Urinary Galectin-3 and CXCL-7 as biomarkers for diagnosing interstitial cystitis and correlation with clinical characteristics

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Abstract

Background: Interstitial cystitis (IC) is a chronic inflammatory disease of the bladder, while both Chemokine (C-X-C motif) ligand 7 (CXCL-7) and Galectin-3 are the molecule related inflammatory diseases.

Objective: This study sought to investigate the potential feasibility of diagnosis of interstitial cystitis by measurement of the urinary level of Chemokine (C-X-C motif) ligand 7 (CXCL-7) and Galectin-3.

Methods: Both interstitial cystitis patients with or without Hunner’s ulcers were recruited in the study, while healthy subjects were involved as controls. Then we collected urine samples from all the involved subjects, and the CXCL-7 level and Galectin-3 level in the supernatants of urine were measured by ELISA. Urinary creatinine concentrations (mg Cr) of involved subjects were measured to normalize the level of CXCL-7 and Galectin-3. Also the clinical data and demographic characteristics of the recruited subjects were collected. The Receiver-operating curve (ROC) analysis was performed to explore the sensitivity and specialty of both CXCL-7 and Galectin-3 to identify interstitial cystitis. And the associations between urinary levels (CXCL-7 or Galectin-3) and the symptom severity (O’Leary and Sant Symptom, OSSI index) were analyzed with linear regression.

Results: The urinary levels of both CXCL-7 and Galectin-3 were significantly increased in interstitial cystitis compared to the healthy controls. The urinary CXCL-7 showed the sensitivity of 59.5% and specificity of 94.6%, at the cut-off value 21.30 pg/mg Cr to discriminate interstitial cystitis from controls, while the urinary Galectin-3 level showed the sensitivity of 62.2% and specificity of 91.9%, at the cut-off value 11.32 pg/mg Cr to discriminate interstitial cystitis from controls. Urinary Galectin-3 level was higher in interstitial cystitis patients with Hunner’s ulcers compared to patients without Hunner’s ulcers. The sensitivity and specificity were 45.5% and 93.3%, respectively for urinary CXCL-7 to discriminate from interstitial cystitis patients with Hunner’s ulcers to patients without Hunner’s ulcers, at the cut-off value of 35.97 pg/mg Cr. In addition, the urinary Galectin-3 level was positive correlated with the symptom severity.

Conclusion: CXCL-7 and Galectin-3 were elevated in the interstitial cystitis patients, both of which could be supplementary biomarker for diagnosis of interstitial cystitis. Moreover, urinary CXCL-7 might serve as the biomarker to decimate interstitial cystitis with Hunner’s ulcers from the without Hunner’s ulcers.

Introduction

Interstitial cystitis (IC) is a chronic inflammatory disease of the bladder, with a prevalence of approximately 10.6 cases per 100000 individuals, which is especially more common in women (approximately 18.1 cases/100000 individuals) than in men.

Although mucosal ulcerations, hemorrhages and extensive inflammation in the bladder have been observed through the histological examination of IC lesions, the pathogenesis of IC remains unclear,
which results in a lack of diagnostic biomarkers and therapeutic strategies. Moreover, multiple symptoms of IC in patients, such as pelvic pain, urinary urgency, and frequency, have overlap with some other bladder diseases, such as infection, overactive bladder, and so on. However, there is no reliable urine biomarker that can discriminate the IC from other bladder diseases, and the diagnosis of IC mostly relies on cystoscopy and histological examination, both of which could be economic burdens. And cystoscopy is an invasive examination and could induce patients’ distress. Therefore, an efficient urine biomarker is needed to improve the accuracy of diagnosis, avoid unnecessary invasive examination, as well as monitor the therapy outcome. However, even though some urine biomarkers have been developed and used to diagnose IC, such as neuronal growth factor (NGF), IL-10, RANTES, eotaxin, CXCL10, there is still no biomarker that can provide both high sensitivity and specialty.

