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1. *UCL*

BACTERIAL URINARY TRACT INFECTION IN PATIENTS WITH OAB SYMPTOMS AND NEGATIVE MIDSTREAM CULTURES EXPOSED THROUGH CULTURE OF THE URINARY SPUN SEDIMENT

Hypothesis / aims of study

The diagnosis of OAB is contingent on the exclusion of tract infection (UTI). Unfortunately, screening out UTI poses a problem of great significance. Standard laboratory methods of MSU analysis operate a diagnostic threshold of $\geq 10^5$ colony forming units (cfu) ml^{-1} of a single known urinary pathogen. But Stamm et al (1) found $\geq 10^2$ cfu ml^{-1} was appropriate for persons with acute frequency/dysuria and that $\geq 10^5$ cfu ml^{-1} missed 50% of infections. The pathophysiology of urinary infection attributes considerable significance to bacterial adherence, notably *E.coli* expressing type 1 fimbriae. If culprit bacteria are adherent to the cells, it may be better to culture the spun sediment, rather than unspun urine as used in routine MSU culture, in order to optimise the likelihood of isolating an offending organism. The dipstick leukocyte esterase and nitrite tests have been calibrated to $\geq 10^5$ cfu ml^{-1}. The OAB literature, reassured by these tests, may not have addressed infection adequately. There have been two meta-analyses of urinary dipsticks, used to assess acute frequency/dysuria, in adults (2). These concluded that dipsticks cannot exclude infection reliably in most clinical settings. At the moment the best surrogate marker of urinary infection, superior to routine culture and dipstick analysis, is the detection of ≥10 white blood cells (wbc) per µl of fresh, unspun urine, examined in a haemocytometer. This has been well validated. Applying this, recent studies in OAB have indentified urinary inflammatory exudates occurring despite negative routine urine cultures(3). Such an inflammatory exudate demands critical scrutiny deploying histopathological, pathophysiological and controlled, comparative observational methods. The exploration of infection, using enhanced culture methods, is also necessary in order to test for an infective cause for the inflammatory signal. This study examined the culture of spun urinary sediments obtained from urine samples taken from OAB patients and normal controls. The data were compared with the results of routine culture of the same specimens and immediate microscopy of a specimen aliquot for white cell expression

Study design, materials and methods

We studied 66 women, mean age 56 (sd=17); 47 had OAB symptoms and provided a CSU; 19 asymptomatic female controls, average age 29 (sd=12), of which 11 provided a meticulous MSU and 8 a CSU. An aliquot of unspun urine was examined immediately by microscopy using a haemocytometer and the urinary white cells evaluated. An aliquot of the urine sample was centrifuged for 5 minutes at 5,000 rpm and the sediment was collected and plated on Columbia blood agar (CBA) and Fastidious Anaerobic agar (FAA) and incubated both aerobically and anaerobically. Another aliquot of urine was sent for routine culture with a threshold of 10^5 cfu/ml. A further urine aliquot was plated immediately on CBA and chromogenic agar and incubated both aerobically and anaerobically, with a threshold of 10^2cfu/ml. The study had 80% power to detect a 40% difference between groups at alpha=0.05. The Fisher exact probability test compared the groups.

Results

- The routine cultures at 10^5cfu/ml were positive in 7 of the 47 patients (15%); all of these (100%) had pyuria.
- The enhanced culture at 10^2cfu/ml was positive in 12 of the 47 patients (26%); 11 of these (92%) had pyuria.
- 6 patients proved positive on both cultures and all of these had pyuria

One out of 19 controls (5%) grew *E.coli* in both cultures, the others were found to be negative. None of the controls has pyuria. (Fisher p=0.03)

In contrast, the spun sediment culture grew bacteria in all samples, with significant differences in colony counts related to OAB and to pyuria:

- The normal controls (N=19) grew 10^2 cfu ml^{-1}; There was no difference between MSU and CSU collected samples
- OAB patients without pyuria (N= 27) grew 10^3 cfu ml^{-1}
- OAB patients with pyuria (N=20) grew 10^4 cfu ml^{-1}

(F=5, df=2, p=0.009)

48% of isolates in the control groups were *lactobacillus* which can be a commensal but sometimes a pathogen. Other prominent control isolates were *enterococcus* (10%) and *streptococcus* (10%). The predominant isolates from control MSUs were *bacteroides*, *corynebacteria, peptostreptococcus* but control CSU were predominately *lactobacillus* and *streptoccocus* In the OAB patients the dominant isolates were *enterococcus* (21%), *streptococcus* (11%) and *lactobacillus* (9%)

Interpretation of results

The urine obtained from patients in this study were carefully collected CSU samples so that contamination was unlikely. The symptom-free control group provided some CSU samples as well as meticulous MSUs but these controls did not differ in pyuria nor bacterial counts but they did differ in the predominant bacterial species found. The CSU specimens had fewer species and more frequent lactobacillus isolates. This implies significant MSU contamination. The data demonstrate a very marked enhancement in the isolation of bacteria when the spun urinary cellular sediment is cultured. The data support the view that ordinary laboratory cultures, which focus on isolating *E.Coli* at a threshold of 10^5 cfu ml^{-1} are insensitive and inappropriate for screening out infection in patients with OAB. Additionally they imply that patients with OAB have a high probability of suffering from a bacterial urinary infection. The probability is increased if pyuria is detected. Pyuria has been found to affect about 35% of OAB patients when sampled by CSU, compared to 0% of asymptomatic controls. It is an inevitable corollary that we must implicate urinary tract infection in the aetiology of overactive bladder symptoms to a far greater extent than has previously been assumed. According to the accepted definition most of the patients in this study, based on MSU culture data, would have received the diagnosis of OAB and not offered treatment for urinary infection. This must be a cause for concern. The sediment culture is evidently sensitive since

bacteria were isolated from controls but in fewer orders of magnitude and the organisms were predominantly the ubiquitous commensal, *lactobacilli*. Some of the control specimens were MSUs and open to contamination but no difference was detected between control CSU and MSU samples

Concluding message

Patients with symptoms of OAB exhibit evidence of pathogenic bacterial urinary tract infection with a frequency markedly in excess to that implied by routine MSU urinanalysis methods. A spun urinary sediment provides a more sensitive culture specimen probably because pathogenic bacteria adhere tenaciously to the urothelial cells. These findings challenge current assumptions about the aetiology and the definition of OAB.

References

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