PROTOCOL: REPLAMOD

PROMOTER: OSR

TITLE: Histological Modifications of Postmenopausal Vaginal Mucosa After Repeated CO_2 Laser Treatment

Aim of the study: To assess the long-term histological and clinical efficacy of MonaLisa Touch procedure for the management of the Genitourinary Syndrome of Menopause (GSM) in postmenopausal female patients.

Study design: This will be a prospective case series.

Background: The Genitourinary Syndrome of Menopause (GSM), previously known as Vulvovaginal Atrophy (VVA), is a condition seen in postmenopausal estrogen-deficient women. This condition includes both histological and clinical modifications at the genital level which can significantly lower quality of life and lead to female sexual dysfunction, social isolation and depression in this population. Clinically, GSM include symptoms related to the lower urinary tract (dysuria, urgency, frequency, nycturia, urinary incontinence or frequent urinary tract infections) and to the vulva/vagina (dryness, itching, or dyspareunia). [1] Histologically, GSM presents alterations in collagen type I fibrils-to-collagen type III fibrils ratio with loss of their trabecular disposition (collagen fibrils become flattened), it results in decreased quantity of elastic fibers and lubricant secretions, reduced vascularization, and thinning/flattening of the vaginal epithelium.[2]
Postmenopausal women who suffer from GSM can be managed firstly with moisturizers and lubricants and if these fail to control the symptoms they can be treated with pharmacotherapy based on vaginal estrogen preparations.[3] Though the effect of local estrogen therapy is often effective, medical adherence among patients is variable (52-74%)[4] and the use of estrogen is contraindicated in women having history of breast or endometrial cancer, thromboembolism, or coronary artery disease.[5] Having an effective non-pharmacologic treatment for the management of GSM would therefore be an attractive option for treatment.
MonaLisa Touch CO_2 fractionated laser is a novel, minimally invasive therapy that helps restore vaginal health. The U.S. Food and Drug Administration (FDA) approved the technology for the excision, vaporization, and ablation of tissues and since then it has been used in many different fields of medicine[6], but it is not approved for the treatment of vulvovaginal atrophy or GSM at the present time. Though observational studies show a possible benefit of MonaLisa Touch in the treatment of vulvovaginal atrophy and GSM [7,8, 9, 10], no studies with long-term follow-up have been performed to date.

We propose a prospective case series of 15 postmenopausal women with bothersome symptoms of GSM treated with MonaLisa Touch CO_2 laser. We hypothesize that even in the long term MonaLisa Touch will significantly improve GSM histologically and clinically from baseline.

Inclusion Criteria:

- Italian speaking and able to give informed consent.
- Menopausal females with absence of menstruation for at least 12 months.
- Presence of GSM symptoms.
- Completion of at least two cycles of three laser treatments sessions in previous years.
- Prolapse stage < II, according to the pelvic organ prolapse quantification (POP-Q) system.
- No pelvic surgery within 6 months prior to treatment.
- Understanding and acceptance to the obligation to return to all scheduled visits and follow-ups.

Exclusion Criteria:

- Inability of give informed consent
- History of vulvovaginal condyloma, vaginal intraepithelial neoplasia (VAIN), vaginal carcinoma, lichen sclerosis, lichen planus.
- History of cancers of the lower genital tract (cervix, uterus, vagina).
- History of pelvic radiotherapy.
- Personal history of genital fistula, a thin recto-vaginal septum as determined by the investigator or personal history of a fourth degree laceration during screening physical exam.
- Any other medical condition that the investigators feel would compromise the study.
- Acute or recurrent urinary tract infection (UTI), or genital infection (e.g. bacterial vaginosis, herpes genitalis, candida).
- Stage III or IV pelvic organ prolapse.
- History of any female sexual disorder.

Power analysis: As this is a prospective case series, no formal power analysis is required.

Efficacy Outcomes:

The **primary outcome objective of the study** is to evaluate the histological modifications of the vaginal mucosa after repeated CO_2 Monalisa Touch laser treatment. We shall evaluate the role of this treatment by calculating the difference in vaginal epithelial thickness between the biopsy obtained before the start of laser treatment and the biopsy obtained after treatment. The latter parameter will be calculated by averaging three measurements 150 micrometers from each other and considering as significant a 20% increase in epithelial thickness compared to baseline. We will then compare the number of papillae, the amount of glycogen present at the epithelial level (by PAS reaction), the ratio between the number of type I collagen fibers ("new" collagen) and type III (old collagen), and the number and size of the vessels present in the subepithelial layer. In addition, the study will analyze the safety of repeated laser treatments verifying the absence of fibrosis formation in the vaginal mucosa.

