Enoxaparin Treatment for Vulvodynia

A Randomized Controlled Trial

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OBJECTIVE: To estimate the effectiveness of enoxaparin—a low-molecular-weight heparin with antiheparanase properties—in treating localized provoked vulvodynia.

METHODS: Forty women with severe localized provoked vulvodynia were randomly and blindly assigned to self-administer either 40 mg enoxaparin or saline subcutaneously for 90 days. Dyspareunia and local sensitivity were evaluated before, at the end, and 90 days after treatment. The most painful focus was biopsied at the beginning of the study and a parallel site at the end of study for mast cells, PGP 9.5 nerve fiber staining, and heparanase quantification.

RESULTS: The enoxaparin-treated women showed a greater reduction in vestibular sensitivity at the end of treatment and 3 months later (29.6% compared with 11.2%, P=.004). Seventy-five percent (15 of 20) of them reported more than 20% pain reduction compared with 27.8% (five of 18) in the placebo group (P=.004). Seven enoxaparin-treated women compared with three in the placebo group had almost painless intercourse at the end of the study. In women who had improvement of sensitivity at the site parallel to the original biopsy site, there was a histologically documented reduction in the number of intraepithelial-free nerve fibers in the enoxaparin group.

CONCLUSION: Enoxaparin reduced the vestibular sensitivity and dyspareunia, concomitant with a reduction in intraepithelial free nerve fibers, in women with localized provoked vulvodynia.

CLINICAL TRIAL REGISTRATION: ClinicalTrials.gov, www.clinicaltrials.gov, NCT00874484.

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LEVEL OF EVIDENCE: I

Vulvodynia is a cause of dyspareunia in women. This underdiagnosed condition inflicts physical pain and emotional distress on millions. During the course of their lifetime, 16% of women suffer for at least 3 months from burning, knife-like pain or pain on contact in the vulvar area.[1] In southeast Michigan, the weighted prevalence of vulvodynia was 8.3% (95% confidence interval 7.0–9.8), remained stable through age 70 years, and declined thereafter.[2]

Vulvodynia is associated with an economic burden to both individuals and society, which is composed of the cost of direct and indirect health care services. It is also related to a relatively low quality of life.[3]

The etiology of vulvodynia remains an enigma, acceptable diagnostic criteria remain subjective, and treatment methods are still empirically based. In a recent review of published studies, success rates for medical treatments of vulvodynia vary between 13% and 67%. This compares with 61–94% for surgical treatment and 35–83% for behavioral treatments.[4] There have been very few randomized placebo-controlled studies on treatment of vulvodynia.[5–8] In two previous studies from our group, vestibular tissues from women with localized provoked vulvodynia showed a significant increase in vestibular mast cells, in subepithelial heparanase activity, and intraepithelial neuroproliferation compared with healthy women.[9,10]

Heparanase is a mammalian endoglycosidase (endo-[beta]-D-glucuronidase) excreted from mast cells.[11] It is capable of degrading heparan sulfate, a

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proteoglycan localized in the cell surface and extracellular matrix that helps to maintain the integrity of connective tissues in many organs.[12] Heparanase cleaves heparan sulfate and releases heparin-binding growth factors, enzymes, and plasma proteins[11] that consequently weaken the composition and structural integrity of the extracellular matrix. In addition, heparanase directly activates endothelial cells and elicits angiogenic responses.[13] Therefore, we suggest that heparanase may be involved in degradation of the vestibular stroma, thus allowing for penetration of nerve fibers through the epithelial basement membrane. It is also possible that nerve fiber proliferation is the result of release of nerve growth factor from mast cells. Intraepithelial hyperinnervation in the vestibule area may be the cause of hypersensitivity to local touch in women with vulvodynia.

The increased presence of heparanase in the vestibule of women with vulvodynia raises the possibility of treating vulvodynia by blocking its enzyme activity. Heparin and enoxaparin, a low-molecular-weight heparin, were found to be powerful inhibitors of heparanase activity.[14,15]

The present study was a clinical and histologic evaluation of the effectiveness of the antiheparanase medication, enoxaparin, in treating localized provoked vulvodynia.

PATIENTS AND METHODS

The study was a randomized; double-masked placebo-controlled study and was approved by the institutional review board (Western Galilee Hospital-Nahariya Helsinki Committee) and registered at www.ClinicalTrials.gov (NCT00874484). Every woman participating in the study signed an informed consent.

