HISTOLOGICAL OUTCOMES IN THE TREATMENT OF VULVOVAGINAL ATROPHY USING PHOTOBIOMODULATION TECHNOLOGY

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Abstract Background

Photobiomodulation (PBM) has become a popular modality to treat a multitude of medical conditions either as a stand-alone or an adjunctive therapy given the technologies' ability to stimulate tissue healing, restore cellular function, and relieve pain and inflammation. Early clinical work using PBM for the treatment of vulvovaginal atrophy (VVA)—a medical condition affecting millions of postmenopausal women—suggests that patients can benefit from this therapeutic modality. While research to date has focused on subjective outcomes such as quality of life (QoL) questionnaires, lack of vaginal histological data post PBM treatment raises skepticism about clinical effectiveness.

Objective

This two-arm, placebo-controlled pilot study investigated histological and clinical outcomes using PBM technology to treat VVA symptoms in postmenopausal women.

Methods

The study included 16 postmenopausal women randomly assigned to two arms, eight active and eight placebo-control subjects. The active arm was treated for 10-minutes, two times per week for four weeks (total of eight sessions) using an intravaginal OTC PBM device administered by the investigator, Dr. Adrian Gaspar, in combination with 15 minutes of Kegel exercises under the direction of a physiotherapist. The placebo-control arm received only 15 minutes of Kegel exercises, two times per week for four weeks (total of eight sessions) under the direction of a physiotherapist. Outcomes included vaginal biopsies at three months post-treatment on six of the eight active-arm patients, in addition to vaginal Visual Analogue Scale (VAS) for dyspareunia and dryness at one-month, and the Female Sexual Function Index (FSFI) on all 16 subjects at one-month.

Results

Histological results at three months post baseline validate impressive change in vaginal health, including significant thickening of the epithelium at the lamina propia, neocollegenesis, and increased angiogenesis and glycogen. Active-arm patients reported no discomfort with treatment device, and no side effects were reported, or thermal injury observed in the histological cuts examined. Subject assessment of vaginal symptoms and mean VAS and QoL FSFI scores in active arm subjects improved significantly one-month post treatment and remained positive at three months. In comparison, placebo-control arm showed negligible QoL FSFI score change after one month.

Conclusion

PBM treatment for VVA symptoms in postmenopausal women shows encouraging subjective and objective improvement. The absence of adverse events, coupled with the ease and affordability of treatment, makes for a viable VVA therapy. Given the size of the study, investigation of clinical outcomes in a larger study population is warranted.

Keywords: vulvovaginal atrophy, vaginal dryness, dyspareunia, sexual dysfunction, pelvic floor disorders, photobiomodulation, LLLT, vaginal rejuvenation

INTRODUCTION

During women's reproductive ages, the vaginal epithelium undergoes changes in response to the level of circulating estrogens, which are found in the pelvic floor musculature and vaginal tissue. When menopause occurs, circulating estrogen levels decrease significantly. A common consequence associated with this is vulvovaginal atrophy (VVA), when estrogen deprivation accelerates the process of deterioration of vaginal tissue. As levels of estrogen decline, the vagina and related tissue lose collagen, elastin, and hyaluronic acid content. This leads to a thinning of the vaginal epithelium, alterations in the function of smooth muscle cells, and a decrease in the density of connective tissue. Vaginal pH increases and alters the vaginal flora, and glycogen production, which is responsible for vaginal secretion, comes to a complete halt [1]. Blood flow to the vagina also

slows, reducing the number of blood vessels in these tissues, which is also associated with decreased fluid secretion during sexual arousal. As a result, women experience decreased lubrication and elasticity and therefore are at increased risk of irritation, tissue bleeding, trauma, and pain during intercourse [2].

Approximately 50% of postmenopausal women have VVA symptoms [3] that can range from mild to debilitating, negatively affecting quality of life [4]. These symptoms usually appear some 2-4 years after the onset of menopause. Unfortunately, VVA is unlikely to improve over time without treatment [5], and if treatment is discontinued, the signs and symptoms of VVA tend to reappear. Some clinical reviews have reported that VVA symptoms significantly contribute to an adverse emotional and physical impact on patients and their partners through unsatisfactory sexual relationships [6,7].

The main therapeutic objective in managing VVA is to relieve genital symptoms as well as try to reestablish the vaginal environment to a healthy condition [8]. The optimal approach for the relief of VVA symptoms needs to take into consideration the patients' preferences, personal needs, and presumed treatment compliance. Given this, it is appropriate to give patients comprehensive and detailed information about the available options.

