

The Value of Histology in Predicting the Effectiveness of Vulvar Vestibulectomy in Provoked Vestibulodynia

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■ Abstract

Objective. This study aimed to determine whether histology can predict response to vestibulectomy in the management of provoked vestibulodynia.

Materials and Methods. Inflammatory cell, mast cell, and nerve fiber counts were determined in prospectively collected vulvar vestibulectomy specimens from 30 women treated surgically for provoked vestibulodynia.

Results. Twenty-three subjects (77%) had a complete early response to surgery. At 3 years of follow-up, this had increased to 28 (93%), with a 29th showing some improvement. No subject had gotten worse after surgery or in the 3 years of follow-up. When comparing patients with an early complete response with those patients who still had symptoms, no difference in lymphocyte counts (27.6 vs. 37.8 per mm²), mast cell counts (110.4 vs. 97.8 per mm²), or stromal nerve fiber counts (16.4 vs. 16.4 per mm²) was found.

Conclusions. Vestibulectomy is a very effective treatment option in women with provoked vestibulodynia who have had failed conservative treatment. Histology is unable to predict which patients will respond to surgery. ■

Key Words: vulvodynia, vestibulectomy, inflammation, mast cells, nerve fibers

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Vulvodynia refers to a vulvar discomfort or pain occurring without any visible clinically relevant findings or without any background neurological lesion [1]. Vulvodynia is classified according to the location of the pain (generalized or localized), by whether the pain is only brought on by a stimulus (provoked) or if spontaneous (unprovoked), or if a mixed picture is present [2]. Localized vulvodynia is then classified according to the anatomical structure it affects, for example, clitorodynia and vestibulodynia. In this study, all the patients experienced provoked vestibulodynia causing severe entry dyspareunia in whom conservative measures had failed to control their symptoms.

A 2012 population-based study found that the lifetime incidence of vulvodynia among sexually active women older than 18 years to be 8% [3]. This disorder affects millions of women worldwide. Although some cases resolve spontaneously, a significant number of cases require medical or even surgical treatment [4–6].

Currently, there are a number of treatment options available for those diagnosed with vulvodynia. The British Society for the Study of Vulval Diseases 2010 guidelines suggests a multidisciplinary approach, grouping treatments into drug-based, psychological/psychosexual therapy, physiotherapy, and surgical management [4]. At present, there is insufficient evidence to confirm that any particular treatment modality has a greater significant benefit than any other [5]. However, a recent systematic review of these studies suggests that the lack of definitive

evidence is due in part to the large placebo effect in vulvodynia interventions [5].

Surgical management is seen to be most effective in patients with longstanding localized provoked pain who have not responded to other types of management [6, 7]. Surgical management can be grouped into posterior vestibulectomy (excision of the vestibule posterior to the paraurethral glands), total vestibulectomy (where the paraurethral glands and the subclitoral glands are removed in addition to the posterior vestibulectomy), and local excision (where only tender erythematous foci within the vestibule are removed) [2]. There is insufficient evidence at present to conclude that one method is superior to any other, with regard to patient outcomes [5, 8].

Previously, a study involving some of us reported that there were no histological parameters that can be used to diagnose provoked vulvodynia, although there was a trend for patients with vulvodynia to have increased nerve counts [9]. This present study, using the same population, aimed to test the hypothesis that long-term outcomes for patients who have undergone vestibulectomy can be predicted on histological evaluation. In doing so, we hope to further understand the histopathology of vulvodynia, which might have clinical implications for the role of biopsy in determining a patient's suitability for vestibulectomy.

MATERIALS AND METHODS

This study follows up patients from a study undertaken 3 years ago, reviewing their outcome in the context of histological markers [9]. The original study consisted of a prospectively collected, retrospectively analyzed, blind study of 30 women with localized provoked vestibulodynia. Patients were selected for surgery on the basis of persistent dyspareunia resulting in apareunia. Thus, all patients were scored as "grade 3" on Marinoff's scoring system [10]. Additional selection criteria required patients to have been screened for possible mental health and primary psychosexual issues, with the exclusion of somatic causes of pain and to be nonresponsive to conservative treatments such as oral amitriptyline and physiotherapy.

