



Provoked vulvodynia: A peripheral neurological pain syndrome

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ABSTRACT

The main cause of vulvar pain and dyspareunia is provoked vulvodynia (PV), also known as vestibulodynia, and formerly termed “vulvar vestibulitis”. It affects 8-10% of women of all ages. Although the etiology remains an enigma, peripheral neurologic mechanism has recently been identified as a factor associated with vulvodynia. The aim of the present review is to present and discuss neuroproliferation as a main cause of PV.

Studies comparing Immunohistochemical staining of specimens from women with PV to controls, and animal models of PV, have been analysed for evidence of inflammation and neuroproliferation.

The density of nerve fibres in the vestibular stroma of women with PV was 10 times greater than that in the vestibular stroma of non-affected women controls. Moreover, the fibres penetrated the basal membrane and continued vertically, reaching close to the surface of the epithelial surface. An increase in the number of stromal mast cells has also been demonstrated in women with PV. Heparanase, discharged from mast cells, degrades the connective tissue and epithelial basement membrane, allowing the proliferating nerve fibres to penetrate the degraded epithelial basement membrane into the epithelium. This intraepithelial hyperinnervation results in local hyperesthesia characteristic of PV. In a mouse model a significant increase has been depicted in the density of nerve fibres in 40% of the mice repeatedly infected with fungal antigen. An increase in mast cells number has been depicted in a mouse model as well.

A peripheral neurologic mechanism leading to neuroproliferation has been recognised in women with PV and animal models. Future research should consider this pathogenesis.

Keywords: Provoked vulvodynia; neuroproliferation; hyperinnervation; mast cell; heparanase; vestibulectomy

INTRODUCTION

Provoked vulvodynia (PV), also called “vestibulodynia” and formerly termed “vulvar vestibulitis”, is the primary cause of vulvar pain and dyspareunia.¹ It affects 8-10% of women of all ages and significantly impairs their quality of life. Its most recognizable symptom is dyspareunia, and it is characterized by extreme symmetric sensitivity at the vestibule, close to the hymenal base. This concealed location of sensitivity has

made diagnosis difficult and has led to myths regarding the development of PV, involving hurtful statements like “it is all in your head.” Recent research has proven that the pain arises from local, organic, and vestibular sensitivity. Approximately 50% women with PV experience sensitivity only in the posterior half of the vestibule.² The others have both anterior and posterior allodynia.

The data provided in Table 1 indicate that idiopathic vulvar pain and vulvodynia have been recognized since the first century

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AD. However, between 1928 and 1976, this syndrome was not reported in the medical literature, possibly due to ignorance owing to failure in curing vulvar pain and vulvodynia. Various treatments have been attempted for PV, but so far, the effective treatment is vestibulectomy, a surgical removal of the vestibule. Although progress in the diagnosis and treatment of PV has been made,^{1,2} the etiology remains unknown. Nevertheless, a breakthrough in understanding the pathogenesis of vulvodynia was achieved with the introduction of a list of evidence-based factors associated with vulvodynia in the 2015 Consensus Terminology and Classification of Persistent Vulvar Pain and Vulvodynia (Table 2).³ Peripheral neurologic mechanism is a remarkable factor associated with vulvodynia;^{1,3} however, the trigger for this mechanism remains unknown. One hypothesis is that laxity of the uterosacral ligaments causes loss of support

to the Frankenhauser's plexus, initiating the development of the pain of PV.⁴ The present review discusses this peripheral neurologic mechanism as a cause of PV.

Peripheral neurologic findings in women with provoked vulvodynia

As shown in Table 3, several studies have reported on hyperinnervation and nerve sprouting in the stroma of the vestibule of women with PV.⁵⁻¹⁵ The proliferating nerve fibres were reportedly nociceptors.¹⁶ Further, increased vanilloid receptor VR1 (TRPV1) density caused an increase in allodynia.¹⁷ To obtain accurate and objective quantitative data regarding hyperinnervation, we applied computerized histomorphometry to images of immunohistochemically stained sections from women with PV and controls; when the immunohistochemical S100 stain was used, the density of nerve fibres in the vestibular stroma of women with PV was 10 times greater than that in the vestibular stroma of controls.⁸ Moreover, these nerve fibres showed collateral sprouting that resembled nerve-ending trauma, which develops in women with chronic inflammation or major trauma such as amputation.

