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Original research article

Effect of CO_2 Laser on the Vaginal Epithelium in Menopausal Women

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ABSTRACT

Vulvovaginal atrophy, caused by a decrease in estrogen, is a problem that affects the quality of life of menopausal women. CO_2 laser treatment is a treatment option that can address this problem. Many qualitative studies show significant efficacy without any serious side effects. There are some small histochemical studies that have observed the impacts of CO_2 laser treatment on vaginal mucosa. The present study aimed to confirm the validity these histochemical findings by using a larger sample size. Menopausal women with pelvic organ prolapse (POP) that required treatment by surgery were recruited and treated with a CO_2 laser for one episode. Then, 1 month after laser treatment, the women would proceed with their scheduled POP- correction surgery, during which 2 pieces of posterior vaginal tissue were excised and sent for histochemical microscopic examination. The thickness of the epithelial cell layer and number of cell rows were each significantly higher in laser-treated tissue when compared with non -laser-treated tissue. Additionally, the degree of superficial cell desquamation in the laser-treated tissue was significantly higher as well. The histochemical microscopic exam involved PAS staining to observe intracellular polysaccharides such as glycogen and collagen. Test results showed a significantly thicker layer of glycogen-containing cells in the laser-treated tissue when compared with non-laser -treated tissue. In conclusion, CO_2 laser treatment has a positive impact on vaginal epithelial cells and the intracellular matrix, which can help restore atrophic vaginal mucosa in menopausal women.

Keywords: CO_2laser; Menopause; Vaginal epithelium

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1. Introduction

The definition of menopause, according to the WHO, is the state in which women permanently stop menstruating due to decreased ovarian function. The onset of this condition leads to significant physiological changes in many organs in the female body. Irregular menstruation is the most common symptom in perimenopausal women. Besides irregular menstruation, the most common symptoms are vasomotor symptoms including hot flushes and night sweating. These symptoms can have a profound, negative effect on quality of life, although they do tend to subside and eventually disappear over time. Unlike genitourinary syndrome of menopause (GSM), the severity tends to occur as women become older. During menopause, a dropping off of the estrogen level causes the vaginal mucosa to become thin, pale and dry. The vaginal maturation index shows a decrease in superficial squamous cells, but an increase in parabasal cells. The glycogen in the vaginal epithelium is reduced and the resident lactobacilli are unable to produce lactic acid. This depletion of glycogen results in an increased vaginal pH, allowing the proliferation of pathogenic bacteria which can lead to increased infections. In addition, the decrease in vaginal blood supply and production of vaginal secretions causes vaginal dryness, burning, itching and pain, all of which can lead to dry dyspareunia in postmenopausal women. Lower urinary tract symptoms (LUTS), such as increased frequency and urgency of urination, urinary incontinence and recurrent cystitis are known to be associated with vulvovaginal atrophy (VVA).

Local estrogen therapy is an effective treatment for GSM. It can restore the dry vaginal mucosa back to its normal state. Many studies have confirmed this effect. It has been found that the use of topical estrogen results in an increase of squamous cells with high glycogen content. Additionally, the number of lactobacilli increases and the vaginal pH decreases. However, estrogen hormones should be used with caution; even if the estrogen treatment is only topical application, it may be absorbed into the bloodstream if used in large quantities. Patients with breast cancer requiring aromatase inhibitor or tamoxifen often have severe symptoms of estrogen deficiency, including both vasomotor symptoms and GSM. In this subset of patients, estrogen therapy should be avoided. They should opt instead for the use of non-hormonal substances, such as a vaginal moisturizer and vaginal lubricant. However, vaginal lubricants have no effect on vaginal health and are typically used only during intercourse. Some vaginal moisturizers claim to help thicken vaginal epithelium, although there have been no long-term or lasting effects observed and they require regular use.

