	59 ± 33	60 ± 34
Parabasal cells % (mean ± SD)	17 ± 33	16 ± 32	21 ± 37	18 ± 34

There were no significant changes after laser treatment ($P < 0.05$).

TABLE 3. Visual Analog Scale for Vaginal Dryness Before and After First Treatment, After Second Treatment and 4 Weeks After the Third Treatment of CO_2 Vaginal Laser Treatment

	Baseline	After first treatment	After second treatment	After third treatment
Vaginal Dryness Score (mean ± SD)	4.5 ± 4	3.2 ± 3*	1.9 ± 3*	1 ± 2*

*Statistically significant data ($P < 0.01$) compared with baseline values.

Pitsouni et al. [19] treated 53 postmenopausal women with moderate to severe GSM with three sessions of vaginal CO_2 laser treatment. In their study, the VMV increased significantly [19]. At the baseline, none of the participants had VMV > 49, but at the 12-weeks' follow-up, 57% of the participants had VMV > 49. In our study, the mean baseline VMV was >49. A VMV of <49 indicates a low estrogenic, atrophic vaginal environment. Similar to our findings, the participants in the Pitsouni's trial that could not overpass the threshold of VMV > 49 still had an improvement in symptoms of GSM. Another study by Athanasiou et al. [14] showed that following the third, fourth, and fifth laser sessions, vaginal dryness completely regressed in 36%, 66%, and 86% of treated women and VMV regained non-atrophic values in 53%, 69%, and 84% of women. In this study, a VMV ≤40 defined atrophy on the vaginal smears. Median VMV was 50 at baseline, which was very similar to our baseline VMV. Athanasiou's study revealed that both VHIS and VMV increased following each subsequent therapy, and 80–100% of participants' scores were above the thresholds of non-atrophic clinical findings. Cruz et al. [18] included 45 women with GSM into a randomized, double-blind, placebo-controlled trial for the evaluation of fractional CO_2 laser compared with topical estrogen [18]. Laser treatment significantly increased the VMV from the baseline value of 42.4–64.5 by 8 weeks and to 58.5 by 20 weeks, while the dryness scores improved significantly as well. No difference was observed in VMV among groups in the different treatment arms. On the basis of these prior studies and our findings, it appears that vaginal CO_2 laser treatment improves vaginal dryness with or without affecting the vaginal cytology. Our study was different from prior studies because the VMV baseline was higher in our study than the threshold for a low estrogenic environment based on vaginal cytology (VMV < 49 or 40) despite the fact that our postmenopausal patients were on average in menopause for 14 years. It is possible that women with very low VMV respond differently to laser treatment than women with scores near or above the threshold levels. In addition, Davila et al. [24] found that symptoms of vaginal atrophy were only weakly correlated with physical findings ($r = 0.14$) and not with maturation value ($r = 0.06$) or age ($r = –0.004$). Davila et al. found a moderate negative association between maturation value and age ($r = –0.375$) which is consistent with the fact that GSM is more common in older postmenopausal women.

One of the strengths of our study is the enrollment of premenopausal as well as postmenopausal women. But there are several limitations to our study. We have used only vaginal cytology as a measure of the effect of laser treatment on vaginal cytology. Other methods have been advocated, which include the karyopyknotic index, the folded-cell index, and the crowded-cell index. Though automated digital imaging analysis of cytospin specimens has been shown to be more sensitive than the classical manual assessment in vaginal cytology, a comparison of the two methods revealed a highly significant correlation between their VMVs results [25]. Therefore, we decided to use VMV, determined to be the classical method for assessment of vaginal health because of its wide acceptance among gynecologists. Another limitation is the relatively low number of premenopausal women enrolled in our study. Vaginal dryness is far more common after menopause than during the premenopausal years. Our finding should be interpreted with caution as this was a small study, powered to detect a 2 unit change in the mean VAS for vagina dryness. A larger sample size may be needed to detect smaller differences or particularly smaller changes in the VMV in the premenopausal group. Additional limitations are the type of laser and the setting used. We have used a CO_2 vaginal laser at a particular setting based on previous publications. But it is likely that these settings may need to be individualized based on the symptom responses observed after each treatment. Also, we have included women with a VAS for vaginal dryness >1, allowing women with mild symptoms rather than just women with moderate to severe symptoms to be in the study. Women with very mild symptoms may respond differently to laser treatment than women with severe symptoms. Vaginal dryness is a component of sexual function, which has a very high placebo response. Providing a control group with sham treatment is essential to knowing whether the obtained result is due to laser or due to placebo. Future studies with sham control are needed to fully assess the placebo effect of laser treatment but in contrast to vaginal dryness, vaginal cytology is not likely to be affected by placebo treatment.

CONCLUSION

In summary, vaginal dryness VAS improved significantly (lower VAS values) in a cohort of premenopausal and postmenopausal women undergoing vaginal CO_2 laser treatment despite no significant change in vaginal cytology. These findings suggest that improvement in vaginal dryness might not be solely the result of changes in vaginal cytology and a healthier vaginal epithelium but that other factors might contribute (vaginal wall remodeling) to the improvement in GSM.

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