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SERVIZIO DI PATOLOGIA DEL TRATTO GENITALE INFERIORE

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METAGENOMIC, TRANSCRIPTOMIC, ELECTRODIAGNOSTIC AND MORPHOLOGICAL EVALUATION OF PATIENTS WITH VESTIBULODYNIA BEFORE AND AFTER TREATMENT WITH TOPICAL ESTRIOL + TESTOSTERON + CANNABIDIOL

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Study Typology : case-control, interventional, prospective trial

1. INTRODUCTION

Vulvodynia is a highly prevalent form of chronic genital pain in women, to such an extent that prevalence studies estimate ranges from 10% to 28% in reproductive-aged women. Localized provoked vulvodynia at the vestibule, known as vestibulodynia (VBD), is the most common manifestation of the disease (about 80%). Women with VBD often describe vulvar pain as a burning, stinging, irritation, rawness, and dyspareunia (difficult or painful intercourse). Most patients with VBD described their pain as "hot," "burning," or "pricking" and that the vestibular area is sensitive to the touch (e.g. during sexual intercourse or tampon use) and that the pain would be increased by rubbing.
The pattern of VBD responses is suggestive of sensory abnormalities in the form of evoked pain (e.g., hyperalgesia or allodynia), suggesting sensitization, an underlying manifestation of neuropathic pain. This prolonged pattern can result in decreased tissue perfusion, muscle dysfunctional overactivity, and the development of myofascial trigger points, resulting in localized or radiating pain and/or intense tenderness. VBD represents a summation and overlapping of various trigger factors (infections, hormonal disturbances, allergies, genetic aspects, psychological vulnerability, and others) with weight and predominance varying from patient to patient. Sex steroid alterations related to estrogen and androgens in VBD has been investigated in previous studies ranging from vestibular tissue immunohistochemistry to circulating microRNA. It was demonstrated that women with VBD compared to controls had reduced concentrations of pregnenolone, progestin and androgen metabolites involved in the steroid hormone biosynthesis. There are indications of a potential difference in the vaginal microbiome of women with VBD compared with control women. In a double-blind study, vaginal samples for bacterial flora and cytokines of patients with VBD were compared with controls, and cultures from control women showed the presence of L. crispatus, which was not present in samples from women with symptomatic VBD or VBD in remission, who alternatively demonstrated the presence of L. gasseri. Recent research has investigated the so-called "priming" of immune cells to assess immune cells responses to repeated immune challenges. Normal immune cells return to a dormant state after provocation, but others remain in a constant state of readiness to respond to the next immune challenge with a heightened proinflammatory response and probably in VBD history the understanding of this process could prevent the development of disease. Unfortunately, the VBD diagnosis always comes too late, when the mechanism of aberrant immune response already has caused the irreversible nervous sprouting in vulvar vestibule. A multifactorial etiology, such as infections, hormone disorders, neuroinflammation, atopic disease, gene polymorphisms that interfere with inflammation and psychogenic factors have been implicated in the development and maintenance of VBD. What is becoming increasingly apparent is that VBD is likely not one disease but several diseases, in which the end points of different factors are the vestibular hypersensitivity and the pelvic floor hypertonic dysfunction. Clustering of patients according to predisposing and precipitating factors provides a means to discover the prominent pathophysiology that may help clinicians to diagnose and select the most appropriate and effective multimodal treatment.

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2. STUDY OBJECTIVES

The Research Hypothesis for the present study is to prospectively compare three main parameters that we consider essential in developing of VBD:
- Inflammatory state
- Hormonal asset
- Microbiological pattern

Our aim is to develop a specific kit in order to identify earlier the most susceptible subjects to develop VBD and build a preventive strategy to avoid the development and/or chronicization of disease. To do so, we will combine data from literature with population transcriptomic and genomic analysis and the state-of-the-art single cell omic analysis on VBD patients and healthy controls. We will perform population RNA-seq analysis on vestibular cells (obtained with a painless scraping) in order to analyze the expression of specific genes with particular focus on hormonal receptors and also to evaluate the composition of immune infiltrates by computational deconvolution of the data. In conjunction we will analyze the composition of the vestibular microbiome employing new generation metagenomic analysis approach (i.e. shotgun sequencing technology) amongst women with diagnosis of VBD and healthy age matched controls.

Moreover, we aim to evaluate in VBD group the vestibular effects of a topical compound contains estriol+ testosterone + cannabidiol (CBD) on inflammatory state, hormonal asset and microbiological pattern through DNA and RNA-seq analysis as a second step of the study. The premise is that hormone supplementation can restore vestibular trophism optimizing microbiological environment and topical CBD for its significant analgesic, anti-inflammatory, anti-neuropathic activities can achieve significant improvement in pain and other disturbing sensations in patients with VBD.