A laser (light amplification by stimulated emission of radiation) is a device capable of emitting a high-intensity continuous beam by stimulating the release of photons from stimulated atoms or molecules. In the medical field, the first laser therapy was used to treat eye disorders, and in 1962, the laser's efficacy for penetration was found to be useful for the treatment of skin diseases. For more than a decade of continuous advancements in laser technology, and up until now, lasers have been widely used in medical practice. There are many different types of lasers, classified in part by the active medium used. Some types used in the medical field include solid–state lasers (neodymium, erbium, holmium and YAG), gas lasers (CO_2 and He-Ne), liquid–state lasers (dye), excimer (chlorine, fluorine with argon, krypton or xenon) and semiconductor lasers. Different lasers have different wavelengths and emit different levels of energy. Lasers can also be classified according to the nature of the emitted light. A laser may thus be a continuous-wave or pulsed laser. In 1983, a study using pulsed-laser-induced photothermolysis found that a pulsed laser was able to provide target selectivity and could help reduce damage to nearby tissue. This type of photothermolysis was later developed as fractional photothermolysis, designed to cause a peeling of the surface tissue, while resulting in fewer side effects [1]. The carbon-dioxide laser has a wavelength of 10,600 nanometers, a wavelength which can be absorbed by water. When using an energy density of 5 joules per cm^2 with a pulse duration of less than 1 millisecond, proteins will break down. The heat causes the collagen to shrink rapidly to a third of its original length. The shrinkage of these fibers is the first mechanism resulting in skin tightening and moisturizing. Subsequently, the tissue repair process will lead to the production of the enzyme collagenase and break up the collagen. The processes of cell regeneration and neocollagenesis will be stimulated and continue for at least 6 months [2].

The efficacy of the CO_2 laser has continued to improve to date. The fractional CO_2 laser, which emits a multiple micro-column beam, appears to promote the wound-healing process. This technology is able to control the area of ablation caused by thermal damage within a very small column. It is designated a microablative column (MAC) and is able to leave the surrounding normal healthy tissue unscathed. The bridge between the ablated and non-ablated tissue plays an important role in promoting wound healing following fractional CO_2 laser therapy. Previous immunohistochemical studies have found that in the early stage of post-treatment by fractional CO_2 laser, heat shock proteins (HSPs) are activated, particularly HSP47 (collagen chaperone). These proteins facilitate the production and secretion of various cytokines. Transforming growth-factor beta (TGF-β) is a cytokine that stimulates matrix collagenase into producing new collagen. Basic fibroblast growth factor (bFGF) stimulates the angiogenic activity of endothelial cells, and extracellular growth factor (EGF) stimulates re-epithelialization. Platelet-derived growth factor (PDGF) stimulates production of the extracellular component of fibroblasts, and vascular endothelial growth factor (VEGF) stimulates vasculogenesis and angiogenesis. Neocollagenesis was found to persist for about 3 months after treatment. All of these changes in cytokine signaling can fully restore epithelial structure and remodel the dermis [3, 4].

The vagina is a fibromusculo-elastic, tubular structure with no glands. It has three layers: the tunica mucosa, tunica muscularis and tunica adventitia. In the reproductive woman, the tunica mucosa consists of an epithelial cell layer underlined by a thick lamina propria. The epithelial cell layer is made up of 4 different cell layers, approximately 400 microns thick. The basal cell layer is a single layer of columnar-like cells, 10 microns thick. The parabasal cell layer consists of 2 layers of small polygonal cells with a thickness of 14 microns. The third layer is the intermediate cell layer with a variable number of cell rows - usually 10 - and a variable thickness of about 100 microns. The top layer is the superficial cell layer with a variable number of large polygonal stratified cell rows. It usually has 10 rows with a thickness of around 200 microns [5]. The lamina propria is a layer of moderately dense connective tissue composed of abundant elastic fiber and an extensive venous plexus. It lies immediately below the epithelial layer. The lamina propria is about 1000 microns thick. The hypoestrogenic state affects the maturation of the vaginal squamous epithelium. It causes the epithelial cell layer to become thinner, mainly due to a reduction in the number of superficial and intermediate cell rows. The collagen and elastic content in the lamina propria are also decreased in the hypoestrogenic state.