We have also proposed an objective histopathological criterion for establishing PV diagnosis: the total calculated area of nerve fibres (per the same microscopic field) is 10 times higher in women with PV than in controls.⁸ Although tissue analysis is not currently required for PV diagnosis, a morphological diagnosis may be useful for research purposes.

In another study, to further characterize PV hyperinnervation, we stained the tissue excised during vestibulectomy of women with

Table 1. Idiopathic vulvar pain: Historic terminologies*	
Period, author	Term or terminology
First Century AD, Soranus	Satyriasis in females
1880, Thomas	Excessive hypersensibility of the nerves
1889, Kellogg	Sensitive points
1889, Skene	Super-sensitiveness of the vulva
1928, Kelly	Exquisitely sensitive in hymeneal ring
1976, Weisfogel	The burning vulva
1976, ISSVD	The burning vulva syndrome
1978, Dodson and Friedrich	Psychosomatic vulvovaginitis
1978, Tovell and Young	Vulvodynia or pudendagra
1983, ISSVD task force	Vulvodynia or burning vulva syndrome
1983, Friedrich	Vestibular adenitis
1983, Woodruff and Parmley	Infection of the minor vestibular glands
1986, Peckham	Focal vulvitis
1987, Friedrich	Vulvar vestibulitis syndrome
1988, McKay	Classification: Vestibulitis and dysesthetic vulvodynia
1997, Bornstein	Vestibulodynia
1999, ISSVD	Terminology: Generalized and localized vulvar dysesthesia
2001, ISSVD	Terminology: Provoked and spontaneous vulvar dysesthesia, each with subsets of generalized and localized
2003, ISSVD	2003 ISSVD terminology
2015, ISSVD, IPPS, ISSWSH	2015 consensus terminology
ISSVD: International Society for the Study of Vulvovaginal Disease, IPPS: International Pelvic Pain Society, ISSWSH: International Society for the Study of Women's Sexual Health	

Table 2. Potential factors associated with Vulvodynia as per the 2015 Consensus Terminology and Classification of Persistent Vulvar Pain and Vulvodynia

• Comorbidities and other pain syndromes (e.g., painful bladder syndrome, fibromyalgia, irritable bowel syndrome, and temporomandibular disorder [level of evidence, 2])
• Genetics [level of evidence, 2]
• Hormonal factors (e.g., pharmacologically induced) [level of evidence, 2]
• Inflammation [level of evidence, 2]
• Musculoskeletal (e.g., pelvic muscle overactivity, myofascial, and biomechanical) [level of evidence, 2]
• Neurologic mechanisms:
o Central (spinal and brain-related) [level of evidence, 2]
o Peripheral neuroproliferation [level of evidence, 2]
• Psychosocial factors (e.g., mood, interpersonal, coping roles, and sexual function) [level of evidence, 2]
• Structural defects (e.g., perineal descent) [level of evidence, 3]
*The factors are listed as per alphabetical order.

severe PV using protein gene product (PGP) 9.5.¹⁰ PGP 9.5 detects the thinnest unmyelinated sensory C fibres (<1 mm thick). We demonstrated that in women with PV, the innervation not only occurred in the stroma (Figure 1a) but also penetrated the basal membrane and continued vertically for >50% of the distance to the epithelial surface (Figure 1b), almost reaching the epithelial surface. Very thin intraepithelial nerve fibres ending within the basal layers of the epithelium were also detected. Parallel sections incubated with only secondary antiserum were not

stained. Control tissues showed no intraepithelial innervation. As such, in addition to the increased density of nociceptors, the nociceptors were located much closer to the mucosal surface. This combination can explain the severe allodynia experienced by women with PV.

