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ORIGINAL RESEARCH

Does One Measure Fit All? The Role of Experimentally Induced Pain Tests in the Assessment of Women with Provoked Vestibular Pain

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Purpose: A diagnostic algorithm was recently suggested to address the underlying mechanisms of provoked-vestibulodynia (PVD). It delineates four subgroups (Hormonal-associated, Augmented-anterior, Hymenal-associated and Hypertonicity-associated), each manifesting a distinctive vulvar pain-hypersensitivity regarding location (circumferential vs posterior-only vestibulodynia) and pain characteristics. We aimed to explore the significance of various experimentally induced vulvar pain measures in the manifestation of pain hypersensitivity in each subgroup.

Methods: Women with PVD (n = 113) and 43 controls reported pain intensity provoked during vaginal penetration and tampon insertion. Vestibular tenderness (anterior and posterior) was assessed by Q-tip test, and pressure stimulation delivered to the puborectalis assessed muscle tenderness. Pain thresholds were measured using a vulvar-algesiometer. These measures were compared between patients and controls and among the PVD subgroups. Correlations between the clinical and experimentally induced-pain measures were assessed. Finally, to address whether the association between experimentally induced-pain measures and dyspareunia severity is mediated by hypertonicity, the conditional indirect effect was analyzed in each subgroup.

Results: Compared to controls, augmented vulvar pain-hypersensitivity and hypertonicity were observed among patients ($p < 0.001$). ANOVA revealed no subgroup differences in dyspareunia severity. Nevertheless, some experimentally induced-pain measures were differently correlated with dyspareunia intensity in each subgroup, allowing discrimination of subgroups according to the unique findings of vulvar pain-hypersensitivity. The degree of pelvic floor muscle-hypertonicity mediated the association between vulvar pain-hypersensitivity and dyspareunia severity, emphasizing the key role of hypertonicity in distinguishing between subgroups.

Conclusion: The findings offer more evidence of variations among PVD subtypes, demonstrating that insertional dyspareunia may originate from dissimilar alterations in the mucosal and muscular tissues. The results also emphasize the significance of utilizing a wide battery of tests to capture different experimentally induced-pain measures, revealing the unique patterns of vulvar pain-hypersensitivity in each subgroup.

Keywords: vulvodynia, provoked vestibulodynia, insertional dyspareunia, experimentally induced pain measures, subgroups

Introduction

Vulvodynia is defined as vulvar pain of at least three months without a clear identifiable cause,[1] consistent with the definition of primary pain syndrome according to the IASP (International Association for the Study of Pain).[2,3] The 2015 ISSVD, ISSWSH, and IPPS (International Society for the Study of Vulvovaginal Disease, the International Society for the Study of Women's Sexual Health, and the International Pelvic Pain Society, respectively) consensus terminology and classification categorized vulvodynia based on pain location, onset, and temporal pattern.[1] Provoked vestibulodynia (PVD), a sub-category of vulvodynia, is the leading cause of insertional dyspareunia.[4,5] Nevertheless, the current

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conceptualization of PVD as primary pain disorder,[2] with its diagnosis made based on symptoms and pain evoked by touching the vestibule, might hinder the understanding of its multifaceted nature and the presentation of pain symptoms secondary to an underlying cause (ie, secondary pain syndrome [3]).

In the last decade, an alternative approach for defining etiologies of insertional dyspareunia manifested as PVD has been proposed.[6–8] This approach aims to characterize different subgroups based on patient's medical history as well as physical examination findings, including vestibular location of pain (entire vestibule vs posterior-only hypersensitivity).[6–11] However, this algorithm still lacks confirmatory evidence, allowing its assimilation into the clinical setting. Recently, Lev-Sagie et al [10] provided evidence to support this approach, which may hold the potential to incorporate this algorithm into clinical practice to improve patients' outcomes. These findings allow characterizing PVD patients based on the particular location of the vestibular hypersensitivity and other clinically assessed factors, suggesting four subgroups representing distinct phenotypes of insertional dyspareunia that may also reflect local or central alterations in sensory signal-processing.[9,10,12] These subgroups include Hormonal-associated vestibular tenderness-women with circumferential (ie, anterior and posterior) vestibular tenderness and vestibular atrophy induced by concurrent or recent use of hormonal contraception; Augmented anterior/upper (AA) vestibular tenderness-circumferential vestibular tenderness without vestibular atrophy, possibly due to vestibular neuroproliferation and/or pro-nociception; Hymenal-vestibular tenderness-posterior-only vestibular tenderness and existence of a hymenal constriction-ring; and Hypertonic vestibular tenderness-posterior-only vestibular tenderness, associated with enhanced pain evoked by pelvic floor muscles (PFM) palpation as well as muscle hypertonicity.

