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ORIGINAL ARTICLE

Evaluation of vestibular biopsy features in patients affected by fibromyalgia, by vulvodynia or by their association

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Key words

Vulvar Biopsy • Histology • Provoked vulvodynia • Fibromyalgia

Summary

Objective. To evaluate the histologic features of vestibular biopsies from patients affected by fibro myalgia (FM), or vulvodynia (VD), or the their association (FM-VD) in order to facilitate differential diagnosis among conditions that present sexual pain with similar clinical characteristics.
Study Design. Fourty-four women already diagnosed with FM were recruited to evaluate the presence of sexual pain not owing to FM. Fourteen women affected by sexual pain of unknown origin who came to our department requesting treatment were also recruited. All subjects were interviewed regarding their history of pain and examined in order to exclude vaginal conditions. Sexual pain did not show the characteristics of VD in 18 FM women; in the remaining 22 women VD resulted as associated with FM. All fourteen self-referred women were diagnosed with VD. All subjects underwent a posterior vestibular biopsy at the fourchette under local anesthesia. Tissue specimens were processed for histologic examination and immunostained for S-100protein and CD34. Statistical analysis was performed with the Pearson's Chi-square test.
Results. Data analysis showed a statistically significant prevalence of inflammation in the VD group. Analysis of the histologic features showed that the concomitant presence of inflammation, nerve bundles, and fibrosis (often mild) is prevalent in VD. Fibrosis is highly frequent and often moderate/severe in FM and it is rarely associated to inflammation and nerve bundles. FM-VD women show intermediate grading.
Conclusions. Our findings show different histologic characteristics in vestibular biopsy in patients affected by Fibro Myalgia, by Vulvodynia or by their association that could be useful to facilitate the differential diagnosis between conditions of sexual pain with similar clinical characteristics.

Introduction

Fibromyalgia (FM) is a syndrome of chronic (3 months or longer) widespread musculoskeletal pain syndrome of unknown origin characterized by hyperalgesia and allodynia and affecting the upper, lower, right and left quadrants of the body [1]. Vulvodynia (VD) is also a syndrome of chronic pain of unknown origin characterized by hyperalgesia, mild erythema and oedema of the vestibular mucosa in addition to entry dyspareunia and burning pain when minimal pressure is applied at the vulvar vestibule [2]. It is distinguished as: localized (at the vestibule) or generalized (at the vulvar and perineal area); unprovoked (pain begins spontaneously) or provoked (pain elicited by to light pressure); and primary (onset at first intercourse) or secondary (onset after any period of normal sexual functioning) It was first named vulvar vestibulitis. Later the suffix "itis" was excluded from the terminology because studies found a lack of association between the bioptic specimens and inflammation [3]. Although the etiology of VD remains unknown, a recent review points out that a number of investigations are suggestive of a possible role of inflammation in the genesis of the disorder [4]. All the conditions of chronic pain of unknown origin tend to associate. VD is reported as co-occurring with FM, irritable bowel syndrome (IBS), and chronic fatigue syndrome (CFS) while women with vulvodynia are 2.78 times more likely to

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report CFS and 2.15 times more likely to report FM than controls [5 6]. In an extensive mail survey, generalized and localized chronic pain conditions were found at an estimated frequency of 36% in the population at large; among those, 27.1% screened positive for multiple conditions and vulvodynia was associated with every one of the other conditions [7].

Pain with intercourse is the iconic symptom of VD but it is also very common in FM because diffuse musculoskeletal pain at the tights, lower back and the perineum makes lovemaking very uncomfortable and the threshold at which sensory inputs become painful is lower that normal in FM resulting in hyperalgesia [8]. For these reasons fibromyalgic dyspareunia can mask the co-occurrence of VD and the overlapping symptoms between these two conditions represent a diagnostic challenge [9].