Women with primary PV (identified at their first attempt at intercourse or physical contact) reportedly have a higher degree of neuroproliferation than those with secondary PV.¹¹ In such cases, the hyperinnervation may be congenital. In another study

Table 3. Studies documenting nerve fibre proliferation in provoked vulvodynia

Authors	Number of patients	Number of controls	Type of study	NFP* in patients	NFP* in controls	p value
Weström and Willén ⁵	47	6	Descriptive	44	0	<0.05
Bohm-Starke et al. ⁶	13	8	Prospective	5.65 ^a	1.125 ^a	<0.001
Tympanidis et al. ⁷	12	8	Prospective, case-control	32.9±18.5 ^b	13.0±9.2 ^b	0.0015
Bornstein et al. ⁸	40	7	Retrospective (S100 staining)	4190 µm ²	425 µm ²	0.01
Halperin et al. ⁹	24	16	Case-control	19±79.1 ^c	0 ^c	<0.0001
Bornstein et al. ¹⁰	7	7	Retrospective (PGP 9.5 staining)	Intraepithelial 2.0±0	0.71±0.488	0.001 ^d
				Stroma 2.0±0	0.14±0.378	0.001 ^d
Goetsch et al. ¹¹	10 primary	4	Neural hyperplasia	Primary 8	All negative	0.01
	10 secondary			Secondary 3	4	
Leclair et al. ¹²	42 primary	-	Retrospective neural hyperplasia (0-3)	Primary ~2.5 mean (0-3)	Secondary ~2.0 mean (0-3)	OR=3.01 p=0.02
	46 secondary					
Brokenshire et al. ¹³	23 early responders	27 late responders	Case-control	16.4 (0-40)/mm ² (early responders)	16.4 (0-52)/mm ² (late responders)	NS (only patients)
Liao et al. ¹⁴	10	7	Case-control (PGP 9.5 staining)	Tender 167% ^e	Control 100%	0.021
				Tender 169%	Non-tender 100%	0.027
	5	5	Case-control (TRPV4 axon density)	Tender 139%	Control 100%	0.011
				Tender 115%	Non-tender 100%	0.002
Tommola et al. ¹⁵	27	15	Case-control (PGP 9.5 staining)	6.3 (0.0-15.8)/mm	2.0 (0.0-12.0)/mm	0.006
			Case-control (NF2F11 staining)	63.0%	0.0%	-

NFP: Nerve fibre proliferation, PGP: Protein gene product, OR: Odds ratio, NS: Not significant, TRPV4: Transient receptor potential vanilloid-4, NF2411: Neuron specific neurofilament, SD: Standard deviation
^aValues are expressed as mean number of nerve fibres per section.
^bValues are expressed as mean ± SD. Area is expressed as µm² of immunostaining.
^cValues are expressed as mean ± SD, calculated per 10 high power fields (HPFs). Nerve fibre area was an independent variable significantly associated with provoked vulvodynia.
^dSemi-quantification, 0-3; result in mean ± SD.
^eValues are expressed in percentage increase of nerve fibre number

involving 10 women with PV, sensitive areas contained increased numbers of mechanoreceptive nociceptor axons relative to nontender foci and to that in 7 controls.¹⁴ The sensitive areas also contained increased T-cell, macrophage, and B-cell numbers. Local renin-angiotensin system protein levels were increased because more T-cells and B-cells expressed angiotensinogen. In another study, the hyperinnervation was more pronounced in foci with marked B-cell but not T-cell infiltration.¹⁵

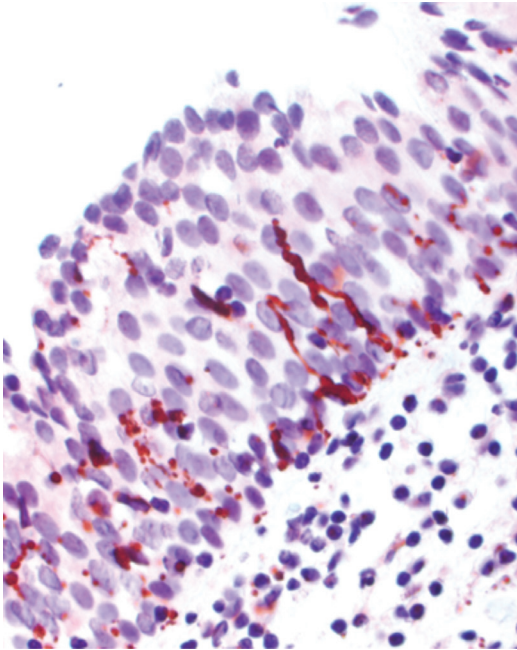


Figure 1a. PGP 9.5 stain in a tissue specimen from a woman with provoked vulvodynia showing nerve fibres intruding into the epithelium to >50% of its depth. Magnification: $\times 400$
PGP: Protein gene product