Various experimentally induced-pain tests are available to assess PVD patients to confirm diagnosis and evaluate treatment efficacy.[13] These tests aim to mimic the experience of insertional dyspareunia and can be delivered to different areas of the vestibule. They obtain mucosal and/or muscular hypersensitivity [7,14] via several stimulus modalities that evoke pain as well as the presence of hypertonicity.[15] However, their actual capacity to simulate the clinical pain experience has not been fully addressed. In addition, it is unknown to what extent these tests discriminate between women with PVD and pain-free women and, furthermore, distinguish between the various PVD subgroups.

Given the proposed allocation of PVD into subgroups,[7–11] it is possible that different tests are manifested in various ways because each measure represents a distinct facet of pain experience. Yet, little is known about whether the various vulvar-pain measures are associated with each other as well as with the pain experienced during intercourse. In line, it is unknown which of the tests is required to confirm the specific characteristics of vestibular hypersensitivity as well as to assist in the classification of the patient into the appropriate subgroup.

Taken together, this study aimed to explore whether experimentally induced-pain tests indeed correspond to the various characteristics of vestibular hypersensitivity, as well as to examine their significance in revealing how the subgroups manifest vulvar pain hypersensitivity. Specific goals were to 1. compare a broad set of tests that evaluate vulvar-pain hypersensitivity in women with and without insertional dyspareunia; 2. assess whether various pain measures differ among the four suggested PVD subgroups; 3. explore the role of PFM hypertonicity (PFHT) in enhancing vulvar pain, and 4. investigate whether the severity of dyspareunia is associated with the various test results.

Materials and Methods

Study Population

A prospective longitudinal cohort study was conducted from November 2016 to January 2019 in a public vulvar clinic. The cohort included women diagnosed with PVD and women without dyspareunia (control group); all were evaluated by the same gynecologist specializing in vulvovaginal disorders (the first author). The current cross-sectional research involved a secondary analysis of data obtained during the initial assessment.

Women presenting with dyspareunia were evaluated at the clinic and enrolled if they met the diagnostic criteria for PVD, consented to participate in the study, and agreed to attend follow-up visits. Controls responded to an advertisement in social media, explaining the study and specifying absence of dyspareunia and other vulvovaginal complaints as required inclusion criteria. Following an interview, controls were recruited based on denying dyspareunia, vulvar pain, and other chronic pain disorders. The study was approved by the local Institutional Review Board and was conducted

according to the Declaration of Helsinki. All participants provided written informed consent after receiving a detailed explanation of the study.

Inclusion criteria for PVD patients were age 18–45, history of ≥3 months of vulvar pain, suggestive of PVD (pain during attempted vaginal penetration and/or pain with tampon insertion), self-reported sexual provoked pain during vaginal penetration or attempted penetration, with an intensity >3 on the 0–10 Numerical Pain Scale (NPS) or complete avoidance of penetrative intercourse due to pain. In addition, localized vestibular tenderness on examination, with pain scores >1 in response to Q-tip stimulation on the 0–10 NPS, was required. Exclusion criteria included patients experiencing pain from other identifiable causes such as vulvovaginitis, dermatitis, skin disorder, etc., major health conditions (cardiovascular, diabetes, etc.), or a diagnosis of an active and unstable psychiatric disorder. Additional exclusion criteria included pregnancy, lactation, spontaneous pain without any provoking physical contact, or mixed vulvodynia (ie, a combination of spontaneous vulvodynia and pain provoked by physical contact).

Patient Evaluation Protocol

During medical consultations, we collected comprehensive data, including personal, gynecological and medical histories, sexual functioning, and verification of inclusion and exclusion criteria. Self-report of pain intensity ratings during sexual intercourse or other vaginal penetration over the last 4 weeks was obtained using a 0–10 NPS. Patients underwent a gynecological exam (details below) and were provided a link to complete online questionnaires at their convenience, which included detailed medical background and sociodemographic data.

Patients were categorized into PVD subgroups as described elsewhere [10] (also detailed in the Results section, see below "allocation into four subgroups").

The study compared the following measures across all participants, both cases and controls:

1. Vestibular tenderness was assessed by the Q-tip test, which involved touching the vestibule with a moistened cotton-tip applicator (Q-tip) on four defined points (2, 4, 8, and 10 o'clock). The Q-tip test was performed twice, once to localize vestibular tenderness at each point (yes/no) and again, to quantify pain intensity (using NPS) at each point, with 0 corresponding to no pain and 10 being the worst possible pain. Pain induced at the 10 and 2 o'clock vestibular locations was defined "anterior vestibule" hypersensitivity, while pain provoked at the 4 and 8 o'clock locations was categorized as "posterior vestibule" hypersensitivity. "Circumferential vestibular tenderness" was defined as the presence of both anterior and posterior vestibular pain-hypersensitivity.

2. Pressure-pain thresholds were obtained in four defined vestibular locations (2, 4, 8, and 10 o'clock) using an algesiometer (Digital force gauge, series 2, Mark-10 Corporation, Copiague, NY, USA). An algesiometer is a probe that applies localized pressure to vestibular mucosa with gradually increasing power. The patient was asked to report the point of initiation of pain while the examiner quantified the mechanical pain threshold, measured in KgF. Higher algesiometer scores indicate a lower vestibular pain sensitivity as expressed by higher pain threshold.