To our knowledge, no patognomonic histologic features are currenctly extablished for genital pain in vulvar vestibulitis or in fibromyalgia and a number of studies of the bioptic vestibular specimens from VD women report not homogeneos findings. Some authors found a statistically significant increase of immune-reactive nerve fibers in the papillary dermis and some degrees of mixed chronic inflammatory infiltrate in the dermis of VD patients [10]. In one histological evaluation of the vestibule, nerve density appears to statistically be significantly higher in VD than in controls, and inflammatory cells appear to localize around superficial minor vestibular glands while inflammation is associated with a calculated area of nerve fibers ten times higher than expected [11]. Statistically significant proliferation of peripheral nerve bundles but no differences regarding inflammation or vascular proliferation were found in bioptic vestibular specimens from VD compared to controls [12]. One group of scientists found more chronic inflammation in specimens from secondary VD than in those from primary VD and higher nerve density in primary than secondary VD and than biopsies from controls have more CD8-positive than CD4-positive T cells, while samples from localized provoked vulvodynia (LPV) have more CD4-positive T cells [13 14]. An extensive literature overview reports evidence of sub-epithelial infiltration of T lymphocites found in vestibular tissues of women with LPV and that similar aspects are seen in healthy women and therefore cannot be considered as diagnostic criteria [15]. In recent studies, immuno-histo-chemistry of biopsies from LPV patients demonstrates organized vestibule-associated lymphoid tissue that was more pronounced than in controls [16]. Pro-inflammatory response measured in fibroblast strains derived from LPV vestibules demonstrated a higher pro-inflammatory response under inflammatory stimuli than in controls [17]; one paper reports that the majority of vulvodynia patients suffered from a benign secondary rather than idiopathic mast cell disorder, that did not show the criteria of neoplastic mast cell disease, and that prominent blood vessels were detected in the subepitelial stroma of approximately 1/3 of women with vulvodynia [18].

In short, most papers ranging from 2 decades ago to today support that neural hyperplasia and inflammatory cells are often found in vestibular biopsies from VD women. To verify whether these features are also common to FM women affected by sexual pain or whether the histologic patterns differ from one condition to the other we evaluated the histology of vestibular mucosa specimens from women affected by FM, VD and their association, respectively. We were hoping to obtain one or more histologic patterns in order to differentiate conditions of sexual pain that present similar clinical characteristics.

Materials and methods

Subjects

Forty-four women already diagnosed with FM by the Pain Laboratory at the State Medical School in Siena and who reported dyspareunia were recruited to explore the presence of sexual pain not due to FM. Fourteen women affected by sexual pain, who referred themselves for diagnosis and treatment, were also recruited to explore the presence of sexual pain of unknown origin. We only admitted women who reported: presence of sexual pain for at least six months, stable heterosexual relationship and otherwise healthy.

Intake

All subjects were seen at the Sexual Medicine Outpatient Unit (University Hospital, Siena) by the study gynaecologist and sex therapist (AG). They were asked about the first onset of dyspareunia, subsequent occurrences, nature of stimuli eliciting vestibular pain, pelvic and perineal localization of pain, duration after onset, and years of adequate sexual functioning if any. In addition, all were examined in order to exclude gynaecological or dermatological conditions and to evaluate the presence of oedema and erythema. After the visual observation of the external genitalia, a vestibular biopsy was performed to confirm or to exclude vulvar dysplasia, early lichen sclerosus or dermatologic conditions and to establish a correct diagnosis of vulvodynia of unknown origin in case the clinical examination is not revealing [19 20].

Sampling and preparation of tissues

Vestibular biopsy specimens of approximately 5x3 mm were taken at 6 o'clock on the posterior vestibular wall with a biopsy forceps after a 5 minutes application of lidocaine/prilocaine (EMLA, Astra Zeneca Italia). No suture was needed. The 6 o'clock area was chosen because it usually shows marked hypereactivity in patients with provoked vulvodynia. The specimens were formalin fixed and paraffin embedded. Sections were cut perpendicularly to the surface epithelium, with a nominal thickness of 4µm and stained with Hematoxylin-Eosin. In addition, the S-100 and the CD34 immunohistochemical stainings were performed to highlight the peripheral

Fig. 1. Dermal inflammatory infiltrate (H&E. Original magnification x40).

