The Role of Immunoglobulin E in the Pathogenesis of Ketamine Related Cystitis and Ulcerative Interstitial Cystitis: An Immunohistochemical Study

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Background: A previous study revealed elevated serum Immunoglobulin E (IgE) in ketamine related cystitis (KC) patients. IgE might participate the pathogenesis of different types of bladder pain syndromes, including KC and interstitial cystitis (IC).

Objectives: To investigate the IgE expression in KC and IC bladder tissue.

Study Design: Prospective evaluation.

Setting: The study was conducted in a tertiary teaching hospital, Hualien Tzu Chi Hospital.

Methods: We investigated the bladder IgE with immunofluorescence staining and quantification. The active mast cells were measuring using tryptase. The symptoms and urodynamic study results were recorded. Double immunofluorescence staining of tryptase and IgE was also performed. Sixteen KC patients, 10 ulcerative IC patients, and 20 non-ulcerative IC patients participated. The history and urodynamic parameters were investigated in these patients. The bladder mucosa was biopsied during cystoscopic hydrodistention. Bladder biopsies were also taken from 22 patients with bacterial cystitis and 12 healthy controls.

Results: Bladder IgE was positive in 15 (93.8%) KC patients, 9 (90%) ulcerative IC patients, one (5%) non-ulcer IC patient, 8 (36.4%) bacterial cystitis patients, and 2 (16.7%) controls ($P < .001$). The bladder IgE was greater in the patients with KC than in the others ($P < .001$). After excluding KC patients, bladder IgE was significantly higher in the patients with ulcerative IC than the others ($P < .001$). The bladder IgE was significantly correlated with pain on a visual analogue scale (r2 = 0.156, $P = .017$) and maximum bladder capacity (r2 = 0.423, $P < .001$). Tryptase expression did not show a significant difference between KC, ulcer IC, and non-ulcer IC ($P = 0.222$). Double immunofluorescence staining showed co-expression of tryptase and IgE.

Conclusions: IgE-mediated inflammation played a significant role in the pathogenesis of KC and ulcerative IC.

Key words: Immunoglobulin E, ketamine cystitis, interstitial cystitis

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S ince the European Society for the Study of Interstitial Cystitis proposed the diagnostic criteria and classification for interstitial cystitis in 2008, scientists have used the term bladder pain syndrome (BPS) to describe patients with painful urinary symptoms (1). BPS is diagnosed on the basis of chronic pelvic pain or discomfort perceived by the patient to be related to the urinary bladder and accompanied by at least one urinary symptom such as persistent urge to void or frequency of voiding (1). Patients with bladder diseases with demonstrable pathophysiology as the cause of the symptoms must be excluded from

the BPS diagnosis (1). The European Association of Urology guidelines suggests that the patients with BPS could be classified according to the cystoscopic finding of Hunner's lesion and the histopathologic results of a bladder biopsy (2).

An earlier study reported some clinical differences between ulcerative and non-ulcerative interstitial cystitis/bladder pain syndrome (IC/BPS) (3). However, the pathogenetic differences between ulcerative and non-ulcerative IC/BPS remain unclear. Ketamine related cystitis (KC) is an emerging clinical syndrome characterized by severe bladder pain and small bladder capacity, but the actual pathophysiology of KC is unclear (4). Based on its clinical characteristics, KC might also be considered within the spectrum of BPS.

Although the pathophysiology of BPS has been investigated for many years, it is still not well understood. The most common abnormal histopathologic findings in the bladders of BPS patients are epithelial denudation, mononuclear cell inflammation, and an increase in mast cells (5). Neurogenic inflammation, urothelial dysfunction, and central sensitization are considered possible pathogenic mechanisms of BPS (6-8). C-Fiber afferent nerve upregulation and releasing neuropeptides such as substance P, nerve growth factor, and calcitonin gene-related peptide could directly increase mast cell degranulation in the urinary bladder and induce inflammation (9). However, our previous study found increasing serum immunoglobulin E (IgE) levels in the patients with KC (10). Does IgE mediated inflammation also participate in the pathogenesis of bladder inflammation in the BPS and KC patients? The aim of the current study was to investigate the role of bladder IgE in the pathogenesis of BPS among patients with KC, and ulcerative and non-ulcerative IC.