3. The tampon test [16] assessed penetrative pain. Patients were instructed to insert, with the assistance of an applicator, an original, regular Tampax® tampon fully into the vagina, above the level of the hymeneal ring, and to remove the applicator from the vagina. Then, they were asked to remove the tampon by applying traction to the tampon-string without any lubrication. Patients recorded the degree of pain during the entire insertion-removal experience using the NPS.

4. Muscular pain was assessed by applying pressure to the puborectalis muscles (each side separately, at 5 o'clock and 7 o'clock locations, for about 5 seconds) using the examiner's index finger. Patients then rated their pain intensity using the NPS.

5. Pelvic floor muscle-hypertonicity (PFHT) was rated by the physician's assessment of hypertonicity (none = 0, mild = 1, moderate = 2, and severe = 3) of the puborectalis during muscle palpation.

Statistical Analysis

Statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL, USA, version 26). Data were checked for normality, outliers, and missing values. Non-normal distribution variables were analyzed using non-parametric tests, the Wilcoxon-Signed Ranks and the Mann–Whitney tests for group comparisons. Correlation of non-normally distributed variables was carried out using Spearman rank tests or the Pearson tests when normally distributed, and Sidak test was used to control for

multiple analyses. ANOVA was performed to compare the variations in vulvar pain hypersensitivity across the PVD subgroups. Conditional indirect effect of experimental provoked-pain (X) on dyspareunia severity (Y) through the mediator pelvic floor muscle-hypertonicity (PFHT) at different values of the moderator PVD subgroups (W) was performed, using IBM SPSS statistical package V.27.0 and the PROCESS macro (V.4.2), model 58.[17] To test for the significance of the indirect effects, bootstrap CIs using the percentile method (based on 5000 resamples) were used with a 0.05 criterion for rejection (ie, 95% CI considered statistically significant when the percentile bootstrap 95% CIs do not include zero, see Figure 1). Statistical significance was set at $p < 0.05$.

Results

The study included 156 women, of whom 113 were diagnosed with PVD, while the remaining 43, reporting no vulvar pain or dyspareunia, served as the control group.

Allocation into Four Subgroups

To further explore the manifestations of various vulvar pain measures and their association with dyspareunia, we divided the women into the four described subgroups.[10] All patients reported experiencing provoked pain in response to Q-tip

