Vestibular Mast Cell Density in Vulvodynia: A Case-Controlled Study

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Objectives: To identify whether mast cell densities in vulvar biopsies from the vestibule are associated with vulvodynia.

Methods: We enrolled 100 women aged 19 to 59 years with confirmed vulvodynia cases, 100 racially matched controls, and 100 black control women. All had vulvar biopsies performed at the 7 o'clock position of the vestibule, which were then immunostained to detect c-KIT protein. The numbers of c-KIT positive mast cells per ×400 magnification field were manually counted, and *t* tests and logistic regression were used to assess the association with case-control status.

Results: Of the biopsies, 235 were adequate samples for c-KIT testing for mast cells. The mast cell density was substantially lower in black control women (13.9 ± 10.9) in comparison to white control women (22.5 ± 13.2 $p < 0.001$): hence the analysis was confined to white cases and racially matched control women. Compared with racially matched controls, cases were younger, more likely to be married, and reported a higher household income. The average number of mast cells per ×400 magnification field overall was 19.1 ± 13.2 (range, 0–62). There was no difference in the mast cell count between racially matched cases (22.4 ± 13.9 per ×400 field) and controls (22.5 ± 13.2) in either the univariate or multivariable analyses. Within the group of cases, there was no difference in mast cell density based on the presence or absence of a variety of urogenital symptoms.

Conclusions: No difference in mast cell density in biopsies of the vestibule was found between white cases and racially matched controls. Black control women have a lower mast cell density compared with white control women.

Key Words: mast cells, vulvodynia, inflammation, ethnicity

(*J Low Genit Tract Dis* 2016;20: 275–279)

Vulvodynia is a disorder characterized by ongoing pain on the vulva for at least 3 months' duration that lacks a clear identifiable cause.[1] A large population-based study has identified that the incidence of new onset vulvodynia in women who are sexually active and older than 18 years is 4.2% per year with incidence rates differing by age, ethnicity, and marital status.[2] In another population-based study, the prevalence of vulvodynia was 8.3% with vulvodynia being more prevalent in younger women, those who were married, and those who were self-identifying as Hispanic, whereas a slightly lower prevalence was noted in black women.[3] The etiology of the disorder is still unknown, reflected in the fact that multiple treatment modalities are available and that no specific treatment method has been shown to have any greater benefit over another.[4,5]

Histopathologic studies of vestibular biopsy tissue in women with and without vulvodynia reveal contradictory results, with some demonstrating increased lymphocytic infiltrates in the vestibule of patients with vulvodynia,[6,7] and with others showing a similar degree of inflammation between patients and healthy controls[8,9] or no active inflammation at all.[10] Several studies have reported that mast cells in the vestibular mucosa are increased compared with healthy women,[6,7,11–14] whereas others have not confirmed this finding.[9,15] Previous studies comparing vulvodynia patients to control women have been limited by relatively small sample sizes and have assessed primarily white women.

Mast cells have a sentinel location and are found at the interfaces of the skin mucosal tissue with the environment. They are considered to play an important regulatory role in various pain disorders because of the substances they release and their proximity to the sensory neurons.[16,17] Difference in mast cell quantity or functions have been reported in patients with interstitial cystitis, fibromyalgia, atopic dermatitis, chronic pelvic pain, and chronic regional pain syndromes.[17] Because of the potential of an underlying mast cell–dependent mechanism that could account for the pain in vulvodynia, the objective of our study was to assess the association of mast cell density in the vestibule with the presence of vulvodynia, using a larger sample of clinically confirmed cases and racially diverse controls.

MATERIALS AND METHODS

Participants were women presenting to one of 2 vulvar specialty clinics in Ann Arbor (HH) and Chelsea, Michigan (BR), who complained of pain (burning or irritation) at the vaginal introitus for at least 3 months and were clinically confirmed to have vulvodynia in the office by cotton swab testing at the vestibule and lack of another diagnosis that might account for the pain. On examination, we were able to categorize 69 women with vulvodynia as generalized (26/69) or localized (43/69) cases based on the location of vulvar sensitivity, but all were sensitive at the introitus where the biopsy was obtained. Two control groups were recruited, including a group of 100 women racially and age-matched to the cases, and 100 black women age-matched to the cases. Recruitment of controls was either by attendance at the office for a gynecologic examination or via response to an online or newspaper advertisement. Eligibility criteria for the control groups included no introital pain, no pain with intercourse, and no history of vulvodynia. The absence of vulvodynia in controls was confirmed by in-office examination.

