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GENOMIC EVALUATION OF PATIENTS WITH VESTIBULODYNIA: A CASE-CONTROL STUDY

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Study Typology : case-control , 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. A genetic aspect related to vestibulodynia appears to be pivotal. Some studies have suggested several potential mechanisms that could underlie a genetic predisposition for vulvodynia, including an influence on the risk of recurrent Candida or bacterial vaginosis infections or a prolonged response to infection or inflammation, an altered inflammatory response, or an increased risk to gonadal hormonal milieu changes associated with oral contraceptives.

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.

References

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2. STUDY OBJECTIVES

The Research Hypothesis for the present study is to prospectively compare four main parameters that we consider essential in developing of VBD:

-Increased sensitivity to pain

- Inflammatory state

- Hormonal asset

- Microbiological pattern

Our aim is to develop a specific test to identify earlier the most susceptible subjects to develop VBD and build a preventive strategy to avoid the development and/or chronicity of disease. Moreover, we postulate to obtain data for clustering the patients with the goal to tailor the most appropriate treatment.

To do so, we will analyze the DNA obtained from a sample of patients with VBD compared with a healthy control group. We will use the technique of Shallow Genome

Sequencing (SGS), also known as low pass whole genome sequencing, that is a new and high-throughput technology to achieve genome-wide single nucleotide polymorphisms (SNPs) genetic variation accurately. Shallow SGS can be used to build a custom reference panel for a specific population. SGS will be associated with the approach of "genotype imputation" that allows the reconstruction of human haplotypes already known, through methods of inferential statistics that along specific algorithms, exploits the databases of human population genetics (e.g. dbsnp or 1000 Genome Project) to reconstruct haploblocks with a certain degree of significance. The collected data will be processed through a biostatistics analysis to correlate the presence of certain SNPs in cases of VBD respect the control group, to explain the molecular aspects of this pathology and discover any predictive markers in association of anamnestic and objective clinical data.

3. DESCRIPTION OF RESEARCH DESIGN

3.1 Overall Study Plan

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.

3.2 Study Duration

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.

4. SELECTION AND WITHDRAWAL OF SUBJECTS

The study population will include women with VBD.

4.1 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) for VBD group
- 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) for VBD group
- Presence of VBD for at least 3 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.

4.2 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 with concomitant vulvar dermatosis or other vulvar disorders
- Symptoms or signs in the past related to VBD, only for Control group

4.3 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.

5. OUTCOMES

Outcomes includes features and correlations in VBD patients respect control group of:

- Shallow Genome Sequencing of DNA pattern obtained from a blood sample
- Vestibular microbiome asset obtained from a vestibular sample
- 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
- 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, only in VBD group
- Vulvoscopy with evaluation of vestibular cotton swab test
- Taking blood samples for DNA analysis (about 3ml)
- Taking vaginal secretion at vestibular site for microbiome 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

The sample size of 60 subjects (30 for VBD group and 30 for Control group) was chosen for this study based on this being a pilot study, and this sample size will be enough to prospectively document genetic correlations

8. FUNDING

This study will be supported by the Associazione Italiana Vulvodinia, a non-profit Italian association whose mission is to improve the health and quality of life of women experiencing vulvodynia and chronic vulvar pain.