Vestibular Mucosa Thickness Measured by Ultrasound in Patients Affected by Vestibulodynia: A Case-Control Study

Filippo Murina, MD, Sara Barbieri, MD, Chiara Lubrano, MD, and Irene Cetin, MD

ABSTRACT

Introduction: A multifactorial etiology has been implicated in the development and maintenance of vestibulodynia (VBD), and atrophic changes of the vestibular mucosa have been observed in many patients.

Aim: To assess the vestibular mucosa thickness in patients with VBD by comparing this sample with a control group of healthy fertile women and postmenopausal patients with symptoms of genitourinary syndrome of menopause (GSM).

Methods: Vestibular mucosa thickness was measured with a 20 MHz ultrasound probe (DermaScan C, Cortex Technology, Denmark), including both the epidermis and dermis.

Main outcome measures: All women were evaluated by anamnesis, physical examination, and self-report symptoms. Thickness of the vestibular mucosa (expressed in micrometers) was determined by the B-mode, excluding the hyperechogenic entrance echo and hypoechogenic subcutis. Clinical data related to VBD and GSM were recorded using a 0- to 10-point visual analog scale related to dyspareunia and vulvar pain/burning (0 = no pain; 10 = worst possible pain).

Results: A total of 85 patients were recruited: 24 with VBD, 20 with GSM-related symptoms, and 20 matched controls. Vestibular mucosa thickness measurements were not significantly different between the VBD (mean ± DS: 1,092.5 ± 226.1 μm) and GSM groups (1,059.7 ± 221.5 μm), while the parameter was significantly lower ($P < .01$) than the control group (1,310.6 ± 250.0 μm). Correlation analysis in the VBD and GSM groups between low vestibular mucosa thickness and symptom intensity (burning/pain and dyspareunia) showed a significant correlation.

Conclusion: Patients with VBD have a vestibular mucosa with a lower thickness than healthy women of the same age, with an almost identical value to that found in postmenopausal women. Furthermore, a low vestibular mucosa thickness in the VBD and GSM groups showed a significant correlation with burning/pain intensity and dyspareunia severity. **F Murina, S Barbieri, C Lubrano, MD, et al. Vestibular Mucosa Thickness Measured by Ultrasound in Patients Affected by Vestibulodynia: A Case-Control Study. Sex Med 2021;XX:XXX–XXX**

Key Words: Vestibulodynia; Vulvar Mucosa; Dyspareunia; Vulvodynia; High-Frequency Ultrasound

INTRODUCTION

Vulvodynia is classified as vulvar pain lasting at least 3 months, without a clear identifiable cause, which may include potential associated factors.[1] 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.[2,3] Localized provoked vulvodynia of the vestibule, known as vestibulodynia (VBD), is the most common manifestation of the disease, accounting for approximately 80% of cases.[4]

Individuals with VBD report localized hypersensitivity and pain of the vulvar vestibule to the touch (eg, during sexual intercourse or tampon use). This pattern of responses is suggestive of sensory abnormalities in the form of evoked pain (eg, hyperalgesia or allodynia).

This is consistent with research biopsy studies that have demonstrated increased innervation of the vulvar vestibule and

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increased subepithelial heparinase activity and cytokines that have been associated with neuroinflammatory processes.[5] Furthermore, the discomfort inherent in VBD is always associated with pelvic floor muscle overactivity, with the development of myofascial trigger points, resulting in localized or radiating pain and/or severe tenderness.[6]

A multifactorial etiology, including infections, hormone disorders, neuroinflammation, atopy, genetic polymorphisms that interfere with inflammation, and psychogenic factors, has been implicated in the development and maintenance of VBD.[4] It has become increasingly apparent that VBD is probably not a single but rather a variety of disorders, in which the common end point is vestibular hypersensitivity and hypertonic pelvic floor dysfunction.

Although there are likely multiple causes of vestibular pain, the possibility of a relationship between VBD and hormonal factors has been raised. Several studies have shown that combined hormonal contraceptives (CHCs) increase a woman's risk of developing VBD, but other studies have not demonstrated this effect.[7–9] It should be noted that these trials are uneven for the age at first use, duration of use, or type of CHCs used. The vulvar vestibule is the area of exquisite tenderness in patients with postmenopausal hypoestrogenism, dyspareunia, and vulvar pain related to the genitourinary syndrome of menopause (GSM)[10]; its non-keratinized squamous epithelium exhibits atrophic features such as pallor, petechiae, erosions, and thinning. One non-randomized not controlled trial showed that postmenopausal women with vulvar or vestibular complaints report statistically significant reduction of symptoms by applying the topical estrogen directly to the vestibule.[11] Our experience has shown that many women with VBD show vestibular disorders such as atrophy, thinning, and pallor, which are quite similar to those observed in women with GSM-related symptoms. The objective of this study was to investigate whether women with VBD differ from healthy controls and postmenopausal women regarding the morphology of the vulvar vestibular mucosa. We hypothesize that a women with VBD exhibit a thinner vestibular mucosa related to patients' symptoms.