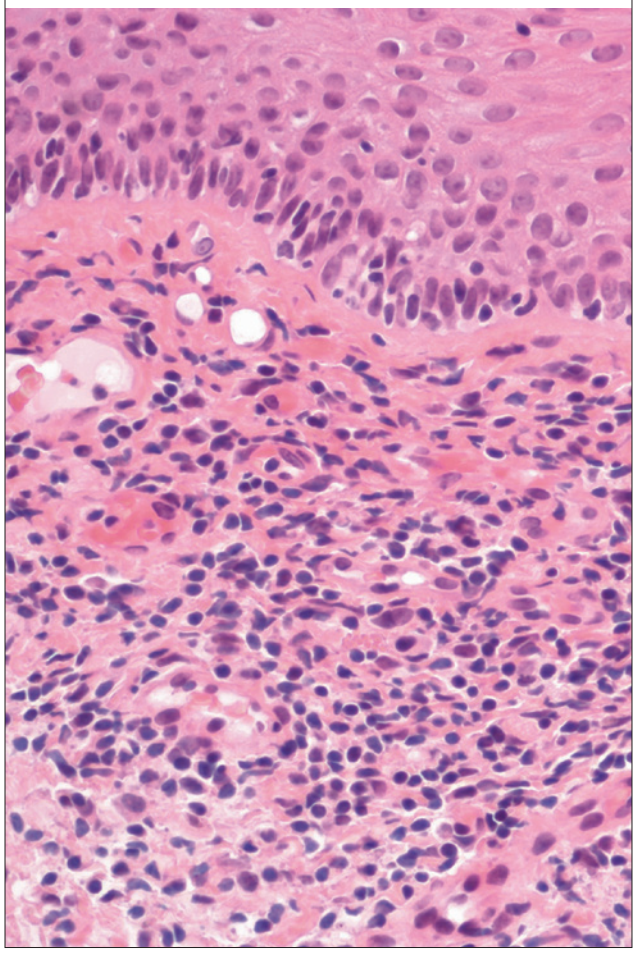
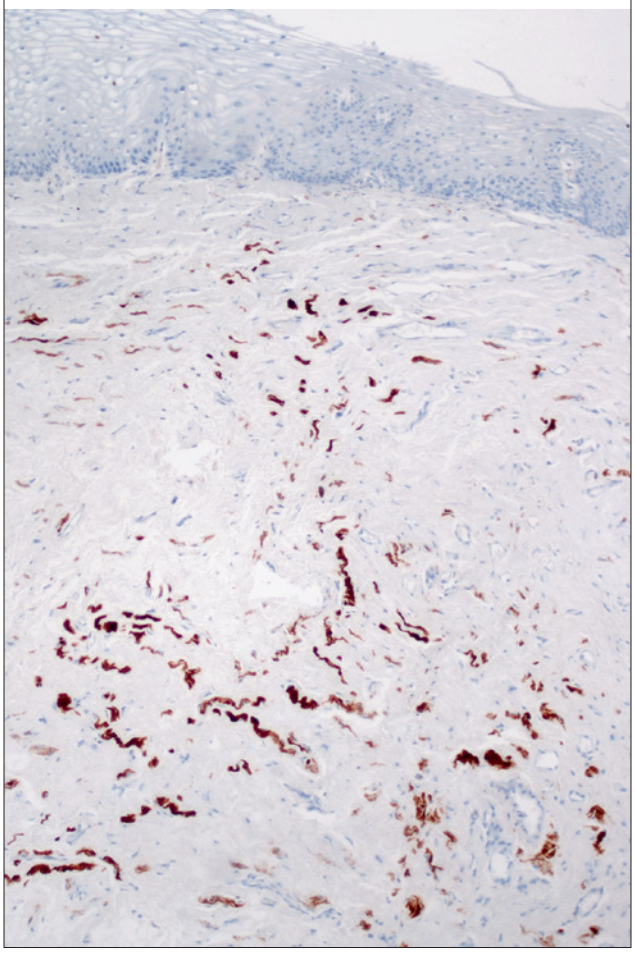


Fig. 2. Increased peripheral nerve bundles in sub epithelial connective tissue (S100 Immunohistochemical stain. Original magnification x10).



nerve bundles and the endothelial cells lining vascular spaces, respectively.

The study pathologist (GT), blind to each woman's clinical symptoms, examined all the specimens to evaluate the squamous epithelium and the sub epithelial connective tissue, oedema, exocytosis, inflammatory infiltrate, vascular spaces, and peripheral nerve bundles. All features were evaluated against the normal histologic parameters of the vestibular tissues and structures (Figs. 1-3). Further, they were scored as none (normal), mild, moderate, and severe.

Statistical analysis

The comparison among groups was performed by means of Pearson's Chi-square test. Level of significance was set at $p < 0,05$. All analysis were performed using Epi-Info version 3.3.2 (Italian).

Results

Dyspareunia - Sexual pain did not show the clinical characteristics of vulvodynia in 18 women with FM (mean age 50,1 years; range 27-65). On the other hand, association with sexual pain of unknown origin and not related to secondary effects of FM was confirmed in the remaining 22 FM women (mean age 42,9 years; range 30-61) thus constituting the new FM-VD group. All 14 self-referred women were diagnosed with VD (mean age 38,6 years; range 21-62).