Methods

The institutional review board and ethics committee of the Buddhist Tzu Chi General Hospital (IRB number 98-50 and 101-61) approved the study. Each patient was informed of the study rationale and procedures, and written consent was obtained before the bladder procedures.

From 2012 to 2014, BPS patients, including those with IC and KC, who were admitted to Hualien Tzu Chi General Hospital for cystoscopic hydrodistention were enrolled in this study. The diagnosis of IC was based on the National Institute of Diabetes and Digestive and Kidney Diseases criteria for IC. The IC patients were classified into ulcerative and non-ulcerative types ac-

cording to the cystoscopic finding of Hunner's lesions (1). The patients with KC were clinically diagnosed based on symptoms of bladder pain, urinary frequency, urgency, and previous recreational use of ketamine for at least 6 months. The frequency of ketamine use in KC patients was at least twice per week, and the dose was at least 3 g every time. The BPS patients with concurrent urological problems such as acute bacterial cystitis, urolithiasis, stress urinary incontinence, or neurogenic voiding dysfunction were excluded. The patients with a history of neoplasms, asthma, autoimmune diseases, and evidence of parasitic infection were also excluded. The patients with current respiratory tract symptoms (such as rhinorrhea or sneezing), itchy skin, or any symptoms that might be induced by allergic diseases were also excluded.

All patients underwent comprehensive medical history reviews after admission to the hospital. The visual analogue scale (VAS) scores for bladder pain were recorded. All patients underwent video-urodynamic studies to confirm the diagnosis and rule out the co-existence of other bladder diseases, and the cystometric bladder capacity (CBC) was recorded. Blood samples were also collected to investigate serum IgE levels using a solid-phase immunoassay (Phadia Specific Immunoglobulin FEIA). All patients underwent cystoscopic hydrodistention under general anesthesia at an intravesical pressure of 80 cm of water, and the maximal bladder capacity was recorded.

Random cold-cup biopsies of the posterior bladder wall for patients with KC and non-ulcer IC were obtained after cystoscopic hydrodistention. The bladder biopsies for the ulcer IC patients were performed at the sites just around the Hunner's lesion. Each specimen was 2 mm in diameter and contained mucosal and submucosal tissues. The pathology department performed the histopathology staining, and our laboratory performed the immunochemical staining analyses on the specimens. The bladder histologies were reviewed by a single pathologist who was masked to the clinical results. Bladder inflammation and eosinophil infiltration were graded on a scale of none, mild, moderate, or severe. In addition, the non-IC patients admitted to the hospital for anti-incontinence surgery or non-lower urinary tract surgery were also enrolled for the comparative study. During the operation, random bladder biopsies were obtained from only the patients without systemic infection symptoms, and the specimens were investigated for IgE. The patients with evidence of pyuria and bacteria in their urine cultures were consid-

ered as bacterial cystitis, and the patients with sterile urine were considered as normal controls.

Immunochemical Staining for Bladder IgE and Tryptase

The IgE expression in the bladder was assessed with immunochemical staining and the mast cell activation in the bladder was measured with tryptase. The bladder specimens were immersed and fixed in an ice-cold solution of 4% formaldehyde in phosphate buffered saline (PBS) (pH 7.4) for one hour. Next, they were rinsed with ice-cold PBS containing 15% sucrose. Biopsy specimens were embedded in optimum cutting temperature medium and stored at -80°C. Four sections per specimen were cut using a cryostat at a thickness of 8 μm and collected on new saline III-coated slides (Muto Pure Chemicals Co., Ltd., Tokyo, Japan). Sections were post-fixed in acetone at -20°C and blocked with rabbit serum. The sections were incubated overnight at 4°C with primary antibodies to anti-human IgE (Thermo Scientific, Fremont, CA, USA) and to anti-human tryptase (Chemicon, Temecula, CA, USA), respectively. After rinsing the sections with 0.1% Tween-20 in PBS, goat anti rabbit-IgG conjugated fluorescein isothiocyanate secondary antibodies (ANA Stec, Fremont, CA, USA) were applied to the sections and incubated for one hour. Finally, the sections were counterstained. Negative controls included the isotype of the primary antibody. We obtained the mean, maximum, range, and standard deviation (SD) of the staining intensity and the percent positive area measurements using 4 random hot spots within each specimen. The number of positively stained cells/total cells per unit area (4 μm²) were counted. The percentage of positive cells was calculated per 100 total cells. Double immunofluorescence staining of IgE and tryptase to identify mast cells was also performed. The procedure was performed as given above, with 2 primary antibodies (anti-human IgE and anti-human tryptase) and 2 secondary antibodies (tetramethylrhodamine isothiocyanate conjugated swine anti-rabbit antibody for IgE and fluorescein isothiocyanate conjugated rabbit anti-mouse antibody for tryptase) (DakoCytomation Denmark A/S, Glostrup, Denmark).