The 30 subjects were identified and entered into the study when their vestibulectomy specimens reached the pathology laboratory. These were collected during the years 2007 to 2009. The subjects were consecutive, and no exclusions were made.

Posterior vestibulectomy was performed by the same surgeon (R.P.), as a day procedure under general

anaesthetic. A crescent-shaped incision was made around the introitus from 2- to 10-o'clock (i.e., commencing just below the paraurethral glands) and extending posteriorly 10 to 20 mm beyond the hymenal edge. A proximal incision was then made along the junction of the hymen and vagina; thus, the hymenal remnant was totally removed. The posterior vaginal wall was then freed by sharp dissection, mobilized, and then brought down to cover the defect. The vaginal wall was sutured down in 2 layers. If there was any paraurethral tenderness, the paraurethral glands were also excised separately. The vestibulectomy specimens measured a mean of 40 × 40 mm, and their shape resembled small aprons. No purely local excisions were performed.

At the time of pathologic grossing, 3-mm punch biopsy samples were obtained from the center of the vestibulectomy specimens. The sites of the punch biopsies were selected by drawing 2 bisecting lines through the specimens to reduce bias (Figure 1). After the punch biopsies were performed, the remainder of the specimens were cut into 3-mm strips and blocked entirely.

Routine stained slides for hematoxylin and eosin (H&E), para-amino salicylic acid, and immunoperoxidase (IPX) for CD117 and PGP9.5 were prepared from the punch biopsies. Slides were then coded, so that the pathologist (J.S.) was blinded to the identity of the patients.

The pathologist assessed the site (mucosa, skin), presence of minor vestibular glands, any identifiable diseases, and, using a counting grid, counted inflammatory cells and nerve fibers. Lymphocytes, plasma cells, and polymorphs in the epithelium and stroma were

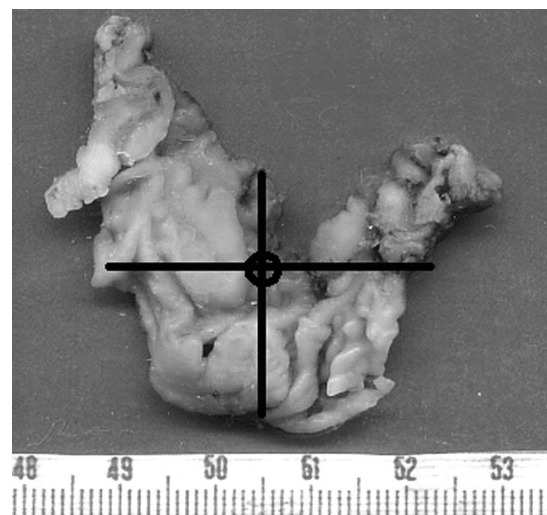


Figure 1. Apron-shaped vestibulectomy specimens in a subject. Punch biopsy performed where the black lines intersect.

counted on H&E slides. Yeast and fungi were looked for on the para-amino salicylic acid stain. Mast cells were counted on CD117 IPX. Nerve fibers were counted in the epithelium and stroma on PGP9.5 IPX. A langerin IPX was performed on one of the subjects to investigate that any of the PGP9.5 staining was due to dendrites from Langerhans cells. The remainder of the specimens were then assessed for any histological evidence of vulvovaginal disease.

Follow-up of the patients occurred in the postoperative period initially at 6 weeks to confirm adequate healing before resumption of sexual intercourse and then 3 months later to assess the degree of improvement of dyspareunia symptoms. The patients were again reviewed 3 years after vestibulectomy.

All 30 patients were interviewed in person at the initial 2 postoperative visits and then over the telephone at the 3 years follow-up. On each occasion, patients were graded according to the severity of the dyspareunia using Marinoff's scoring system (Table 1) [10]. At 3 years of follow-up, patients were asked about, in addition to pain on intercourse, any changes in sexual practices, change of partners and so on. All interviews were conducted by the operating surgeon (R.P.).

Statistical analysis was performed using the *t* test and Wilcoxon unpaired tests.