As it relates specifically to VVA, vaginal estrogens have historically been considered the gold standard of treatment for the relief of VVA symptoms. Topical estrogen administration has been widely preferred compared to the systemic modality when genital symptoms are the only complaint, and, moreover, low-dose vaginal estrogen administration has been proven to reach optimal effectiveness with minimal side effects and systemic absorption [9].

More recently, non- or minimally invasive energy-based procedures that correct and restore the structure of the vagina and surrounding tissues have become a viable treatment option to address VVA. These modalities include CO_2 laser-based and radio-frequency (RF)-based technologies and erbium:yttrium-aluminum-garnet (Er:YAG) lasers. These energy sources heat the connective tissues of the vaginal wall and induce collagen contraction, neocollagenesis, vascularization, and growth factor infiltration, which act to regenerate the elasticity and moisture of the vaginal mucosa [10]. While clinical studies and patient feedback have shown encouraging results, the need for in-office treatments and the high cost to patients ($1000 to $3000 per treatment) present significant barriers to their widespread adoption

Photobiomodulation (PBM), also known as low-level light therapy or LLLT, is the process whereby the application of non-coherent light-emitting diodes (LEDs) at specific wavelengths is exposed to tissue, altering the metabolic activities of cells. Evidence indicates that light-energy in the red (630-750 nm) and near infrared (NIR) (700 to 1200 nm) wavelengths activates cytochrome c oxidase, with absorption peaks being at ~660 nm and ~850nm [11], and then is absorbed by the mitochondria to increase adenosine triphosphate (ATP) production, release nitric oxide (NO), and induct various transcription factors [12, 13,14]. Ultimately, PBM causes increases in protein synthesis that triggers increased cell proliferation and migration, as well as modulation of growth factors, inflammatory mediators, and increased tissue oxygenation [15]. This combination of cellular effects is the foundation of the ability of PBM to induce healing and repair at the cellular level. Regarding connective tissue, PBM also appears to affect fibroblasts by stimulating their proliferation, migration, and collagen synthesis.

Karu et al. concludes that to date there is vast statistical material that has been amassed proving that PBM has a positive effect on tissue and there is no significant difference in cell stimulation, regardless of whether the light source used was generated by a laser or an LED [16], provided the dosimetry parameters of the LED (wavelength, power density, energy, and treatment time) are optimized for therapeutic use. Since lasers are used to cut, burn, or coagulate tissue to induce a healing response, PBM allows cells to accelerate their natural healing response. And because the light is diffused and does not cut, burn, or coagulate tissue like lasers do, the energy delivered through LEDs is associated with no known adverse effects [17,18].

Taking into consideration all the above, we hypothesized that a home-use PBM device would be a strong option for the treatment of VVA conditions since the use of PBM owns many of the treatment characteristics that would benefit VVA symptoms. A recently published QoL study supporting PBM as an effective vaginal treatment modality for stress incontinence

and sexual dysfunction and a study looking at PBM for the treatment of Vulvar Lichen Sclerosus further supports our argument that PBM as a viable treatment option for VVA [19, 20]. However, in order to gain widespread acceptance and treatment adoption, histological data that supports clinical efficacy is critical. With confirmatory data, we believe an OTC PBM solution is a viable treatment solution for a large population of postmenopausal women suffering from VVA.

MATERIAL AND METHODS

Study Design

This was a prospective pilot study conducted between June and July 2017 at a private OB/GYN clinic in Mendoza, Argentina, under the direction of Dr. Adrian Gaspar, OB/GYN, and through the coordination of Dr. Gustavo Leibaschoff, OB/GYN. All patients provided informed consent before study participation.

Patient Enrollment

Patients were evaluated for study eligibility by the assessment of inclusion and exclusion criteria, medical history, and physical examination. Eligible patients were postmenopausal women with serum estradiol levels of 20 pg/ml or less, BMIs under 28, more than two years from menses, aged 45 to 69 years old, and self-reported symptoms of VVA. Main exclusion criteria were active sexually transmitted disease, urinary tract infection, neurological disorder; history of cancer, chemotherapy or radiation therapy; and morbidly obese.

