

Vulvovestibular Syndrome and Vaginal Microbiome: A Simple Evaluation

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Abstract

Background: The vulvovestibular syndrome (VVS) is a chronic, inflammatory, multifactorial, chronic inflammation of the female urogenital access.

Methods: The aim of this anecdotal, observational, retrospective, case-control study was to comparatively evaluate the most common bacterial strains (*Lactobacillus spp.*, *Klebsiella spp.*, *Gardnerella spp.*, and *Streptococcus spp.*) and fungi (*Candida spp.*, *Penicillium spp.*, and *Aspergillus spp.*) in vulvodinic women, and in women without gynecological symptoms (control group).

Results: We found that vulvodinic patients had statistically lower *Lactobacilli* and higher total *Fungi* concentration.

Conclusions: Our preliminary study is useful to further clarify the etiopathology of vulvodinia and suggest new therapeutic strategies for approaching the VVS.

Keywords: VVS; Vulvodinic; Lactobacilli; Fungi; Bacteria; Women

Introduction

Vulvovestibular syndrome (VVS) or vulvodinia or vestibulodynia, is a worldwide spread chronic syndrome of unknown etiology that affects approximately 20-30% of the women, with moderate/severe life quality impairment due to chronic submucosa vulvar inflammation in the vulvar vestibule area,

which is often ulcerated with burning pain, edema and erythema [1].

In the 2015, the International Society for the Study of Vulvovaginal Disease (ISSVD), the International Society for the Study of Women's Sexual Health (ISSWSH), and the International Pelvic Pain Society (IPPS) defined, during an international meeting, the vulvodinia as "vulvar discomfort, most often described as burning pain of at least 3 months duration, occurring in the absence of relevant visible findings or a specific, clinically identifiable, neurologic disorder" [2].

The patients complain vaginal discharge or vaginal dryness and pelvic floor muscle hypertonicity, with positivity to cotton swab test and/or Friedrich's three diagnostic criteria (severe pain on vestibular touch, tenderness to pressure localized within the vulvar vestibule, and vestibular erythema of various degrees) [3, 4]. The VVS is thus included in neuropathic pain group, where the nerve endings network of the submucosal vestibular space named "peripheral awareness", causes hyperalgesia (an exaggerated response to a painful stimulus) and allodynia (pain due to application of a normally non-painful stimulus) [5].

However, the risk factors associated with this clinical condition can be endogenous (e.g. pregnancy and lactation, yeast or other genital tract infection, variations in hormone levels) or exogenous (e.g. sexual intercourse with a specific partner, oral contraceptives, vaginal surgery or laser treatment, smoking, stress) triggers that could induce a chronic localized stimulation and/or proliferation of the peripheral nerve fibers in the vestibule, resulting in an increased local sensitivity [6, 7].

The healthy vaginal microbiota with predominant *Lactobacillus spp.* (10^7 to 10^8 colony forming units (CFU)/g of vaginal fluid in premenopausal woman) is putatively stated as milestone against bacterial vaginosis, yeast infections, sexually transmitted infections, urinary tract infections and HIV infection [8, 9]. In fact, the lactic acid produced by *Lactobacilli* and present in two different isomers (L- and D-lactic acid) has antimicrobial, antiviral and immunomodulatory properties: lowering variably the environmental pH; dampening pro-inflammatory responses elicited by Toll-like receptor (TLR) agonists from cervico-vaginal epithelial cells, producing antimicrobial compounds (e.g. H_2O_2 , bacteriocins) that inhibit the growth of pathogenic bacteria, including *Salmonella*, *Chlamydia*, and *Trichomonas* [10-19]. The L-lactic acid, which is produced by both bacteria and vaginal epithelial cells, can induce release of pro-inflammatory cytokines by vaginal epithelial cells. Also, the D-lactic acid (produced mainly by bacteria)

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Table 1. Patient's Characteristics

	Patients	Controls
No. of patients	50	50
Mean age (years \pm SD)	33.6 \pm 2.75	30.5 \pm 3.12
Mean weight (kg \pm SD)	57.5 \pm 5.11	55.8 \pm 3.19
Mean height (cm \pm SD)	162 \pm 5.97	157 \pm 3.44
Smokers	18	21
Currently in a stable relationship	32	27
Mean pain severity (\pm SD)	7.1 \pm 1.23	0
Electromyographic biofeedback (μ V \pm SD)	127.8 \pm 12.5	12.7 \pm 8.1

is supposed to have some role not yet adequately investigated in this syndrome [20]. Several studies on the microbiome in VVS patients showed a prevalence of *Streptococcus*, *Gardnerella*, *Enterococcus*, and *Candida albicans spp.* [7-22].

