Estrogen and Progesterone Receptors in Patients with Bladder Pain Syndrome

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Objective: *Analyze if bladder mucosa of patients with bladder pain syndrome (BPS) have more estrogen receptor (ER) and progesterone receptor (PR) than the normal population.*
Material and Method: *Between July 2009 and December 2010, 15 female patients with chronic bladder pain syndrome and 10 female patients without bladder pain were enrolled in the present study. Three pieces of trigonal bladder mucosa were biopsied and sent for estrogen receptor and progesterone receptor immunohistochemistry staining by the Benchmark automated machine. The results were reported as positive and negative and then compared between the two groups.*
Results: *Estrogen receptor was found in 14 out of 15 patients in the BPS group (93%) and in 7 out of 10 patients in the control group (70%). Progesterone receptor was found in 10 out of 15 patients in the BPS group (66.7%) and 5 out of 10 patients in the control group. Both were not significantly different with p = 0.267 and p = 0.678, respectively.*
Conclusion: *The authors concluded that ER and PR might not play a role in the etiopathogenesis of BPS/IC. However, other receptors should be further investigated about their role in this type of pain.*

Keywords: *Bladder pain syndrome, Interstitial cystitits, Estrogen receptor, Progesterone receptor*

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Bladder pain syndrome (BPS) refers to a symptom of pain in the urinary bladder region, which is usually accompanied with lower urinary tract symptoms such as urgency and urinary frequency. The patient has to be excluded from confusable diseases using the symptoms. Interstitial cystitis (IC) is a common cause of chronic bladder pain and represents a special type of chronic inflammation of the urinary bladder, which is clinically diagnosed by exclusion. Many studies have reported that patients with IC have low quality of life and have to pay high costs for the treatment. Therefore, because of its economic burden, it is considered to be an important disease in many countries[1].

The exact cause of BPS is still not clearly defined. There are many different hypotheses about the cause of BPS, such as glycosaminoglycan (GAG) layer defect in bladder urothelium that renders the bladder mucosa more fragile, abnormal mast cell activation, alteration of autonomic responses, and CNS processing of afferent stimuli. Because BPS is found more commonly in female, the authors wonder whether ER and PR might take part in the etiology of this disease. If they do, there should be a role of ER or PR antagonists in treating BPS. There are few recent studies implicating the role of female sex hormones in the pathogenesis of the disease but no conclusion has been established. The objective of the present study was to determine whether bladder mucosa of patients with BPS might have more ER and PR than in normal subjects.

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Material and Method

The present study was approved by the Ethics Committee on Human Experimentation involving Human Subjects of Faculty of Medicine, Ramathibodi Hospital, Mahidol University. Between July 2009 and December 2010, 15 female patients with chronic bladder pain syndrome were enrolled in the present study. The inclusion criteria were (1) patients who were more than 18 years of age, (2) patients who had bladder pain syndrome, which had pain related to filling phase;

the pain may be located at suprapubic area, urethra, pelvic, vagina, or perineum, (3) patients with abnormal voiding symptoms, (4) patients with pain at least one year, and (5) patients having been considered to undergo cystoscopy with hydrodistension by qualified urologists in Department of Surgery, Ramathibodi hospital, Mahidol University. There were 10 patients in the control group including those who underwent vesicovaginal fistula repair, open cystolithotomy, and open nephroureterectomy with bladder cuff excision. All patients in the control group had normal voiding symptoms. The exclusion criteria were (1) patients aged less than 18 years, (2) patients with severe medical disease and/or contraindicated for general anesthesia, and (3) patients refused to take part in the present study. All patients were approached by a research team, and informed consent was obtained before performing the operation. Patient information was collected including age, lower urinary tract symptom, point of tenderness, duration of disease, and pain score in terms of visual analog scale. The results of ER and PR were reported as either positive or negative comparing with positive of breast cancer specimen in each slide.