Study Device

The PBM device is an intravaginal Class II multi-modality medical device (Fig. 1 vSculpt, Joylux, Seattle, WA, USA) that delivers a combination of PBM with red and infrared LEDs at 662+/-20nm and 855+/-30nm wavelengths, coupled with gentle therapeutic heat at 42° degrees Celsius, and sonic technology within the 75 to 110 Hz range (Fig. 1). The device is inserted into the vaginal canal for 10 minutes per treatment session. The total energy density deposition of the device is 22 J/cm2.

Methods

Two groups of eight women, 16 total, were randomized and blinded to either the active group or placebo-control group.

Fig. 1

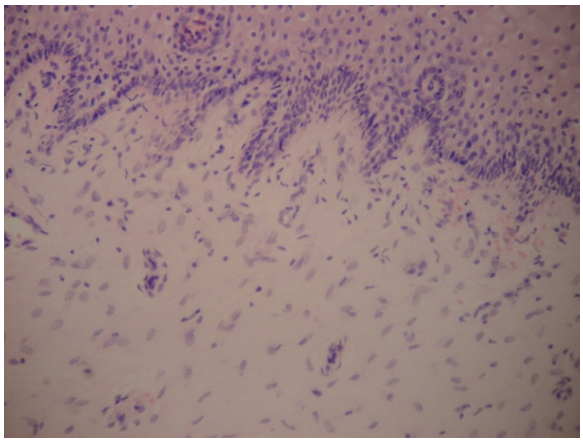
The active group patients underwent a routine examination to assess the vaginal tissue health using the VAS (visual analogue scale) for dyspareunia and vaginal dryness at baseline, one-month, and three months post-treatment. Vaginal biopsies using Tischler Morgan forceps were taken on six of the eight women in the active group at baseline and three months post-treatment at the left lateral wall at the junction of the middle and lower third of the vagina. Basic H&E (hematoxylin and eosin) staining methods were used for the biopsies. These patients completed a validated sexual function QoL questionnaire at baseline and three months post-treatment. Following baseline assessment, the active group then underwent an in-clinic PBM treatment for 10 minutes, followed by 15 minutes of Kegel exercises under the guidance of a pelvic floor physiotherapist. Treatment sessions were performed biweekly for one month for a total of eight treatment sessions.

The placebo-control group completed a validated sexual function QoL questionnaire at baseline and three months post-treatment. They underwent 15 minutes of Kegel exercises under the guidance of a pelvic floor physiotherapist in-clinic biweekly for one month, for a total of eight treatment sessions.

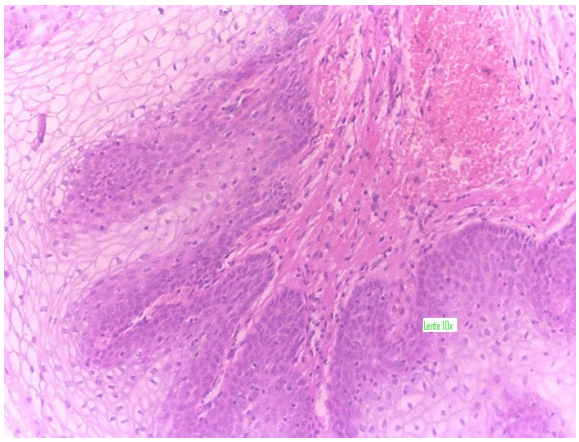
RESULTS – HISTOPATHOLOGY OUTCOMES

Histological samples assessed mucosal thickness, neocollagenesis, vascularization, and overall vaginal health.

