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# WHAT CAN URINE TELL US ABOUT INTERSTICIAL CYSTITIS/PAINFUL BLADDER SYNDROME? PRELIMIMARY DATA FROM PROTEOMIC COMPARATIVE STUDY.

Hypothesis / aims of study

The interstitial cystitis/painful bladder syndrome (IC/PBS) has a large spectrum clinical presentation and sometimes the diagnosis is not an easy task. All guidelines regard IC/PBS based the diagnosis in exclusion criteria. Thus, there are several patients that suffers for long periods without properly diagnosis. The main problem is the lack of reliable marker or test that could help clinicians to identify the disease.

In the present study, we performed a shotgun proteomics study to show the difference between the urinary protein profile in control subjects and patients with IC/PBS to identify possible specific proteins and networks associated with IC/PBS.

Study design, materials and methods

We collected 50mL of urine from mid stream first urine in the morning from 5 normal women (control) and from 5 women with IC/PBS. The urine was processed and the protein was quantified for each sample using a BCA protein assay (Sigma-Aldrich, Saint Louis, USA). Pools were prepared normalizing the protein content, using a same amount of protein for each group (control and IC/PBS). After trypsin digestion, the $MS^E$ analysis the NanoUPLC tandem nanoESI acquisition was performed in a Xevo G2 Qtof (Waters, UK) in alternate low (precursor ion scan) and high collision energy (fragment ion scan) from *m/z* 50-2000. The protein identification was performed using ProteinLynxGlobalServer (PLGS) with $Identity^E$ software, searched against the Uniprot databank, version 2012_01, human, with manually revised proteins (UniProtKB/Swiss-Prot).The Statistical analysis was performed using PASW 18.0 for Windows.

Results

We identified 528 proteins in the IC/PBS group and 743 proteins in the control group. Out of the 528 proteins in IC/PBS group, 32 were exclusive, 78 were up-regulated. Out of the 743 proteins in the control group, 247 were exclusive and 16 up-regulated. There was 402 proteins that were similarly distributed in both groups. When we analyzed individually the 110 exclusive and up-regulated proteins in IC/PBS urinary sample, the following protein functions were highlighted: cell death, acute phase and apoptosis. Table 1 shows the main protein identified. In the Control group the exclusive proteins were mainly associated with cell proliferation, glycosaminoglycan biosynthesis process and cell adhesion (Table 2).

The difference in protein expression was investigated for novel protein-protein interactions. In the graph of biological function proteins form urinary samples of IC/PBS patients present negative regulation for cell adhesion and up-regulation for acute inflammatory response.

Interpretation of results

This preliminary study demonstrated that urinary proteomic could help to identify significant difference in urinary proteins. It allows us to determine that IC/PBS patients present an absence of some proteins related to cell proliferation, glycosaminoglycan biosynthesis and cell adhesion. Furthermore, the IC/PBS women presented increase proteins related to acute phase and apoptosis. These findings are clearly associated with pathological events previously described in IC/PBS etiology and could be used as future markers in clinical diagnosis.

Concluding message

This preliminary study indicates a significant qualitative and quantitative difference in urinary proteins between women with and without IC/PBS. In a near future, it may be applied as a new technology to determine biomarkers and help understanding the IC/PBS physiophatology.

Table 1. Exclusive and up-regulated proteins in urinary sample of IC/PBS women.