In the BPS group, the patients were performed cystoscopy with hydrodistension under general anesthesia in the usual manner. After that, the urologists biopsied three pieces of bladder mucosa at trigonal area using cold cup biopsy forcep. Tissues were put into formaline solution and sent to the pathologist. In the control group, after finishing the operation, bladder mucosa at trigone was biopsied three pieces and the tissues were put into formaline solution and also sent to the pathologist. Bleeding was checked and stopped before the urinary bladder was closed.

About the immunohistochemistry staining process, bladder tissues were cut in 4-micron sections and incubated at 70 degree Celsius for one hour. The tissue was then deparaffinized with ES prep and instilled with reaction buffer solution. Then, the slides were retrieved with Cell Condition-1 solution (Ventana1 Medical System Inc.), which was tris/EDTA. Subsequently, anti-human progesterone receptor (1:500) or anti-human estrogen receptor (1:300) was added to the slide for 40 minutes then incubated with ultraview multimer Ig comprising of goat anti-mouse IgG, goat anti-mouse IgM, goat anti-rabbit IgG and goat serum caseine, and incubated with ultraview diamine benzidine tetrahydrochloride hydrate (DAB) chromagen. After that, the slides were counterstained with Hematoxylin II for four minutes. All slides were examined and reported blindly by the same pathologist.

Statistical analyses were done with SPSS version 11.5. Patients information was reported as mean and SD and compared by using unpaired t-test ($p<0.05$). Estrogen receptor and progesterone receptor were compared using Chi-square test ($p<0.05$).

Results

Twenty-five patients were enrolled in the present study, 15 in the BPS group and 10 in the control group (Table 1). Mean age of patients in the BPS group was 45±12 years old (range 21-61 years old), whereas that in the control group was 52±12 years old (range 32-71).

In BPS patients, 10 of 15 patients (67%) had pain at suprapubic area and 5 of 15 patients (33%) had pain at the urethra. Mean duration of symptoms was 3±1.7 years (range 1-6 years). Regarding lower urinary tract symptoms, frequency was found in all patients in the study group. Ninety-three percent of patients (14 in 15 patients) voided more than 10 times a day. Nocturia was found in 13 of 15 patients (87%). Other symptoms found in the study group were urgency, dyspareunia, stress urinary incontinence, and acute urinary retention. According to visual analog scale, mean pain score was 8.2±1.6 (range 5-10).

Estrogen receptor was found 14 in 15 patients in BPS group (93%) and 7 in 10 patients in the control group (70%). Progesterone receptor was found 10 in 15 patients in BPS group (66.7%) and 5 in 10 patients in the control group (50%). Both were not significantly different with $p = 0.267$ and $p = 0.678$, respectively.

Discussion

Bladder pain syndrome (BPS) or painful bladder syndrome (PBS) is defined as suprapubic pain related to bladder filling accompanied by other symptoms such as increased daytime and nighttime frequency, in the absence of other pathology. Interstitial cystitis (IC) represents a special type of chronic inflammation of the bladder and may be a subgroup that encompasses the patients with typical histologic and cystoscopic features.

BPS is supposed to be due to multifactorial etiologies such as infection, inflammation, autoimmune disease, glycosaminoglycan (GAG) layer defect in

Table 1. The number of patients with positive ER and PR

	BPS group	Control group	p-value
ER positive	14/15 (93.0%)	7/10 (70%)	0.267
PR positive	10/15 (66.7%)	5/10 (50%)	0.678

bladder urothelium leading to more fragile bladder mucosa, abnormal mast cell activation, alteration of autonomic responses, and CNS processing of afferent stimuli and pelvic floor muscle dysfunction. Despite many theories to explain the pathogenesis, there is little confirmation about it.

In the present study, the authors wondered whether the number of estrogen and progesterone receptor might be greater in patients with BPS/IC because there are many lines of evidences, not only the study in animals but also in humans, suggesting that these hormones may play a part in the etiopathogenesis of the disease. BPS/IC is believed to have an association with pelvic floor myofascial pain syndrome, which explains that why pelvic pain is the result of pelvic floor muscle spasm and hyperirritability, producing local and referred pain. Hormones such as estrogen, progesterone and relax in are believed to be involved in musculoskeletal pain through effects on muscle fibers, nociceptors, neuroinflammation or some other mechanisms.

