231

Hsieh J H[1], Chen C Y[1], Kuo H C[1]
1. *Buddhist Tzu Chi General Hospital and Tzu Chi University*

INCREASED APOPTOSIS AND DECREASED JUNCTION PROTEIN EXPRESSION OF UROTHELIUM DUE TO SUBUROTHELIAL INFLAMMATION IN PATIENTS WITH INTERSTITIAL CYSTITIS/PAINFUL BLADDER SYNDROME

Hypothesis / aims of study
Interstitial cystitis/painful bladder syndrome (IC/PBS) is a chronic bladder condition characterized by bladder pain, frequency and nocturia. There is no clear definition of IC/PBS as the clinical symptoms and histology are not specific. Recent findings have proposed several pathophysiological mechanisms including epithelial dysfunction, activation of mast cells, neurogenic inflammation, autoimmunity and occult infection. However, the triggering factor that leads to disease is still unclear. One of the most common findings in IC/PBS patients is denudation or thinning of the bladder epithelium, suggesting an altered regulation of urothelial homeostasis. This study investigated the relationship between suburothelial inflammation and urothelial dysfunction in IC/PBS.

Study design, materials and methods
Thirteen women with characteristic symptoms of IC/PBS and proven by cystoscopic hydrodistention were enrolled in this study. Another 6 women with stress urinary incontinence without irritative bladder symptoms served for the controls. Bladder biopsies at three sites were taken immediately after cystoscopic hydrodistention for the diagnosis of IC/PBS and from normal controls during anti-incontinence procedures. All patients were not previously treated for IC/PBS before the bladder procedure. The IC/PBS patients were assessed by Oleary-Sant symptom score and visual analog scale (VAS) pain score. Functional bladder capacity (FBC) was obtained from the three day voiding diary. The maximal bladder capacity (MBC) was recorded during cystoscopic hydrodistention at the intravesical pressure of 80 cm H_2O and the degree of glomerulation after hydrodistention was recorded as grade 0 to 4 representing none, mild, moderate and severe degree.Three bladder biopsies were obtained in all patients with IC/PBS just after cystoscopic hydrodistention. The immunofluorescence (IF) staining of ki-67, E-cadherin and TUNEL staining were performed in all bladder tissues from IC/PBS (n=13) and controls (n=6). Mast cell activation was also analyzed by IF of tryptase and the fluorescence of intensity was measured by Image J. The expressions of urothelial dysfunction in IC/PBS were compared with the control bladders.

Results
There was significantly down-regulation of the ratio of ki-67 positive cells/total cells in IC/PBS compared to the controls (0.885±1.06 v 1.23±1.28, p=0.011). TUNEL staining revealed significant increase of the number of apoptotic cells in IC/PBS bladders compared with controls (4.69±5.80 v 0.06±0.13, p=0.013). The results of IF also showed significantly reduced in the distribution of E-cadherin in IC/PBS bladders compared with controls (5.84±6.37 v 61.1±16.0, p=0.006). The tryptase signal of IC/PBS was significantly increased comparing with the controls (8.46±5.78 v 1.15±0.43, p=0.000). Decreased expression of E-cadherin was significantly correlated with visual analog pain score in IC/PBS patients. Co-existence of increased apoptosis, decrease of E-cadherin expression and mast cell activation was noted in all 13 patients with IC/PBS compared with the mean value of the controls. In addition, 11 of the 13 IC/PBS patients had a decrease of urothelial proliferation compare with the controls. (Table 1) While we correlated the expressions of TUNEL, ki-67, E-cadherin, tryptase and clinical presentations of IC/PBS patients, the correlation was non-significant except that of the E-cadherin and VAS.