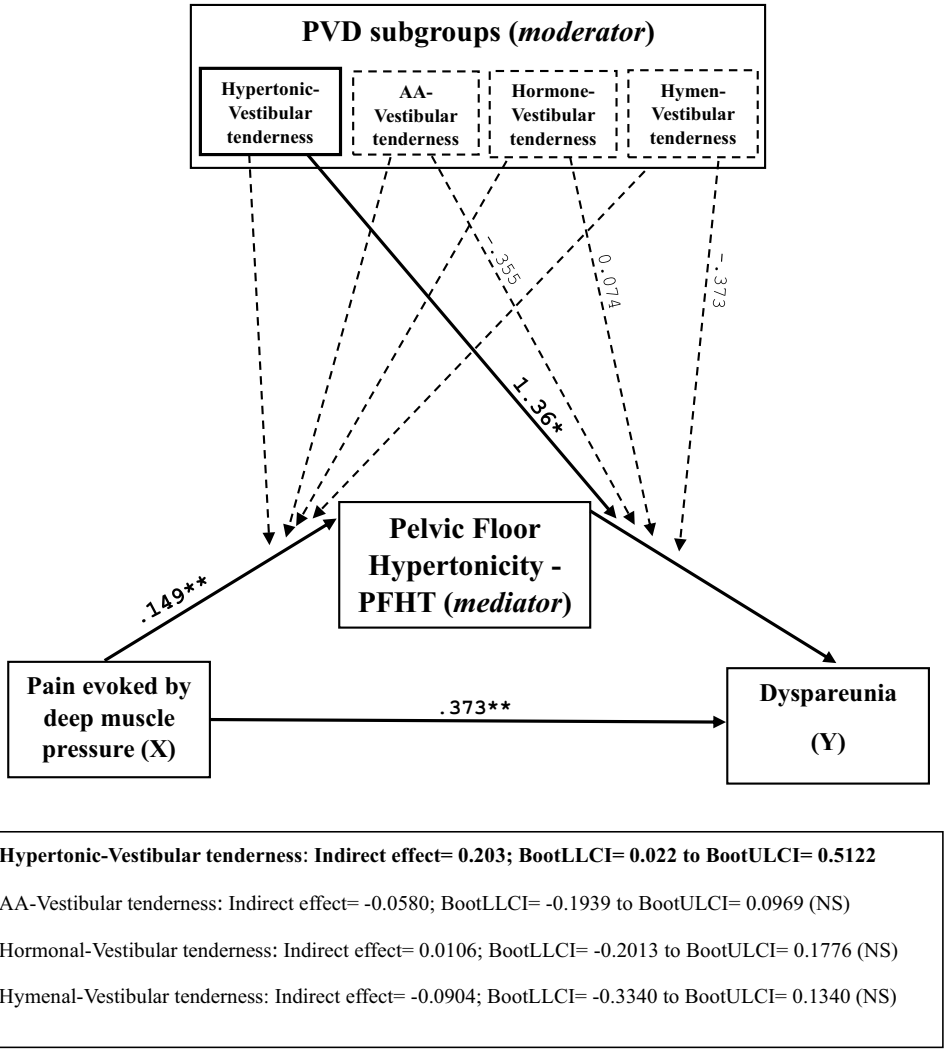

Figure 1 The indirect effects of experimental provoked pain on dyspareunia severity through pelvic floor hypertonicity (PFHT) at different values of the PVD subgroups. The model shows significant unstandardized regression coefficients. Dashed lines highlight non-significant relationships ($p < 0.05$); NS (nonsignificant indirect effect when the 95% CIs contain zero); *$p < 0.05$; **$p < 0.01$; PVD: provoked-vestibulodynia; PFHT: pelvic floor hypertonicity.

stimulation at the posterior vestibule (4 and 8 locations), while 41/113 patients (36%) also reported anterior vestibular hypersensitivity, expressed as provoked pain >1 in response to the Q-tip test at the 2 and 10 locations. Thus, these women were characterized as having circumferential vestibular tenderness. Patients were further subdivided according to vestibular pain localization and other criteria based on a previously proposed algorithm for PVD classification [6,9] (for more details, see [10]). Women with circumferential vestibular tenderness, associated with vestibular atrophy induced by hormonal contraception, were classified as Hormonal-associated vestibular tenderness (n = 21). The remaining 20 patients who had circumferential vestibular tenderness, lacking vestibular atrophy, were grouped as Augmented anterior (AA) vestibular tenderness, suggesting that vestibular hypersensitivity may be attributed to vestibular neuroproliferation and/or pro-nociception.[6]

The 72/113 patients who experienced pain in response to Q-tip provocation applied only to the posterior vestibule, were defined as having posterior-only vestibular tenderness and were further divided into two subgroups based on the existence of a hymenal constriction-ring, representing rigidity of the connective tissue. Accordingly, 37 women with hymenal-rigidity were termed Hymenal vestibular tenderness, and the remaining 35 patients were labeled Hypertonic vestibular tenderness, consistent with the original algorithm,[6] and given that they demonstrated enhanced pain ratings evoked by PFM palpation and muscle hypertonicity.[10]

Comparison of Vulvar Pain Measures Between Patients and Controls

Patients and controls did not differ regarding marital status (73% of patients vs 69.8% of the controls reported being in a stable intimate relationship), gravidity, and parity. Notably, patients were significantly younger (mean age, 26.2 ± 4.1 vs 32.5 ± 6.7 ($p < 0.001$)), but this age difference may result from the different recruitment process and has no clinical significance, given all women were in their reproductive years.

To explore whether the various experimentally induced pain tests could distinguish penetrative dyspareunia, we compared the various measures between patients and controls. Patients demonstrated an augmented pain response as obtained by the various experimentally induced pain tests as well as higher PFHT (Table 1).

Correlations Between Insertional Pain and Vulvar Pain Measures Among Patients and Controls

To test for relationships between insertional dyspareunia and the assessed vulvar pain hypersensitivity measures, we separately analyzed these correlations among patients and controls. As shown in Table 2, among patients, the intensity of dyspareunia was associated with higher pain ratings for the Q-tip and muscle-deep pressure measures. Pain thresholds and pain provoked by tampon insertion were not correlated with dyspareunia severity. As for the controls, none of the assessed experimentally induced-pain measures were associated with the severity of pain experienced during intercourse.

Table 1 Comparison of Vulvar Pain-Hypersensitivity Measures Between Patients and Controls

Measure	Patients (n=113)	Controls (n=43)	p
Pain evoked during vaginal intercourse (NPS 0–10)	7.87±1.71	1.00±.98	<0.001
Q-tip pain at the anterior vestibule (NPS 0–10)	2.80±2.30	0±0	<0.001
Q-tip pain at the posterior vestibule (NPS 0–10)	6.21±1.94	0.36±.73	<0.001
Pain threshold – anterior vestibule (Algesiometer, KgF)	0.35±.19	0.59±.22	<0.001
Pain threshold – posterior vestibule (Algesiometer, KgF)	0.15±.09	0.43±.19	<0.001
Tampon-test (NPS 0–10)	4.14±3.57	0.05±.0.21	<0.001
Pain evoked by muscle pressure (NPS 0–10)	5.91±2.07	0.60±.82	<0.001
Tonus level of pelvic floor muscles (none=0, mild=1, moderate=2, severe=3)	2.40±.0.68	0.58±0.95	<0.001

Abbreviation: NPS, numerical pain scale.