MATERIALS AND METHODS

Subjects

This case-control study compared women with VBD, patients with GSM-related symptoms, and control women without vulvar pain. All women aged 18 years and before menopause (cessation of menstruation for 12 months) with VBD who presented to our unit of lower genital tract disease were invited to participate. Cases with VBD met the criteria of the International Society for the Study of Vulvovaginal Disease,[1] including vulvar pain, burning, irritation localized to the vestibule during vaginal intercourse and activities exerting pressure on the vestibule (eg, inserting a tampon insertion, wearing tight jeans or pants) that had been present for at least 3 months, with no other demonstrable cause for their symptoms.

The postmenopausal group included women who had complaints compatible with the symptoms of GSM (vulvovaginal irritation, burning, and pain at sexual activity) and no history of topical or systemic hormone therapy or selective estrogen receptor modulators for menopausal symptoms in the past 3 months. The control group included healthy fertile asymptomatic women without any vulvovaginal conditions who attended our hospital for cervical cancer screening programs. Exclusion criteria were as follows: women who were using hormonal contraceptives in the past 6 months, vulvar dermatosis or other vulvar disorders, and active vulvovaginal infections at the time of their gynecological examination. The study was approved by the local ethics committee, and all the women provided informed written consent.

All enrolled participants completed a detailed questionnaire to assess demographics including age, marital status, ethnic origin, age for onset of menopause, use of contraception, prescription drug history, and vaginal infections. Clinical data related to VBD and GSM were recorded using a 0- to 10-point visual analog scale related to dyspareunia and vulvovaginal pain/burning (0 = no pain; 10 = worst possible pain). A vestibular cotton swab test was also performed. It consisted of a small cotton-tipped applicator which was lightly rolled over the surfaces of the vestibule (mean of values at the 1, 3, 5, 6, 7, 9, and 11 o'clock locations) by asking the subject to report pain intensity through a numerical rating scale, in which 0 represented “no pain” and 10 represented “worst pain imaginable.”

Ultrasound Measurements

Before any other vaginal examination was performed, vestibular mucosa thickness was measured by ultrasound (B-scan ultrasonography) with a 20-MHz validated system[12] (DermaScan C, Cortex Technology, Denmark) producing cross-sectional images of the skin, down to a depth of approximately 15 mm. The thickness of the vestibular mucosa (expressed in micrometers) was determined by the B-mode, excluding the hyperechogenic entrance echo and hypoechogenic subcutis. Lines were established to include both the epidermis and dermis, along the entrance echo on the surface of the epidermis and the underside of the dermis (along the interface with the subcutaneous tissue), using automated edge detection software from the DermaScan C device. After storage of the cross-sectional vestibular mucosa imaging, skin thickness was calculated using computer-assisted image analysis software. Vestibular measurement was performed on days 7-13 from the first day of the menstrual cycle (ie, during the follicular phase) in PVD and healthy participants, and in the absence of menstruation or unknown bleeding.

Statistical Methods

Quantitative variables (ie, demographics), if normally distributed, were described as the mean ± standard deviation. The

Student t-test was used to detect any differences in demographic and clinical characteristics. The Mann-Whitney U-test was used to compare vestibular mucosa thickness between the study groups (VBD, GSM, and healthy women). Pearson's chi-squared test was used for the purposes of describing the correlation between subjective report of pain (burning/pain and dyspareunia) and vestibular mucosa thickness, respectively. Statistical significance was set to (P, 0.05) for all comparisons. The Statistical Package for the Social Sciences program(version 21, SPSS Inc, Chicago, IL) were used to analyze the data.