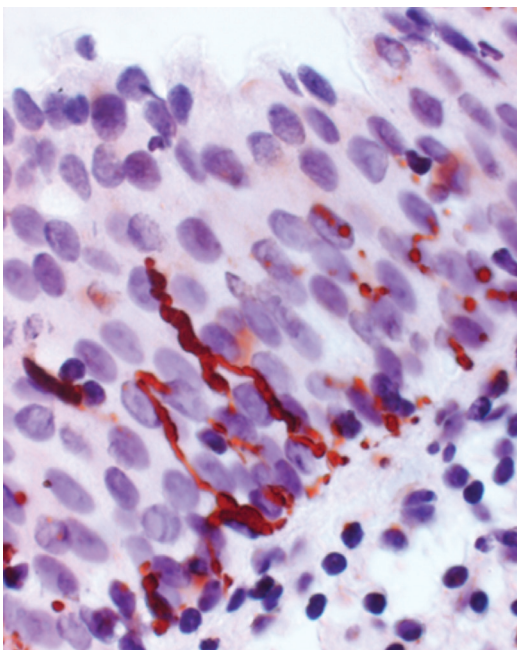


Figure 1b. Same as **Figure 1a**, but with magnification: $\times 600$

Inflammation and provoked vulvodynia

Acute inflammation may lead to chronic sensitization that persists even after the inflammation resolves.^{18,20} However, results regarding cytokine, prostaglandin E2 levels, T-cell, B-cell, natural killer cell, and macrophage numbers have been inconsistent in women with PV.²¹ Studies on proinflammatory vaginal or plasma cytokine profiles in vulvodynia are ongoing.²²⁻²⁴

Some women with vulvodynia have reported recurrent yeast infections;²⁵ this, together with the reduced systemic number of natural killer cells in women with vulvodynia compared with that in controls,²⁶ led to the hypothesis that deficiency in natural killer cell numbers is correlated with recurrent *Candida* infections in these women. Vulnerability to *Candida* infection associated with the reduced capability to stop the resulting inflammation has been described; however, a causal relationship between *Candida* infection and vulvodynia development cannot be determined via these observations.

Mast cells and hyperinnervation

Findings concerning hyperinnervation in women with PV prompted a search for agents that could enhance nerve growth. One of the potential agents may be mast cells. Mast cells discharge various mediators from their granules, such as histamine, tryptase, nerve growth factor (NGF), and bradykinin.^{27,28} Some of these directly cause pain, whereas the others cause pain by stimulating peripheral neurons. An increase in the number of mucosal mast cells has been demonstrated in women with PV,²⁹⁻³¹ and in an animal model of vulvodynia.³² We assessed women with severe PV undergoing vestibulectomy and compared them with those of a similar age undergoing vestibular biopsy due to other conditions.³³ Mast cell detection was conducted not only using Giemsa stain (Figure 2) but also immunostaining for c-KIT (Figure 3) and mast cell tryptase.^{8,10,33} c-KIT is a membrane proto-oncogene encoding a type 3 tyrosine kinase protein receptor. In contrast, tryptase is a specific protein present in granules secreted from mast cells. Positive tryptase staining indicates that the mast cells were activated and degranulated. Using both c-KIT and tryptase staining, we observed a significant number of mast cells in the superficial dermis, mostly activated, in women with PV compared to that in controls. An increase in the number of stromal mast cells has also been demonstrated in animal models of PV.²⁹⁻³¹ In another study, although the number of mast cells was not increased, they were activated in sensitive vestibular areas, as indicated via chymase discharge.¹⁴ Chymase is one of three peptidases capable of converting angiotensin I to angiotensin II, which is a promoter of axon sprouting and regeneration.³⁴

While some studies have not demonstrated an increase in mast cell numbers in women with PV, these studies were limited