Nowadays, fractional CO_2 lasers are widely used for restoration and remodeling in many parts of the human body, including the face, neck and chest. A tissue biopsy within 48 hours after treatment is performed to confirm these changes, and the process of re-epithelialization and recovery of the basement membrane persists for at least 3 months [6]. In addition, it is assumed that the connective tissue around the scar and other aging skin will decrease, as in the atrophic vaginal mucosa. Accordingly, a specific probe has been developed to facilitate the use of the CO_2 laser in the vagina. As previously mentioned, the vaginal epithelial-layer thickness in premenopausal woman is about 400 µm, so the epithelial thickness of menopausal woman with low estrogen would likely be about 100-200 µm, owed mainly to the loss of the superficial cell layer (~200 µm) [5]. The variables of the GSM treatment protocol (output power, exposure time, distance between single points, stack level and total energy fluence) have been studied and adjusted by the manufacturer (SmartXide2 V2LR, Monalisa Touch, DEKA, Florence, Italy). The use of 30 W of power with a dwelling time of 1000 µs produces an epithelial vaporization crater with a depth of about 200 µm. Using the MAC technique, this is very close to the limit of coagulation (10 µm), and well-preserved lateral epithelial linings are detectable. An ex-vivo histologic study in 5 postmenopausal women was able to demonstrate the safety of this therapeutic protocol and showed immediate initial-tissue remodeling of the vaginal mucosa [7]. Neocollagenesis and neoglycogenesis within the vaginal connective tissue and epithelium, resulting in the restoration of the vaginal mucosa from atrophy, had been strongly supported by a in-vivo microscopic and ultrastructural-modification study [8]. A series of tri-monthly treatments using this 30 W power setting resulted in significant improvement in both the vaginal health-index score (VHIS) and female sexual-function index (FSFI) without any severe adverse events [9-12]. However, the manufacturer recently changed its standard protocol settings, increasing the power from 30 W to 40 W. There have been some studies, including one by the authors of the current study, that have confirmed the efficacy and safety of this 40 W power setting for GSM treatment [13-16]. In 2017, there was a retrospective case-control study, the purpose of which was to compare the efficacy of the 30 and 40 W protocols in 50 postmenopausal women with GSM. This study concluded that both power levels could significantly improve GSM symptoms and atrophic values in the clinical findings. Moreover, this improvement seemed to be independent of which power level, 30 or 40 W, was used. Until now, histological studies using 30 W protocols have been available, but only with small sample sizes [7, 8]; regarding 40 W protocols, no histological study has been published in any peer-reviewed journal to the best of the authors knowledge. The focus of this study was on identifying the effects of fractional CO_2 laser treatment, using a 40 W power level, a dwelling time of 1000 µs and a spacing of 1000 µm, on the histochemical morphology of vaginal mucosa in postmenopausal women.

2. Material and Methods

This study protocol was approved by the Human-Research Ethics Committee of Thammasat University (Medicine).

From May of 2019 to January of 2021, 30 women with pelvic organ prolapse (POP) who had planned for transvaginal surgery at Thammasat university hospital were recruited. The inclusion criteria were: the woman was postmenopausal, they must not have taken any type of hormonal therapy for at least 6 months, that they would be able to stay in the lithotomy position for at least 5 minutes continuously and that they had their point D in their POP-Q measurements (Fig. 1) located at least 2 centimeters above their hymens (-2). The exclusion criteria were having severe perineal or vaginal infection.

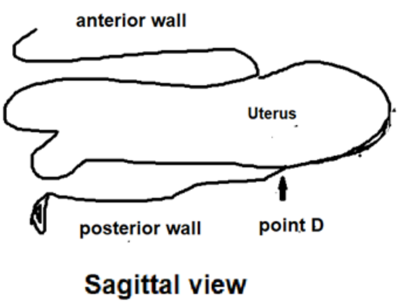

Fig. 1. Point D in POP – Q measurement.

