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1. *UCL*

SPUN URINARY SEDIMENTS AND GIEMSA STAIN REVEAL A CHRONIC INFLAMMATORY INFILTRATE IN PATIENTS WITH SYMPTOMS OF OAB

Hypothesis / aims of study

A vital step in the sequence leading to the diagnosis of overactive bladder (OAB) is the exclusion of urinary tract infection (UTI). A number of recent publications have cast doubt on the veracity of normal midstream urine (MSU) culture and urinary dipstick analysis for accomplishing this step. Since 1968 it has been known that the best surrogate method of diagnosing urine infection, in symptomatic patients, is the identification of pyuria of ≥10 white blood cells (wbc) µL-1, counted by microscopic examination of fresh unspun urine in a haemocytometer. This has been very well validated[1]. Studies using CSU with enhanced culture methods have shown that the detection of ≥10 wbc µL-1, is more sensitive than MSU culture for detecting infection in patients with acute frequency and dysuria (1). Being laborious and requiring certain skills, fresh urine microscopy is not a usual method for screening patients with OAB symptoms. Nevertheless, it has been reported elsewhere that new patients presenting with OAB symptoms manifest pyuria 34% of the time (95% CI 32% to 37%) with only 35% of these proving MSU culture positive at 10^5 colony forming units (cfu) ml^{-1} (95% CI 29% to 41%). Asymptomatic controls showed pyuria 7% of the time (95% CI 1% to 14%)(2). These data imply that a third of patients with OAB symptoms exhibit microscopic pyuria and two thirds of these are culture negative. There are several options available for elucidating the nature of this phenomenon but basic first steps would be a morphological description of the inflammatory exudate; confirming that it comes from the bladder; and accommodation of the variance in urinary dilution. The morphology can be addressed by differential staining; bladder origin can be ensured by catheter sampling of urine and dilution effect can be countered to a degree by centrifuging the samples. The Shandon Cytospin© is used to concentrate the cells from small volumes of urine. Cells are focalised into a small area on a glass slide with the excess fluid absorbed onto a cardboard filter. Thus a reproducible technique places cells from 0.5 ml of urine onto glass slides, forming 5 mm diameter circles of single cell depth. This permits closer scrutiny and some quantification. The Giemsa stain is a classical blood film stain for peripheral blood smears. Erythrocytes stain pink, platelets show a light pale pink, lymphocyte cytoplasm stains sky blue, monocyte cytoplasm stains pale blue, and leucocyte nuclear chromatin stains magenta. This stain thus allows differential inflammatory cell analysis of the Cystopsin preparation. A CSU specimen, because it avoids contamination, may legitimately be studied by enhanced culture methods, which use a diagnostic threshold of 10^2 cfu ml^{-1}.

Study design, materials and methods

This was a blinded, observational cohort study of patients with symptoms of overactive bladder and normal asymptomatic controls. CSU samples were obtained, apart from males who provided MSU samples. Aliquots of fresh urine were examined by haemocytometer to evaluate pyuria. Aliquots were sent for routine culture (threshold 10^5 cfu ml^{-1}) and an enhanced culture (threshold 10^2 cfu ml^{-1}). Five drops of urine filled disposable cuvettes were placed in the Shandon Cytospin©. The centrifuge was run at 850 rpm for 5 minutes using high acceleration. The preparations were stained by the Giemsa method. Neutrophils and lymphocytes throughout the preparation were counted. The enhanced culture was achieved as follows: One Pre dried ChromID CPS (Biomerieux) culture plate and two pre dried blood agar culture plates (horse) were immediately inoculated with 200 µL of fresh unspun urine and incubated. The Chrome plates were incubated aerobically for 24 hours at 35-37^{o}C and the blood plates were incubated anaerobically at 35-37^{o}C for five days. The data was examined by ANOVA at the 95% level of confidence.

Results

178 patients with OAB symptoms (94% female) and 21 controls (77% female) were studied. The controls were younger (mean age 34 sd=9 versus mean age 57 sd=19). 75 (42%) OAB patients showed pyuria (95%CI = 36 to 48), no controls had pyuria. The OAB pyuria & OAB no-pyuria groups were similar in age & sex. 46 patients (28%) proved enhanced culture positive (10^2 cfu ml^{-1}) as compared to 32 (18%), who were routine culture positive (10^5 cfu ml^{-1}). One control was routine culture positive only. Of the 46 patients with positive enhanced culture, 32 (70%) showed pyuria. Of the 32 positive on routine culture 25 (75%) had pyuria. Only 3 OAB patients (1.7%) and 1 (5%) control showed no inflammatory cells in the spun sediment. The mean total inflammatory cell count per spun preparation was substantially higher in OAB patients (mean=29, sd=33) compared to controls (mean=6, sd=5.6) (t=8, df=197, p<0.001 mean diff = 22, 95% CI 17 to 28). A sub-analysis showed that OAB patients without pyuria, nevertheless showed an increased total inflammatory cell count in the spun preparation compared to controls (F=23, df=2, p<0.001). see figure 1 The lymphocyte: neutrophil ratio differed between OAB Groups: 1:1 in OAB but 2:3 in OAB & pyuria (F=4.8, df 167, p<0.001).

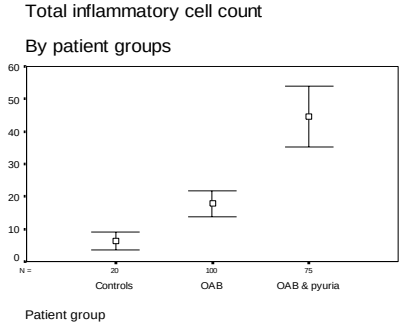

Figure 1

Interpretation of results

These data used CSU or meticulous MSU in men so the probability that the urinary cell content originated from the bladder is very high. The previous identification of pyuria in 36% to 48% of OAB patients is re-confirmed. The use of spun urinary sediment has unearthed evidence of a quantitatively markedly increased urothelial inflammatory cell response in OAB patients whether exhibiting pyuria or not. Pyuria, if identified implies an increased inflammation with more acute inflammatory cells. OAB without pyuria appears to be associated with a mixed acute and chronic inflammation of the urothelium

Concluding message

It would seem that OAB symptoms are purely a manifestation of cystitis.

References

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2. Malone-Lee J, Ghei M, Lunawat R, Bisahara S, Kelsey M. Urinary white cells and the symptoms of the overactive bladder. Neurourol Urodyn 2007;26(5):656-7

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