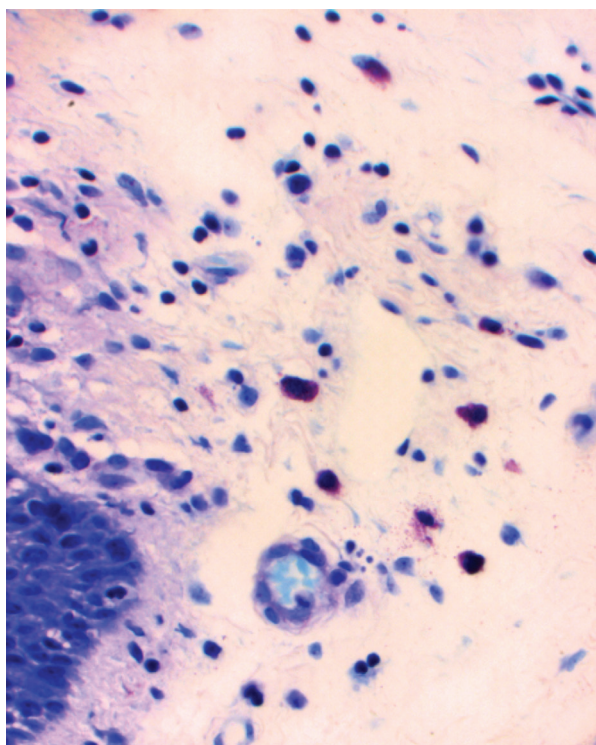


Figure 2. Giemsa staining of a specimen from a woman with provoked vulvodynia showing mast cells in the subepithelium. Magnification: $\times 600$

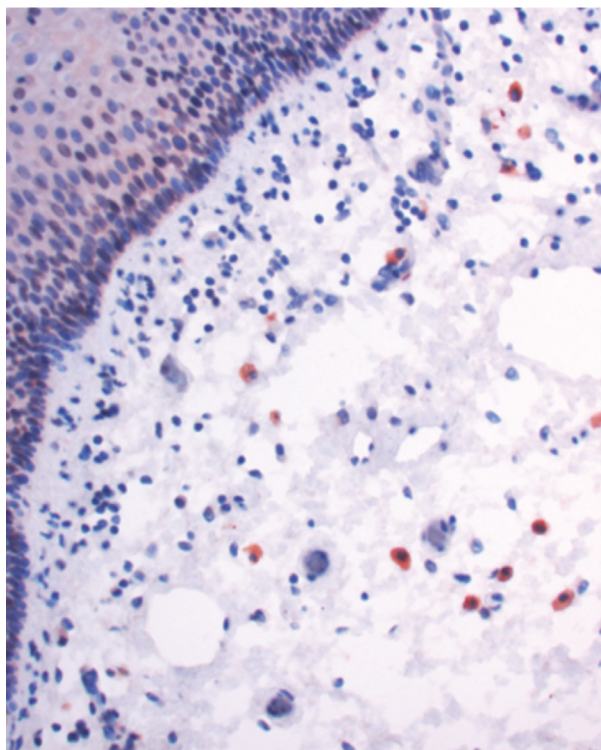


Figure 3a. CD117 (c-KIT) staining in a specimen from a woman with provoked vulvodynia showing mast cells in the subepithelium, among other inflammatory cells. Magnification: $\times 400$
 c-KIT: gene encoding the receptor tyrosine kinase protein known as tyrosine-protein kinase, CD117: Cluster of differentiation 117

because they only used Giemsa staining or c-KIT staining but not a marker of mast cell activation, such as tryptase.³⁵ Another limitation was that these studies sampled only one fixed area of the vestibule, regardless of whether that focus was sensitive. In another study that compared tender and non-tender foci, mast cell numbers were significantly increased in the tender foci.¹¹

Heparanase (HPSE) is another enzyme secreted by mast cell granules.³⁶ We immunostained surgical specimens from women undergoing vestibulectomy and from controls for HPSE and demonstrated the presence of this enzyme in the subepithelial cells of the vestibule in women with PV (Figure 4).^{10,33} HPSE is a mammalian endoglycosidase (endo- α -D-glucuronidase) that can cleave heparan sulfate, a proteoglycan that stabilizes the connective tissues,³⁶ thereby releasing heparin-binding growth factors, enzymes, and plasma proteins that further weaken the extracellular matrix. Conceivably, HPSE degrades the connective tissue and epithelial basement membrane, allowing proliferating nerve fibres to penetrate the degraded epithelial basement membrane to the epithelial surface. This intraepithelial hyperinnervation results in local hyperesthesia characteristic of PV.