After the subject signed the informed consent and answered the vulvovaginal symptom questionnaire (VSQ), laser therapy was administered with a power setting of 40 W, a dwelling time of 1000 µs, dot spacing of 1000 µm and a stack parameter of 1-3. The specific 90° vaginal probe (Fig. 2) was gently inserted into the vagina until reaching the vaginal apex. The probe was then moved out a distance of 1 cm. The 90° emission side was pointed toward the left-lateral posterior vaginal wall and the laser was then emitted along the left lateral posterior wall by moving the probe out in 1 cm increments until it had come completely out through the vaginal opening (Fig. 3).

Fig 2. The specific 90º vaginal probe.

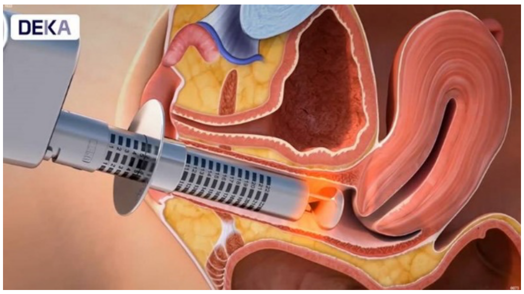

Fig. 3. The 90° emission side was pointed to the left lateral posterior vaginal wall. (with permission from Deka).

Immediately after the procedure, the subject was asked whether she had experienced any side effects and was checked for any abnormal lesions resulting from the laser treatment. The patient was advised to not clean her vagina with any washing solution or have any sexual intercourse for 3 days following treatment. One month later, the transvaginal surgery for POP was carried out as scheduled. On the day of admission, any side effects or abnormal lesions would be recorded.

During the posterior colporrhaphy, a 1x1 cm square section was cut from the left lateral posterior vaginal wall at about 1 cm from point D (Fig. 4) and put into a plastic bag containing 4% formaldehyde. This bag was then labeled with the subject's hospital number and either A or B, chosen randomly, so as to keep the excision site unknown to the pathologist. The right lateral posterior vaginal wall at the same level was cut in the same manner and labeled with the remaining letter, A or B.

For the purpose of histochemical assessment, each tissue sample was fixed and stained with hematoxylin and eosin (H&E) for the evaluation of:

1. Epithelial cell thickness: to measure the vaginal wall thickness from the basal cell layer to the superficial cell layer, in units = µm.
2. Number of epithelial cell rows: to count the epithelial cell rows from the basal cell row to the superficial cell row, in units = number of rows.
3. Degree of superficial-cell desquamation: to assess the degree of desquamation of the superficial cell, in units = degrees (0= no, 1= mild, 2= marked).

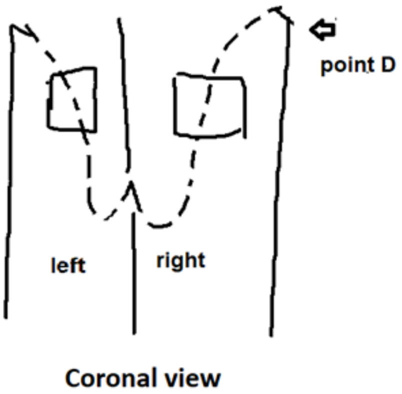

Fig. 4. A 1x1 cm square from the left and right lateral posterior vaginal wall at about 1 cm from point D would be cut in treated and untreated specimens.

A periodic acid–Schiff (PAS) stain was used to observe the intracellular polysaccharide component for the evaluation of:

1. Thickness of the glycogen-containing cell: to measure the PAS-stained cells which contained glycogen inside, in units = µm.
2. Length of connective tissue papillae: to measure the depth of the connective tissue and capillaries which protrude into the epithelial layer, in units = µm.

All data were analyzed using SPSS version 26. General characteristics such as age, parity and presenting symptoms were descriptively analyzed. The normality of the distributions were tested. The statistical difference between the means of the treated and untreated measurements with a normal distribution was analyzed using paired samples *t*-test with the threshold for statistical significance set at p-value ≤ 0.05. The median of the differences between untreated and treated desquamation degree was analyzed with related sample Wilcoxon-signed Rank test with a statistical significance of p-value ≤ 0.05.