Secondary objectives of the study will include an evaluation of the clinical effects associated with repeated Monalisa Touch CO_2 laser treatments. Questionnaires to address these secondary objectives will include the following:

- Vaginal Health Index (VHI) [11] which will objectively measure the severity of vaginal atrophy
- Visual Analog Scale (VAS) which will subjectively measure the severity of vaginal symptoms
- Female Sexual Function Index (FSFI)[12] questionnaire which will assess female sexual function
- Urinary Distress Inventory-6 (UDI-6) and the International Consultation on Incontinence Questionnaire – Urinary Incontinence (ICIQ-UI) which will evaluate the extent of urinary symptoms
- 5-point Likert[13] scale to assess the impact of GSM symptoms on quality of life (QoL)

Study Design: All participants will be screened to ensure inclusion/exclusion criteria are met. A biopsy will be taken from each patient one month before the first laser treatment and one month

after the last laser treatment. The MonaLisa treatment will be performed monthly for 3 months. MonaLisa treatment with fractional microablative laser system (SmartXide 2 V 2 LR, MonaLisa Touch, DEKA, Florence, Italy) will be performed to the following energy settings and treatment parameters: dot power 30 W, dwell time 1000us, dot spacing 1000um, smart stack parameter from 1 to 3 and emission mode deka pulse. For subjects with concomitant vulvar symptoms, vulvar treatment will be done with the dot power reduced to 26 W, dwell time 800us, dot spacing 800um, and the smart stack parameter of 1.

During the procedure, an external introducer ring will be inserted into the vaginal canal, the markings on the probe will be aligned with the edge of the ring to measure withdrawal of the probe after a series of pulses will be delivered, and the probe will be rotated of 60°. Laser power could be adjusted before and during the treatment to assure patient comfort. The procedure will be performed in the outpatient clinic, not requiring any specific preparation, analgesia, or anesthesia. Participants will be advised to refrain from engaging in sexual intercourse or bathe for 48 hours after the MonaLisa treatment.

Biopsy samples will be analyzed with Hematoxylin- Eosin and Trichrome staining, PAS reaction for glycogen and immunohistochemistry for CD34, a vessel marker. All clinical questionnaires and VHI will be assessed at baseline and at one month after the last session.

Any medical changes will be reported to the research team who will then evaluate for any impact on the study. Any adverse effects reported by participants will also be recorded and managed accordingly.

Fifteen subjects will be considered for the study. All materials will be kept confidential and maintained by the research coordinator in a locked cabinet.

The participants will keep the research team aware of any changes in medical history, and if any adverse effects are detected.

Data Collection:

- Biopsy baseline visit -Visit 1:
    - Inclusion/Exclusion criteria;

    - Collection of patient anamnestic information (age, parity, years since menopause, previous pathologies, previous surgical interventions, BMI, use of estrogenic therapies, sexual activity);
    - VAS, FSFI, ICIQ-UI, UDI6 e Likert questionnaires completion;
    - Informed Consent signing;
    - Bioptic procedure;
    - VHI completion from research coordinator;
- Laser treatment visits (three visits at one month interval) - Visit 2, 3, 4
    - Monalisa Touch Laser procedure
    - Adverse events including inability to complete the procedure due to discomfort or constricted vagina.
- Follow-up visit (1 month from the last laser treatment) – Visit 5
    - VAS, FSFI, ICIQ-UI, UDI6 e Likert questionnaires completion;
    - Bioptic procedure;
    - VHI completion from research coordinator;

Data Management: To ensure the protection of each subject's personal health information (PHI), one master Excel file containing subject PHI including name and medical record number will be kept on a password-protected and SharePoint computer. The participant shall be given a unique identification number used for the purpose of de-identified data collection and analysis.

Any hard-copy forms for data collection will be kept in a locked cabinet in the PI's office, which shall be locked at all times, at Urogynecology department at Ospedale San Raffaele Milan, Italy. All forms will contain de-identified information. Identification numbers will correspond to the subjects listed in the master Excel file.

Summary: GSM is a progressive and devastating condition in menopausal women, and is associated with bothersome irritative symptoms compromising the quality of life if this population. MonaLisa Touch laser treatment has shown initial promise in reversing postmenopausal vaginal atrophy, but its efficacy in treating symptoms of GSM long term is lacking. This study is designed

to investigate the long term feasibility of using Mona Lisa laser therapy to treat GSM symptoms in postmenopausal women.

References:

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