The aim of this observational, retrospective, case-control study was to assess the vaginal flora in patients affected by chronic VVS, compared with the pattern of the healthy, asymptomatic women (control group), working on the hypothesis that some vaginal infection bacterial or mycotic could be a risk factor for the onset of VVS.

Materials and Methods

A total of 50 women (aged 19 and 45 years) affected by symptomatic vulvodynia, including burning pain, edema and erythema of the vulvar vestibule, resulting vulvovaginal dryness and hypertonic pelvic floor since at least 6 months, spontaneously appealed to our "Second Opinion Medical Consulting Network" (Modena, Italy) (Table 1). The Second Opinion Medical Network is a consultation referral web and Medical Office System recruiting suddenly a wide panel of real-time available specialists, to whom any patient affected by any disease or syndrome and not adequately satisfied by the diagnosis or therapy can apply for an individual clinical audit [23].

All the enrolled patients had dyspareunia treated with different topical and/or systemic therapies with little or no benefit (wash out period of previous therapies for up to 20 days).

The VVS diagnosis was confirmed through the swab test, performed with a cotton swab touched to the vaginal vestibule, and the degree of erythema is determined by visual inspection of the vulvovaginal glandular outlets by the examiner, the higher the score, the more severe the symptoms.

The quantification of the intensity of the pain is deter-

mined by the numeric rating scale (NRS) which scored the intensity of the symptoms: each patient, during the visit, is asked to indicate, with a number, the pain severity: from 0 (no pain) until 10 (most intense pain imaginable).

The hypertonia of anus elevator muscle is evaluated either manually (subjective perception) or by electromyographic biofeedback (BFB), through a vaginal probe into the anus, transducing electromagnetic signals to a specific software (following Spano's method) [24]. In this observational, retrospective, case-control study, we adopted some well-defined exclusion criteria: 1) pregnant or lactating women, 2) women with ongoing antifungal, antibiotic or antiviral therapy, 3) women using oral contraceptives, and 4) women using vaginal diazepam suppositories for high-tone pelvic floor dysfunction.

We included 50 healthy women of childbearing age, with no previous medical history of vulvodynia and no complaints, problems or current or past infection in the genital tract (Table 2).

The intestinal flora was screened about *Bacteria* and *Candida* positive in stool samples on plates, accordingly with American Society of Clinical Microbiology [25]. The fecal samples of VVS and health patients were collected in transport medium with specific sticker. Subsequently, the bacterial flora was evaluated after 48 h of incubation under appropriate conditions using a selective agar plate. In particular, we used LBS Selective Agar (Becton Dickinson, Franklin Lakes, New Jersey) for *Lactobacilli*; -CHROMagar Orientation (Becton Dickinson, Franklin Lakes, New Jersey) for *Escherichia coli*, *Klebsiella spp.*, *Enterobacter spp.*, and *Enterococcus spp.*; -Orientation CHROMagar Candida (Becton Dickinson, Franklin Lakes, New Jersey) for *Candida spp.*; and -Sabraud Glucose Agar (Becton Dickinson, Franklin Lakes, New Jersey) for the *Fungi*.

A further metabolic test on organisms isolated by the iden-

Table 2. Inclusion and Exclusion Criteria of Enrolled Patients

Inclusion criteria	Exclusion criteria
Dyspareunia	Pregnant or lactating women
Positive SWAB test	Ongoing antifungal, antibiotic or antiviral therapy
The hypertonia of anus elevator muscle	Oral contraceptives
Previous topical or systemic therapies	Vaginal diazepam suppositories