Interpretation of results
The results of this study revealed that chronic inflammation in suburothelium is consistently associated with increased urothelial apoptosis, decreased urothelial cell proliferation and significantly reduced E-cadherin in the IC/PBS patients. The severity of urothelial dysfunction as shown by reduced E-cadherin expression is significantly correlated with clinical pain score in patients with IC/PBS. This result suggests a link between suburothelial inflammation, increased urothelial cell apoptosis, decrease junction protein expression and clinical symptoms in IC/PBS bladder. The decreased signal of ki-67 and increased signal of TUNEL in urothelium might play an important role in the pathogenesis of IC/PBS and clinical presentation of bladder pain. Although ki-67 did not correlate well with VAS in linear regression analysis, the result of TUNEL assay and VAS showed a trend of positive correlation between the apoptotic cell number and bladder pain severity in IC/PBS. Although the correlation between TUNEL and ki-67 with VAS pain score and MBC are not significant, the trend that a higher apoptosis rate and lower E-cadherin expression is associated with higher VAS in IC/PBS patients did exist. Moreover, All IC/PBS patients had abnormally increased mast cell activation, decreased E-cadherin expression, increased TUNEL staining, and most of them also had a lower proliferation in urothelium. These evidences indicate the link between chronic suburothelial inflammation and abnormal urothelial differentiation.

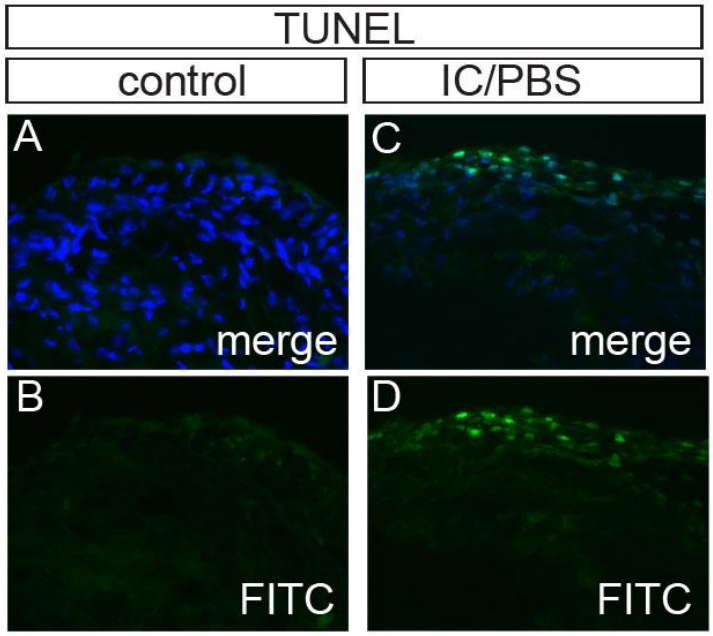
Concluding message
The growth rate and junctions between urothelial cells in bladder tissue of IC/PBS are abnormal. Highly activated mast cells exist in urothelium and suburothelium in IC/PBS. These results suggest abnormal urothelial function in IC/PBS is associated with chronic inflammation of the bladder and cause clinical symptoms as well as bladder pathologies.

Table 1. Expression of E-cadherin, ki-67, TUNEL and mast cell activation in IC/PBS patients and controls.

	IC/PBS (n= 13)	Control (n=6)	P value
E-cadherin	5.84 ± 6,37	61.1 ± 16.0	0.006
Ki-67	0.541 ± 0.70	1.23 ± 1.28	0.016

TUNEL	4.69 ± 5.80	0.055 ± 0.13	0.013
Mast cell activation	8.46 ± 5.78	1.15 ± 0.43	0.000

Figure 2. The number of apoptosis cells increased in IC/PBS patients compare with the controls.



Specify source of funding or grant	**National Science Council of Taiwan**
Is this a clinical trial?	**No**
What were the subjects in the study?	**HUMAN**
Was this study approved by an ethics committee?	**Yes**
Specify Name of Ethics Committee	**Institutional Review Board of Buddhist Tzu Chi University and Tzu Chi General Hospital**
Was the Declaration of Helsinki followed?	**Yes**
Was informed consent obtained from the patients?	**Yes**