RESULTS

A total of 85 patients were recruited into this study: 24 with VBD, 20 with GSM-related symptoms, and 20 matched controls. The mean ages of patients with VBD, GSM, and controls were 31.63 ± 6.34 years, 58.55 ± 7.97, and 36.10 ± 8.34 years, respectively. Oral contraceptive use (never used: VBD = 13 and controls = 13; CHCs: VBD = 11 and controls = 7) and length of menstrual cycles were not significantly different between the VBD and control groups. Furthermore, in the GSM group, time since menopause was <5 years in 6 women (30%) and >5 years in 14 women (70%). No significant differences (in any clinical characteristics) were found between the VBD and GSM groups (Tables 1 and 2). Vestibular mucosa thickness measurements were not significantly different between the VBD (1,092.5 ± 226.1 μm, mean ± DS) and GSM groups (1,059.7 ± 221.5 μm, mean ± DS) with a difference of only 32.8 μm (2.5%). Instead, the parameter was significantly lower ($P < .01$) than the control group (1,310.6 ± 250.0 μm, mean ± DS) with a difference of 218.1 μm (16.6%) and 250.9 μm (19.1%) for the VBD and GSM groups, respectively (Figure 1).

Correlation analysis in the VBD and GSM groups between low vestibular mucosa thickness and intensity of symptoms (burning/pain and dyspareunia) showed a significant correlation (Table 3).

In addition to analyzing the correlation of data in all cases and matched controls, we considered 2 subsets of vestibular mucosa thickness: low thickness, <1,100 μm; normal thickness >1,100 μm. The cutoff value of 1,100 μm was assumed because higher than mean vestibular mucosa thickness value of 1,092.5 μm that we found in the VBD group.

DISCUSSION

The first evidence of our study is that patients with VBD have a peculiar vestibular mucosa profile with a lower thickness compared with healthy women of the same age and with an almost identical value to that found in postmenopausal women (Figure 2).

To the best of our knowledge, this is the first study to report the measurement of vestibular mucosa thickness (epidermis and dermis) in patients with VBD through a non-invasive 20-MHz ultrasonographic evaluation that has been shown to be correlated with histological skin thickness measurements.[13]

Table 1. Distribution of symptoms and signs in patients with VBD and GSM

Parameter	VBD group (n. 24)	GSM group (n. 20)	P value
Burning/pain	5.42 ± 2.52	6.90 ± 1.33	NS
Dyspareunia	7.42 ± 2.30	7.95 ± 1.57	NS
Swab test	7.13 ± 1.94	5.40 ± 1.85	NS

VBD = vestibulodynia; GSM = genitourinary syndrome of menopause.
Data recorded through a 0- to 10-point visual analog scale (VAS) and presented as the mean values ± standard deviation.

Johannesson et al[14] have shown that vestibular biopsy specimens remain unaffected in patients with VBD, compared with the controls.

Our data do not support the results of Johannesson et al,[14] in which the vestibular biopsy specimens showed that the epithelial morphology remained unaffected in patients with VBD, compared with the controls. Contradictory results obtained may be mainly explained by the target of measurement. In our study, we also evaluated the dermis of the vestibular mucosa, whereas Johannesson et al have focused the measurements on epithelial tissues structures such as interdermal papilla distance, distance from the dermal papilla top to the epithelial surface, and the distance from the basal layer to the surface epithelium.

Menopause is associated with a severe decline in circulating sex hormones, particularly with regard to the estradiol level, but also androgens have a relevant role.[10] It is expected that the hypoestrogenic and hypoandrogenic state contributes to progressive atrophy of tissues in this area of the body due to the high proportion of estrogen receptors in the lower genital tract; but why is the vestibular mucosa thin in patients with VBD?

The vulvar vestibule is a site extremely sensitive to estrogens and androgens; it mainly surrounds the vestibular glands. Thus, predominant postmenopausal symptoms such as vaginal dryness and dyspareunia may be primary and more pronounced at this site, rather than the proximal or upper vagina.[15] We hypothesized that the vestibule may be involved in the decreased absorption of sex hormones (estrogens and androgens) in patients with VBD, both for a quantitative and/or qualitative receptor abnormality and lower hormone levels.

A recent study showed that the expression of estrogen receptor beta (ERβ) was significantly more pronounced in samples from patients with VBD than in healthy controls,[16] whereas Eva et al[17] reported a patchy and reduced presence of ERα. In addition, Goldstein et al[18] have identified polymorphisms of the androgen receptor gene genetic polymorphism in the vestibular androgen receptor in patients with VBD.

These experimental data support the hypothesis of dysregulation of estrogen and androgen signaling in patients with VBD.