Women were considered for enrollment if they were aged 18–50 years, desired vaginal intercourse, had an available sexual partner, met Friedrich's first two criteria[16] (Table 1) for vulvar vestibular syndrome, or suffered from levels II or III dyspareunia according to Marinoff[17] (Table 2). Enrollment was limited to women using an effective form of contraception, women who were postmenopausal, or had been surgically sterilized. Women were excluded if they had generalized vulvodynia (constant vulvar pain, unrelated to provocation), had known hypersensitivity to heparin or enoxaparin, a positive pregnancy test, were pregnant or lactating, or planned to become pregnant during the study period. Women were also excluded from the study if they were chronic users of narcotics, had hepatic disease or clinically significant abnormal liver function tests, anticipated not being available for the entire duration of the study, had any coexisting significant medical condition that was likely to interfere with study procedures (eg, cardiovascular, hematologic, central nervous system, pulmonary, renal), were scheduled to undergo any invasive surgical procedures during the study period, had a history of gastrointestinal or any bleeding, abnormal coagulation studies, or were found to have a systolic blood pressure greater than 180 mm Hg or less than 90 mm Hg at screening.

Table 1. Friedrich's Criteria for Diagnosing Vulvar Vestibulitis Syndrome

No.	Criterion
1	Severe pain in the vulvar vestibule on touch or attempted vaginal entry
2	Tenderness to pressure localized within the vulvar vestibule
3	Vulvar erythema of various degrees

Data from Friedrich EG Jr. Vulvar vestibulitis syndrome. J Reprod Med 1987;32:110–4.

Table 2. Levels of Dyspareunia

Level	Definition
I	Causes discomfort but does not prevent sexual intercourse
II	Sometimes prevents sexual intercourse
III	Completely prevents sexual intercourse

This table was published in Marinoff SC, Turner ML. Vulvar vestibulitis syndrome: an overview. Am J Obstet Gynecol 1991;165:1228–33. Copyright Elsevier.

Forty women with severe localized provoked vulvodynia were recruited to the study. They were randomly and blindly assigned into one of two groups to receive either enoxaparin or placebo. The allocation sequence was generated at random without restrictions by an uninvolved assistant and kept in sealed envelopes. The participants and the gynecologists who evaluated them were blinded to the group assigned at all stages of the study. The solutions to be self-injected were prepared by the hospital's pharmacist in similar, numbered, and ready-to-inject vials. Before treatment, the women were questioned about possible inclusion and exclusion criteria, filled in questionnaires for those with localized provoked vulvodynia, and underwent a physical examination, complete blood count, liver function tests, urine analysis, pregnancy test, and blood pressure measurement.

The questionnaires that were used for that study included a modified Brief Pain Inventory[18] and short form McGill Pain Questionnaire[19] and the International Society for the Study of Vulvovaginal Disease vulvo-

dynia questionnaire.[20] All were translated into Hebrew. They were validated at a previous study of our group [7]

Women found suitable for the study underwent a Q-tip test to confirm the diagnosis of vestibulodynia and for scoring the sensitivity in seven foci around the vestibule. They also underwent colposcopic examination of the cervix and vulva. A biopsy was then taken from the most painful focus of the vestibule for evaluation of the number of mast cells, amount and localization of nerve fibers, and the presence of heparanase. After completing evaluation, the women started daily self-administration of either 40 mg enoxaparin or the same volume of saline (for the placebo group) subcutaneously in the abdominal region for 90 days. In addition, all women were instructed to consume a "low oxalate diet" with calcium citrate supplements for the duration of the treatment phase. This was done to give all participants a similar background nutrition and because the diet has been regarded in the past as a treatment of localized provoked vulvodynia.[21]

Clinical pain scores and vulvar sensitivity by Q-tip test were repeated at the end of the treatment phase and 3 months later; these were compared with the clinical pain scores at the beginning of the study. Subjective evaluation was carried out by comparing personal data from questionnaires filled in 90 days after completion of treatment (end of study) with similar questionnaires filled in before treatment.

Clinical evaluation was performed by two experienced vulvar experts blinded to the patient's treatment group assignment using the pain intensity scale (the 11-point [0–10] pain intensity numerical rating scale)[22] in seven foci throughout the vestibule (the Q-tip test) and by comparing patients' responses to questionnaires evaluating pain during intercourse or other activities (riding a bicycle or horse), before treatment, and at the end of the study. The effect of treatment on heparanase and neuroproliferation was measured by comparing immunostaining of biopsies taken from the most painful focus at the beginning of the study with those taken from a symmetrical vestibular site at the end of the study.