The differences in clinical parameters among the patients with KC, ulcerative IC, and non-ulcerative IC were analyzed using the Kruskal-Wallis test. The bladder IgE quantification results between the BPS, bacterial cystitis, and normal control patients were also analyzed. The post-hoc analyses applied Scheffé's method. The correlation between IgE and the clinical parameters was analyzed using linear regression. The histologic differences were compared using the chi-square test. All calculations were performed using SPSS for Windows, version 16.0 (SPSS, Chicago, IL). A P value < 0.05 was considered statistically significant.

RESULTS

A total of 10 patients with ulcerative IC (all women), 20 with non-ulcerative IC (16 women and 4 men), 16 with KC (6 women and 10 men), 22 with bacterial cystitis (10 women and 12 men), and 19 normal controls (14 women and 5 men) were enrolled. The mean ages of the patients with ulcerative and non-ulcerative IC were 56.4 ± 15.2 and 44.2 ± 16.5 years, respectively. KC patients, those with bacterial cystitis, and normal controls were 28 ± 5.2, 52.8 ± 14.2, and 46.3 ± 18.1 years, respectively. All patients underwent complete clinical examinations and cystoscopic hydrodistention with bladder biopsies. Among the BPS patients, the KC patients had significantly higher VAS pain scores and smaller CBC and MBC (all P < 0.001) (Table 1). In the histopathology review, 11 (78.6%) KC patients and 4 (40%) patients with ulcerative IC had moderate to severe bladder inflammation. None of the patients with non-ulcerative IC had moderate or severe inflammation (P < 0.001). Similarly, moderate to severe eosinophil infiltration in the bladder was found in 12 (75.1%) KC patients and 4 (40%) patients with ulcerative IC. No eosinophil infiltration was observed in the patients with non-ulcerative IC (P < 0.001).

The KC patients had a significantly higher mean serum IgE level than the patients with ulcerative and non-ulcerative IC (P = 0.016) (Table 1). The mean serum IgE level in patients with ulcerative IC did not differ from that of patients with non-ulcerative IC (P = 0.181). The serum IgE levels correlated significantly with VAS (r2 = 0.107, P = 0.01), CBC (r2 = 0.132, P = 0.011), and MBC (r2 = 0.079, P = 0.018). After excluding the KC patients from the BPS patients, the mean serum IgE level of the remaining BPS patients did not significantly correlate with the VAS, CBC, or MBC (P = 0.081, 0.127, and 0.157, respectively).

Immunohistochemical staining for bladder IgE was positive in 15 of 16 (93.8%) KC patients and 9 of 10 (90%) patients with ulcerative IC. In contrast, only one of 20 (5%) patients with non-ulcerative IC, 8 of 22 (36.4%) bacterial cystitis patients, and 2 of 19 (10.5%) normal controls were positive for bladder IgE. The bladder IgE positive rate was significantly higher in patients with KC and ulcerative IC than in the other

Table 1. *Clinical symptoms and immunofluorescence staining results in thepatients with bladder pain syndrome and non-bladder pain syndrome.*