Table 2 Comparison of Correlations Between Dyspareunia Intensity Report and Experimentally Induced Pain Hypersensitivity Measures in Patients and Controls

	Patients (n=113)	Controls (n=43)
Q-tip anterior vestibule (NPS, 0–10)	**r=0.285, p=0.003**	r=000, p=1.0
Q-tip posterior vestibule (NPS, 0–10)	**r=0.506, p<0.001**	r=0.126, p=0.421
Algesiometer-anterior vestibule (KgF)	r=0.032, p=0.774	r=0.019, p=0.905
Algesiometer-posterior vestibule (KgF)	r=0.136, p=0.166	r=−.230, p=0.138
Muscle pain (NPS, 0–10)	**r=0.406, p<0.001**	r=−.060, p=0.704
Tampon-test (NPS, 0–10)	r=0.030, p=0.778	r=000, p=1.0

Note: Bold values represent significant correlation after controlling for multiple analyses.
Abbreviation: NPS, numerical pain scale.

Correlations Among the Various Vulvar Experimentally Induced Pain Measures

To further explore the nature of the associations among the various vulvar pain hypersensitivity measures, we constructed a correlation matrix analysis among patients and controls. Higher pain intensity evoked by the Q-tip at the posterior vestibule was correlated with higher pain evoked by deep muscle pressure ($r = 0.342$, $p < 0.001$ in patients, $r = 0.484$, $p = 0.001$ in controls). However, no correlations were observed between pain thresholds and Q-tip ratings at either the anterior or posterior vestibule in both groups.

Pain thresholds measured at both the anterior and posterior vestibule demonstrated significant intercorrelations within both the patient and control groups ($r = 0.271$, $p = 0.005$; $r = 0.684$, $p < 0.001$, respectively), revealing that for this measure, a variability in mucosal sensitivity was noted in the entire vestibule. Regarding pain ratings evoked by Q-tip provocation, a significant correlation was found between pain reports at both the anterior and posterior vestibule ($r = 0.488$, $p < 0.001$) only in the patients' group.

As for the tampon-test, only in patients, higher Q-tip ratings obtained at both anterior and posterior vestibule were correlated with enhanced pain ratings evoked by tampon insertion ($r = 0.266$, $p = 0.009$, $r = 0.356$, $p < 0.001$, respectively).

The intricate correlations observed among the patients and the controls inspired us to further compare these experimentally induced pain measures across the four dyspareunia subgroups.

Comparisons of Pain Measures Among the Four Subgroups

ANOVA revealed that dyspareunia intensity (Figure S1), posterior vestibule provoked pain intensity (Q-tip, Figure S2), pain evoked by muscle pressure (Figure S3) and the Tampon test (Figure S4) did not differ among the four subgroups. However, both the AA-vestibular tenderness and Hormonal-vestibular tenderness subgroups exhibited significantly higher pain sensitivity at the anterior vestibule from Q-tip stimulation compared to the other two subgroups (Figure S2). In contrast to the observed group-differences regarding pain evoked by Q-tip at the anterior versus posterior vestibule, no such group-differences were found regarding the anterior and the posterior vestibule pain thresholds (Figure S5).

Correlations Between Experimentally Induced Pain Measures and Dyspareunia Intensity

To further address which experimentally induced pain measure represents dyspareunia severity (ie, self-reported sexual penetration provoked pain), we conducted an additional correlation analysis for each subgroup that included the relevant measures. Table 3 shows that those with circumferential vestibulodynia demonstrated a different set of correlations between self-reported insertional pain and experimentally induced pain measures, distinguishing between the Hormonal and AA subgroups. Similar picture was observed regarding the two posterior-only subgroups, distinguishing between the Hypertonic and Hymenal.

Table 3 Correlations Between Experimentally Induced Pain Measures and Insertional Pain Intensity

	Posterior-Only Vestibulodynia		Circumferential Vestibulodynia	
	Hypertonic (n=35)	**Hymenal (n=37)**	**Hormonal (n=21)**	**Augmented Anterior (n=20)**
Q-tip anterior vestibule (NPS, 0–10)	r=0.283, p=0.505	r=0.239, p=0.648	r=0.512, r=0.106	**r=0.581, p=0.048**
Q-tip posterior vestibule (NPS, 0–10)	**r=0.585, p=0.003**	r=0.381, p=0.128	r=.495, p=0.137	**r=0.602, p=0.034**
Muscle pain (NPS, 0–10)	**r=.724, p<0.0001**	r=.214, p.757	r=0.384, p=0.415	r=0.508, p=0.153

Note: Bold values represent significant correlation after controlling for multiple analyses.
Abbreviation: NPS, numerical pain scale.

Mucosal pain intensity evoked by the Q-tip test at the posterior vestibule was correlated with dyspareunia exclusively in the Hypertonic and AA subgroups. Additionally, pain evoked at the anterior vestibule by the Q-tip test was correlated with dyspareunia only in the AA-vestibular tenderness subgroup.