PATIENT 1



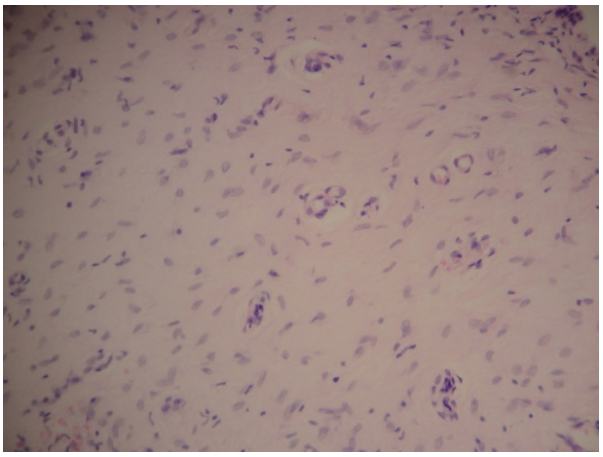
Patient 1
Baseline



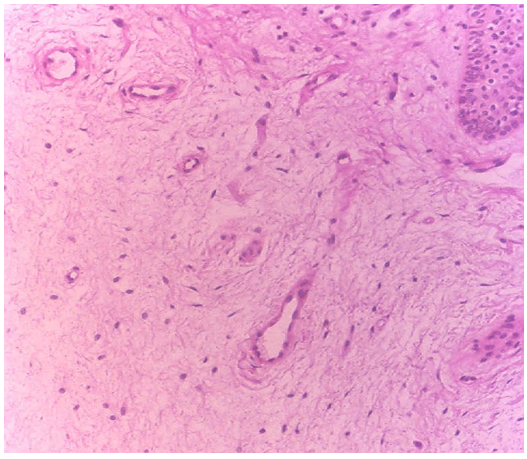
Patient 1
3 Months Post-Tx

Case 1: Increased the presence of papillae, glycogenic load and thickness of the epithelium. Acanthosis and parakeratosis. Increased cellularity, angiogenesis, a complete restorative reaction at the level of the extracellular matrix. 3 months after the treatment.

PATIENT 2



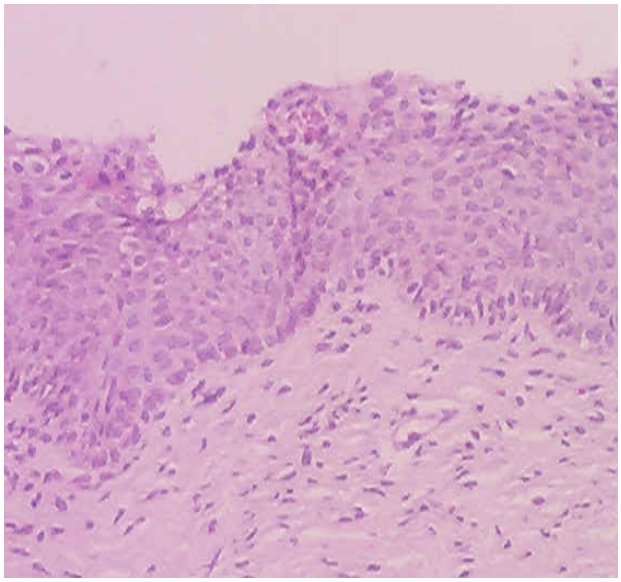
Patient 2
Baseline



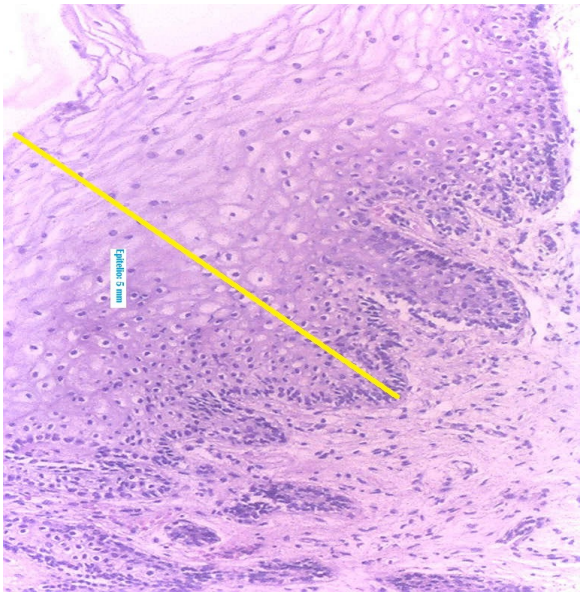
Patient 2
3 Months Post-Tx

Case 2: Increased cellularity, more organization of connective tissue and increase vascularization.