All patients were instructed to fill in a log book with side effects, including occasions with easy bruising and abnormal bleeding. The log book was examined during each visit. In addition, the women were given a phone number of the study coordinator with the request to inform him of any side effect as soon as it occurs. In addition, every 2 weeks, the coordinator has initiated a phone call to every patient to find out of any untoward side effects. Blood tests for activated partial prothrombin time and antifactor Xa level were drawn 1 and 6 weeks after the initiation of treatment to rule out the therapeutic effect of the low-molecular-dose heparin. Complete blood count for platelets count and hemoglobin level was drawn 1 and 6 weeks and 3 months after the initiation of treatment.

Vulvar biopsy was performed using a "Tishler-Kevorkian Cervical Biopsy Punch, Surgical Gyne." Specimens were immediately immersed in 4% buffered formalin. Paraffin blocks were made for each vestibular specimen. A 5-micrometer-thick section of each block was stained by hematoxylin and eosin. In addition, parallel sections were stained by Giemsa for mast cell detection. Sections were analyzed by light microscopy at 10×10 and 10×40 magnifications. Immunostained specimens were examined by a senior pathologist in a blinded fashion, that is, without prior information as to whether the examined slide belonged to a patient treated with enoxaparin or placebo.

Parallel, serial 5-micrometer sections were cut from the same paraffin blocks, deparaffinized, and rehydrated with xylene and alcohol in decreasing concentrations. The specimens were adhered to the slide and microwaved for 20 minutes. We then rehydrated the slide in citrate buffer (pH 6) and stained for CD117, PGP 9.5, and heparanase. Endogenous peroxidase produced by the inflammatory cells was blocked by incubation with 3% hydrogen peroxide at room temperature for 10 minutes. The sections were incubated in 10% goat serum for 60 minutes and then incubated with the primary antibody for 1 hour at room temperature. Primary antibodies used were CD117 in a 1:50 dilution and PGP 9.5 diluted 1:100. Histostatin plus kit 3-amino-9-ethylcarbazole substrate was used for streptavidin–biotin peroxidase signaling. Positive cells, granules, and nerve fibers

Table 3. Semiquantitative Score of Histologic Measurements in Localized Provoked Vulvodynia

No. of Mast Cells Stained by CD117	Stromal Free Nerve Fiber Density Stained by PGP 9.5	Intracytoplasmic Heparanase Density	Intraepithelial Nerve Fiber Density by PGP9.5
0=none	1=low	0=none	0=none
1=1–8	2=intermediate	1=low	1=focally positive
2=9–16	3=high	2=intermediate	2=positive
3=more than 16		3=high	3=highly positive

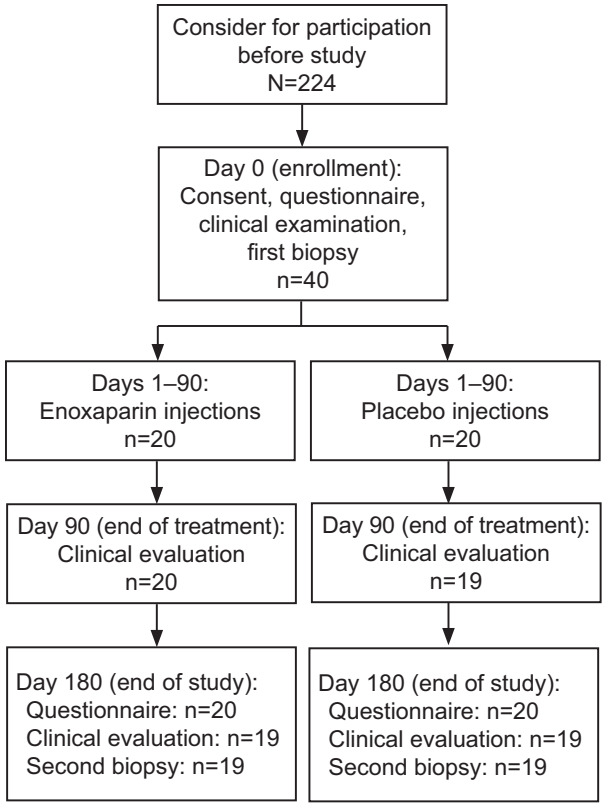

Fig. 1. Women with localized provoked vulvodynia participating in the study.