3.Results and Discussion

Among the 30 subjects, the mean age was 66.9 $\pm$ 8.1 years, and the median of parity was 3 (range 2-7). All subjects had at least 1 vaginal delivery. The most common presenting symptom was vaginal bulking (73%), and 18% had LUTS. Only 9% presented with another symptom, such as sexual dysfunction. A combination of anterior compartment and apical compartment prolapse was the most frequent diagnosis in this group.

During and immediately after the laser treatment, only 2 women complained of mild vaginal discomfort, though they did not need any treatment. Also, at 1-month post-treatment, neither of these 2 women reported any complications.

Table 1 shows the histochemical microscopic results. The mean thickness of the epithelial cell layers in the treated site was 92.3$\pm$36.3 µm, significantly thicker than the mean thickness at the untreated site (75.1$\pm$39.6 µm, p = 0.008). There were a significantly greater number of epithelial cell rows and glycogen-containing cells at the treated site than at the untreated site (both p-values < 0.0001). Additionally, 13.3% of the untreated sites had no desquamation of superficial cells (degree=0), while 70% had mild desquamation (degree =1), and 16.7% had marked desquamation (degree=2). As compared to the degree of desquamation at the treated site, only 3.3 % had no desquamation and 26.7% had marked desquamation. The median of differences between the degree of superficial desquamation at the treated and untreated sites was not significantly different (p-value = 0.34). The depth of the connective tissue and capillaries protruding into the epithelial layer was not significantly different between the two sites (p-value = 0.151).

Table 1. Histochemical microscopic results (N=30).

	Treated site	Untreated site	*P*-Value
Epithelial thickness (mean $\pm$ SD.,microns)	92.3 $\pm$ 36.3	75.1 $\pm$ 39.6	0.008
Number of epithelial cell rows (mean + SD., rows)	17.4 $\pm$ 6.0	13.8 $\pm$ 5.1	0.000
Thickness of glycogen containing cells (mean + SD.,microns)	123.6 $\pm$ 54.7	87.1 $\pm$ 54.1	0.000
Length of connective tissue papillae (mean + SD.,microns)	83.1 $\pm$ 40.8	72.3 $\pm$ 44.9	0.151

* Paired sample *t* - test, *P*-value < 0.05

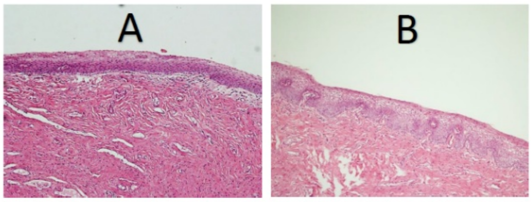

Fig. 5. The H&E-stained vaginal tissue from the same subject.

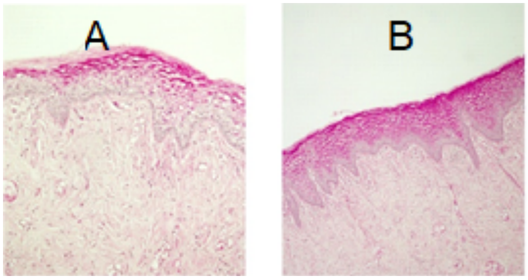

Fig. 6. PAS-stained vaginal tissue from the same subject.

Fig. 5 shows histochemical evidence from the H&E-stained vaginal tissue of Subject No.13. Slide A shows a thin epithelial cell layer with a smooth basement epithelial layer, but with no connective tissue papillae protruding into the epithelial cell layer. Slide B, by contrast, shows an irregular basement cell layer caused by numerous papillae invading the epithelial cell layer, and a thicker superficial cell layer. In Fig. 6A, the PAS staining from the untreated site shows a thin layer of glycogen-containing cells stained pink, as compared to Fig. 6B, where it can be seen that the treated site has a thicker glycogen-containing stained cell layer and a significant number of connective-tissue papillary projections.