	KC (N = 16)	Ulcerative IC (N = 10)	Non-ulcerative IC (N = 20)	Bacterial cystitis (N = 22)	Normal controls (N = 12)	*P*-value	*P*-value (excluding KC)
VAS	8.1±0.9	7.7±2.3	5.2±2.9	N/A	N/A	0.001	0.025
CBC (mL)	60.5±29.2	171.5±55.6	301.3±130.0	N/A	N/A	<0.001	0.006
MBC (mL)	168.7±74.2	480.0±168.7	607.5±164.9	N/A	N/A	<0.001	0.057
Bladder IgE Positive Quantification	15(93.8%) 4.97±4.27	9 (90%) 1.83±1.68	1 (5%) 0.05±0.21	8 (37.5%) 0.47±0.68	2 (10.5%) 0.20±0.48	<0.001 < .0001	<0.001 <0.001
Serum IgE (IU/mL)	599.8±678.6	278.9±635.9	81.8±105.0	N/A	N/A	0.016	0.181
Activated mast cells	8.63±6.20	7.90±5.63	12.87±8.39	N/A	N/A	0.222	0.112

CBC: cystometric bladder capacity, IC: interstitial cystitis, KC: ketamine related cystitis, MBC: maximal bladder capacity, VAS: visual analogue scale.

patients ($P < 0.001$) (Fig. 1). The quantitative bladder IgE results showed significantly greater mean IgE in the KC patients than in the patients with ulcerative IC, non-ulcerative IC, bacterial cystitis, and the normal controls ($P < 0.001$) (Table 1). After excluding the KC patients from the BPS group, the patients with ulcerative IC had significantly higher bladder IgE than the remaining patients did ($P < 0.001$). The number of active mast cells did not show a significant difference between the KC, ulcerative IC and non-ulcerative IC patients. ($P = 0.222$, Table 1).

Among all BPS patients, bladder IgE was significantly correlated with VAS ($r^2 = 0.119$, $P = 0.007$), CBC ($r^2 = 0.277$, $P < 0.001$), and MBC ($r^2 = 0.333$, $P < 0.001$).

After excluding KC patients, bladder IgE remained significantly correlated with VAS ($r^2 = 0.124$, $P = 0.019$) and CBC ($r^2 = 0.192$, $P = 0.006$) but not MBC ($P = 0.114$). However, the bladder IgE was not significantly correlated with VAS, CBC, or MBC in the KC patient group alone or in the ulcerative IC/BPS patient group. In addition, the BPS patients with moderate and severe eosinophil infiltration had significantly higher bladder IgE than did the patients with no or mild eosinophil infiltration (4.16 ± 3.96 vs. 1.01 ± 2.47, respectively, $P = 0.014$). Double immunochemical staining of the bladder mucosa in patients with KC showed co-expression of tryptase and IgE. The locations of IgE and tryptase were identical (Fig. 2, A and B).
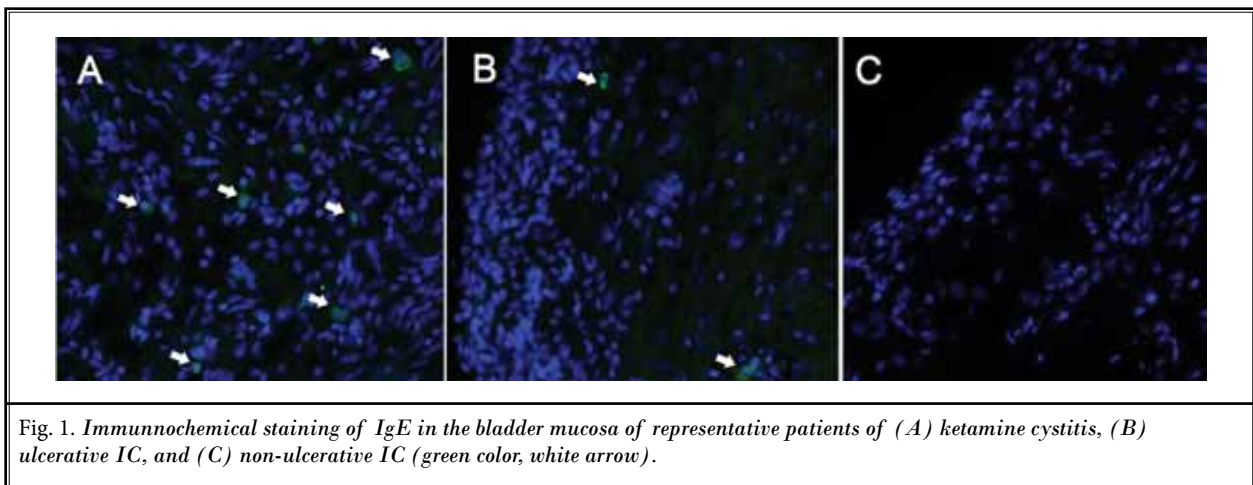

