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Innovations

Isolation, culture, and activation of vestibular mast cells from patients with localized provoked vulvodynia: a pilot study

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Localized provoked vulvodynia (LPV), also known as provoked vestibulodynia (PVD), is a condition characterized by pain localized to the vulvar vestibule, a specialized ring of tissue surrounding the vaginal opening, upon light pressure or contact.[1] Affecting approximately 14 million people in the United States, LPV exerts a profound impact on sexual health, relationships, and overall quality of life. For many patients, the condition leads to significant sexual dysfunction, including dyspareunia, avoidance of intimacy, and psychosexual distress. Despite its prevalence and debilitating consequences, LPV remains underdiagnosed and lacks effective management strategies, due in part to an incomplete understanding of its underlying pathophysiology. A deeper understanding of this pathophysiology could pave the way for more cutting-edge management strategies.

Histological studies consistently demonstrate 2 key features in the vestibular tissue derived from LPV patients: Nerve proliferation and accumulation of mast cells.[2] Beyond their traditional role in allergic reactions, mast cells have been increasingly recognized for their involvement in chronic pain and inflammation. When activated, mast cells degranulate, releasing mediators such as cytokines, growth factors, and neuropeptides, which in turn can promote nerve sprouting and/or sensitize sensory nerve endings. The peptide bradykinin, for example, has been strongly linked to inflammatory and neuropathic hyperalgesia. It acts on receptors expressed on nociceptors, lowering their threshold for activation and sustaining peripheral sensitization. Nerve growth factor (NGF), on the other hand, is known to promote the growth and survival of sensory nerve fibers. Persistent release of these mediators from mast cells could create a microenvironment in the vulvar vestibule that perpetuates both nociceptor sensitization and hyperinnervation, thereby amplifying pain in response to otherwise innocuous stimuli.[3] This interaction provides a plausible mechanistic link between mast cell degranulation and the hyperinnervation and pain observed in LPV.

Despite this, most of the knowledge on the properties of mast cells is derived from animal models.[4] While valuable, these models fail to capture the specific biology of human mast cells in the unique environment of the vulvar vestibule. The absence of a relevant and validated *in vitro* model of vestibular mast cells has represented a critical barrier to advancing our understanding of mast cell-nerve interactions in the context of LPV.

To address this gap, the aim of this study was to isolate and culture viable mast cells from the vestibule of LPV patients and perform functional assays to better understand the general function of human vestibular mast cells. Vestibular tissue was obtained from 5 LPV cases undergoing vestibulectomy at the University of Rochester Medical Center's Obstetrics and Gynecology practice between July 2024 and October 2025. Control labial tissue was similarly obtained from one subject undergoing an elective labiaplasty at the same practice. All subjects provided informed consent, and the research was approved by the University of Rochester Institutional Review Board (IRB #42136). Detailed demographic data can be found in Table S1. The average age of patients at time of surgery was 28.2 ± 10.4 years. These patients met Friedrich's criteria, denied the use of corticosteroids and non-steroidal anti-inflammatory medications, had no chronic inflammatory illnesses other than LPV, and were negative for any current vulvovaginal infections (Figure S1). Controls met the same criteria but were negative for Friedrich's criteria. During surgery, a modified vestibulectomy with vaginal vault advancement was performed, extending from approximately the 2 to 10 o'clock positions (Figure S2).

The mast cell isolation workflow is summarized in Figure S2. The excised tissue was weighed, cut into small 10 mm pieces, and treated with dispase overnight at 4 °C. The next day, the epidermis was removed from the dermis, and the dermal pieces were mechanically digested to form a uniform mass. The homogenized tissue was then enzymatically digested at 37 °C for 1 hour in a

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digestion medium containing collagenase, hyaluronidase, and DNAse. Isolated cells were separated from the remaining tissue by 3 steps of filtration. The resulting cell suspension was then stained with 4′,6-diamidino-2-phenylindole to exclude dead cells, and anti-FcεRI-PE and anti-CD117-APC, both of which are cell surface markers of mast cells. The cells were analyzed by flow cytometry to identify $CD117^{+}/Fc\varepsilon RIa^{+}$ cells for positive selection of mast cells (Figure S3). Isolated mast cells were then cultured in Basal Iscove's medium, supplemented with 10% fetal bovine serum and antibiotics. Human stem cell factor (100 ng/mL) and human interleukin-4 (20 ng/mL), established growth factors required for the survival and proliferation of mast cells in culture, were added to the mast cell medium for their long-term culture. In addition to isolating mast cells from vestibular tissue of LPV patients, mast cells were also isolated from the labial tissue of one subject without vulvar pain whilst undergoing an elective labiaplasty to serve as an approximate control.

Over a 3-week period, the number of mast cells increased approximately 3-fold, providing an adequate number of cells for experimentation. Degranulation was experimentally induced with Compound 48/80, a synthetic mast cell degranulator, and cell supernatants were collected and analyzed for both bradykinin and NGF levels using enzyme-linked immunosorbent assays.

Enzyme-linked immunosorbent assay data revealed that mast cells activated with Compound 48/80 released significantly higher levels of both bradykinin (Figure S4A) and NGF (Figure S4B) compared to non-activated mast cells ($P < .0001$; 2-way analysis of variance (ANOVA) with Šídák's multiple comparisons test). Non-degranulated mast cells showed minimal or no bradykinin and NGF release, indicating that degranulation is required for mediator secretion. Interestingly, the amount of bradykinin, but not NGF, released from degranulated labial mast cells was significantly lower compared to vestibular mast cells (Figure S4A; $P = .011$; 2-way ANOVA with Šídák's multiple comparisons test). These findings demonstrate that human vestibular mast cells are both viable in culture and functionally active, capable of releasing mediators directly implicated in nociceptor sensitization and chronic pain.

While these results establish mast cells as contributors to the maintenance of chronic vulvar pain, several limitations should be noted. First, this pilot study was limited to tissue derived from 5 LPV patients, and variability in mast cell mediator release may exist across LPV patients. Another limitation is the absence of control vestibular tissue derived from subjects without vulvar pain, which would provide a baseline comparison for understanding whether the mast cell phenotype observed in LPV is truly disease specific. Because subjects without vulvar pain do not undergo vestibulectomies, control vestibular samples are difficult to obtain. Thus, mast cells isolated from the labia of one control subject served as the closest available comparison. Additionally, while bradykinin and NGF release were confirmed, other mast cell–derived mediators of interest were not assayed. A more comprehensive analysis of the mast cell secretome using RNA sequencing would be critical in fully elucidating the role of mast cells in LPV.

Taken together, this study demonstrates the successful isolation, culture, and activation of vestibular mast cells from patients with LPV. By establishing the first *in vitro* model of vestibular mast cells, this work offers a novel and physiologically relevant method for advancing mechanistic insight in vulvodynia. Specifically, this model can serve as a platform for the preclinical testing of mast cell-targeted therapeutics, such as ketotifen fumarate and soluble epoxide hydrolase inhibitors, on mast cell degranulation and mediator release in patient-derived cells, thus offering a translational path toward human trials and ultimately improving the sexual health and quality of life of patients affected by this debilitating condition.

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Conflicts of interest

M.L.F., the corresponding author of this manuscript, serves on the Editorial Board of the Journal of Sexual Medicine. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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