PATIENT 3



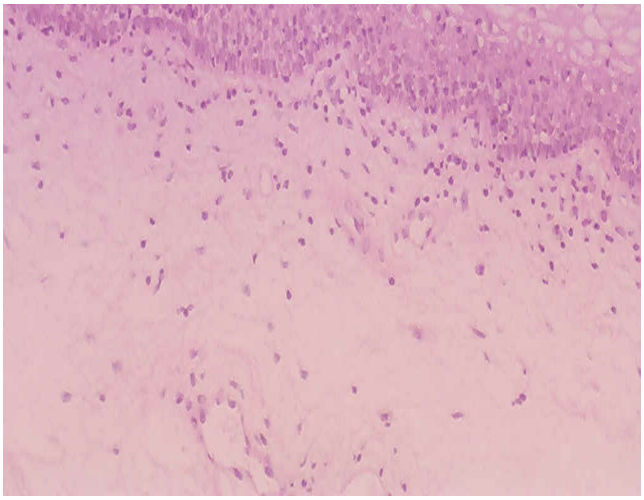
Patient 3
Baseline



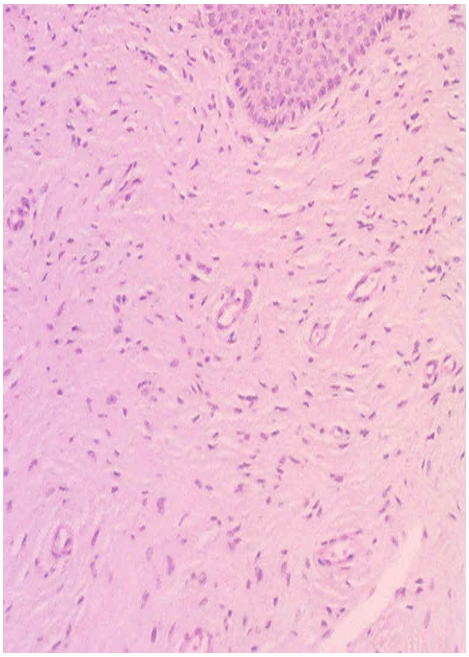
Patient 3
3 Months Post-Tx

CASE 3: Noticeable thickening and glycogen changes at the level of the epithelium and lamina propria.