Histology – During the pathological examinations four cases out of 58 showed scanty sub-epithelial connective tissue unsuitable for evaluation and were thus eliminated reducing the total sample to 54 (18 FM, 22 FM-VD, and 14 VD). Early signs of lichen sclerosus or dermatosis were not found in any specimen. The intensity of nerve and vascular proliferation, of connective tissue oedema, and of lymphocitic exocytosis (Tab. I), as well as the prevalence of epithelial hyper- and hypo-plasia (Tab. II) were similar in the three groups.

However, marked differences were observed in the intensity of inflammation that was more prevalent in VD, (and to a lesser degree in FM-VD), with respect to FM. In addition, moderate or severe fibrosis was more frequent in FM and in FM-VD and it was mostly mild in VD (Tab. I).

Tab. I. Intensity of the different hystological features in the three study groups.

	None	Mild	Moderate	Severe
Inflammation				
FM	67%	11%	6%	17%
FM-VD	36%	18%	36%	9%
VD	7%	36%	43%	14%
Fibrosis				
FM	6%	28%	22%	44%
FM-VD	18%	32%	32%	18%
VD	7%	79%	7%	7%
Nerve bundles				
FM	67%	22%	6%	6%
FM-VD	55%	36%	5%	5%
VD	43%	29%	21%	7%
Lymphocytic exocytosis				
FM	83%	17%	0%	0%
FM-VD	68%	32%	0%	0%
VD	50%	50%	0%	0%
Vascular proliferation				
FM	0%	17%	28%	56%
FM-VD	0%	23%	18%	59%
VD	0%	14%	29%	57%
Oedema				
FM	67%	22%	11%	0%
FM-VD	55%	27%	14%	5%
VD	36%	43%	21%	0%

FM: Fibromyalgia (N=18); VD: Vulvodynia (14) FM-VD: combination of the two [22].

To perform the statistical analysis we clustered the intensity scores given to each item. Among the three groups, the statistical analysis shows a significant difference in the frequency of inflammation (Chi square = 11,84; p = 0,002) which was prevalent in the VD group (Tab. II). To determine whether any associations among the evaluated histologic features might be representative of one or more of the study groups, we analyzed all possible combinations. The Pearson's Chi-square test showed significant differences among the three groups in the concomitant presence of inflammation and nerve bundles (Chi-square 6,29, p = 0,043); in the concomitant presence of inflammation, nerve bundles, and fibrosis (Chi-square 7,04, p = 0,03); in the concomitant presence of inflammation, nerve bundles and blood vessels (Chi-square 6,29, p = 0,04). The associations reported above are prevalent in VD (Tab. III).

Discussion

Our initial aim was to verify whether vestibular neural hyperplasia and the inflammatory cells, identified in many papers, could be a consistent histological feature to differentiate between VD specimens and specimens from FM and FM-VD women. In accordance with the literature, our patients showed different degrees of neural hyperplasia and inflammation as expected; in addition, the investigation of three similar but not identical clinical conditions of genital pain produced more complex findings.

Our data indicate that inflammation has a statistically significant higher frequency in the biopsies from VD than in those from FM or FM-VD. This contradicts a paper reporting low expression of inflammatory markers with the same intensity degree found in vestibular biopsies from vulvar vestibular syndrome (VVS) patients and controls, indicating no active inflammation [21]. Other scientists found a relative inability to down regulate pro inflammatory mediators detected in a sub group of VVS women [22]. The same AA found that gene polymorphism of one trigger for complement activation was prevalent in women with primary VVS and it could be a predisposing factor for autoimmune dysregulation [23]. The presence of higher inflammatory intensity in our VD

Tab. II. Specimens hystologic features.

Histology	FM (18) n. (%)	FMVD (22) n. (%)	VD (14) n. (%)	Chi-square (p)
Inflammation	6 (33,3)	14 (63,6)	13 (92,8)	11,84 (p=0,002)
Nerve bundles	6 (33,3)	10 (45,4)	8 (57,1)	1,82 (p=0,401)
Vascularproliferation	18 (100)	22 (100)	14 (100)	*
Hypotrophic squamous epithelium	1 (5,5)	1 (4,5)	2 (14,2)	1,32 (p=0,517)
Hypertrophic squamous epithelium	9 (50,0)	11 (50,0)	7 (50,0)	0,00 (p=1,00)
Connettive tissue oedema	6 (33,3)	10 (45,4)	9 (64,2)	3,05 (p=0,218)
Fibrosis	17 (94,4)	18 (81,8)	13 (92,8)	1,90 (p=0,386)
Lymphocytic exocitosis	3 (16,6)	7 (31,8)	7 (50,0)	4,06 (p=0,131)