Cabral CA et al (2003)[2] investigated the effects of female sex hormones on rat vesical extracellular matrix which were evaluated by analyzing glycosaminoglycan (GAG) and collagen composition under different hormone conditions including prepubertal female rat, adult intact female rat, adult oophorectomized female rat, adult male, and adult sham operated female. They found that viscoelastic properties are different among those five groups and oophorectomy performed at a prepubertal age may lead to greater vesical wall stiffness. Robbins MT et al (2010)[3] assessed the effect of oophorectomy and estrogen replacement in nociceptive responses to bladder distention in a rat model and found that an acute decrease in estrogen results in increased visceromotor responses, whereas estrogen alone is not sufficient to produce increased nociceptive responses. These data suggest that the pronociceptive effects of estrogen may be the result of a mismatch between peripheral vs. central and/or genomic vs. non-genomic effects, which occur during rapidly decreasing estrogen level. Ying Cheng et al (2009)[4] investigated whether estrogen could directly modulate signaling pathways within bladder sensory neurons. Their results provide new insights into the complex effects of estrogens on bladder nociceptor signaling, revealing a pattern of activation of p38 and extracellular signal-related kinase (ERK) and mitogen-activated protein kinase (MAP kinase) in lumbosacral dorsal root ganglia. These may have a role in pelvic visceral pain and raise the possibility of developing new way to modulate their function in hyperactivity or pain states. Imamov O et al (2007)[5] found that estrogen receptor β is expressed in bladder urothelium and localize in the basal cell layer of mice bladder. In bladder of ERβ deficit female mice, there are specific morphological changes that bear a strong resemblance to the bladder of patients with IC.

Marcus VO Maroclo et al (2005)[6] investigated whether the menstrual cycle affects urinary glycosaminoglycan (GAG) excretion in normal young women. They concluded that excretion of total urinary GAG during the normal menstrual cycle of young women has a biphasic pattern with significantly higher values occurring in the first half of the cycle. This variation implies modulation by estrogens and consequently it should be considered when comparing the GAG concentration in urine samples from women of childbearing age.

Powell-Boone T et al (2005)[7] studied about menstrual cycle effect to bladder pain sensation in patients with IC. They reported that bladder sensation in females is affected by menstrual cycle and an overall increase in clinical pain is noted in subjects with IC.

Pang X et al (1995)[8] investigated the role of estrogen in the pathophysiology of IC with respect to activated bladder mast cells. They found that bladder mast cells have high affinity to ER and there are a higher number of mast cells present in patients with IC compared with control. They concluded that estradiol could augment mast cell histamine secretion in response to the neuropeptide, substance P, which may be released in certain neuroinflammatory disorders.

Theoharides TC et al (1998)[9] explained the pathophysiology of IC by a defective bladder glycosaminoglycan (GAG) which can be damaged by mast cell-derived proteases. Bladder mast cells are located close to neuronal processes and they can be activated in situ by acetylcholine (ACh) and substance P (SP). Such activation is augmented by estradiol, which acquires significance in view of the fact that human bladder mast cells express estrogen receptors, but few progesterone receptors, which may explain the worsening of IC symptoms during ovulation. Bjorling DE et al (2001)[10] demonstrated that the mucosa is the primary source of nerve growth factor (NGF) in the mouse bladder, and the bladder mucosa also expresses estrogen receptor (ER)-alpha, ER-beta, and the high-affinity NGF receptor tyrosine kinase A. Estrogen may also modulate neurogenic inflammation.

These observations indicate that estrogen has the capacity to influence the onset and course of neurogenic inflammation of the bladder.