In previous, through bioinformatical analysis, we identified several key genes in the pathology of IC, including (C-X-C motif) ligand 7 (CXCL-7) and Galectin3 (Gal-3). CXCL-7 is an important chemoattractant cytokine, which signals through binding to its receptor CXCR-2. CXCL-7 can recruit neutrophil during the inflammatory response, and participate in the cross-talk between neutrophils and platelet. Moreover, CXCL7 can participate in the pathology of some immune dysregulation diseases, such as rheumatoid arthritis. Besides, Gal-3 is a β-galactoside-binding evolutionarily highly conserved lectin of approximately 30 kDa. Gal-3 is located in the tissue micro-environment, extracellular, cytoplasmic, or nuclear, and regulates multiple biological systems including inflammation. And the elevated Gal-3 level has been found in some inflammatory diseases. Watanabe et al found the increased level of serum Gal-3 in idiopathic inflammatory myopathy patients, while Yu et al found the increased serum Gal-3 level in inflammatory bowel diseases. Therefore in this study, we aimed to assess the diagnostic value of both Gal-3 and CXCL-7 in IC by measurement their levels in urine using ELISA, in comparison with controls. A novel diagnostic algorithm was then developed to distinguish IC from controls based on the data we obtained.

**Subjects And Methods**

**Patients.**

Study protocols involving human subjects were approved by the Hebei general hospital institutional ethics committee. All methods were performed in accordance with the relevant guidelines and regulations. From 2017-2020, we enrolled 37 IC patients, and 37 cases of age (within 2 years) and gender matched healthy subjects as the normal control. All the involved patients agreed to the study and signed written informed consent forms were obtained on their behalf.

The diagnostic criteria for IC were based on the guidelines of Diagnosis and Treatment Interstitial Cystitis/Bladder Pain Syndrome (2014), constituted: suprapubic pain, pressure, or discomfort pressure or discomfort related to bladder filling, accompanied with urinary urgency and frequency, for more than 6 months. In addition, cystoscopy examination was applied on the IC patients, who then were divided into the subgroup of IC patients with Hunner’s ulcers (HIC) and subgroup without Hunner’s ulcers (NHIC).
Exclusion criteria of IC patients included as following: acute urinary tract infection, urinary incontinence, neurogenic bladder disease, neurodegenerative disorder(such as Alzheimer's disease, Parkinson's disease, multiple sclerosis), injury/surgery/disease on urinary system(kidney, bladder, urethra), pelvic organ, or nervous system, other inflammatory diseases other than IC(such as Crohn's disease and ulcerative colitis).

Demographic data and clinical variables were collected from the IC patients and healthy subjects, including gender, age, diabetes, hypertension, smoking status, alcohol history, body mass index (BMI). Besides, in the group of IC patients, the data about the O'Leary–Saint symptom index(OSSI), and the Bladder volume were collected to access the IC symptoms.

**Enzyme-linked immunosorbent assay (ELISA)**

Midstream urinary specimens(10ml) were collected from IC patients when diagnosed, and healthy controls when enrolled. All the samples were centrifuged at 3000rpm for 10 min to remove the cell debris, and then the supernatants were stored at -70°C until processing. Urinary Gal-3, CXCL-7 levels were measured using the ELISA method with a kit for Gal-3 (Beyotime biotechnology, Cat. PG363, China), CXCL-7 (R&D Systems, DY393, US) respectively.

In brief, 96-well plates were coated with different capture antibodies (target Gal-3 or CXCL-7), then 100 µl samples or standards were added into the wells accordingly and incubated for 1 hour at room temperature. Thereafter, the wells were aspirated, and 100 µl of the biotinylated detection antibody solution were added into each well, and incubate for 2 hours at room temperature, following the manufacturer's protocol. After incubation, the liquid was aspirated, 100 µl of working streptavidin-HRP solution was added into each well, and incubate for 30 minutes at room temperature. Then 100 µl of TMB substrate solution was added to each well and incubated for 30 min at room temperature. Subsequently, 100 µl of stop solution was added to each well. Finally, the absorbance of each well was measured using a multimode plate reader at 450nm. The standard reagents were used to generate a standard curve, and the Gal-3 or CXCL-7 levels were determined based on the standard curve.