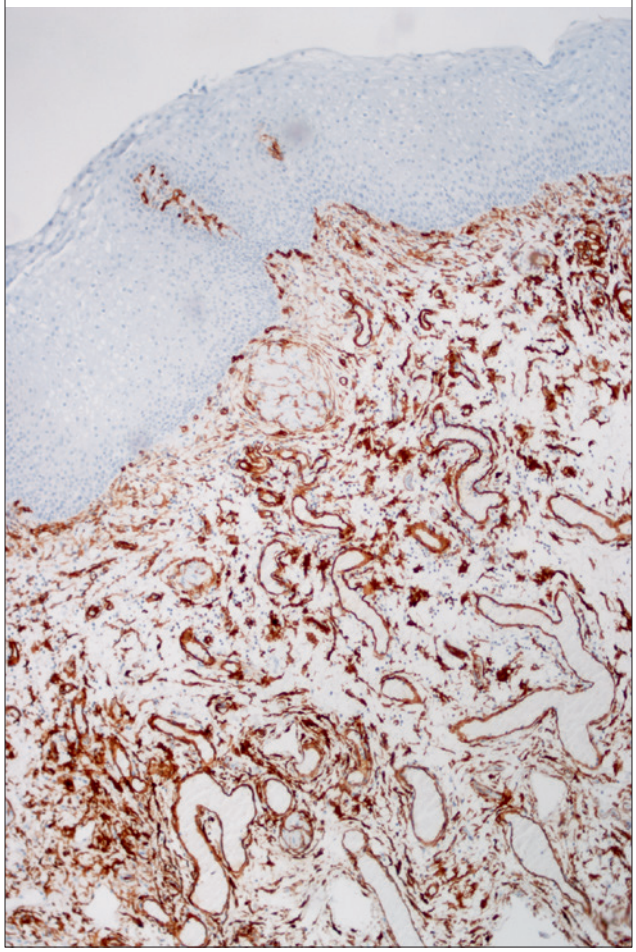
Legend: * Cannot do statistical test

Tab. III. Features associations.

Histology	FM (18) n. (%)	FMVD (22) n. (%)	VD (14) n. (%)	Chi-square (p)
Inflammation+nerve bundles	3(16,6)	6 (27,2)	8 (57,1)	6,29 (p = 0,04)
Inflammation+nerve bundles +fibrosis	3 (16,6)	5 (22,7)	8 (57,1)	7,04 (p = 0,03)
Inflammation+nerve bundles +vascularproliferation	3 (16,6)	6 (27,2)	8 (57,1)	6,29 (p = 0,04)

specimens could morphologically correlate with the humoral findings reported in these investigations.
According to our results, VD women show the highest frequency of histologic features related to the inflammatory response in comparison with FM women who show the lowest frequency, while the values are intermediate in FM-VD. Fibrosis is seen in almost all specimens from FM and from VD patients, but the observation of its grading value shows distinct differences: it is present in FM mostly with moderate and severe frequency but it is present with mostly mild frequency in VD while FM-VD women show intermediate grading (Tab. I). To our best knowledge there are no papers discussing the frequency of fibrosis in biopsies from the genitals or from other body sites in FM patients, therefore we cannot compare or discuss these findings.
It is worth considering that the concomitant presence of inflammation, nerve bundles, and fibrosis (often mild) is prevalent in VD (Tab. III). High frequency of fibrosis (Tab. II) (often moderate/severe) rarely associated to inflammation and nerve bundles (Tab. III) is found in FM. These different patterns may represent useful histologic characteristics to facilitate the differential diagnosis.
We believe that the data obtained are clinically relevant. Of course our preliminary findings will need to be confirmed by further investigations. These should use a simple random sampling with the simple size calculation on the basis of the expected results obtained from this first study.
We are strongly convinced that the differential diagnosis of vulvodynia is based on the history of pain, nonetheless the findings in our study could prove helpful to differentiate conditions with similar clinical characteristics. We hope further investigations will follow in order to clarify the vestibular histology in case of genital pain of unknown origin.

Fig. 3. CD34 immunostain highlighted increased vasculature in sub epithelial connective tissue (CD34 Immunohistochemical stain. Original magnification x10).



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