Table 2. Characteristics of patients with VBD, GSM, and control women

Variable	VBD group (n.24)	GSM group (n.20)	Control group (n.20)
Menstrual pattern (number)			
Normal (28 ± 3)	19		16
Polymenorrhea (<25)	2		3
Oligomenorrhea (>31)	3		1
Time since menopause			
<5 years		6 (30%)	
>5 years		14 (70%)	
History of vaginal infections†	11/24 (46%)	3/20	2/20
Etnicity†			
Caucasian	21 (87%)	19 (87%)	17 (87%)
Hispanic or Latino	3 (3%)	1 (3%)	3 (3%)
Body mass index (kg/m^2)	21.9 ± 3.4	21.9 ± 3.4	21.9 ± 3.4
Nulliparous†	22 (92%)	11 (20)	17
Past oral contraceptive use†	13 (54%)	9 (45%)	13 (65%)
Duration of symptoms (months)*	28.5 (±31.1)	34.5 (±29.1)	
Comorbidities†			
Irritable bowel syndrome	10 (41%)		
Bladder painful syndrome	4 (17%)		
Previous treatments (number)			
Anti-fungal (fluconazole, itraconazole)	12	–	
Topical estriol	6	9	
Anti-depressant (amitriptyline, duloxetine)	15	–	
Physical therapy			
Manual pelvic floor physical therapy	17	–	
Electromyographic biofeedback	8	–	
Electrical nerve stimulation (TENS)	5	–	
No previous treatments	4	–	

VBD = vestibulodynia; GSM = genitourinary syndrome of menopause.
*Data are presented as the mean values ± standard deviation.
†Data are presented as number (%).

However, receptor expression is a dynamic process with upregulation and downregulation related to exogenous and endogenous serum hormone levels.

It has been demonstrated that the vestibular mucosa in healthy women using CHCs displays an altered morphological pattern with shallow and sparse dermal papillae, compared with the

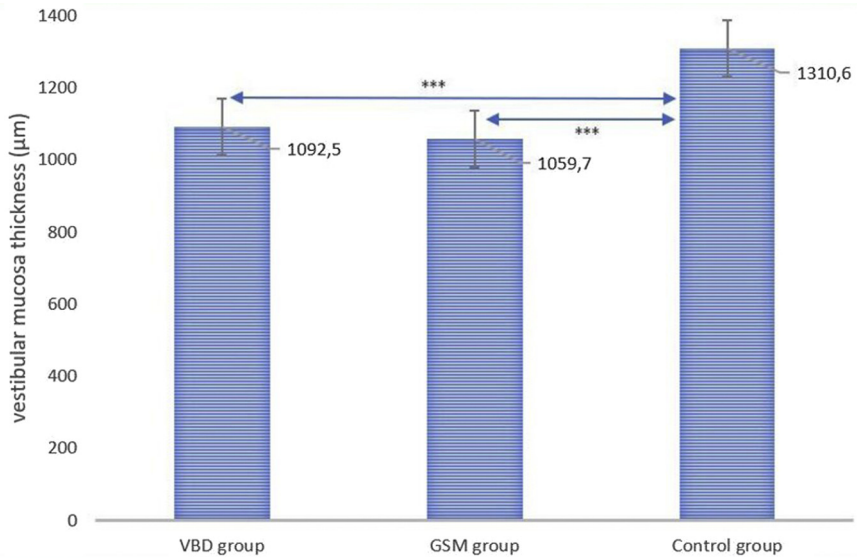

Figure 1. Vestibular mucosa thickness (μm) in study groups, mean, and SD. *** = $P < .001$.

Table 3. Correlation between vestibular mucosa thickness and symptoms

Parameter	VBD group (n. 24)	GSM group (n. 20)	*P* value
Burning/pain	Low Normal	Low Normal	$P < .01$
VAS ≤3	11.8% 28.6%	12.5% 25.0%	
VAS 4-10	88.2% 71.4%	87.5% 75.0%	
Dyspareunia			$P < .01$
VAS ≤3	5,9% 14.3%	33,3% 35.5%	
VAS 4-10	94.1% 85,7%	66.7% 64,5%	

VBD = vestibulodynia; GSM = genitourinary syndrome of menopause. Low = Low vestibular mucosa thickness <1,100 μm and Normal = Normal vestibular mucosa thickness >1,100 μm. *P* values are from chi-square test.
Data recorded through a 0- to 10-point visual analog scale (VAS) and presented as percentage.

follicular phase.[19] Similar findings were observed in women not using CHCs during the luteal phase, indicating a gestagenic effect on the mucosa. These findings are thought to be reflective of an anti-androgenic effect, and they support the data associated with an elevated risk of developing vestibular pain from using pills with low estrogenic and high anti-androgenic potencies.

In this study, we found that the lower vestibular mucosa thickness of women with VBD was independent of the use of CHCs; in fact approximately 55% and 45% of patients had never used or quit for 6 months, respectively. It remains unclear whether CHCs play a causative role in the development of VBD, but we postulate that CHCs exposure may contribute to the disease by eliciting a preexisting vestibular defective sex hormone absorption in patients with VBD.