3. DESCRIPTION OF RESEARCH DESIGN

3.1 Overall Study Plan
This is a two-step trial:

- **First step**. A case-control study will compare women with VBD and control women without vulvar pain. All women aged 18 years and before menopause (cessation of menstruation for 12 months) with VBD that will present to our unit of lower genital tract disease will invited to participate. The control group will include healthy fertile asymptomatic women without any vulvovaginal conditions that will attend to our hospital for cervical cancer screening programs.
  For the purpose of this first step, a vestibular scraping samples will be collected to obtain vestibular cells for omic-analysis and microbiome characterization, in both groups (VBD and Control group).
- **Second step.** VBD patients will move to the second step using a topical galenic product that contains Estriol 0.05 % (15mg) + Testosteron vegetal origin 0.6 % (180mg) + Cannabidiol 3% in argan oil in Pentravan® to 30 ml. Pentravan® is an oil-in-water emulsion with a liposomal matrix that uses the same penetration enhancing ingredients that enable it to establish a greater rate and extent of absorption of the drug than other transdermal bases. The topical drug will be dispensed through the Topi-CLICK® dispenser. Topi-CLICK makes it easy to accurately measure the dose and apply topical medications to the intended area, thereby decreasing the risk of improper dosing and contamination while improving compliance and outcomes of therapy. Every vestibular dose will include two click of Topi-CLICK® dispenser (each click is 1/4 turn of the bottom of the Topi-Click® and dispenses a metered amount of 0.25 ml). Patients will be instructed to apply the cream to the whole of the vulvar vestibule once per day for two months, except menstrual period. A new vestibular scraping samples will be collected to obtain vestibular cells for omic-analysis and microbiome characterization after two months of therapy

3.2 Study Duration
Each eligible subject will participate in the study for approximately 2 months
It is expected this investigator-initiated research study will be completed approximately 6 months following initial approval by the Institutional Ethical Board.

3.3 Institutional Ethical Board Approval (IEB)
Prior to conducting any study-related procedures, the Principal Investigators will each obtain written approval from their respective IEB for the informed consent form, protocol, recruitment materials, and any written information provided to Subjects pertaining to the procedure.

5. SELECTION AND WITHDRAWAL OF SUBJECTS

The study population will include women with VBD.

5.1 Subject Inclusion Criteria

All criteria below must be met for a Subject to be eligible for study participation.

- Women at least 18 years of age and before the menopause (absence of menstruation for 12 months)
- Experience moderate to severe pain (minimum of 5/10 on a numerical rating scale in at least 90 % of attempted sexual intercourse)
- Pain limited to the vestibule during vaginal intercourse and during activities exerting pressure on the vestibule (tampon insertion, tight jeans or pants, cycling, horseback riding)
- Presence of VBD for at least 6 months and diagnosed according to the standardized gynecological examination protocol by one of our staff gynecologists
- Have a stable sexual partner (sexual activity should include some attempted vaginal penetrations to evaluate pain intensity)
- Subject is willing to attempt sexual activity between visits
- Read and signed informed consent.

5.2 Subject Exclusion Criteria

Subjects who meet any of the following criteria shall be excluded:

- Active vulvo-vaginal infections at the time of their gynecological examination.
- Genital bleeding of unknown origin.
- Unwillingness to provide the informed consent to the trial.
- Women who were using or used hormonal contraceptives, topical or systemic hormonal drugs in the past 3 months
- Women with concomitant vulvar dermatosis or other vulvar disorders

5.3 Subject Withdrawal Criteria

The Principal Investigator may discontinue a subject's participation in the study at any time if it is considered in the subject's best interest to do so. Such a decision may be precipitated by adverse events, new onset illness, clinically important changes in vital signs, physical examinations, or laboratory tests. Subjects who are noncompliant with study procedures and visits may also be withdrawn by the Principal Investigator. Subjects may withdraw from participation in the study at any time for any reason. A subject's decision to withdraw will not cause the subject to lose any benefits to which she is entitled. A subject who withdraws prematurely from the study will return to the clinic as soon as possible to undergo the final visit evaluations.
If a subject prematurely withdraws or is withdrawn from study participation, the reason for the withdrawal must be recorded on the case report form (CRF).
Record the primary reason for premature withdrawal according to the following categories:

• Adverse Event: Subject experiences an intolerable event, which may or may not be related to the study medication.
• Withdrawn Consent: Subject withdraws from study participation for personal reasons (exclude adverse experience before indicating this category).
• Concomitant Medication Violation: Subject initiates, discontinues, or changes dosing regimen of concomitant medication in violation of the protocol, which, in the judgment of the Principal Investigator, may adversely affect evaluation of safety.
• Lost-to-Follow-up: Subject does not return for evaluation and no further contact is made by the Subject after three documented phone or email attempts and a final attempt by certified mail.
• Other: Any reason that does not fit in the above 4 categories: the reason will also be recorded on the CRF.

