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# UROTHELIAL METAPLASIA AND OVERACTIVE BLADDER SYMPTOMS – DATA SUPPORTING CHRONIC UROTHELIAL STRESS

**Hypothesis / aims of study**

In recent years there has been an increased interest in the role of the urothelium in the pathophysiology of overactive bladder (OAB) symptoms [1]. This has included a focus on afferent nerves and the influence of adenosine triphosphate (ATP) on puringergic receptors. Data from human experiments are limited but increase of stretch activated release of ATP from bladder urothelial cells in patients with interstitial cystitis has been described [2]. A human urothelial pathology associated with OAB symptoms has not been rigorously defined. A inflammatory response in the urine of OAB patients has been described but the implications as yet unexplained. Animal models have shown increased urothelial release in association with inflammation [3].

It is proposed that one mechanism underlying OAB could be a chronic urothelial inflammation resulting in excessive release of ATP. If correct, a number of consequences could be inferred, and these are testable. Serial bladder biopsies could be examined for inflammatory changes related to symptoms. The urinary sediment could be similarly evaluated for evidence of inflammation. The urine itself could be tested for cytokine or ATP expression in relation to symptoms. The urine could be examined for evidence of sustained urothelial stress.

Many epithelia react to chronic stress by developing metaplasia. In the early stages this is manifest as greater cell turnover. Exfoliated cell deposits show increased ratios of deeper immature cells to mature surface cells. With persistence, the superficial cells become squamous forming squamous metaplasia and eventually squamous keratinisation can occur.

The urothelium is very susceptible to metaplasia, which should be expected as a manifestation of significant prolonged urothelial stress. Nevertheless, urothelial metaplasia is the focus of only 35 publications on Medline. Cystoscopists anecdotally describe it as a frequent macroscopic finding, but sampling and recall bias may influence that claim.

If OAB is associated with chronic urothelial stress it is reasonable to postulate a metaplastic response, its absence would mitigate against the proposition. Metaplasia can be evaluated by differential cell counts of spun urinary sediments, prepared for cytological examination.

This study tested the hypothesis that patients presenting with OAB symptoms would manifest evidence of metaplasia in the urinary deposit that was not found in matched asymptomatic controls

**Study design, materials and methods**

The study had an 87% power to detect a 15% difference in transitional cell proportions between OAB patients and 20 controls. This was a blinded observational cohort study.

Urine samples were obtained by catheter specimens (CSU) apart from males and control subjects who provided clean-catch midstream specimens (MSU). The urine samples were processed in a Shandon Cytospin 2. This centrifuge is used to concentrate the cells from small volumes of urine into a small area on a glass slide with the excess fluid absorbed onto a cardboard filter.

Six drops of urine were placed in disposable cuvettes, which fitted into a sealed, removable rotor head that was placed in the Shandon Cytospin. The centrifuge was run at 850 rpm for 5 minutes using high acceleration. The slides, prepared in this manner, were then stained by the Papanicolaou method.

The stained sediments were examined under light microscopy using a x40 objective with x10 eyepiece. 100 cells were counted and individually identified as (1) superficial umbrella cells (2) deep transitional cells (3) squamous cells (4) inflammatory cells

The data were examined for differences between patients and controls using a t-test for independent samples at the 95% level of confidence

**Results**

95 patients with OAB were sampled and their mean age was 59 (sd=18) There were 21 controls who were significantly younger with a mean age of 31 (sd=11) with a gender difference there were 14 male controls and only 2 male patients. The results of the t-test analysis are shown in table 1.

Table 1 – the results of the t-test for independent samples

| | t | df | Sig. | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower | Upper |
| Umbrella cells | 3.177 | 114 | .002 | 1.94 | .611 | .731 | 3.152 |
| Deep Transitional cells | 4.186 | 114 | .000 | 22.20 | 5.303 | 11.691 | 32.702 |
| Squamous cells | -.250 | 114 | .803 | -1.81 | 7.236 | -16.144 | 12.524 |
| Inflammatory cells | 2.324 | 114 | .022 | 8.00 | 3.442 | 1.180 | 14.818 |

The patients with overactive bladder symptoms demonstrated significantly higher proportions of umbrella cells, inflammatory cells and most notably deep transitional cells in their sediments

**Interpretation of results**

The age and sex of the controls reflects the difficulties experienced in obtaining samples from normal asymptomatic controls. The asymptomatic tend to be younger and men are more willing than women. However, whilst disappointing these differences do not compromise the experiment.

This experiment was designed as a Popperian falsification "What would lead us to believe that OAB is not associated with chronic urothelial stress or inflammation?" One answer would be a failure to detect the ubiquitous manifestation of the urothelial stress, metaplasia. The high proportion of deep transitional cells in the sediments obtained from patients with OAB is indicative of metaplasia so the proposition survives the test.

**Concluding message**

These data support the hypothesis that OAB, in some individuals may be associated with chronic urothelial stress possibly caused by inflammation. They are supportive of the recent trend towards examining urothelial signalling in the pathophysiology of OAB.

**References**

1. Neurourol.Urodyn. 26(6 Suppl), 908-913. 2007
2. J Urol. 166(5), 1951-1956. 2001
3. J Neurophysiol. 99(1), 49-59. 2008



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|---|---|
| ***Specify source of funding or grant*** | **The Whittington Hospital NHS Trust** |
| ***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*** | **The East London Ethics Committee** |
| ***Was the Declaration of Helsinki followed?*** | **Yes** |
| ***Was informed consent obtained from the patients?*** | **Yes** |