Fig. 1. *Immunochemical staining of IgE in the bladder mucosa of representative patients of (A) ketamine cystitis, (B) ulcerative IC, and (C) non-ulcerative IC (green color, white arrow).*
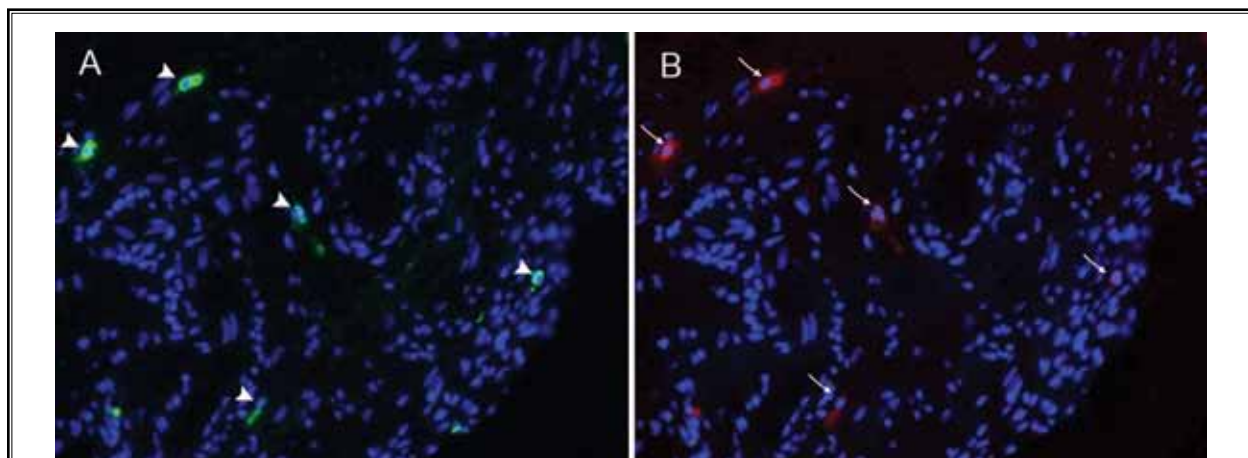
Fig. 2. *Double immunohistochemical staining of tryptase and IgE in the bladder mucosa of a representative patient with ketamine cystitis. (A) Expression of tryptase specific to mast cells (green color, white arrowhead). (B) Expression of IgE (red color, white arrow). The location of IgE expression (B) is identical to the location of the mast cells identified by tryptase staining in panel A.*

DISCUSSION

Although many researchers have investigated the pathophysiology of BPS in the past 20 years, the disease remains a mystery. Current consensus suggests that the etiology of BPS is multifactorial, and the symptom complex results from one or more different pathways (1). Earlier studies suggested that BPS patients should be classified into ulcerative and non-ulcerative IC subtypes according to the cystoscopic findings. The recent guidelines also consider the histopathology findings in the classification criteria for BPS (1). However, clinician understanding of the pathophysiologic differences between the different BPS subtypes remains limited. Previous studies showed increased mast cells in the bladders of patients with ulcerative and non-ulcerative IC (11). Mast cells could be activated by IgE binding with high affinity IgE receptors (FcεRI), then release many inflammatory mediators and proliferate (12). IgE is well known for its essential role in type I hypersensitivity, but neurogenic inflammation also might increase IgE levels without classical hypersensitivity reaction (13,14). The current study provides immunochemical staining evidence of increased IgE in the bladders of KC and ulcerative IC patients but not in non-ulcerative IC. IgE-mediated inflammation might play an important role in the pathogenesis of these diseases via neurogenic inflammation or hypersensitivity.