RESULTS

The early and late responses to surgery and lymphocyte, mast cell, and nerve fiber counts in each subject are shown in Table 2. In summary, 29 of the 30 subjects had a good early response, with 23 having no dyspareunia and 6 with a lesser degree of dyspareunia than before surgery. One patient had no improvement in her symptoms at all. At 3 years of follow-up, 28 had no dyspareunia, 1 was further improved from grade 2 to grade 1 symptoms, and the 30th patient again showed no improvement and remained grade 3. No patient had worse symptoms after the surgery, and no patient deteriorated in the 3 years after the operation. At 3 years of follow-up, only a small number had changed their

Table 2. Response to Vestibulectomy Compared With Lymphocyte, Mast, and Nerve Cell Counts

Patient number	Age	Early response grading	Late response grading	Lymph cells in stroma, per mm ²	Mast cells in stroma, CD117, per mm ²	Nerves in stroma, PGP9.5, per mm ²
1	28	0	0	1	90	20
2	23	1	0	4	80	29
3	36	0	0	35	40	9
4	44	1	0	21	140	40
5	59	0	0	20	230	6
6	32	0	0	6	40	16
7	42	0	0	4	60	52
8	26	0	0	9	60	14
9	57	1	0	4	80	1
10	36	0	0	23	50	13
11	55	0	0	2	30	36
12	27	0	0	85	40	9
13	54	1	0	2	80	12
14	33	0	0	34	230	14
15	33	0	0	23	90	9
16	44	0	0	22	140	26
17	37	0	0	62	220	20
18	54	0	0	100	90	5
19	28	0	0	66	150	19
20	34	0	0	9	130	4
21	23	0	0	3	170	7
22	32	0	0	74	100	4
23	22	0	0	22	130	41
24	48	0	0	6	110	24
25	35	3	3	130	160	0
26	36	0	0	10	130	0
27	26	2	1	34	60	12
28	52	0	0	10	90	3
29	34	1	0	13	60	14
30	36	0	0	8	120	26

sexual partners with no obvious predominance in the group who had a delayed benefit to the surgery. These delayed responders had not used any adjuvant therapies in that time either. When the early complete responders were compared with those who still had symptoms after the operation, there was no difference in any of the histological parameters (Table 3).

When the remaining tissue (after the punch biopsy) was examined, a dense lymphocytic infiltrate could be seen to begin abruptly in a number of patients, whereas others had few lymphocytes throughout their specimens. The dense lamina propria lymphocytic infiltrate was

Table 1. Scoring System Used to Assess Severity of Symptoms

Grade	Description
Grade 0	No dyspareunia
Grade 1	Pain prevents intercourse occasionally.
Grade 2	Pain prevents intercourse on most occasions.
Grade 3	Intercourse not possible at all (apareunia).

Adapted from Marinoff and Turner [10].

Table 3. Analysis of Response to Vestibulectomy Versus Lymphocyte, Mast, and Nerve Cell Counts

	Cure (grade 0)	Residual symptoms	<i>t</i> test	Wilcoxon unpaired
Lymphocytes, per mm ²	27.6 (1–100)	37.8 (2–130)	<i>p</i> = .5	<i>p</i> = .6
Mast cells, per mm ²	110.4 (30–230)	97.8 (60–160)	<i>p</i> = .2	<i>p</i> = .6
Nerve fibers, per mm ²	16.4 (0–52)	16.4 (0–40)	<i>p</i> = .4	<i>p</i> = .6

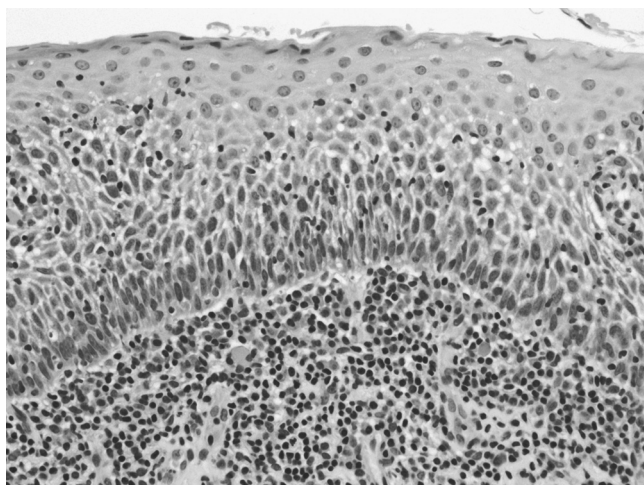


Figure 2. Marked lymphocytic infiltrate of lamina propria associated with epithelial spongiosis and lymphocytosis resembling MALT (H&E).