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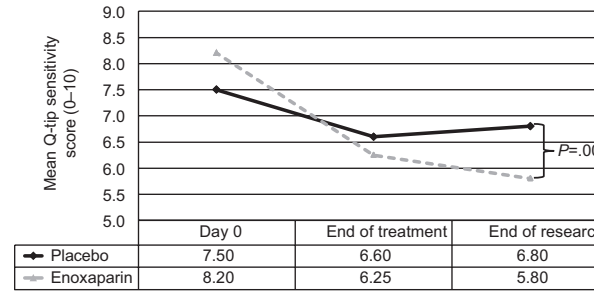

Fig. 2. Q-tip sensitivity scores at enrollment, end of treatment, and end of study.

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stained brown on the background of the blue hematoxylin counterstain.

Formalin-fixed, paraffin-embedded 5-micrometer sections were stained for heparanase using the EnVision protocol.[23] Briefly, slides were deparaffinized, rehydrated, and endogenous peroxidase activity was quenched (30 minutes) by 3% hydrogen peroxide in methanol. Slides were then subjected to antigen retrieval by boiling (20 minutes) in 10 mM citrate buffer, pH 6. Slides were incubated with 10% normal goat serum in phosphate-buffered saline for 60 minutes to block nonspecific binding and incubated (20 hours, 4°C) with antiheparanase 733 antibodies diluted 1:100 in blocking solution. Slides were extensively washed with phosphate-buffered saline and incubated with a secondary reagent according to the manufacturer's instructions. After additional washes, color was developed with the AEC reagent and sections were counterstained with hematoxylin and mounted. The sections were examined by a pathologist blind to the patients' clinical data and were scored according to the intensity of staining (0, no staining; 1, low; 2, intermediate; 3, high staining). Quality control was assured by using specimens that were similarly stained with preimmune serum or by applying the described procedure but without the primary antibody with no detectable staining.

Representative 10×10 and 10×40 microscopic fields were sampled at similar depths. The intensity of mast cells in the Giemsa, CD117, heparanase, and innervation in vestibular tissue were established by a semiquantitative score as shown in Table 3.

In a published meta-analysis of chronic pain studies, a 30% reduction in pain intensity was found to be statistically significant.[24] We calculated the power of the study using two calculations of the one-tailed *t* test: first considering the treatment group alone and second in comparison with the control group. $P<.05$ was considered significant.

If treatment with enoxaparin results in a 30% reduction of pain for the 20 cases, the study will have a power of 97.4% to yield a statistically significant result (based on a mean reduction of 2.0 and standard deviation of 2.4). In comparing pretreatment and posttreatment pain between the 20 case participants

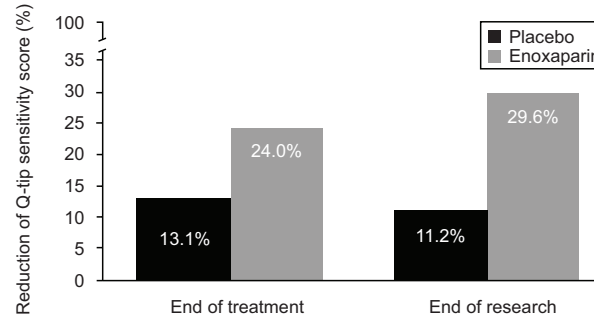

Fig. 3. Reduction of Q-tip sensitivity score (%) among women at beginning of treatment, end of treatment, and end of study.

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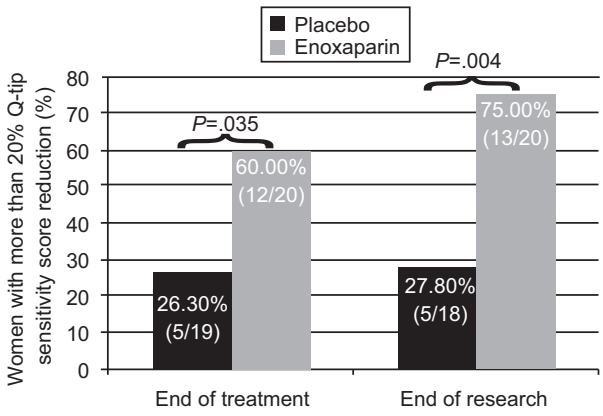

Fig. 4. Percentage of women with localized provoked vulvodynia who experienced a reduction of more than 20% in their Q-tip sensitivity score between beginning of treatment and end of treatment or end of study.