**Statistical analyses**

All the data were analyzed in an R environment for statistical computing and graphics (version 3.6.2). Data of clinical variables and demographic characteristics were expressed as the mean ± SD. Statistical comparisons were performed by student's t-test for clinical variables, demographic characteristics and urinary Gal-3 and CXCL-7 levels.

Receiver operating characteristic curves (ROC) were generated to determine the suitable cut-off value. Moreover, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated respectively to discriminate between the IC group and normal controls, or between HIC and NHIC at the cut-offs. Associations between urinary levels and the symptom severity (OSSI) were tested analyzed with linear regression. Differences with p <0.05 were considered to be statistically significant.
Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Results

Demographic Data of the Involved Patients

A total of 74 patients (37 cases of IC, and 37 cases of normal controls) were recruited in this study (Tables 1 and 2, and Supplementary data), in which 22 cases were the IC patients with Hunner’s ulcers, 15 cases were IC patients without Hunner’s ulcers.

<table>
<thead>
<tr>
<th>Variable</th>
<th>IC patients</th>
<th>Controls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n</td>
<td>75</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Age(y)</td>
<td>45.72 ± 10.98</td>
<td>45.61 ± 15.67</td>
<td>0.193</td>
</tr>
<tr>
<td>Female(%)</td>
<td>71(94.67)</td>
<td>70(93.33)</td>
<td>0.731</td>
</tr>
<tr>
<td>Hypertension n (%)</td>
<td>5(6.67)</td>
<td>6(8.0)</td>
<td>0.754</td>
</tr>
<tr>
<td>Diabetes n (%)</td>
<td>8(10.67)</td>
<td>3(4.0)</td>
<td>0.117</td>
</tr>
<tr>
<td>Smoking history n (%)</td>
<td>5(6.67)</td>
<td>3(4.0)</td>
<td>0.467</td>
</tr>
<tr>
<td>Alcohol history n (%)</td>
<td>2(2.67)</td>
<td>3(4.0)</td>
<td>0.649</td>
</tr>
<tr>
<td>BMI</td>
<td>24.43 ± 5.02</td>
<td>24.99 ± 4.26</td>
<td>0.492</td>
</tr>
<tr>
<td>CXCL-7(pg/mg C)</td>
<td>25.95 ± 11.83</td>
<td>13.30 ± 4.60</td>
<td>0.000</td>
</tr>
<tr>
<td>Gal-3(ng/mg C)</td>
<td>11.52 ± 5.50</td>
<td>7.63 ± 3.27</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Data of clinical variables and demographic characteristics were expressed as the mean ± SD. Statistical comparisons were performed by chi-squared test or student’s t test. Differences with p < 0.05 were considered to be significant.
Table 2
Demographic data and clinical variables of involved IC patients’ cohort

<table>
<thead>
<tr>
<th>Variable</th>
<th>NHIC patients</th>
<th>HIC patients</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n</td>
<td>23</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Age(y)</td>
<td>44.75 ± 9.27</td>
<td>41.82 ± 11.63</td>
<td>0.289</td>
</tr>
<tr>
<td>Female(%)</td>
<td>22(95.5)</td>
<td>49(94.2)</td>
<td>0.641</td>
</tr>
<tr>
<td>Hypertension n (%)</td>
<td>2(8.7)</td>
<td>3(5.8)</td>
<td>0.489</td>
</tr>
<tr>
<td>Diabetes n (%)</td>
<td>3(13.0)</td>
<td>5(9.6)</td>
<td>0.468</td>
</tr>
<tr>
<td>Smoking history n (%)</td>
<td>2(8.7)</td>
<td>3(5.8)</td>
<td>0.165</td>
</tr>
<tr>
<td>Alcohol history n (%)</td>
<td>2(8.7)</td>
<td>0(0)</td>
<td>0.091</td>
</tr>
<tr>
<td>BMI</td>
<td>23.81 ± 4.22</td>
<td>24.70 ± 5.34</td>
<td>0.481</td>
</tr>
<tr>
<td>OSSI index</td>
<td>12.41 ± 2.73</td>
<td>12.65 ± 3.01</td>
<td>0.748</td>
</tr>
<tr>
<td>Bladder volume(ml)</td>
<td>668.33 ± 163.84</td>
<td>700.01 ± 188.05</td>
<td>0.487</td>
</tr>
<tr>
<td>Urinary CXCL7(pg/mg C)</td>
<td>17.95 ± 6.71</td>
<td>29.49 ± 11.92</td>
<td>0.000</td>
</tr>
<tr>
<td>Urinary Gal-3(ng/mg C)</td>
<td>12.31 ± 6.67</td>
<td>11.17 ± 4.93</td>
<td>0.409</td>
</tr>
</tbody>
</table>