associated with epithelial changes of spongiosis, numerous intraepithelial lymphocytes, and occasionally intraepithelial polymorphs on the surface and plasma cells in the lamina propria (Figure 2). However, no basal layer vacuolization, apoptotic keratinocytes, extravasated red blood cells, or hemosiderin were seen in the dermis. Thus, there was no histological evidence of a dermatological condition.

Mast cells in all subjects appeared as fairly evenly distributed single cells (Figure 3). No clusters of mast cells were seen in any subject.

PGP9.5-positive fibers were rarely seen in epithelium but were common in the stroma (Figure 4). The pattern of PGP9.5 fiber staining was different from that of langerin. PGP9.5 fibers were less numerous, longer,

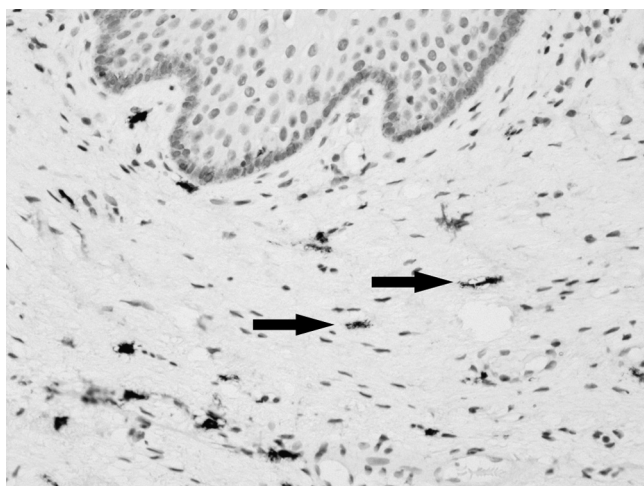


Figure 3. Even distribution of single mast cells (arrowed) found in all subjects (CD117 IPX).

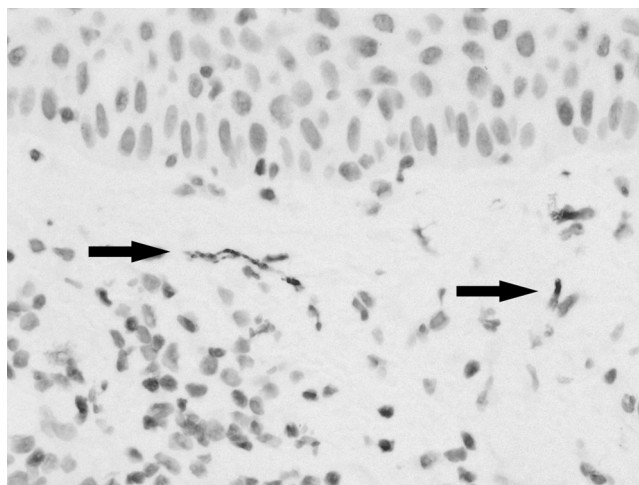


Figure 4. PGP9.5-positive fibers in lamina propria (arrowed) (IPX).

thinner, and never connected to a cell compared with langerin fibers.

DISCUSSION

The clinical results of this study are in line with findings reported in other literature [6, 7, 11] in that there was a high response rate to surgery with a low subsequent rate of symptom recurrence on follow-up. This is quite encouraging and further supports vestibulectomy as a treatment option for vestibulodynia in patients who are nonresponsive to conservative treatment.

Analysis of clinical and histological variables between subjects showed no significance or discernable trends. We are unable, therefore, to describe any histological predictive factors for response to vestibulectomy.