PATIENT 4



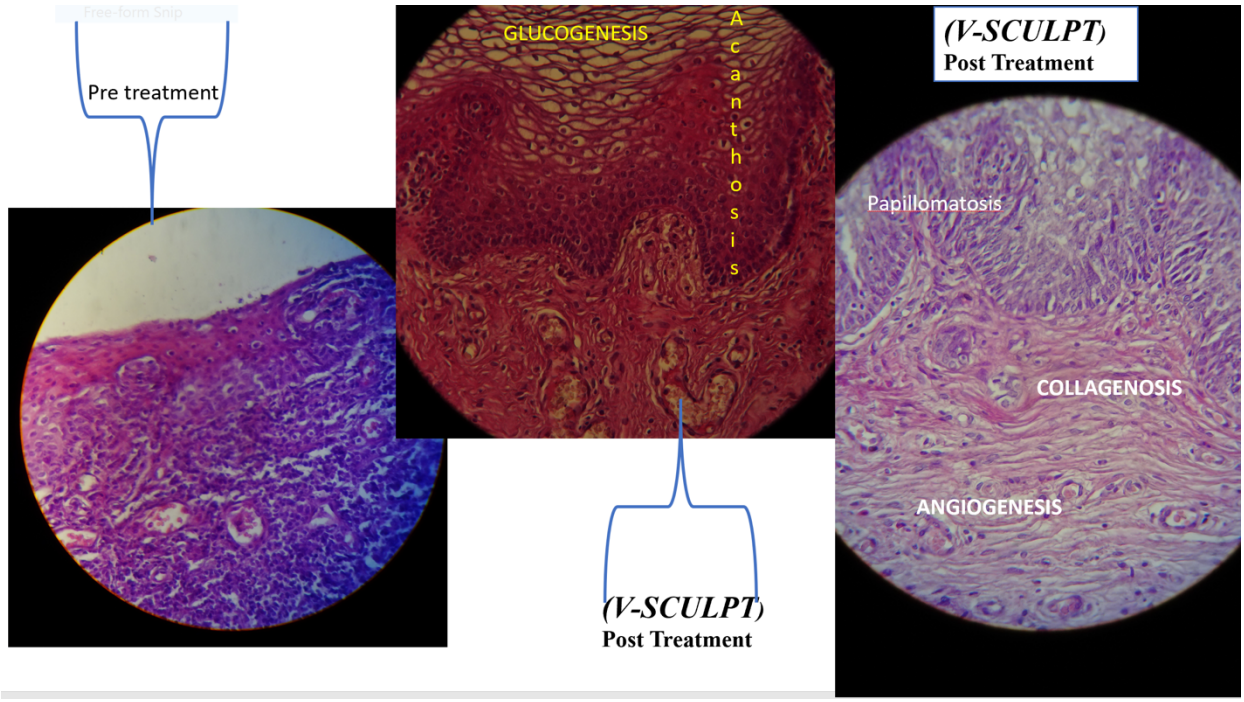
Patient 4
Baseline



Patient 4
3 Months Post TX

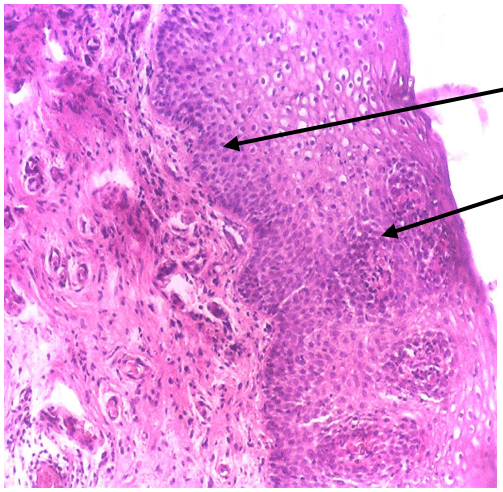
CASE 4: Increased cellularity and vascularization.

PATIENT 5



CASE 5: Increased glycogen load with marked angiogenesis, neocollagenesis, and increased cellularity of the extracellular matrix in the lamina propria.

PATIENT 6:



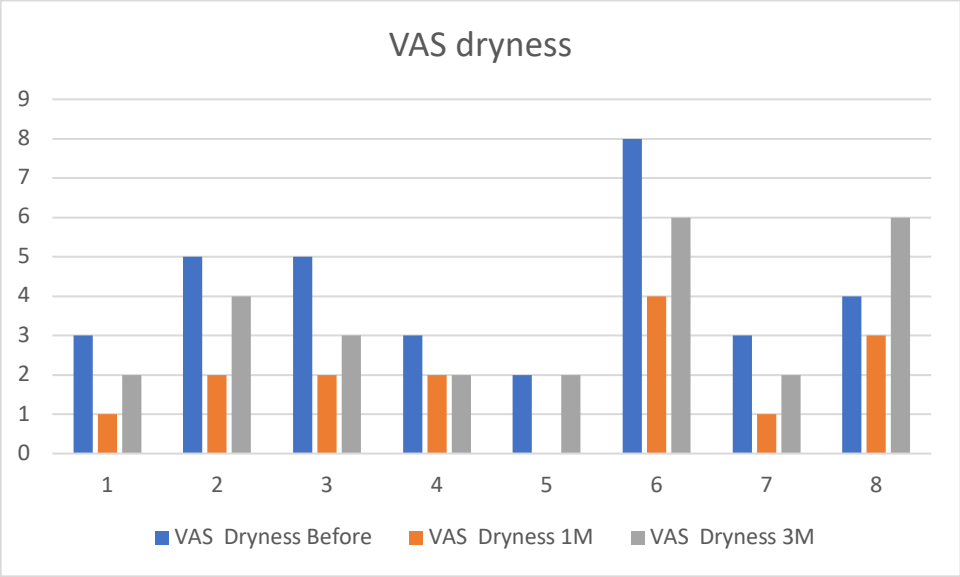
CASE 6: Basal cell hyperplasia and acanthosis of the epithelium.

Patient 6
3 Months Post TX

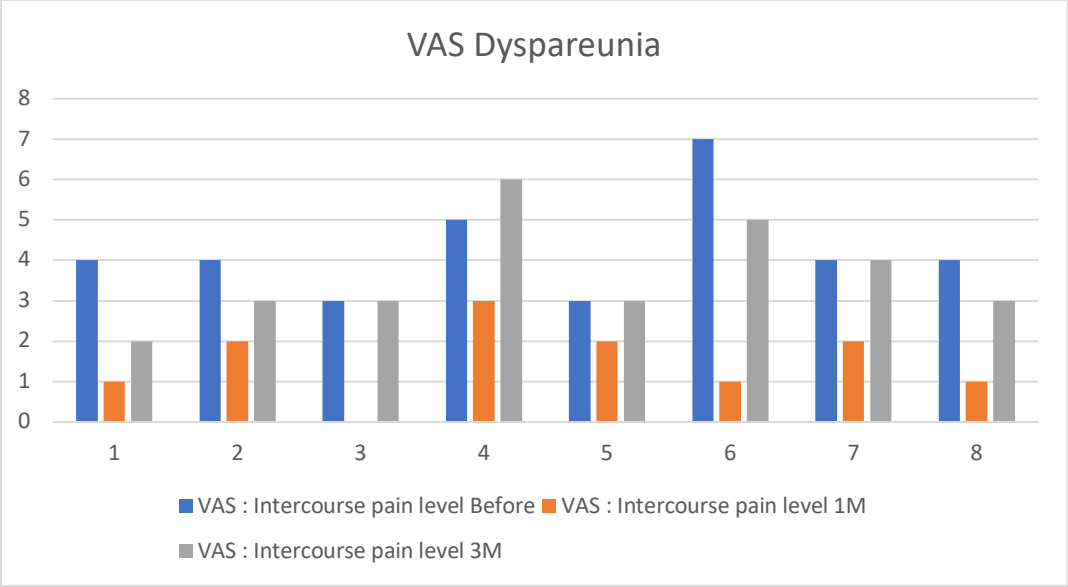
Histological Summary: A sample of histological results at three months post treatment validates the improvement in vaginal health. The epithelial tissue consistently exhibited parakeratosis with an increase in keratinocytes, with acanthosis and an increased glycogenic load. Changes were also observed in the lamina propria consisting of marked angiogenesis and evidence of congestion of tissues with red cells in new vessels, neocollagenesis, increased cellularity of the extracellular matrix and increased presence of papillae. There was no clinical or histologic evidence of thermal injury or scarring during the course of the study or at the 3 month follow up evaluation.