Data of clinical variables and demographic characteristics were expressed as the mean ± SD. Statistical comparisons were performed by chi-squared test or student’s t test. Differences with p < 0.05 were considered to be significant.

The demographic characteristics of IC patients and controls subjects were shown in Table 1. No statistical differences were observed in demographic data and in baseline clinical variables between these two groups, regarding age, gender, body mass index, previous history (smoking status, diabetes, alcohol history). Besides, in the IC group, the mean OSSI was 12.59 ± 3.10 with a mean maximal bladder capacity of 567.68 ± 162.95 mL under anesthesia.

Both the CXCL-7 and Gal-3 levels in urine were measured by ELISA (Table 1 and 2). And our data showed a significant difference in both CXCL-7 and Gal-3 levels between IC patients and healthy controls (Fig. 1). And the in group of IC patients, the level of CXCL-7 was significantly higher in the subgroups of HIC, compared to the NHIC (Fig. 1).

To investigate the diagnostic value of urinary CXCL-7 and Gal-3, the sensitivity and specificity were both calculated by ROC analysis (Fig. 2). For urinary Gal-3, the data showed that the sensitivity and specificity were 62.2% and 91.9% respectively to discriminate IC patients from controls at the optimal cut-off value 11.32 pg/mL (AUC = 79.3%) with a PPV of 88.4% and an NPV of 91.9%, while the sensitivity and specificity were 59.5% and 94.6% respectively for urinary CXCL-7 to discriminate IC patients from controls at the optimal cut-off value 21.30 pg/mL (AUC = 83.3%) with a PPV of 91.7% and an NPV of 70.0%. 

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Further analysis was employed to discriminate from HIC to NHIC (Fig. 1–2 and Table 2). The sensitivity and specificity were 45.5% and 93.3%, respectively for urinary CXCL-7 to discriminate from HIC to NHIC, at the cut-off value of 35.97 pg/mg (AUC = 74.7%), with a PPV of 90.9% and an NPV of 63.6%. However, there was no significant difference in urinary Gal-3 levels between HIC and NHIC.

In addition, regression analysis showed that the urinary Gal-3 level was correlated with OSSI (R$^2 = 0.189$, P = 0.032) in IC group. However, we did not observe the correlation between the urinary CXCL7 level and OSSI (P = 0.198).

**Discussion**

In the present study, we found that urinary levels of CXCL-7 and Gal-3 were significantly increased in IC compared with normal controls. Moreover, the urinary CXCL-7 level was efficient to discriminate HIC from NHIC, while the urinary Gal-3 level was correlated with the symptoms of IC.