Exclusions for both cases and controls included current pregnancy, taking immunosuppressing medication, or a history of vulvar surgery (including laser treatment). Cases were also excluded if they had been treated for at least 1 month in the past 6 months with doses of antidepressants with the potential to affect vulvar pain. All participants completed a 24-page survey inquiring about demographic characteristics, past and current health (asthma, eczema, allergies, smoking history, etc.), medications, parity, urogenital symptoms, vulvar symptoms, and psychological symptoms and diagnoses.

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University of Michigan Medical institutional review board (HUM00049893).
The authors have declared they have no conflicts of interest.
This study was funded in part by a grant from the Institute of Child Health and Human Development of the National Institutes of Health (HD40112).

The in-office examination included external inspection, cotton swab testing for vulvar sensitivity, intravaginal examination, in-office laboratory analysis of vaginal discharge sample, and a vaginal culture for Candida species. In-office laboratory testing included pH determination, amine test (whiff test), normal saline, and potassium hydroxide (KOH) preparations for microscopic analysis. A 4-mm vulvar biopsy was performed using standard aseptic technique under local anesthesia at the 7 o'clock position of the vestibule, within Hart's line. Vulvar biopsy samples were subsequently dehydrated in graded alcohols, cleared in n-butanol, and embedded in paraffin wax. Sections were cut and deparaffinized. Samples were immunostained in one laboratory (CC) to detect c-KIT protein (DAKO cat#:A4502), which is a specific marker of mast cells.

Microscopic analysis of labelled slides was conducted by CC blindly. Numbers of c-KIT positive cells per ×400 magnification field were counted manually. Multiple ×400 fields were counted per slide (range, 2–10 fields per slide) and an average number of c-KIT–positive mast cells was computed and recorded. Inadequate samples (inadequate staining, or poor orientation of samples on the slide with lack of epidermis, basement membrane and dermis sample) were excluded.

Statistical analyses included frequencies of variables (demographics, case control status, vulvar pain characteristics, and histologic findings) followed by determination of associations between demographic or clinical variables and histologic findings with case control status using *t* tests, analysis of variance, and chi-square analyses as appropriate. All analyses were performed on the data for the white women with vulvodynia, the white control women, and the black control women. Women with other racial backgrounds and the black women with vulvodynia were excluded because of the very small numbers in these categories. An assessment of similarities and/or differences noted in the cohort of control women who were black compared with those controls who were white was conducted. For the purposes of the primary analyses, white cases and controls were evaluated because of marked differences in mast cell density between white control and black control women. The relationship between mast cell densities and vulvar pain characteristics among vulvodynia cases were assessed using linear regression, controlling for demographic characteristics.

The sample size for the study was determined using previously reported values for mast cell densities for vulvodynia cases and controls.[11] We determined we would have 80% power to detect a mast cell density difference of 0.5 SD with 78 women per group or a power of 90% to detect a difference of 1.0 SD with 27 women per group, based on values for cases and controls in the literature.

All cases and controls gave informed consent. The study was approved by the University of Michigan Medical institutional review board (HUM00049893).

RESULTS

Over a 3-year period, 300 women were enrolled, including 100 women with vulvodynia, 100 racially matched controls, and 100 black controls. All had vulvar biopsies performed. Of the biopsies, 235 were adequate for c-KIT testing, including 75 white cases with vulvodynia, 65 white control women, 84 black control women, 2 black cases, and 9 women of other or unreported racial background (1 case and 3 controls classified as "other," 1 control classified as "Asian," and 2 cases and 2 controls with missing racial data).

The results were based on only the white women with vulvodynia, the white control women, and the black control women, unless otherwise stated. Table 1 represents the demographic features of white cases and controls and black controls. Cases were younger, more likely to be married, and had a higher household income than racially matched controls but were equally likely to have had intercourse in the previous month. The black control group differed from both white cases and white control women in marital status, education, and income but not in age or likelihood of recent intercourse.

Table 2 presents the vulvar pain characteristics of all women with vulvodynia. Mean vulvar pain score was 8.0 ± 2.0, with more than 80% of cases experiencing pain during and after intercourse, and the mean number of years since first vulvar pain was 5.5 ± 6.7 years.