In our study, we also observed that low vestibular mucosa thickness in the VBD and GSM groups showed a significant correlation with burning/pain intensity and dyspareunia severity.

Herein, we showed that the thinner the vulvar vestibular mucosa, the more painful are the symptoms reported by the patient. What is the meaning of a vulvar vestibule thinner than a normal vestibule? A rich nerve plexus was identified within the vaginal submucosa, which was only composed of sympathetic and parasympathetic axons, with contributions of smaller sensory fibers. The sensory nerve endings of the vulvar vestibule are dense and shallow, making this region more physiologically sensitive.[20]

We can speculate that a thinner and delicate vestibular mucosa is more sensitive to nociception because nerve endings become more superficial, thus altering the transduction of mechanical pressure to facilitate nociception.

Furthermore, these morphological alterations may influence the barrier properties of the vestibular mucosa, making it more susceptible to infections, as well as mechanical trauma.

Study limitations include our sample size is small and, although it is appropriate for a pilot study, it is not a particularly

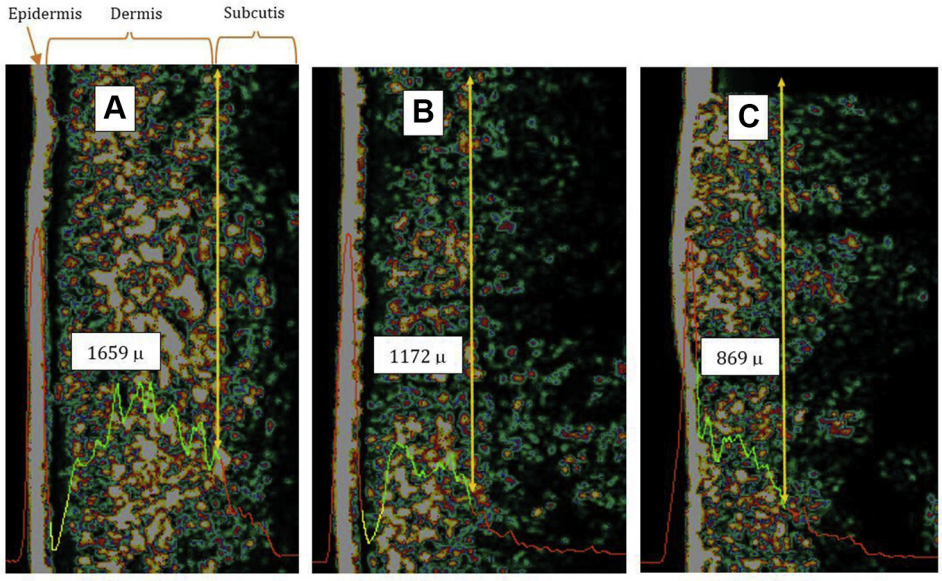

Figure 2. B-mode cross-sectional image of vestibular mucosa with A-mode scan inserted, depicted at the lower 1/3 of the image (the registered A-scan is displayed at the bottom of the scan). The scan line is automatically positioned to the visually assessed anatomically correct interfaces (red + yellow lines = epidermis; green line = dermis; red line = subcutis), with competing echoes and artifacts excluded. Sample of 3 women included in the study: A, control group; B, VBD group; and C, GSM group.

representative sample of all patients with VBD, although we made no distinction between patients recruited. In addition, the thickness categories were established post hoc and ultrasound employing the 20-MHz probe was performed on the vestibular mucosa for the first time, even if the procedure proved to be a reproducible method that has been applied to thickened or thinning skin versus normal skin.[21,22] And finally, the trial has a lack of evaluation of sexual hormones blood levels (estradiol and androgen) and sexual features (ie, arousal and excitation).

CONCLUSIONS

This study supports the need for careful assessment of vestibular trophism in all patients with VBD, as well as the potential utility of treatment options (ie, topical estrogens, testosterone, or fractional laser) that are more often targeted toward specific pathways associated with vestibular atrophy.

In our experience, VBD represents the summation and overlap of various trigger factors (infections, allergies, genetic aspects, psychological vulnerability, etc.) with weight and predominance varying from patient to patient, in which the modifications of vestibular trophism related to hormonal imbalances may play a relevant role, at least in a subgroup of women with VBD.

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STATEMENT OF AUTHORSHIP

Filippo Murina: Conceptualization, Methodology, Investigation, Resources, Writing - Review & Editing, Funding Acquisition. Sara Barbieri: Writing - Review & Editing, Investigation, Formal Analysis. Chiara Lubrano: Investigation, Formal Analysis. Irene Cetin: Conceptualization, Writing - Review & Editing.

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