Recently research has revealed that Gal-3 plays an important role in the pathology of inflammation. Elevated levels of Gal-3 have been identified in T cell, macrophage, and endophagocyte cells, as well as in the endothelial cells. And in inflammation disease, Gal-3 could recruit the leukocyte cells, monocytes, and macrophages, and then promote phagocytosis, release of cytokines, and promote adhesion of human neutrophils. Moreover, previous studies have found that the Gal-3 is stored in both eosinophils and mast cells, promotes rolling and adhesion of eosinophils, activates mast cells through the JNK signaling pathway, and then contribute to inflammation response, while both mast cells and eosinophil play an important role in the IC. The activated mast cell can induce vasodilation and bladder mucosa damage, recruit infiltration of inflammatory cells. Richter et al suggested the clinicopathological parameters of IC, such as detrusor fibrosis, were related to the expression of mast cells in bladder tissues. And Patnaik et al suggested that infiltration of mast cells could be a potential marker of IC. Thus as an activator of immune cells, Gal-3 is a potential biomarker to diagnose IC. And the existence of Gal-3 has been found in urine by some other studies. Balasubramanian et al found the urinary Gal-3 level was in increase with the progression of multiple cancers, including cervical cancer, breast cancer, esophagus cancer. Also urinary gal-3 was increased in patients with heart failure. Moreover, in one study by Kochiashvili, the data showed the IC patients had higher Gal-3 levels in urine. In our study, we found that the urinary Gal-3 level of IC patients had a significant difference from the normal control, which made the Gal-3 a potential biomarker. Furthermore, the urinary Gal-3 level was related to the symptomatic severity (OSSI) of IC patients. We speculated that the Gal-3 level could represent the inflammatory level, and infiltration of immune cells, both of which triggered the further pathology changes of detrusor muscles, such as nerve injury, fibrosis, and then induce multiple core hypersensitive symptoms, such as pain, frequent urination, and urgency. Besides, Gal-3 could be related to the pain symptoms directly. In a study by Ma et al, the data showed that Gal-3 regulated the pain symptoms in peripheral Nerve Injury.
CXCL-7 is another molecule that plays a key role in inflammation. Via binding receptor CXCR2, CXCL-7 recruits neutrophils into the inflammatory site\textsuperscript{21}. The increased CXCL-7 level has been found in some autoimmune diseases. Yeo et al observed the increased synovial CXCL-7 level in the early stage of rheumatoid arthritis\textsuperscript{4}, while Patsouras et al found the plasma CXCL7 levels were significantly higher in patients with antiphospholipid syndrome\textsuperscript{22}. CXCL-7 level was also increased in ulcerative colitis, another autoimmune inflammation disease\textsuperscript{23}. Besides, the existence of CXCL-7 has been identified in the urine\textsuperscript{24}. In fact, Niimi et al have evaluated the CXCLs in the urine samples of IC patients, including CXCL1, CXCL10, CXCL8, and they only found the urinary CXCL10 was significantly increased in the urine of IC patients\textsuperscript{25}. In the present study, we measured the urinary CXCL-7 in IC patients. We found that CXCL-7 is another molecule whose level was increased in the urine of IC patients compared to the normal controls. Moreover, urinary CXCL-7 level in the HIC patients had a significantly higher level compared to the NHIC patients. There existed the dissociation of symptomatic similarity between HIC and NIHC, and the discrepancy between HIC and NHIC in CXCL-7 could contribute to distinguish HIC from NHIC, which make the CXCL-7 to be a more ameliorated urinary marker in IC.

In summary, this is the first time that urinary CXCL-7 and Gal-3 were examined as a possible diagnostic biomarker of IC, and we found the increased urinary level of CXCL-7 and Gal-3, both of which could be a promising diagnostic biomarker for IC with modest sensitivity and considerably high specificity. Moreover, the urinary CXCL-7 could differentiate HIC from NHIC.

References


Figures
Figure 1

Urinary CXCL-7 and Gal-3 levels in IC patients and normal controls. Both urinary CXCL-7 (A) and Gal-3 (B) levels in the IC patients were significantly higher than that in the normal controls. (C) The urinary CXCL-7 levels in the HIC patients were significantly higher than that in NHIC patients. Both urinary CXCL-7 and Gal-3 levels values were normalized by urinary creatinine (/mg creatinine, /mg C), and presented with
mean±SD. Statistical comparisons were performed by student's t test. Differences with p < 0.05 were considered to be significant.

Figure 2

Diagnostic value of urinary CXCL-7 and Gal-3 in IC. A receiver operating characteristic curve analysis was performed to access the diagnostic power of urinary CXCL-7 and Gal-3. To distinguish IC from normal
controls, urinary CXCL-7 (A), Gal-3 (B), yielded AUC values of 0.833, 0.793 with 59.5%, 94.6%, sensitivity and 62.2%, 91.9% specificity, respectively. To distinguish HIC from NHIC, urinary CXCL-7 (C) yielded AUC values of 0.747 with 90.9% sensitivity and 63.6% specificity.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarydataclinicalvariablesoftenlovedsubjects.xlsx