As for muscular provoked pain, enhanced pain report in response to pressure was associated with dyspareunia only in the Hypertonic-vestibular tenderness subgroup.

Testing the Conditional Indirect Effects

To further explore the role of pelvic floor hypertonicity (PFHT) in determining group differences in women with vulvar pain hypersensitivity, as well as investigating its role in affecting the associations between experimentally induced pain measures and dyspareunia, we conducted a conditional indirect effect analysis. The first step was to explore the possible role of PFHT as a mediator of the association between the muscular (deep pressure pain) and mucosal evoked pain measures (Q-tip) in the whole dyspareunia group. No mediating role for PFHT between pain ratings evoked by deep muscle pressure and Q-tip test ratings at the posterior vestibule was found.

Additionally, the conditional indirect effect analysis examined the extent to which the association between experimentally provoked-pain and dyspareunia severity was mediated by elevated muscle tonus at different levels of the moderator PVD subgroups. Figure 1 shows that pain evoked by deep muscle pressure was associated with hypertonicity ($B_{(unstandardized)}$= 0.149, $p < 0.01$) only in the hypertonic subgroup (moderator); hypertonicity (mediator) was associated with dyspareunia ($B_{(unstandardized)}$= 1.36, $p < 0.05$). Moreover, the conditional indirect effect analysis showed a significant direct effect ($B_{(unstandardized)}$= 0.373; LLCI = 0.204 to ULCI = 0.544; $p < 0.01$) and indirect effect of pain provoked by deep muscle pressure (X) on dyspareunia (Y) through elevated tonicity at the hypertonic subgroup only (Indirect effect = 0.203; BootLLCI = 0.022 to BootULCI = 0.5122, significant indirect effect when the 95% CIs not contain zero). Unstandardized coefficients of the paths and the indirect effects of the PVD subgroups are shown in Figure 1.

This further supports the above findings that dyspareunia in patients in the Hypertonic subgroup may be attributed to the muscular component. When the same model was performed to assess how the level of PFHT mediated the association between posterior Q-tip pain and dyspareunia, similar findings were found, further supporting the assumption that in the Hypertonic-subgroup, the presence of PFHT played a significant role in pain hypersensitivity at the posterior vestibule (Hypertonic-PVD: Indirect effect = 0.136; BootLLCI = 0.005 to BootULCI = 0.413) (data not shown).

Discussion

Accumulative evidence emphasizes the complexity of pain in dyspareunia and the need to promote the conception of a mechanism-based treatment approach according to the precise vulvar-pain manifestations.[1,7,9] Therefore, this study explored the relevance of a broad set of experimental tests to distinguish between PVD-subgroups. Such an investigational approach allows us to attain more understanding which of the tests better represent the experience of clinical dyspareunia.

The findings provide more evidence regarding the distinctive nature of each of the four PVD subgroups in terms of the vestibular hypersensitivity characteristics. Furthermore, the results show that vulvar tenderness was manifested by a dissimilar amalgamation of pain manifestations that probably represent different pain origins. These findings may also

suggest that the various experimentally induced pain measures represent a different facet of vulvar pain hypersensitivity, further contributing to the conceptualization of insertional dyspareunia.

Although similar pain intensities of dyspareunia were reported in the entire cohort, each subgroup differed by various components. These included the dominating painful tissue (vestibular mucosa, muscles, and hymenal distention hypersensitivity); the anatomic location (anterior and/or posterior vestibular allodynia); unique combinations of pain response to the various experimental tests (deep muscle pain, threshold and Q-tip) and different associations with muscle hypertonicity. Altogether, these findings may indicate different facets of pathology-related alterations in the vulvar tissues.

The differences between patients and controls regarding their response to experimentally induced-pain tests and muscle tonicity are not surprising.[15,18,19] The very low pain hypersensitivity ratings among controls confirmed that these women, indeed, express a pain-free response to vulvar stimulation and further demonstrate that experimentally induced pain tests allow the attainment of pain provoked during vaginal penetration. On the other hand, due to the "floor effect" characterizing the pain intensity measures in the control group, the measure of pain thresholds seems like the only test allowing comparison between patients and controls. Collectively, these findings further emphasize the potential role of each test to signify dyspareunia severity well beyond patients' self-report of dyspareunia, probably affected by the contextual circumstances as well as the setting.

The wide inter- and intra-variability in pain sensitivity adds to the complexity of its assessment, mainly in chronic pain syndromes. Thus, the fact that these experimentally induced-pain assessments were performed in women with dyspareunia and pain-free controls allows further illumination of vestibular pain-hypersensitivity. Indeed, the variability among the control group implied that complex sensory mechanisms affect vulvar pain response, even among women who do not suffer from painful intercourse. Assuming that even women without dyspareunia may respond to noxious stimulation to some degree [15] and exploring the relationships between these measures among controls may be relevant to further understand the mechanisms of altered pain response in patients.