The average number of mast cells per ×400 magnification field overall for the white women with vulvodynia, white controls, and black controls was 19.1 ± 13.2 (mean ± SD), with a range of 0 to 62 mast cells. However, the mast cell density was substantially

TABLE 1. Demographic and Exposure Characteristics of Cases and Controls

	A. White cases (n = 75)	B. White controls (n = 65)	*p* value A vs B	C. Black controls (n = 84)	*p* value B vs C	*p* value A vs C
Age (mean ± SD)	34.1 ± 9.6	39.0 ± 12.0	0.011	37.0 ± 11.7	0.295	0.095
Married or living as married	79.5% (n = 59)	60.9% (n = 39)	0.009	13.1% (n = 11)	<0.001	<0.001
Never married	17.8% (n = 13)	18.8% (n = 12)		57.1% (n = 48)		
Widowed	0.0% (n = 0)	1.6% (n = 1)		3.6% (n = 3)		
Separated or divorced	2.7% (n = 2)	18.8% (n = 12)		26.2% (n = 22)		
Missing	(n = 1)	(n = 1)		(n = 0)		
Education (mean ± SD in yr)	15.2 ± 2.5	14.6 ± 2.5	0.132	12.9 ± 1.7	<0.001	<0.001
Missing	(n = 3)	(n = 2)		(n = 0)		
Household income						
<$20,000	2.7% (2)	14.5% (9)	0.007	58.5% (48)	<0.001	<0.001
$20,001–$40,000	17.8% (13)	32.3% (20)		24.4% (20)		
$40,001–$60,000	28.8% (21)	19.4% (12)		8.5% (7)		
>$60,000	50.7% (37)	33.9% (21)		8.5% (7)		
Missing	(n = 2)	(n = 3)		(n = 2)		
Intercourse over past month	64.9% (48)	66.2% (43)	0.873	54.3% (44)	0.608	0.183
Missing	(n = 1)	(n = 1)		(n = 3)		

TABLE 2. Vulvar Pain Characteristics of Vulvodynia Cases[a]

Worse vulvar pain ever (0–10 scale) (mean ± SD)	8.0 ± 2.0
Pain with first tampon	18.9% (n = 14)
Pain with first intercourse	35.5% (n = 27)
Age first vulvar pain (mean ± SD)	27.6 ± 10.2 yr
Years since first vulvar pain (mean ± SD)	5.5 ± 6.7 yr
Pain with insertion	87.7% (n = 64)
Pain with thrusting	81.4% (n = 57)
Pain after intercourse	82.9% (n = 58)
Constant pain	50.7% (n = 34)
Intermittent pain	68.7% (n = 46)
Exacerbators	
Intercourse	82.5% (n = 66)
Partner touch	57.5% (n = 46)
Menses	33.8% (n = 27)
Sitting	30.0% (n = 24)

[a]n = 67–80, based on missing data.

lower in black control women (n = 84, 13.9 ± 10.9 mast cells per ×400 magnification field) when compared with white control women (n = 65, 22.5 ± 13.2 mast cells per ×400 magnification field, $p < 0.001$). A number of variables were assessed to try to determine whether they explained some or all of the difference in mast cell density by racial background. None were found that could explain the reason for the low mast cell density in vulvar biopsies among black control women compared with white control women, including age, marital status, household income, smoking status, presence of allergies, asthma, irritable bowel disorder, fibromyalgia, interstitial cystitis, ever being pregnant or number of pregnancies, ever douching or recently douching, contraceptive use (OCPs, IUD, Depo-Provera), baths versus showers, sanitary protection, history of yeast infections, having a current partner, history of abuse, level of stress, or worry. Because of this marked difference in mast cell density among controls of different racial backgrounds and because only 2 cases were black, we confined the analysis to white cases (n = 75, 97.4% of all cases) and white controls (n = 65).

The average number of mast cells was 22.4 ± 13.9 for white vulvodynia cases and 22.5 ± 13.2 for white controls with no difference detected ($p = 0.975$) by univariate analysis (Figure 1). When age, marital status, and household income were controlled, there were still no significant differences noted ($p = 0.613$). When further controlled for whether the women had intercourse in the past month, again, no significant differences were noted ($p = 0.410$).

Within the group of vulvodynia cases, mast cell density was evaluated to determine whether it varied according to the presence or absence of a variety of reported symptoms (Table 3). No significant differences were found in mast cell density with any of the comparisons made.