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and 20 control participants, we assumed the reduction in pain to be 1.75 points greater in the treatment group than in the control group. As such, the study will have a power of 83.9% (based on standard deviation estimates of 2.5 and 1.50, respectively) to yield a statistically significant result. The statistical power will be reduced by 15% when nonparametric tests are used.

Quantitative data were described by mean and standard deviation, median, and range. Qualitative data were described by frequencies and percentages (ordinal variables were described as quantitative data and qualitative data).

We used independent sample *t* test or Wilcoxon rank-sum test for comparison of quantity variables between groups, as appropriate. The comparison of qualitative variables (including ordinal variables) was done by χ^2 test or Fisher's exact test, as appropriate.

In each group, changes over time were examined by paired sample *t* test or Wilcoxon signed rank test for quantity variables and by Wilcoxon signed rank test for ordinal variables. Differences of pain evaluation were calculated over time and the groups compared by independent sample *t* test or Wilcoxon rank-sum test, as appropriate. The correlation between the reduction of sensitivity at the site parallel to the original biopsy site and the reduction of number of intraepithelial nerve fibers in the first and second biopsies was examined by Spearman's correlation coefficient test.

RESULTS

As shown in Figure 1, 224 women from the vulvodynia referral clinics of two of the authors (JB and LA) were offered enrollment to the study. Enrollment lasted from July 1, 2009, to October 30, 2010. Sixty-three were found to have an exclusion criterion; 121 other women refused to participate for the following reasons: fear of undergoing biopsies of the vestibule (64 women), difficulty in transportation to the study center (17 women), preference of other treatments (40 women), and 40 women were recruited (ages 19–39 years), 20 in each group. Figure 1 depicts that one woman in the placebo group dropped out at the midtreatment phase. Another woman in the placebo group refused to have a clinical evaluation and a biopsy at the end of the study. One woman in the enoxaparin group refused to undergo the final biopsy. The groups were comparable at the onset of study in terms of age (P=.35), marital status (P=.451), intimate relations (P=.451), living with a partner (P>.99), sexual activity (P=.748) and pain at first intercourse

Table 4. Subjective Evaluation of Pain Reduction From Before to the End of the Study

Situations Examined	% Reduction in Placebo Group (Wilcoxon Signed Rank Test)	% Reduction in Enoxaparin Group (Wilcoxon Signed Rank Test)	*P* Compared Between Groups	Test to Compare Between Groups
During penetration	12.6 (P=.053)	15.1 (P=.004)	.196	Wilcoxon rank sum
During intercourse	4.4 (P=.238)	28.9 (P=.006)	.057	*T* test
After intercourse	44.8 (P=.003)	38.6 (P<.001)	.296	*t* test
Vestibular touch with a finger	28.1 (P=.016)	40.7 (P=.003)	.367	Wilcoxon rank sum
Tampon insertion	0	31.1 (P=.048)	.115	Wilcoxon rank sum
Wearing tight pants	18.3 (P=.282)	36.8 (P=.094)	.404	Wilcoxon rank sum
Riding a bike	29.6 (P=.188)	22.9 (P=.50)	.089	Wilcoxon rank sum
Crossed legs sitting	46.7 (P=.102)	26.9 (P=.352)	.178	Wilcoxon rank sum
Voiding without intercourse	38.1 (P=.133)	47.6 (P=.250)	.400	Wilcoxon rank sum
Voiding after intercourse	32.4 (P=.077)	52.2 (P<.001)	<.111	*t* test

Table 5. Self-Estimation of Pain Intensity Before and at the End of Study

	Placebo			Enoxaparin		
Pain Intensity	Beginning	End	*P*	Beginning	End	*P*
Pain so intense unable to have full intercourse with penetration	5 (26)	2 (10.5)	.055 2-sided	3 (15)	4 (20)	.037 2-sided
Pain so intense must stop intercourse	6 (31.6)	5 (26.3)	.027	9 (45)	3 (15)	.019
Intense pain but able to have full intercourse with penetration	8 (42.1)	6 (33.3)	1-sided*	8 (40)	5 (25)	1-sided*
Almost painless intercourse	0 (0)	3 (15.8)		0 (0)	7 (35)	
Painless intercourse	0 (0)	0 (0)		0 (0)	0 (0)	
Not sexually active lately	0 (0)	2 (10.5)		0 (0)	1 (5)	

Data are n (%) unless otherwise specified.
* Wilcoxon signed rank test.