The histology of vulvodynia naturally separates into 2 groups as follows: lymphocyte-rich vulvodynia and lymphocyte-poor vulvodynia. It is difficult to determine the significance of any changes observed in those experiencing vulvodynia, as previous studies have demonstrated histological variance in the asymptomatic population [12, 13]. The study of vulvodynia is complicated by the difficulty in obtaining samples from a truly normal population for use as controls.

The histology of dense lymphocytic infiltrates in our study is reminiscent of mucosa-associated lymphoid tissue (MALT) in Waldeyer's ring. If this is MALT in the vestibule, lymphocyte counts related to vestibulodynia would be overwhelmed. Our understanding of MALT and its role in the vulva is incomplete, but MALT would be expected to be significant owing to contact of antigens at the site. The histopathology of vulvodynia remains an area of controversy, with findings which support both an

inflammatory [14] and a noninflammatory [15] disease state in the literature. Studies suggesting inflammation may be confounded by the amount of MALT present in the area sampled.

The appearance of the mast cell distribution of a grid of evenly spaced single mast cells was similar in all subjects. No mast cell clusters, the hallmark of many mast cell diseases, were seen. We therefore believe that mast cells are unlikely to play a role in the pathogenesis of vulvodynia, as has been previously observed [15].

Evidence that increased nerve counts are found in the vestibules of patients with vestibulodynia compared with normal subjects has been accumulating for the past 15 years [16]. One explanation of the good response to surgery is that it removes hyperinnervated tissue; however, our study did not show an association between the response of patients to surgery and the number of nerve fibers present in the stroma.

The pathogenesis of the disease remains unclear, with systemic and local involvement being considered. The high incidence of concomitant pain and lower pain threshold in those experiencing vulvodynia point toward possible central pain dysfunction [17–19]. We are unable to conclude on the site of the fundamental problem; furthermore, we see no role for cell counts in the determination of treatment response.

The limitations of our study include the small number of subjects, the single surgeon, the subjectivity of the patients' response, possible confounding factor of previous amitriptyline treatment, limited amount of tissue examined, changes in sexual practice, and placebo response.

All operations performed by the same surgeon reduced variance between patients' responses as a result of surgical factors but only gave a look at response to one surgeon's work. We could not assess whether different techniques could increase the response rate; however, there is no evidence in the literature to suggest that one surgical method is superior to any other with regard to patient outcome [2, 5, 8].

Pain is a subjective marker of disease. We have tried to standardize scoring of patients' responses to surgery using scoring systems; however, this does not eliminate the subjective nature of the response (one patient's "severe" might be another patient's "moderate").

All patients were first tried on conservative therapy including neuromodulation (amitriptyline). Because all our patients had been nonresponsive to neuromodulation, a question that arises is were we only seeing those patients with lower nerve fiber counts? It is not known whether

amitriptyline works better in those with higher nerve fiber counts.

Our study design of examining only a 3-mm punch biopsy in a larger specimen achieved objectivity. However, this was at the cost of missing any focal changes in the larger specimen.

At 3 years of follow-up, patients were asked about any changes in sexual practices, change of partners, and so on. Only a small number had changed their sexual partners with no obvious predominance in the group who had a delayed benefit to the surgery. We do not know why improvement so far from the time of surgery occurred in the delayed responders. One would expect this group of delayed responders to have a higher proportion of new partners (with different penile sizes, sexual techniques, etc.), but this was not the case. These delayed responders had not used any adjuvant therapies in that time either.

Having the patients interviewed by the surgeon involved could introduce a bias in patients reporting the success of the procedure. However, these private patients had all been experiencing vestibulodynia for a long time before surgery and certainly would have voiced their disappointment if their dyspareunia persisted.

In conclusion, vestibulectomy represents an effective treatment option, with a high curative rate in those patients who are unresponsive to conservative treatment. Histological examination of the vestibulectomy specimen including inflammatory cell, mast cell, and nerve fiber counts cannot be used to predict positive response to surgery. Thus, as the overall positive response rate to vestibulectomy is so good and the histology is not helpful in predicting outcome, there is no point in performing a biopsy of the vestibule in these patients preoperatively.

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