DISCUSSION

In our study, we found no difference in mast cell density in biopsies of the vestibule when comparing cases and racially matched controls. This is in line with other studies that have reported on similar densities of mast cells in the vestibular mucosa of both patients and healthy controls.[9,15] An explanation provided in the recent study of Tommola et al[15] was that increased numbers of mast cells are usually found in acute inflammation, and because vulvodynia is a chronic condition, mast cell counts are not increased. The authors reported that their patients had a long history of disease which they suggest could partly explain why they did not find an excess of mast cells. The patients in our study also

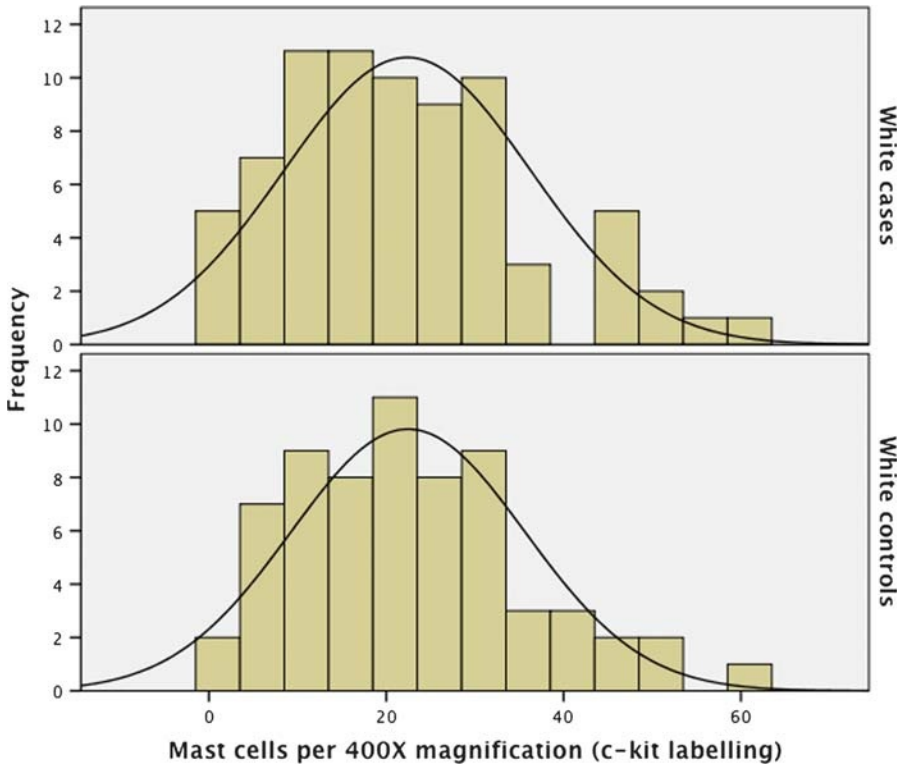

FIGURE 1. Distribution of mast cells per ×400 magnification field among white cases and white controls.

TABLE 3. Mast Cell Density Among White Cases With Differing Pain Characteristics

	Mast cell density (per ×400 magnification field) mean ± SD	P^a	P^b
Level of worse pain ever (terciles)		0.809	0.753
1–7	21.3 ± 12.7		
8–9	22.9 ± 16.5		
10	23.9 ± 12.6		
Pain after intercourse			
Yes	21.1 ± 13.1	0.090	0.078
No	29.8 ± 12.2		
Pain with first tampon			
Yes	29.1 ± 17.9	0.095	0.103
No	21.4 ± 13.0		
Pain with first intercourse			
Yes	19.7 ± 11.6	0.215	0.201
No	24.1 ± 14.9		
Intermittent pain			
Yes	20.9 ± 13.0	0.479	0.327
No	23.6 ± 15.3		
Continuous pain			
Yes	23.9 ± 15.6	0.491	0.385
No	21.4 ± 12.9		

[a]Univariate analysis (*t* tests or analysis of variance).

[b]Linear regression, controlled for age, marital status, and household income.

had a long history of vulvodynia as the mean number of years since first vulvar pain was similar to that reported in the study of Tommola et al.