(*P*=.451). More women in the placebo group had level III dyspareunia but the difference was not significant (36.8% compared with 15%, *P*=.155). The two groups were comparable in terms of vulvodynia symptoms at baseline: feeling of constant irritation in the vaginal region (*P*>.99), pain or burning sensation without prior intercourse (*P*=.767), frequency of intercourse last 2 months (*P*=.134), frequency of intercourse since first painful intercourse (*P*=.304), and self-estimation of pain (*P*=.830). There were no significant treatment side effects.

There was good treatment compliance in both groups. In the enoxaparin group, 98.2% of the injections were administered, and in the placebo group, 96.6%; these differences are not statistically significant (*P*=.496) (data not tabulated). Figures 2 and 3 show the clinically evaluated scores of vestibular sensitivity in each time period using the Q-tip test and the percentage of pain reduction at the end of treatment and 3 months later. The enoxaparin group showed a greater reduction in Q-tip pain scores at the end of treatment than did the placebo group (24% compared with 13.1%, respectively, *P*=.018) and again 3 months later (29.6% compared with 11.2%, respectively, *P*=.004). At the end of the study (3 months after completion of treatment), 75% (15 of 20) of women in the enoxaparin group compared with 27.8% (five of 18) of women in the placebo group had more than 20% pain reduction (*P*=.004) (Fig. 4).

Data collected from questionnaires at the beginning and at the end of the study were compared with respect to vulvar pain and discomfort. Table 4 shows that women in the enoxaparin group reported a greater percentage of pain reduction compared with the placebo group during intercourse (28.9% compared with 4.4%, *P*=.057), tampon insertion (31.1% compared with 0%, *P*=.115), and voiding after intercourse (52.2% compared with 32.4%, *P*<.111); however, these differences were not statistically significant.

Table 5 shows pain intensity during intercourse at the beginning of the study and 3 months after end of treatment in each group. Seven women in the enoxaparin group compared with three of the placebo group had nearly painless intercourse at the end of the study. Altogether, 47.4% (nine of 19) of the enoxaparin group compared with only 25% (four of 16) of the placebo group reported improvement of pain during intercourse (*P*=.156). After immunostaining, the number of mast cells shown in the Giemsa stain, CD117, intracytoplasmatic heparanase, stromal, and intraepithelial-free nerve fiber density in vestibular tissue was evaluated by a semiquantitative score as described in Table 3.

Figures 5 and 6 show biopsies taken from one patient of the enoxaparin-treated group, demonstrating a decrease in intraepithelial nerve fibers in the biopsy taken at the end of the study compared with the biopsy at the beginning. As shown in Table 6, the

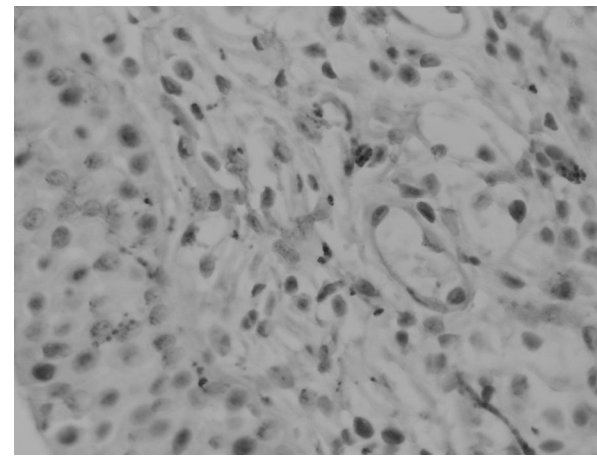

Fig. 5. PGP9.5 staining for unmyelinated nerve fibers before beginning of treatment (original magnification 10×40).
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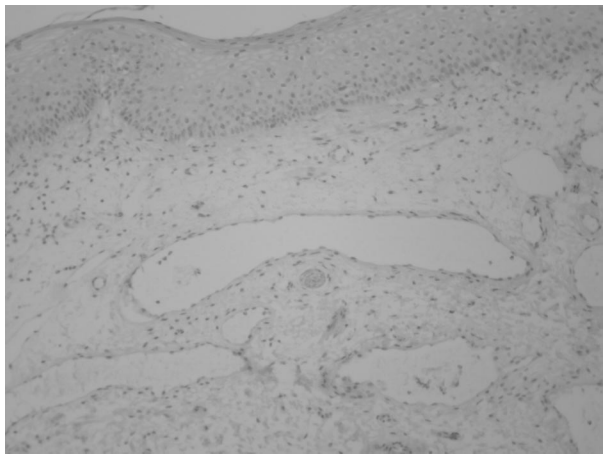

Fig. 6. PGP9.5 staining for unmyelinated nerve fibers at the end of study (original magnification 10×40).