The idea of dividing patients presenting with provoked vestibular pain-hypersensitivity into defined subgroups, according to the presumed pathophysiology, has been proposed [7,8] without experimental validation. This expert's opinion mainly relies on clinical observations of the location of vestibular pain. Some present allodynia in the entire vestibule (circumferential vestibular tenderness), while others demonstrate allodynia only at the posterior vestibule. It was also proposed that tenderness throughout the entire vestibule might be the result of an intrinsic pathology within the vestibular mucosa (ie, representing mucosal feature), whereas hypersensitivity confined to the posterior vestibule might be associated with an extrinsic pathology, such as hypertonic pelvic floor muscle dysfunction [7,8] possibly causing altered neurodynamics and tissue hypoxia.[9] Considering that PVD is likely not just one disorder but rather a collection of symptoms of diverse disease processes,[9] four PVD subgroups were previously described, which partially parallel the algorithm suggested in the literature.[6–8,10] This conceptualization expanded the aforementioned algorithm by 1) using the term "Augmented Anterior" rather than "neuroproliferation"- given that no histological studies are available to support this proposed pathophysiology; 2) the exclusive observation that a large portion of those with posterior-only allodynia also demonstrates a constricting hymenal ring.[10]

While posterior vestibular allodynia was evident in all patients, anterior allodynia was evident only in the Hormonal-mediated and AA-subgroups. The posterior vestibular tenderness was greater than the anterior tenderness, evident in the control group as well. This may be attributed to a combination of mucosal pathology causing circumferential vestibular tenderness and an additional pathology causing enhanced posterior pain-hypersensitivity such as PFHT. Tenderness of the anterior vestibule may result from mucosal neuro-proliferation, as proposed in the original algorithm,[6] or from systemic pain hyperresponsiveness.[12] This assertion should be further confirmed, for example, using biopsies.

In line with the proposed contribution of PFHT to the augmentation of posterior vestibular-hypersensitivity,[7,8] enhanced pain response to the Q-tip at the posterior vestibule was correlated with higher pain ratings evoked by deep muscle pressure in both patients and controls. Additionally, higher pain intensity evoked by muscle pressure was correlated with dyspareunia severity only in the Hypertonic subgroup, and so was hypertonicity. The results of the mediation analysis further supported these findings. These findings collectively reinforce the hypothesis that dyspareunia in this subgroup may stem from PFHT, which could represent either a primary or a conditioned response, associated with

deep muscular pain hypersensitivity and increased posterior vestibular mucosal tenderness. Moreover, these results support the notion that posterior-only hypersensitivity is comprised of two distinct subgroups: those with PFHT and those with a constricting hymenal ring.

As for the Hymenal subgroup, despite presenting with posterior vestibular tenderness, they predominantly experienced distention-provoked pain, probably due to anatomic restrictive tissue. Although it might be assumed that the posterior vestibular component is secondary to responsive muscle hypertonicity, no association with muscular alterations was observed in this subgroup. Whereas in the Hypertonic subgroup, various pain measures were inter-correlated, the Hymenal subgroup did not manifest any associations. In this aspect, pain measures in this subgroup were more similar to the control group than any of the other three subgroups.

As for the significance of each vulvar experimentally induced pain measure, this study illuminated the unique contribution of each measure as representing an independent facet of vulvar hypersensitivity. The different expressions of each measure in the subgroups emphasize that combining all PVD patients prevents discerning the relevance of each experimental-pain measure due to the "dilution" of scores. For example, when measuring anterior vestibular tenderness, those with posterior-only tenderness lower the mean rating of the entire cohort. It also highlights that the information attained by utilizing a battery of experimental tests is required to properly characterize the individual elements contributing to dyspareunia. Such a personalized approach is especially important to advise tailored treatment to improve the efficacy of therapeutic interventions. Not all experimental pain tests were found to be relevant to estimate pain severity in women with introital dyspareunia. For example, a higher rating of penetrative pain was correlated with pain provoked by the Q-tip and with muscular pain induced by palpation but not with pain thresholds or the tampon-test pain ratings.

The Cotton-swab test is accepted as the standard diagnostic tool for PVD,[7,13] although it was claimed that the patient's response is affected by the type and degree of pressure delivered by the examiner.[20,21] The fact that Q-tip pain intensity evoked at the posterior vestibule was associated with penetrative pain in the entire cohort adds to the accumulative evidence of the Q-tip test as a sensitive test. Notably, this test allows us to distinguish the two circumferential groups (the AA and Hormone subgroups) from the posterior-only subgroups.