Table 3. Bacterial Species in the Health Patients (Controls) and in VVS Patients

Bacterial species	Controls, mean ± SD (CFU/mL)	VVS patients, mean ± SD (CFU/mL)	Statistical relevance
<i>Lactobacillus spp.</i>	236.00 ± 54.13	158.69 ± 28.32	P < 0.05
<i>Klebsiella spp.</i>	35.40 ± 5.36	174.03 ± 27.21	P < 0.05
<i>Candida spp.</i>	2.00 ± 1.47	37.53 ± 6.71	P < 0.05
<i>Gardnerella spp.</i>	1.30 ± 1.20	1.11 ± 25.12	P < 0.05
<i>Streptococcus spp.</i>	1.35 ± 1.05	1.51 ± 29.31	P < 0.05
Mycoplasma	1.51 ± 1.11	1.42 ± 26.11	P < 0.05
<i>Fungi spp.</i>	25.60 ± 6.02	230.13 ± 92.14	P < 0.05

tification system of Cristal BBL (Becton Dickinson, Franklin Lakes, New Jersey) was added to the screening. The test coefficient of variation was < 9%. The results are being expressed in colony forming units per milliliter (CFU/mL) of stool. The test was performed by Functional Point Laboratory (Bergamo, Italy), a clinical and virology laboratory that adheres to international quality control standards and is accredited as an official laboratory within the National Health System.

Statistical analysis

The statistical analysis was performed using the Student’s t-test. Statistical significance was set at a P value < 0.05, and all data and graphics were analyzed using the R software, version 3.1.2 [26].

Results

The results of bacterial isolations were expressed in colony

forming units (CFU)/mL (Table 3). The selected *Fungi* belonged, in all the samples, to the genera of *Aspergillus* and *Penicillium spp.* The *Lactobacilli* were significantly (P < 0.05) reduced in VVS patients, compared to the health women (controls). Conversely the microbiological charge of *Klebsiella*, *Candida* and *Fungi spp.* were significantly (P < 0.05) higher in the VVS population compared to the control group. We extended the study to *Gardenerella*, *Streptococcus spp.* and *Mycoplasma* that were dominant in the samples of symptomatic women (Fig. 1).

Discussion

Several studies evidenced the doctor-patient communication gap, and the increasing number of patients that usually wander around the medical websites looking for proper answers to their health problems [27, 28]. However, their search often becomes compulsive and obsessive and often ambiguous and frustrating [29]. Palmieri et al [30] describe this borderline or even pathological behavior as the “Web Babel Syndrome” - a

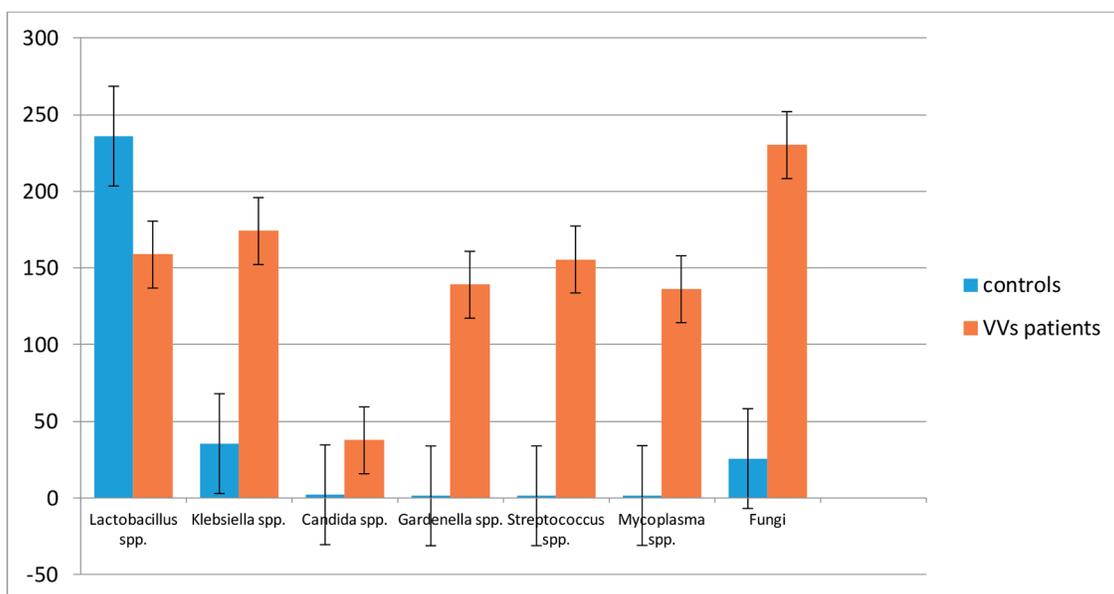


Figure 1. Comparison of bacterial population in the studied clinical cases. The value is expressed in CFU/mL ± SD.