RESULTS – OTHER CLINICAL OUTCOMES

Improvement of VAS for dryness was noticeable in all 8 of the women one-month post-treatment, with average dryness scores 60% stronger than baseline. At three months post-treatment, VAS improvement was still positive in 75% of patients. Only one patient returned to her baseline score, and one patient was one point lower than baseline.

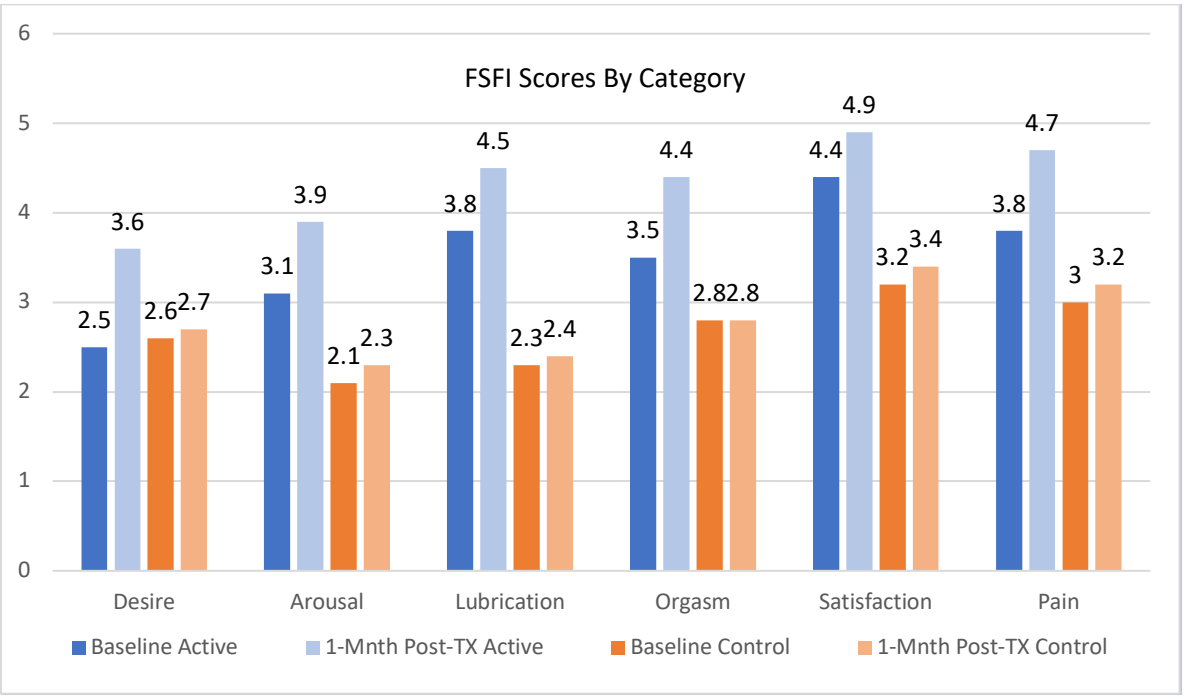


Improvement of VAS for dyspareunia was also noticeable in all 8 of the women one month post-treatment, with average improvement being 68% better than baseline. At three months post-treatment, VAS improvement was still positive in 50% of the patients. Of the remaining 4 women, 3 returned to their baseline scores, and the same woman who was one point higher with her dryness score at 3 months post-treatment was also one point higher with her dyspareunia score 3 months post-treatment, indicating other factors may have been in play.