| **Protein** | **Description** | **Gene** | **Score** | **p-Value** | **Fold Change** | **Function** |
|---|---|---|---|---|---|---|
| Q13510 | Acid ceramidase | ASAH1 | 1334,356 | ,0495 | 1,7 | Cell death |
| P07339 | Cathepsin D | CTSD | 5513,86 | ,0495 | 1,5 | Cell death |
| P06865 | Beta-hexosaminidase subunit alpha | HEXA | 213,7733 | ,0495 | 2,2 | Cell death. |
| P07686 | Beta-hexosaminidase subunit beta | HEXB | 296,7381 | ,0495 | 2,8 | Cell death. |
| P19652 | Alpha-1-acid glycoprotein 2 | ORM2 | 6037,606 | ,0495 | 2,5 | Acute phase/ inflammatory immune response |
| P01009 | Alpha-1-antitrypsin | SERPINA1 | 8327,304 | ,0495 | 1,6 | Acute phase/ inflammatory immune response |
| P01011 | Alpha-1-antichymotrypsin | SERPINA3 | 1971,545 | ,0495 | 1,8 | Acute phase/ inflammatory immune response |
| P00738 | Haptoglobin | HP | 4194,774 | ,0495 | 2,8 | Acute phase/ inflammatory immune response |

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|---|---|---|---|---|---|---|
| P00734 | Prothrombin | F2 | 4163,33 | ,0495 | 1,3 | Acute phase/ inflammatory immune response |
| Q7Z4R8 | UPF0669 protein C6orf120 | C6orf120 | 298,9371 | only in cystitis | | Apoptosis |
| Q9BRT3 | Migration and invasion enhancer 1 | MIEN1 | 1332,216 | only in cystitis | | Apoptosis. |
| P12830 | Cadherin-1 | CDH1 | 703,5341 | ,0495 | 2,7 | Apoptosis |
| P06396 | Gelsolin | GSN | 2094,463 | ,0495 | 1,3 | Apoptosis. |
| P80188 | Neutrophil gelatinase-associated lipocalin | LCN2 | 11728,19 | ,0495 | 1,8 | Apoptosis. |

Table 2. Exclusive proteins in control group.

| **Protein** | **Description** | **Gene** | **Score** | **p-Value** | **Fold Change** | **Function** |
|---|---|---|---|---|---|---|
| P08779 | Keratin, type I cytoskeletal 16 | KRT16 | 591,5092 | only in control | | Positive regulation of cell proliferation |
| P15153 | Ras-related C3 botulinum toxin substrate 2 | RAC2 | 482,9939 | only in control | | Positive regulation of cell proliferation |
| P26992 | Ciliary neurotrophic factor receptor subunit alpha | CNTFR | 192,7764 | only in control | | Positive regulation of cell proliferation |
| Q16610 | Extracellular matrix protein 1 | ECM1 | 482,093 | only in control | | Positive regulation of cell proliferation |
| P25942 | Tumor necrosis factor receptor superfamily member 5 | CD40 | 289,8728 | only in control | | Positive regulation of cell proliferation |
| P31431 | Syndecan-4 | SDC4 | 744,3799 | only in control | | Glycosaminoglycan biosynthetic process |
| P35052 | Glypican-1 | GPC1 | 502,2459 | only in control | | Glycosaminoglycan biosynthetic process |
| P51654 | Glypican-3 | GPC3 | 210,9776 | only in control | | Glycosaminoglycan biosynthetic process |
| O75487 | Glypican-4 | GPC4 | 717,5419 | only in control | | Glycosaminoglycan biosynthetic process |
| P35858 | Insulin-like growth factor-binding protein complex acid | IGFALS | 131,1168 | only in control | | Cell adhesion |
| P05026 | Sodium/potassium-transporting ATPase subunit beta-1 | ATP1B1 | 332,3071 | only in control | | Cell adhesion |
| O00533 | Neural cell adhesion molecule L1-like protein | CHL1 | 327,8996 | only in control | | Cell adhesion |
| P29279 | Connective tissue growth factor | CTGF | 404,955 | only in control | | Cell adhesion |
| Q14393 | Growth arrest-specific protein6 | GAS6 | 369,4051 | only in control | | Cell adhesion |
| P11047 | Laminin subunit gamma-1 | LAMC1 | 256,402 | only in control | | Cell adhesion |

Disclosures
**Funding:** None **Clinical Trial:** No **Subjects:** HUMAN **Ethics Committee:** CEP **Helsinki:** Yes **Informed Consent:** Yes