Currently, the fractional CO_2-laser has not been FDA-approved for use in treating GSM. Furthermore, several professional societies, such as the American College of Obstetricians and Gynecologists (ACOG) and the International Urogynecological Association (IUGA) have stated that this procedure should be used with caution, on account of the lack of sufficient data from RCTs to support the long-term safety and efficacy of the treatment [17-19]. However, it has nonetheless become increasingly popular over the last several years. The first study by Salvatore et al. in 2014 showed that vaginal laser treatment administered in 3 applications at 4-week intervals could significantly improve VVA symptoms [20]. Each application of this procedure is carried out in the office, lasts less than 5 minutes and requires no local anesthesia. There has been a steady increase in the number studies similar to the 2014 study that support the efficacy and lack of side effects of CO_2 laser therapy for GSM treatment [12, 15, 21-26]. There is histological evidence to support the restorative effect of vaginal laser therapy with a power setting of 30 W, a dwelling time of 1000 µs and a dot spacing of 1000 µm. An ex vivo study demonstrated how the remodeling of vaginal connective tissue led to the consequent renewal of the vaginal mucosa [7, 27]. The in-vivo histological studies by Zerbinati et al. [10] in 5 women with GSM also showed morphological changes that supported the effectiveness of fractional CO_2 laser therapy in the restoration of the vaginal mucosa [8]. Both ex-vivo and in-vivo histologic evidence has been reported in studies conducted among a small series of subjects using a power setting of 30 W. The current study used a power setting of 40 W in 30 postmenopausal subjects. One month after the laser application, the H&E and PAS histologic staining of the untreated and treated sites on the same subject were then compared. The proliferation of vaginal epithelial cells was represented by a significant increase in thickness and number of epithelial cell rows. The resulting significant increase in glycogen-containing cells, including a striking desquamation of the superficial cells, confirmed the thermal effect of the laser on the maturation and differentiation of the epithelial cells. We did not find any significant change in the length of connective tissue papillae, possibly because our study only covered one session of laser therapy with a one-month follow-up. Thus, more time may be needed in order to observe any renewal of the connective-tissue layer. Future research may be needed in order to follow the standard protocol of the manufacturer (SmartXide^2 V^{2}LR, Monalisa Touch, DEKA, Florence, Italy): a power setting of 40 W, a dwelling time of 1000 µs, a dot spacing of 1000 µm with the smart stack 1-3 and a treatment interval of 4 weeks for 3 sessions. Hopefully, results might then confirm morphological modification of the mucosal recovery with complete clarity. Furthermore, additional research studies may be required in order to confirm the longevity of this repair process at the cellular level.

The FDA has issued a warning on the use of fractional CO_2 laser therapy for GSM, and more evidence is needed, especially regarding the long-term effects of this laser therapy. Thus far, this vaginal laser device has been widely used as an alternative treatment for GSM, especially for certain specific populations, such as breast cancer survivors. Some women in this population may go into menopause at an early age, when severe GSM can have a significant impact on quality of life. There are studies reporting on the improvement of VVA symptoms and sexual function in breast cancer patients [11, 24], and the North American Menopause society had recommended vaginal laser therapy as an alternative GSM treatment for breast cancer survivors [28]. We should not routinely recommend this treatment as an alternative treatment for all GSM cases, but in some situations, it can offer improved quality of care by the physician and help improve the quality of life of the patients.

In the market, there are various types and protocols of energy-based devices for the treatment of GSM. Our study involved the effects of treatment using the SmartXide^2 V^{2}LR with a power setting of 40 W, a dwelling time of 1000 µs and a dot spacing 1000 µm with the smart stack 1-3 for one single session only.

4.Conclusion

Our study has provided histologic evidence for restorative changes in the vaginal mucosal structure after one month following thermal injury by use of the fractional CO_2 laser at a power setting of 40 W. The use of this laser incurred no serious side effects or complications whatsoever. In the future, research using an improved study design with a complete standard protocol and a long-term follow-up period may yield additional useful information.

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