This proposed mechanism raised the hypothesis that blocking HPSE activity might halt PV development. Such blockade may be achieved by an anti-HPSE agent such as unfractionated or low-molecular-weight heparin; heparin is reportedly a powerful inhibitor of the activity of HPSE on antithrombin-binding

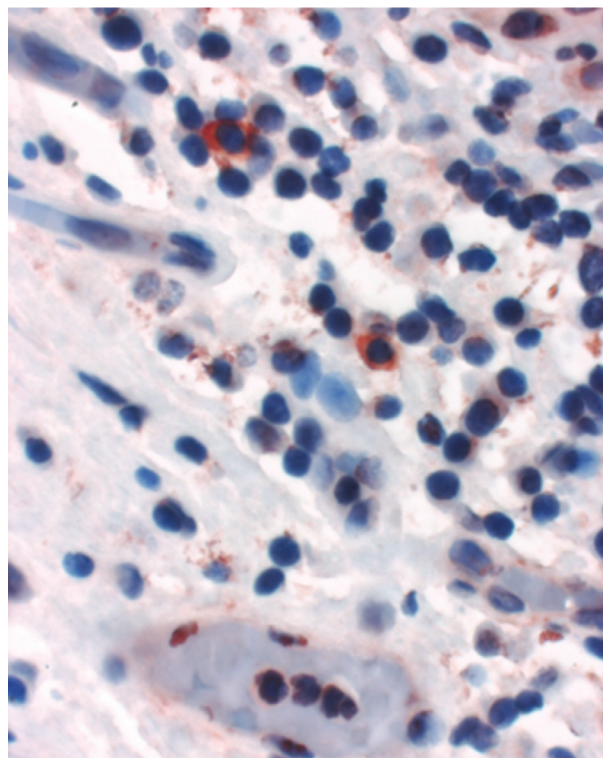


Figure 3b. Same as **Figure 3a**, Magnification: $\times 600$

oligosaccharides.³⁷ We therefore conducted a randomized controlled trial of an anti-HPSE agent (enoxaparin) for the treatment of PV.³³ This treatment led to a greater reduction of clinical and subjective pain scores in women treated with

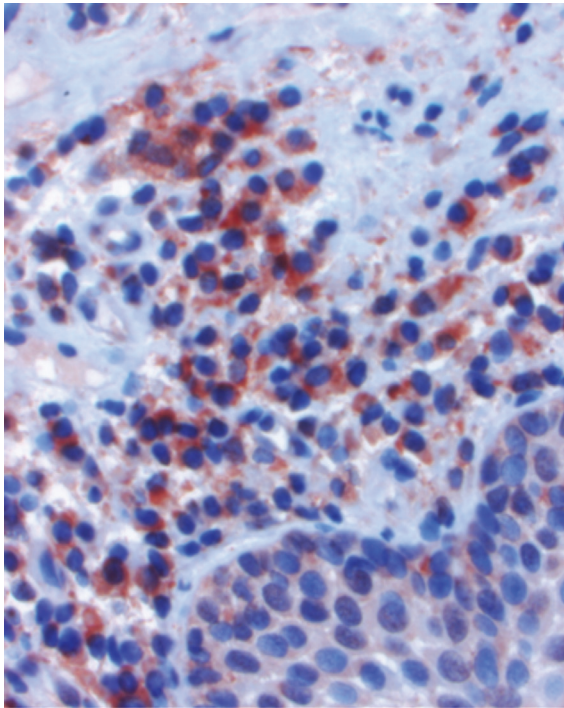


Figure 4a. Heparanase expression in a specimen from a woman with provoked vulvodynia. Positive cytoplasmic staining is seen in the subepithelial layer, close to the epithelial basement membrane. Magnification: $\times 400$

