GENERAL GYNECOLOGY

Immunoreactivity of oxytocin receptor and transient receptor potential vanilloid type 1 and its correlation with dysmenorrhea in adenomyosis

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OBJECTIVE: We sought to investigate the expression and localization of oxytocin receptor (OTR) and transient receptor potential vanilloid type 1 (TRPV1) in women with and without adenomyosis.

STUDY DESIGN: Ectopic and homologous eutopic endometrium from 50 women with adenomyosis and endometrium from 18 women without adenomyosis were used for immunohistochemical analysis of OTR and TRPV1. Microscopic evaluation assessed the presence and localization of OTR and TRPV1 throughout the menstrual cycle in both eutopic endometrial and endometriotic tissues of women with adenomyosis and compared them with normal endometrium.

RESULTS: Compared with normal endometrium, immunoreactivity of OTR and TRPV1 were significantly increased in ectopic endometrium. Both OTR and TRPV1 immunoreactivity were positively correlated with the severity of dysmenorrhea and found to be significant predicators for dysmenorrhea severity.

CONCLUSION: These findings suggest that OTR and TRPV1 may be involved in dysmenorrhea and its severity in adenomyosis and may be potential therapeutic targets.

Key words: adenomyosis, dysmenorrhea, immunohistochemistry, oxytocin receptor, severity, transient receptor potential vanilloid type 1

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Adenomyosis, characterized by the benign invasion of endometrial glands and stroma deep and haphazardly into the myometrium, is a common gynecologic disorder with a poorly understood pathogenesis.[1] Its presenting symptoms include a soft and diffusely enlarged uterus with dysmenorrhea, menorrhagia, and subfertility.[2] Treatment of adenomyosis has been a challenge, with hysterectomy being the treatment of choice.[1] Although the disease is hormone sensitive, progestogenic agents are not very effective; gonadotropin-releasing hormone agonists induce suppression of adenomyosis, yet their use is restricted by short duration.[1] In addition, the symptoms often recur after discontinuation of gonadotropin-releasing hormone agonist therapy.[3] The use of levonorgestrel-containing intrauterine device to treat adenomyosis has been reported to be promising,[4] yet the information on its long-term efficacy is scanty.

While approximately 35% of adenomyotic cases are asymptomatic,[5] dysmenorrhea is the most prevalent symptom, besides abnormal uterine bleeding, and is arguably the most debilitating to the patient. In women with endometriosis, persistent dysmenorrhea after optimal surgery or dysmenorrhea of long duration may be indicative of adenomyosis.[6,7]

There are studies that report higher myometrial infiltration depth is associated with dysmenorrhea in adenomyosis.[8] Other studies report that adenomyosis-related symptoms are variable, nonspecific, and related to other associated pathologic conditions.[9,10] What determines the frequency and severity of dysmenorrhea in adenomyosis remains a conundrum.[11] No biomarker for dysmenorrhea or its severity in adenomyosis has been identified so far.

One culprit that has long been suspected in causing dysmenorrhea, not necessarily adenomyosis related, is prostaglandins (PGs).[12] Since cyclooxygenases (COXs) are the rate-limiting enzymes that catalyze the initial step in formation of biologically active PGs from arachidonic acid, including $PGF_{2\alpha}$, PGE_2, and PGI_2, nonsteroidal antiinflammatory drugs and selective COX-2 inhibitors are often used to treat endometriosis-associated dysmenorrhea as a first-line therapy. Indeed, increased ex-

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pression of COX-2, which converts arachidonic acid to PGs, in adenomyosis has been reported.[13]

In endometrial cells, the production of $PGF_{2\alpha}$ can be promoted by oxytocin, the action of which is mediated by oxytocin receptor (OTR).[14] OTR has been shown to be overexpressed in smooth muscle and epithelial cells of endometriotic lesions,[15] suggesting its possible involvement in endometriosis and perhaps in endometriosis-associated symptoms.

At the peripheral endings of primary nociceptors, PGE_2 and its E prostanoid$_1$ receptor, in concert with PGI_2 receptors (I prostanoid receptors), sensitize the capsaicin receptor, transient receptor potential vanilloid type 1 (TRPV1), eventually leading to hyperalgesia or pain[16] and perhaps also to dysmenorrhea. Both PGE_2 and $PGF_{2\alpha}$ can up-regulate COX-2 expression through activation of F-series prostanoid receptor in an autocrine/paracrine manner.