According to the study of Diaa E. Rizk et al (2001)[11], who investigated evidence of ER and PR in patients with incontinence, it was found that immunostaining for estrogen and progesterone receptor are similar in trigonal and lateral wall samples. In another study from the same author about the presence of receptors in the layers of the urinary bladder, the preliminary results showed positive immunostaining for estrogen receptors in the detrusor muscle as well as the mucosa of the bladder in the receptor positive group. Therefore, the authors decided to do a biopsy of bladder mucosa only at trigone to minimize possible bleeding. Not only premenopausal women but also postmenopausal women were included in our control group, because the same previous study reported that there was no significant differences in immunostaining for estrogen or progesterone receptors in the trigonal mucosa of pre- versus post-menopausal women.

In the present study, the authors hypothesized whether the bladder mucosa of patients with BPS/IC might be different from bladder mucosa of patients without bladder pain symptoms regarding ER and PR. The authors concluded that ER and PR might not play a role in the etiopathogenesis of BPS/IC base on our study. However, other receptors should be further investigated about their roles of etiopathology.

Conclusion

Even though estrogen was believed to play a role in the pathophysiology of BPS/IC, estrogen receptor and progesterone receptor of patients with BPS were not found to be significantly different in bladder mucosa compared with patients with normal voiding symptoms.

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Potential conflicts of interest

None.

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ตัวรับเอสโตรเจนและโปรเจสเตอโรนในเยื่อบุผิวกระเพาะปัสสาวะของผู้ป่วยกลุ่มอาการปวดกระเพาะปัสสาวะ (bladder pain syndrome)

ปกเกศ อึ้งภากรณ์, พนัส เฉลิมแสนยากร, สุพรรณี นิลสกุลวัฒน์, วชิร คชการ

วัตถุประสงค์: *เพื่อศึกษาหาตัวรับเอสโตรเจนและโปรเจสเตอโรนในเยื่อบุผิวกระเพาะปัสสาวะของผู้ป่วยกลุ่มอาการปวดกระเพาะปัสสาวะ (bladder pain syndrome) เปรียบเทียบกับกลุ่มที่ไม่มีอาการ*

วัสดุและวิธีการ: *ทำการศึกษาผู้ป่วยที่มีอาการปวดกระเพาะปัสสาวะ ที่มารับการรักษาที่โรงพยาบาลรามาธิบดี ตั้งแต่เดือนกรกฎาคม พ.ศ. 2552 ถึงเดือนธันวาคม พ.ศ. 2553 จำนวน 15 ราย ทำการตัดชิ้นเนื้อเยื่อบุผิวกระเพาะปัสสาวะส่งตรวจตัวรับเอสโตรเจนและโปรเจสเตอโรนโดยใช้วิธีย้อม immunohistochemistry เทียบกับกลุ่มควบคุมเป็นผู้ป่วยที่ไม่มีอาการแต่ได้รับการผ่าตัดเปิดกระเพาะปัสสาวะจากสาเหตุอื่น จำนวน 10 ราย นำข้อมูลผลการตรวจตัวรับเอสโตรเจนและโปรเจสเตอโรนของทั้ง 2 กลุ่มมาเปรียบเทียบกัน*

ผลการศึกษา: *ผลการตรวจชิ้นเนื้อเยื่อบุผิวกระเพาะปัสสาวะพบว่าตัวรับเอสโตรเจนให้ผลบวกในกลุ่มทดลอง 14 ใน 15 ราย คิดเป็น 93% และให้ผลบวกในกลุ่มควบคุม 7 ใน 10 ราย คิดเป็น 70% ($p = 0.267$) ตัวรับโปรเจสเตอโรนให้ผลบวกในกลุ่มทดลอง 10 ใน 15 ราย คิดเป็น 66.7% และให้ผลบวกในกลุ่มควบคุม 5 ใน 10 ราย คิดเป็น 50% ($p = 0.678$)*

สรุป: *ตัวรับเอสโตรเจนและโปรเจสเตอโรนในผู้ป่วยกลุ่มอาการปวดกระเพาะปัสสาวะ (bladder pain syndrome) ไม่มีความแตกต่างจากกลุ่มที่ไม่มีอาการอย่างมีนัยสำคัญทางสถิติ จึงไม่น่าจะมีผลโดยตรงต่อการเกิดโรคกลุ่มอาการปวดกระเพาะปัสสาวะ*