On the other hand, there are studies[6,7,11–14] that have reported on the excess mast cell density in the vestibular mucosa of patients with vulvodynia in comparison to healthy controls and several mechanisms have been suggested to explain this finding. In the studies of Bornstein et al,[12,13] it has been hypothesized that there is an unknown topical stimulus driving mast cell proliferation and degranulation, which in turn induce nerve proliferation and sensitize the peripheral neurons causing pain. Another proposed mechanism that might cause increased mast cell density proposed in the studies of Goetsch et al[6] and Leclair et al[7] is that of neurogenic inflammation. In this process, stimulated nociceptors cause neurons to release neuropeptides that attract and activate local mast cells.[16]

A recent systematic review by Chatterjea and Martinov[17] highlighted the importance of mast cells in vulvodynia and reported that certain mediators at the mast cell nerve synapse may play a major role in the mast cell–mediated mechanisms of vulvar pain. Chatterjea and Martinov suggest that mast cell nerve proximity in the tissues facilitates neuroimmune cross-talk relevant to the modulation of pain. They report that both mast cells and neurons have the potential to secrete nerve growth factor,[18] which in turn induces neuronal growth and reduces the subsequent neuronal firing threshold, leading to increased pain perception.[19–21] They also report that certain mediators at the mast cell–nerve synapse, such as substance P, have the effect of priming the mast cells to degranulate upon repeated application of lower doses of substance P.[22] In our study, we measured only the mast cell density in cases compared with controls and did not measure the mediators secreted by the mast cells. Thus, although mast cell density is not increased in vulvodynia, our data do not exclude a pathogenic role based on increased or abnormal function.

Our study has shown that there was no difference in the density of mast cells in biopsies from the vulvar vestibule when comparing white cases and controls but a marked difference between white and black control women. There are reports in the literature that white women have a higher prevalence[3,23] and incidence[2] of vulvodynia in comparison to black women. The effect of racial background on the prevalence/incidence is unclear, but suggested explanations include sociocultural differences or differences in genetic disposition.[23–25]

Our study has shown that the mast cell density in the vestibular mucosa was substantially lower in black control women in comparison to white control women. There are reports in the literature that there are microanatomical differences between black and white skin.[26] A systematic review by Wesley and Maibach[27] reported that racial differences in skin properties may explain the racial disparities seen in certain skin disorders. In the study of Sueki et al,[28] it was shown that mast cells in black skin compared with white skin contain larger granules and demonstrate ultrastructural and immunophenotypic differences. It was suggested that the ultrastructural differences in the mast cell granules between black and white skin possibly reflect differences in their rates of mast cell degranulation/regranulation.[28] However, we have found no study reporting on the differences between mast cells derived from the vestibular mucosa of white women in comparison to black women. Further study on the differences in mast cell density between black and white women is needed and may cast light on the difference in vulvodynia prevalence/incidence in these groups.

The studies that have reported on the increased mast cell density in the vestibular mucosa of patients with vulvodynia in comparison to healthy controls[6,7,12–14] have not reported on the racial background of their participants. If we had included in our analysis the black controls with the lower mast cell density, there may have been a difference observed in the mast cell density in cases compared with controls, but this would have reflected the difference related to racial background only and would not represent a valid finding related to case status. The use of racially unbalanced cases and controls in the studies reported in the literature might explain some of the discrepancies seen in the literature regarding vulvar mast cell density and vulvodynia.

Our study has certain limitations. First, this is a cross-sectional study and, therefore, can only indicate that at this time in their disease, women with vulvodynia do not have a greater density of mast cells in their vestibular tissue compared with those without. Second, we performed 4-mm punch biopsies at the 7 o'clock position of the vulvar vestibule in cases and controls. Whether findings differ over a broader area of the vestibule is unclear. Finally, we did not measure mast cell activity or mediators excreted by the mast cells. There are reports that certain mediators at the mast cell–nerve synapse may play a role in the mast cell–mediated mechanisms of vulvar pain that is not reflected in the relative number of mast cells present.

The main strength of our study was its large sample size with the inclusion not only of white cases and white control women but also of a large number of black women recruited for a second control arm. This made it possible to assess the contribution of racial background in the vestibular mucosa histologic findings. In addition, we documented that most of our patients had experienced a severe level of vulvodynia (mean vulvar pain score of 8 on a 10-point pain scale).

CONCLUSIONS

In summary, we found similar mast cell density in vulvar biopsy specimens taken from asymptomatic white women and white women with vulvodynia. In addition, among healthy control

women, mast cell density was substantially lower in black women compared with white women. These findings underscore the importance of accounting for ethnic background in studies of vulvodynia and suggest that mast cell density is similar in white cases and controls.

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