3. OUTCOMES

Primary outcomes includes features in VBD patients respect control group and changes after application of Estriol 0.05 % (15mg) + Testosteron vegetal origin 0.6 % (180mg) in argan oil in Pentravan to 30 ml + Cannabidiol 2% in Pentravan® to 30 ml in VBD group only, of:

- Vestibular RNA expression pattern and microbiome asset.
- Evaluation of current perception threshold (CPT) testing, a technique which quantifies the sensitivity of vestibular nerve fibers. The CPT values will be measured using the Neurometer CPT/C electrodiagnostic neurostimulator (Neurotron, Inc., Baltimore, MD), which emits constant alternating sinusoid waveform current stimuli at frequencies of 2000 Hz (specific for large, myelinated Ab fibers), 250 Hz (specific for Ad fibers), and 5Hz (specific for C fibers), at intensity levels from 0.001 to 9.99mA. Vulvar vestibule CPT values (1=0.01 mA) will be determined using a G-trode Vaginal/ Rectal Electrode (Neurotron, Inc., Baltimore, MD).
- Vestibular mucosa thickness performed by ultrasound measurements (B-Scan) with a 20 MHz validated system (Derma Scan C, Cortex Technology, Denmark), producing a cross sectional images of the skin down to a depth of approximatively 15 mm

Secondary outcomes include evaluation at baseline and after therapy of changes in VBD group of:

- 0-10 point visual scale (VAS) related to vulvar burning/pain and dyspareunia
- Changes on validated instruments: Female Sexual Function Index (FSFI), Vulvar Pain Functional Questionnaire (V-Q)
- Vaginal EMG measurements, collected at states of rest and during several exercises of the pelvic floor through an EMG device with a vaginal sensor (Myotonus plus©-London-UK)

6. Clinical Procedures

Clinical procedures throughout the study are described in the sections below.

6.1 Informed Consent

Each potential study Subject must provide written informed consent and authorize release of her protected health information before any study procedure is conducted.

6.2 Study Day 0 . Subjects Screening and Visit 1

Candidates for enrollment will be screened within 15 days prior to enrollment. Before initiation of any test procedures, Subjects will be fully informed of the study plan, procedures, and risks involved in participating in the study. Each potential Subject will be required to read and to indicate her understanding by signing and dating the ICF prior to initiation of any screening procedures.
Screening procedures will consist of the following:

- Physical examination and medical history will be collected
- Evaluation of symptoms: 0-10 point visual scale (VAS) related to dyspareunia and vulvo-vaginal pain/burning
- Completion of validated questionnaires FSFI and V-Q
- Vulvoscopy with evaluation of vestibular cotton swab test
- Sampling of vestibular cells obtained with a painless scraping for -omic analysis
- Assessment of vestibular CPT evaluation using the Neurometer CPT/C, electrodiagnostic neurostimulator (Neurotron, Inc., Baltimore, MD)
- Vestibular mucosa thickness performed by ultrasound measurements (B-Scan) with a 20 MHz validated system (Derma Scan C, Cortex Technology, Denmark), producing a cross sectional images of the skin down to a depth of approximatively 15 mm
- Assessment of vaginal EMG measurements, collected at states of rest and during several exercises of the pelvic floor through an EMG device with a vaginal sensor (Myotonus plus©-London-UK)
- Only VBD patients will receive topical galenic product that contains Estriol 0.05 % (15mg) + Testosteron vegetal origin 0.6 % (180mg) + Cannabidiol 3% in argan oil in Pentravan® to 30 ml. They will be trained to apply the cream to the vulvar vestibule once a day for 60 days

6.3 Study Day 60± 3 days: only for VBD that received topical treatment with Estriol 0.05 % (15mg) + Testosteron vegetal origin 0.6 % (180mg) + Cannabidiol 2% in argan oil in Pentravan® to 30 ml.

Patient who completed the treatment, will receive:

- Evaluation of symptoms: 0-10 point visual scale (VAS) related to dyspareunia and vulvo-vaginal pain/burning
- Completion of validated questionnaires FSFI and V-Q
- Vulvoscopy with evaluation of vestibular cotton swab test
- Sampling of vestibular cells obtained with a painless scraping for -omic analysis

- Assessment of vestibular CPT evaluation using the Neurometer CPT/C, electrodiagnostic neurostimulator (Neurotron, Inc., Baltimore, MD)
- Vestibular mucosa thickness performed by ultrasound measurements (B-Scan) with a 20 MHz validated system (Derma Scan C, Cortex Technology, Denmark), producing a cross sectional images of the skin down to a depth of approximatively 15 mm
- Assessment of vaginal EMG measurements, collected at states of rest and during several exercises of the pelvic floor through an EMG device with a vaginal sensor (Myotonus plus©-London-UK)

7. Statistical Methods

7.1 Determination of Sample Size

We used http://statulator.com program which calculated sample size for paired differences. With power of 80% and level of significant of 5%, for detecting a mean of the differences of VAS scale of 1.5 (20%) between pairs, assuming the standard deviation of the differences to be 2 we will need to recruit 40 participants, 20 for each group.