TRPV1 has been shown to play a role in inflammatory hyperalgesia in animal models.[17] TRPV1 is expressed by sensory neurons and integrates multiple noxious stimuli on peripheral terminals or primary sensory neurons, such as heat (>43°C), acid (pH <5.9), and inflammatory mediators.[18] TRPV1 activation also leads to local release of sensory neuropeptides, including calcitonin gene-related peptide and substance P, which, in turn, activate their effector cell receptors and contribute to the process of neurogenic inflammation. TRPV1 also is expressed in nonsensory cells, although its significance is unclear.

We hypothesized that, as in endometriosis, both TRPV1 and OTR expression are increased in adenomyosis, which may be responsible for dysmenorrhea in adenomyosis. Hence, in this study we sought to investigate the expression and localization of TRPV1 and OTR in eutopic and ectopic endometrium of women with adenomyosis and in endometrium in women without adenomyosis. We also sought to determine the relationship, if any, between the amount of menses, uterus size, and severity of dysmenorrhea and TRPV1 and OTR immunoreactivity.

MATERIALS AND METHODS

Patients and tissue samples

Fifty women with adenomyosis (excluding endometriosis) seen at Shanghai Obstetrics/Gynecology Hospital, Fudan University, from 2004–2005, were recruited for this study. Their diagnoses were made by transvaginal ultrasound before surgery and histologically confirmed postoperatively. All patients' ectopic, along with their homologous eutopic, endometrial tissue samples were collected after hysterectomy and fixed in 10% buffered formalin and routinely processed for paraffin embedding. For controls, we also collected, after informed consent, endometrial tissue samples through curettage from 18 women with tubal infertility or surgically diagnosed benign ovarian cysts, but without any clinical history or signs of endometriosis, adenomyosis, or myoma as per medical history, symptoms, gynecologic and sonographic examination before the surgery, laparoscopic examination, and histology after the surgery. None of these women reported dysmenorrhea. The selection of the controls was based solely on menstrual phase and age besides disease status.

All women in both study and control groups were premenopausal and had regular menses (lengths varied from 21–35 days), with no hormone therapy or intrauterine device use for ≥6 months prior to the surgery or tissue collection. The menstrual phase of the patient at the time of surgery was determined based on the day elapsed since the last period. All endometrial samples were grouped either in proliferative or secretory phase based on Noyes' dating criteria. In women with adenomyosis and without, exactly half were in the proliferative and the other half were in the secretory phase.

For each patient with adenomyosis, the following information was collected through reading medical charts and interviewing: age at surgery, uterus size (calculated as $\pi D_1 D_2 D_3/6$, where D_1 = the distance from fundus to the internal os of the cervix, D_2 = transverse diameter at the level of the cornua, and D_3 = anteroposterior diameter at the level of cornua), report of dysmenorrhea, severity of dysmenorrhea, duration of dysmenorrhea, amount of menses, duration of menstruation, gravidity, and parity. Their amount of menses during menstruation was grouped into 3 classes: light, moderate, and heavy, depending on whether they changed their sanitary pads <3, between 3–6, or >6 times a day, respectively.[19] The severity of dysmenorrhea was classified as mild (pain but no interference with routine daily life or work and no need for analgesics), moderate (pain interfering with routine daily life or work to some extent and relief of pain after taking analgesics), and severe (pain seriously interfering routine daily life or work and no relief of pain after taking analgesics).

This study was approved by our institutional ethics review board.

Immunohistochemistry

Serial 4-μm sections were obtained from each paraffin-embedded tissue block, with the first resultant slide being stained for hematoxylin-eosin to confirm pathologic diagnosis and the subsequent slides stained for OTR and TRPV1. Routine deparaffinization and rehydration procedures were performed.

The rabbit polyclonal antibodies against OTR (ab13051; Abcam, Cambridge, United Kingdom) and TRPV1 (ab63083; Abcam) diluted to 1:100 and 1:200, respectively, were used as primary antibodies. For antigen retrieval, the slides were heated at 98°C in an EDTA buffer (pH 9.0) for a total of 30 minutes and cooled naturally at room temperature. Sections were then incubated overnight with the primary antibody at 4°C. After slides were rinsed, the biotinylated secondary antibody, Supervision TM Universal (antimouse/rabbit) Detection Reagent (HRP) (GK500705; Shanghai Gene Tech Co, Shanghai, China), was incubated at room temperature for 30 minutes. The bound antibody complexes were stained for 3–5 minutes or until appropriate for microscopic examination with diaminobenzidine and then counterstained with hematoxylin and mounted.

Immunoreactivity staining was characterized quantitatively by digital im-