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differences between the groups were not statistically significant. However, in women with an improvement of sensitivity at the site parallel to the original biopsy site, there was a correlation with the reduction in the presence of intraepithelial nerve fibers in the enoxaparin group (n=13, R=0.437, P=.068, one-sided), but no such correlation was found in the placebo group (n=8, R=−0.091, P=.415, one-sided) using the Spearman correlation coefficient test.

DISCUSSION

The hypothesis of the current study was that neuroproliferation and penetration of nerve fibers into the epithelium increase local sensitivity in patients with localized provoked vulvodynia. Consequently, strengthening the stroma by inhibiting heparanase may prevent neuroproliferation. The results show that the hypothesis may be valid. A greater reduction of pain scores, as determined by clinical and subjective evaluations, was demonstrated in women treated by enoxaparin compared with a placebo control group. The pain during intercourse has been reduced more in women who were treated by enoxaparin that with placebo (28.9% compared with 4.4%, P=.057). This reduction has a clinical importance, because the current medical treatment of localized provoked vulvodynia is unsatisfactory. Pain reduction was correlated with the reduction in the presence of intraepithelial-free nerve fibers in the enoxaparin group but not in the placebo group. Because the dosage and treatment periods were empirical, it was an important step in validating the proposed neuroproliferative etiology of vulvodynia and possibly in discovering new possible treatment for this pain disorder either as monotherapy or in combination with other treatments.

The strength of the study is that it was randomized, double-blind, and placebo-controlled and that all women participating in the study had severe dyspareunia. Thus far, few studies have evaluated treatment effectiveness for vulvodynia in this manner. Current treatments rely on experience with other pain syndromes that may have a different etiology from vulvodynia, which would introduce reservations regarding the effectiveness of current treatments for this particular pain syndrome.

The moderate effectiveness of enoxaparin in this study raises the possibility that it can be helpful in a specific subgroup of women with localized provoked vulvodynia, those in the early stages, in which the enoxaparin might hinder neuroproliferation and subsequent progression to a chronic irreversible pain condition. It may also be valuable to further examine the specimens of this study to identify histologic and molecular factors that predicted response, because there were nearly twofold the number of "complete responders" in the treatment group.

However, enoxaparin treatment is, for some patients, an inconvenient option. It requires daily self-injections and may enhance bruising and bleeding. In addition, its cost is not negligible. Nevertheless, although the response rate is not overwhelming, even if treatment only applies to 20% of women with localized provoked vulvodynia, if it works well in that subgroup, it would be valuable. Many current treatments have hardly obtained similar

Table 6. Immunohistochemistry Findings of Biopsies of Women With Localized Provoked Vulvodynia Before and at the End of the Study

	Mast Cells CD117		Intraepithelial Nerve Fiber Density PGP9.5		Stromal-Free Nerve Fiber Density PGP9.5		Intracytoplasmatic Heparanase Density	
	Placebo	Enoxaparin	Placebo	Enoxaparin	Placebo	Enoxaparin	Placebo	Enoxaparin
Improvement	27.7	21.1	66.6	47.3	50	47.37	50	31.6
No change	61.1	52.5	33.4	42.1	38.9	42.1	44.4	47.3
Worsening	11.2	26.4	0	10.6	11.1	10.53	5.6	21.1

Data are %.

results, and most women prefer to avoid undergoing vestibular excision.

Heparin has shown effectiveness also in the relief of interstitial cystitis.[25–28] This is of particular interest in light of the possibility of a common pathogenesis and etiology between vulvodynia and interstitial cystitis[29] and recent documentation of a clinical correlation between the two pathologies.[30,31] An open-label trial[28] examined the intravesical instillation of lidocaine, heparin, and bicarbonate on women with interstitial cystitis and painful bladder syndrome. Thirteen (57%) of the 23 sexually active women who reported dyspareunia at the outset reported resolution of dyspareunia after treatment.

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