The total FSFI score improved in all 8 patients. Mean total FSFI score increased 25% over the treatment period, from 21.1 to 26.0. Biggest increase in individual mean scores was in the Desire category with +44%, followed by Arousal, Orgasm, and Pain all seeing a 24% increase. In comparison, the placebo-control group saw negligible change, with post-treatment mean total score increasing only 5% over baseline, from 16.0 to 16.8.

FSFI Domains	Desire	Arousal	Lubrication	Orgasm	Satisfaction	Pain	Total FSFI Score
Baseline Active	2.5	3.1	3.8	3.5	4.4	3.8	**21.1**
1 Mth Post TX Active	3.6	3.9	4.5	4.4	4.9	4.7	**26.0**
Baseline Control	2.6	2.1	2.3	2.8	3.2	3	**16.0**
1 Mth Post TX Control	2.7	2.3	2.4	2.8	3.4	3.2	**16.8**



DISCUSSION

Decreased vaginal tissue and muscular function is basically a result of damage to the vagina at a nerve, muscular, cellular, and connective tissue level caused primarily by pregnancy and aging. There are promising advancements with in-office laser and radio-frequency devices to treat the symptoms of VVA, but the large patient expense ($3,000+) and the need for ongoing treatments to achieve and maintain results presents a barrier for widespread adoption. There is an opportunity for a simple, effective, and affordable treatment to help a broader population of women suffering these difficult conditions, a convenient way for all women to maintain their results—from an in-office procedure or an at-home solution—in the privacy of their home.

As discussed, photobiomodulation (PBM) using non-coherent light-emitting diodes (LEDs) is the process whereby the

application of light energy modifies the metabolic activities of tissue cells. It is an important and increasingly utilized concept in healthcare for a wide variety of conditions and injuries—facial rejuvenation, wound healing, pain management, and reduction of inflammation, among others—based on being noninvasive and painless and having the ability to induce healing in damaged tissue by affecting processes at the cellular level [21]. Light in the red spectrum (630-700 nm), including some near-infrared portions (700-1200 nm), also prevents apoptosis, modulates inflammatory and antioxidant responses, and stimulates angiogenesis [22,23,24]. PBM also appears to affect fibroblasts by stimulating their proliferation, migration, and collagen synthesis. Given that PBM has a positive impact on tissue repair in other body areas, there is considerable promise in using PBM as a treatment option for VVA and other pelvic floor disorders.

vSculpt is a class II OTC medical device made by Joylux, Inc. approved by a CE Notified Body and Health Canada and currently under 510(k) review in the US with the Food and Drug Administration (FDA) for stress urinary incontinence clearance. vSculpt is primarily a PBM device using red and NIR light in the 662+/-20nm and 855+/-30nm wavelengths, but also uniquely adds gentle heat (42°C) and vibration (75-110Hz) to provide a new treatment option for women suffering pelvic floor disorders. In an early study using quality-of-life questionnaires (QoL) and objective measurements such as the 1-hour pad weight test, vSculpt has been shown to objectively and subjectively improve the quality of life for women in the areas of vaginal muscle tone and tightness, stress urinary incontinence, and improved vaginal lubrication and sexual function [18].

vSculpt provides a home-use device that is simple to use, effective in providing both relief and healing, and is affordable for the patient suffering from VVA. The early clinical diagnosis results support an ability to improve quality of life, the primary treatment goal of the vSculpt device. With the histological data, we now conclude that the structural change happening within the vaginal tissue supports the clinical effectiveness of this multi-modality therapy.

Conclusion: The use of PBM in treating postmenopausal women suffering from symptoms of VVA showed improvement in both objective and subjective symptoms. The subjective improvements correlated to the related histological changes, showing an improvement in vaginal atrophy. Specifically, the histological samples three months post-treatment showed angiogenesis, increased vascularization in the lamina propria, neocollagenesis, increased thickness of the epithelium, and an increase the cellularity in the ECM, with no thermal damage effects. Finally, it is interesting to emphasize that the histological changes were maintained at 12 weeks, which suggests that if these patients continued the treatments at home, they could further improve or maintain the clinical benefits beyond the initial treatment protocol. To confirm this theory, we would need to run a prolonged, double blind study with a greater number of patients.

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