psychological imbalance affecting young and elderly patients, especially those with multiple synchronous diseases who receive from their caregivers heterogeneous and misleading informations or advices, including confused, contradictory statements and prescriptions [31]. To deal with this problem, the Second Opinion Network aims to be a useful “problem-solving” support revisiting each diagnostic and therapeutic step and properly re-addressing tailored treatments and prognoses, as well as preventing unnecessary investigational procedures and unhelpful and expensive medical and surgical interventions, such as the diagnosis, risk factors, pathological mechanism and therapy of VVS [32].

In the clinical expression of VVS, many pathological mechanisms are involved [22]. The inflammatory aspect has a key role in generating the main symptoms of this syndrome [33]. The correlation between the immune system and bacteria is significant: the poor growth of *Lactobacilli* can suggest a pro-inflammatory immune background; the overgrowth of *Klebsiella spp.* is a probable responsible of pathological condition associated with VVS. The elevated detection of *Streptococcus spp.* in the women with VVS may also be involved in these mechanisms. This microorganism, which also produces lactic acid, is usually found at low levels in apparently healthy women, while high vaginal concentrations have been associated with a clinical condition known as aerobic vaginitis [34]. In addition, a case report evidenced an association of group B *Streptococcus* with vulvar pain, common VVS symptom [35]. Ventolini et al, in a preliminary clinical trial, hypothesized a vaginal flora alteration and an immunological response involving *Candida albicans* in VVS patients [36]. Indeed, they reported an increase of IL-17 in patients with vaginal candidiasis, reaching their pick 14 days after the infection, and a decrease of interleukin-12 (IL-12) and seven-fold of the macrophage inflammatory protein 1 beta (Mip-1b) in post-infection due to *Candida a.* These data suggest that the *Candida* superinfection might be the first cause of vulvodynia symptom or as immunological response in VVS. Accordingly, with previous animal models investigation where the seeding of fungi in the vaginal flora induced a local and systemic inflammatory state [36, 37]. The fungal flora in our case-control study is represented by *Candida*, *Aspergillus* and *Penicillium spp.* and since is significantly altered, it is reasonably supposed to trigger or boost the organ dysfunction.

The use of probiotics to populate the vagina and prevent or treat this alteration has been, in the past, anecdotally reported, but only recently we have an evidence-based clinical benefit of their administrations with supplementation of antimicrobial treatment to improve cure rates and prevent recurrences. It has been known that *Lactobacilli* produce bacteriocins that can inhibit the growth of pathogens, including some associated with bacterial vaginosis, such as *Gardnerella vaginalis* [38]. Indeed, Mastromarino et al [39] found, *in vitro*, that *Lactobacillus gasseri* 335 and *Lactobacillus salivarius* FV2 were able to coaggregate with *Gardnerella v.* When these strains of *Lactobacilli* were combined with *Lactobacillus brevis* CD2 in a vaginal tablet, adhesion of *Gardnerella v.* was reduced by 57.7%, and 60.8% of adherent cells were displaced. Boris et al found that the adherent properties *Gardnerella v.* were similarly affected by *Lactobacillus acidophilus* [40]. Cauci et al

observed also that vaginal concentrations of IL-1 β were higher in women whose vaginal microbiota was dominated by *Gardnerella spp.*, supposing a pro-inflammatory immunity, induced by the association between vaginal IL-1 β levels and bacterial vaginosis, often characterized by increased concentration in *Gardnerella* and reduced concentration of *Lactobacilli* [41].

Another effective method for reducing the bacterial burden and the microbiota imbalance is the vaginal irrigation with carbonated saline by Palmieri et al [42], who investigated the therapeutic effect of 7% CO₂ enriched water washout (250 mL saline plus CO₂ volume protocol) in 40 women complaining, in the last 3 years, vaginosis and vaginitis, daily for 2 weeks of treatment. The outcomes reported definitive symptoms improvement (reduction of burning pain, itching) in 15 patients after 6 days, 10 in 4 days and two in 2 days.

Conclusions

Our preliminary data suggest a role of the fungal pathogenic flora and reduced *Lactobacilli* population in vulvodynic patients and stimulate further options of local and systemic medical treatments.

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