Since the first report of KC in 2007, the pathogenesis of KC has attracted the interest of many urological researchers (4). A previous animal study showed that enhanced purinergic receptor P2X1 was involved in the pathogenesis of KC (15). Recent animal and human studies also showed increased cyclooxygenase-2 and nitric oxide synthase (NOS) in the bladders of patients with KC (16,17). However, these studies do not explain why 73% of patients who abuse ketamine did not develop urinary symptoms even with high-dose ketamine use (18).

Previously, we found increased numbers of mast cells in the bladders of patients with KC (19). Additionally, the mast cell expression and MBC correlated in a positive manner (19). We also found abnormally elevated serum IgE in 55% of KC patients. The patients who had recently used ketamine had significantly higher serum IgE levels (10). The immunohistochemical staining results of the current study showed increased expression of bladder IgE in patients with KC compared with that of patients with ulcerative IC, non-ulcerative IC, bacterial cystitis, and normal controls. The double immunohistochemical staining also showed co-expression of mast cells and IgE. These data suggest IgE mast-cell-mediated inflammation, either via neurogenic inflammation or hypersensitivity may play a significant role in the pathophysiology of KC and explain, in part, why only some ketamine abusers developed KC.

Recently, consideration of the pathogenesis of ulcerative and non-ulcerative IC has triggered heated debate. Logadottir et al (20) reported increased bladder wall nitric oxide production in ulcerative IC patients

compared to undetectable nitric oxide in non-ulcerative IC patients. Gamper et al (21) found increased mast cell activation in the bladders of patients with ulcerative IC in contrast to non-ulcerative IC. However, our current study showed no significant difference between ulcerative and non-ulcerative IC. The non-ulcerative IC patients in the current study had increased mast cells activation, and it is consistent with our and some others researchers' previous studies (5,19). The difference might have resulted from using different diagnosis criteria of IC between our study and Dr. Gamper et al's study (21). The results of this study showed increased bladder IgE expression in ulcerative IC compared to that in non-ulcerative IC. In addition, bladder IgE was significantly correlated with VAS and CBC in these IC patients. These data suggest that IgE, in combination with mast cells, contributes to the pathogenesis of ulcerative IC but not non-ulcerative IC. The mast cells activation in ulcerative IC and non-ulcerative IC might result from different pathogenesis pathways. A recent study showed a high expression of T- and B-cells markers in the bladders of patients with ulcerative IC and significantly increased IgA and IgG in their urine but not in their blood (22). IgE in combination with mast cells could recruit and activate T and B cells (12), induce a local immune response in the bladder, and produce upstream pathogenesis in ulcerative IC.

In the current study, serum IgE was abnormally elevated in 9 of 16 KC patients and only one of 10 ulcerative IC patients. In contrast, most of the KC and ulcerative IC patients were positive for bladder IgE. Bladder IgE in ulcerative IC differed significantly from that of non-ulcerative IC, but this difference was not observed when comparing serum IgE levels. The half-life of IgE is weeks to months if it is bound to cells in tissues, but it is only about 6 hours when it is free in the serum (23). Bladder IgE could be a better biomarker for patients with BPS than serum IgE levels.

The main limitation of this study is the small number of cases. Due to the gender distribution, the ulcer and non-ulcerative IC patients were mostly women in the current study. The patients with IC were also usually older than the KC patients. The healthy controls were stress urinary incontinence patients, all women. The non-matched age and gender in different groups might reduce the strength of the results. The diagnosis of KC should be established by urine ketmine test instead of only subjective history of ketamine abuse. The number of eosinophils should also be quantified to obtain a more objective result. Histopathology interpretation by a single pathologist can lead to significant bias, especially with a non-validated scale. Further study to confirm hypersensitivity in the bladder could be accomplished by invesitgating the IgE receptors on mast cells such as FcεRI receptors or IgE receptors on epithelial CD23 cells. In vitro stimulation testing or patch testing of KC patients or patients with ulcerative IC could also be helpful in elucidating hypersensitivity reactions.

Conclusion

Greater bladder IgE was found in patients with KC and ulcerative IC than in patients with non-ulcerative IC and normal controls. The increase in bladder IgE correlated with the clinical symptoms. IgE mediated inflammation plays a role in the pathogenesis of KC and ulcerative IC.

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