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1. *UCL*

URINARY ATP A TEST TO REPLACE DIPSTICKS AND MICROSCOPY FOR PYURIA

Hypothesis / aims of study

The best surrogate marker of urothelial inflammation available is the detection of pyuria by the microscopy of a fresh unspun specimen of urine (1). This is laborious, skilful and requiring the ready availability of a microscope. Recent data have indicated that the sensitivity of pyuria for detecting infection is around 66% (95% CI 54% to 77%) (2). It has also been shown that the urine dipsticks used in routine clinical analysis lack sufficient sensitivity for the clinical needs. These findings present a problem because the need for the easy detection of urothelial inflammation in clinical practice is becoming increasingly important. There is a growing body of data that point to inflammation of the urothelium as a very important aetiological component in the pathology of OAB symptoms. These developments are clouded by the knowledge that the methods used to detect urothelial inflammation and urinary infection, when assessing patients with OAB, are woefully inadequate. There is no doubt that we need replacement tests rather urgently.

There is considerable interest in the role of ATP signalling in the human detrusor. Early experiments contrasted with animal studies in finding minimal ATP expression in normal human detrusor. However a number of publications implied that ATP may have a more prominent role in diseased states and there is now good evidence that inflammation of the human urothelium is associated with increased ATP expression by urothelial cells.

Provided that ATP is not metabolised too rapidly, there should be scope for its detection in the urine. If the expression of ATP arises in response to inflammation the levels of urinary ATP should vary in concordance with the presence inflammation.

In order to test the hypothesis that urinary ATP could act as an effective, clinically applicable, marker of urothelial inflammation a sequence of experiments were proposed. (1) Urinary ATP concentration were compared between controls, and patients with OAB with reference to the amount of pyuria (2) An urinary ATP decay curve was plotted from time of sampling for 24 hours. (3) The first experiment was repeated so as to correct for urinary dilution by expressing levels of ATP in relation to urinary creatinine. Creatinine is excreted into the urine at a constant rate The reference range for men is 9-21 mmol/ 24hr and 7-14 mmol/ 24hr for females

Study design, materials and methods

This was a blinded, controlled observational study. Patients with OAB symptoms provided a catheter specimen of urine and normal controls provided meticulous MSU samples. Pyuria was counted by immediate urine microscopy. Aliquots were stored frozen at -20^{o}C. Using a batch process a luciferin-luciferase assay was used to quantify the ATP released into the urine samples. The data were not normally distributed but positively skewed. These normalised on log transformation and so analysis was conducted using ANOVA at the 95% level of confidence.

Results

172 patients (157=F, 15=M, mean 57, sd=19) and 20 controls (9=F, 11=M, mean age=34, sd=-11). Of the 172 patients who had OAB symptoms 99 (58%) had pyuria and 73 (42%) did not have pyuria. The patients had a mean 24-hour frequency of 8 (sd=3), incontinence episodes of 0.6 (sd=.99) and urgency score of 2.7 (sd=2.7), normal urgency score being zero.
The decay curve for urinary ATP is shown in figure 1. The ATP concentration varied in relation to the presence of pyuria (F=3.6, p=.04) See figure 2.The ATP did not differ between patients with zero pyuria and controls. The expression of ATP as a ratio of the urinary creatinine did not influence these relationships implying that urinary concentration was less significant than expected

Figure 1
Urinary ATP decay with proportion of initial value against time. There appears to be a trend of an overall decrease, with a steeper drop occurring between 2 and 6 hours, after which time, it levels off again

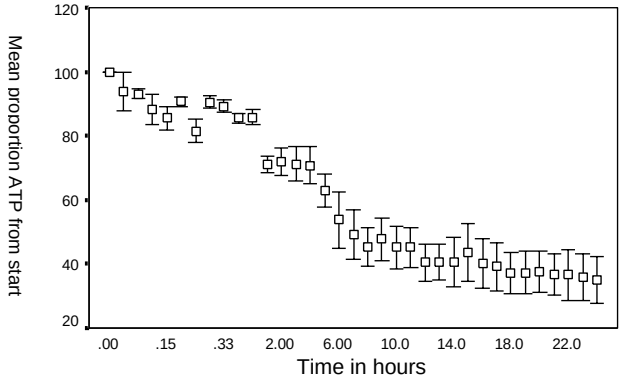
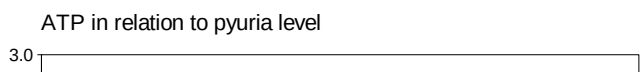


Figure 2
ATP in moles x10^{8} according to pyuria. It is notable that the level of ATP proves To be a sensitive marker of pyuria



Interpretation of results

ATP is an easily measured marker of inflammation that is secreted into the urine. Whilst there is a decay in the concentration with time following collection this is not of a magnitude that it would impede useful clinical application. The correction of the concentration of ATP for urinary creatinine did not alter the relationships described in these data. This may reflect the parsimony affecting fluid intake amongst patients experiencing OAB symptoms

Concluding message

Urinary ATP assays should be considered for development for replacing urinary dipsticks and microscopy of the urine to count pyuria

References

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Was this study approved by an ethics committee?	**Yes**
Specify Name of Ethics Committee	**Moorfields and Whittington Research Ethics Committee**
Was the Declaration of Helsinki followed?	**Yes**
Was informed consent obtained from the patients?	**Yes**