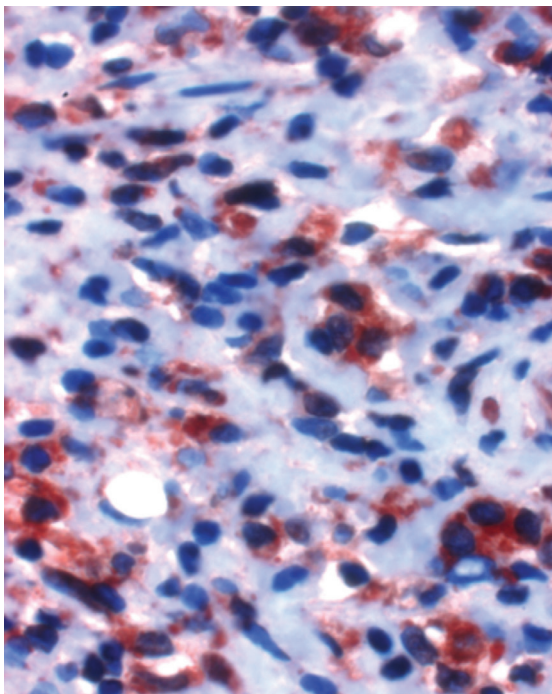


Figure 4b. Same as Figure 4a, Magnification: $\times 600$

enoxaparin than in a placebo-controlled group. Pain reduction was correlated with a diminishing number of intraepithelial-free nerve fibres in the enoxaparin group but not in the placebo group.³³ The anti-HPSE therapy may have worked by strengthening the stroma, thereby inhibiting neuronal sprouting and proliferation.

Genetic predisposition to neuroproliferation and provoked vulvodynia

Consequent to our studies associating mast cell increase and activation, HPSE excretion, and hyperinnervation with vulvodynia, we examined a possible genetic predisposition of women with PV to these phenomena.³⁸ We examined several single nucleotide polymorphisms (SNPs) in *TRPV1*, *NGF*, and *HPSE* genes in 65 women with moderate or severe primary PV, comparing them to 126 healthy, ethnically matched controls. Genotyping analyses revealed a novel, statistically significant and elevated prevalence of SNPs in *TRPV1* and in the promoter region of *NGF* in women with PV than in controls. The amino acid substitution resulting from SNPs in *TRPV1* modifies the functional properties of the channel and induces increased *TRPV1* protein expression due to an increased copy number.³⁹ Women with PV exhibit lower thresholds for heat pain as well as increased expression of the receptor.^{17,40}

Our finding of a polymorphism in *NGF* is further supported by another study that demonstrated increased blood levels of *NGF* in response to *Candida* antigen stimulation in women with vulvodynia than in controls.⁴¹ In another study, an increase in *NGF* levels was detected in vestibular tissues from women with PV, but only in areas with abundant B-cells.¹⁵ *NGF* plays a role in the development of peripheral nerves by promoting neuronal growth and survival.^{42,43} *NGF* produces pain directly by acting on the nerves and indirectly by affecting mast cells.⁴⁴ *NGF* also increases *TRPV1* expression.⁴⁵ Additionally, hyperalgesia was shown to be extensively mediated by *NGF*-positive regulation of the *TRPV1*-promoter system via Sp1 and Sp4 transcription factors.^{34,46}

Animal models of provoked vulvodynia

Animal models of PV have been developed to monitor the progression of PV. Hyperinnervation has been elicited in the vestibule of mice and rodents in which vulvodynia was induced via various topical vestibular stimuli. Hyperinnervation persisted even after the initial local inflammatory reaction subsided. In an established mouse model,⁴⁷ a significant increase in the density of nerve fibres, immunostained by PGP 9.5, developed in 40% of the mice repeatedly infected with *Candida albicans* strain SC5314 or by zymosan (a mixture of fungal antigens). There was

a 300-400% increase in the number of nerve fibres in the mice that developed hyperinnervation. Furthermore, an elevation in the density of peptidergic fibres, as revealed by calcitonin gene-related peptide (CGRP) levels was depicted in the allodynic group. The hyperinnervation was observed throughout the lamina propria, with some thin nerves penetrating the lamina propria beneath the epithelium. An increase in sympathetic innervation, as revealed by the vesicular monoamine transporter 2 levels, was shown in allodynic mice. No inflammation was noted in the tissues. The finding of hyperinnervation and allodynia in only 40% of the treated mice was attributed to the variable genetic predisposition of the mice to vulvodynia.

In another mouse vulvodynia model, allodynia was achieved through topical application of the proinflammatory hapten oxazolone.⁴⁸ In oxazolone-sensitized female ND4 Swiss mice, a single labial oxazolone challenge provoked vulvar mechanical hyperalgesia for up to 24 h. This was accompanied by neutrophil influx, increased inflammatory cytokine gene expression, and increased density of cutaneous CGRP and PGP9.5-immunostained nerve fibres. Moreover, a 3-day consecutive oxazolone challenge caused vulvar hyperalgesia that was persistent for up to 5 days, even after the initial local inflammatory reaction subsided.