The pain threshold is an experimental pain test representing the degree of stimulus evoking the first pain sensation. As such, it reflects a different facet of pain perception, signifying the degree of stimulus required to distinguish between non-painful and painful sensations. Hence, it was assumed to serve as an additional indicator of sensory alteration of the vestibular mucosa that could be relevant to distinguish between subgroups. In contrast to the differences in pain evoked by the Q-tip in the anterior versus posterior vestibule, the evaluation of pain thresholds did not contribute to subgroup distinction. The vulvalgesiometer has been used in prior studies,[19,22] including for an assessment of treatment outcomes,[23] providing valuable information about the sensory alterations in vulvar tissues. It has been suggested that using psychophysical properties which stimulate different sensory fibers involving temporal aspects of pain such as pressure stimulation and vaginal distention are relevant in exploring pain hypersensitivity in PVD.[15] While two studies [19,22] reported that pain ratings obtained by vulvalgesiometer were correlated with dyspareunia intensity, Aerts et al [24] did not find any significant correlations between these measures. Pain thresholds capture a single facet of the pain experience not expressed by pain ratings evoked by the Q-tip test. Hence, both tests do not fully mimic the pain experience provoked by intercourse, which is affected by friction and movement, as well as by the context.

The issue of how much each test accurately depicts PVD is also relevant to the tampon test suggested as an alternative measure of dyspareunia.[16] This measure was proposed as an accurate, easily accessible, and cost-effective primary outcome test due to its limited participant burden, strong methodologic properties, and excellent construct validity. Moreover, pain evoked by intercourse and cotton-swab test pain ratings were found to be correlated. In the current study, tampon-test pain ratings were not correlated with dyspareunia severity in any of the four subgroups. These findings suggest that in a portion of women, this measure may not serve as a sensitive assessment tool to evaluate vulvar pain hypersensitivity.

The key role of muscle hypertonicity in pain experience and response was also observed in the present study. Higher levels of PFHT were observed among patients in the Hypertonic-vestibular tenderness subgroup as compared to the other three subgroups. Furthermore, PFHT was associated with augmented dyspareunia. It seems that PFHT, possibly serving as a protective mechanism for potential pain experience or harm, may cause hypersensitivity at the posterior vestibule. The suggested protective mechanism may have evolved due to primary anatomical elements or as a secondary

conditioned response to pain.[25–27] We assume that such hypersensitivity at the posterior vestibule, derived by PFHT among those patients, is "diluted" when analyzing the entire cohort as one group. The role of muscle plasticity and resistance was also suggested as determining the level of tonus.[27,28] For example, Benoit-Piau et al reported that pain during intercourse was associated with muscle power and its elasticity but not with the tonus.[29] It can be assumed that anticipation of painful intercourse among patients who perceive vulvar-pain as harmful induces a cascade of neuronal activity and secretion of neurochemicals, including adrenalin, that collectively affect the amygdala and other brain regions that involve stress and pain modulation. Consequently, the muscles respond by increasing their strength, which is represented by hypertonicity.[30,31] Augmentation may also occur with the experience of danger and stress in the context of vaginal penetration.[32] The reaction model was suggested by Spano & Lamont,[33] who emphasized that distress from penetration together with elevation of stress hormones attenuate lubrication and sexual desire, resulting in enhanced tonicity caused as a protective mechanism, also expressed by avoidance from penetration.[28]

This study has several strengths; first, it utilized a wide array of experimental tests, which were evaluated in all patients and in a large group of pain-free women. Second, it has an adequate sample size of women in which the diagnosis of PVD was confirmed. Third, all participants were evaluated by a single gynecologist, reducing possible inter-observer bias. Nevertheless, it has several limitations, including relatively small groups (AA and hormonal), a non-blinded evaluation, lack of a standardized, objective method to assess PFHT and absence of uniformity regarding the time in the menstrual cycle, possibly introducing variability in pain ratings. In addition, examination by a single gynecologist may reduce methods and results generalizability, due to the difficulty to replicate them.

In summary, the current study further supports classification of patients with introital dyspareunia and vestibulodynia into subgroups. These findings may promote the exploration of different mechanisms in provoked vestibular pain. In addition, analyzing the relationships between the various experimental pain measures allows for a better understanding of the role of pain evoked by different modalities at different vestibular locations, as well as from the vestibular mucosa or PFM. Although our findings support the existence of four subgroups, it is probable that more subgroups remain to be discovered.

Conclusion

The distinct characteristics observed among the women in the various subgroups underscores the importance of personalized intervention strategies targeting specific pain mechanisms unique to each subgroup. Future longitudinal studies will establish which pain parameters most sensitively reflect vestibular hypersensitivity and which of these parameters change in response to a particular intervention.

Abbreviations

AA, Augmented anterior; NPS, numerical pain scale; PFHT, pelvic floor muscles hypertonicity; PFM, pelvic floor muscles; PVD, provoked-vestibulodynia.

Data Sharing Statement

The datasets analyzed for the current study are available from the corresponding author on reasonable request.

Ethics

The study was approved by the Institutional Review Board of the Clalit Health Organization, COM1-16-89. All patients approved their participation and signed informed consent form. Clinical trial registration: NCT02712814, https://clinicaltrials.gov/study/NCT02712814?term=ahinoam%20lev%20sagieandpage=2andrank=18

Disclosure

The authors report no conflicts of interest in this work.

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