In another study performed by the same group,⁴⁹ oxazolone-challenged mice developed significant tactile hypersensitivity that persisted for >3 weeks, even after exposure to vestibular allergen was discontinued. The hypersensitivity only disappeared 6 weeks after the exposure. In the allergen-challenged mice, >3-fold increase in cutaneous vestibular CGRP nerve intensity was detected compared with that in vehicle-challenged and untreated controls. A 10-fold increase was noted in RNA expression for transcripts encoding *NGF* and the nerve/mast cell synapse marker cell adhesion molecule 1. This indicates that the local environment supported neuronal growth and maintenance. These sites were characterized by increases in mast cell numbers as well as regulatory CD4, CD25, FoxP3, and T cell infiltration. Local depletion of mast cells by intralabial administration of the mast cell degranulation compound 48/80 led to reduction in both nerve density and tactile sensitivity.

In a placebo-controlled study,⁵⁰ an increased number of CD68-immunoreactive cells was demonstrated in the distal vagina of C57BL/6 mice after microinjection of complete Freund's adjuvant (CFA) versus saline. Inflammation and innervation density were assessed at 7 and 28 days following the single administration or 14 days following the repeated administration of CFA or saline. Increased mast cell numbers were demonstrated. Hyperinnervation was evident in CFA mice via staining with PGP 9.5, CGRP, substance P, vasoactive intestinal peptide, and neuropeptide Y.

DISCUSSION

The 2015 Consensus Terminology and Classification of Persistent Vulvar Pain and Vulvodynia regards the peripheral neurologic mechanism as one of several factors associated with vulvodynia.³ The data reviewed in this study support the hypothesis that neuropathy or hyperinnervation is the actual cause of allodynia in women with PV. Several groups, including ours, have successfully shown that neuroproliferation developed only in women with PV and not in healthy women. Subsequently, removal of the hyperinnervated tissue may explain the successful outcome of the surgical excision of the vestibule-vestibulectomy, in women with PV. Non-invasive treatment approaches, aiming at preventing or reducing the effect of hyperinnervation may be developed in the future.

In animal models, hyperinnervation persisted for many days, even after the topical application of an anti-irritant and the initial local inflammatory reaction subsided. In terms of the etiopathogenesis of neuroproliferation, several researchers have demonstrated a correlation between hyperinnervation and the increased presence of mast cells in local tissues and their activation in women with PV. Consequently, we hypothesized that mast cell activation triggers the increase in the numbers of nerve fibres, with granule discharge of various mediators such as *NGF*, histamine, bradykinin, tryptase, prostaglandins, serotonin, and leukotriene. These mediators can sensitize C fibres and induce their proliferation, causing pain. *NGF* may be secreted by both mast cells and neurons and can induce neuronal growth and reduce the subsequent neuronal firing threshold, causing increased pain perception.²⁹ The SNPs in *NGF* and TRPV1 demonstrated by us may be the reason for the uncontrolled production of *NGF*, leading to excessive neuroproliferation in some women with PV. This is a vicious cycle as *NGF* also attracts mast cells and stimulates its own synthesis via *NGF* receptors on the mast cell membranes and nerves alike. Nerve sprouting and penetration of the newly formed nerve fibres into the epithelium is then made possible by HPSE.

Alternatively, another potential etiological mechanism for the increased mast cell numbers in women with PV is neurogenic inflammation. In this regard, stimulated nociceptors cause neurons to discharge neuropeptides that draw and stimulate local mast cells. Indeed, electron microscopic studies have shown that mast cells are closely related to sensory nerve fibres containing neuropeptides that induce mast cell degranulation. A similar mechanism has been reported in interstitial cystitis/bladder pain syndrome, a condition that may occur simultaneously with PV.

In addition, pelvic organ prolapse has been associated with vulvodynia.⁵¹ Further studies may explore the association between

pelvic organ prolapse, laxity of the uterosacral ligaments and PV, and possibly the peripheral neurologic mechanism of PV.

CONCLUSION

In conclusion, vestibular neuroproliferation has been repeatedly depicted to be a substantial associated factor of PV.

ETHICS

Peer-review: Externally peer-review.

DISCLOSURES

Conflict of Interest: There are no conflicts of interest to declare.

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