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1. *UCL*

ENHANCING THE DETECTION OF PYURIA IN FRESH URINE FROM PATIENTS WITH OVERACTIVE BLADDER BY USE OF THE STERNHEIMER-MALBIN STAIN

Hypothesis / aims of study

A growing body of data from experiments on urinary tract infection and overactive bladder (OAB), imply that the initial assessment of patients with lower urinary tract symptoms (LUTS) would be greatly enhanced if the investigation involving microscopy of a fresh, unspun sample of urine in a haemocytometer could be universally adopted. The finding of >=10 white blood cells (wbc) μl^{-1} of urine remains the most effective surrogate method for diagnosing urinary infection and is much superior to dipsticks and midstream urine (MSU) culture (1;2). It is utopian to propose widespread adoption at this time for several reasons: (1) Suitable microscopes are unavailable in many clinics (2) Microscopy of unstained urine requires some training (3) White cells lyse rapidly in the urine after collection, resulting in gross underestimation after transport to central laboratories. These problems should be surmountable. It may be possible to discover a preservative that would permit accurate delayed analysis. A wet stain could enhance the microscopic image, ease interpretation and open the door to automated analysis by combining digital microscopy with microcomputer image analysis. Such a development would be an important enhancement for clinical care of patients with LUTS. The Sternheimer-Malbin stain is for unfixed, wet urinary sediment. It comprises of a preferential nucleus-seeking dye and a preferential cytoplasm-seeking dye providing a readily detectable difference between nuclear cellular material and cytoplasmic cellular material. It also stains urinary casts, other mucoid material and bacterial elements (3). It would seem to be an excellent candidate for helping to advance this technique. There are no published data on its application for screening patients with LUTS. This study measured the effect of the Sternheimer-Malbin stain in improving microscopic urinanalysis.

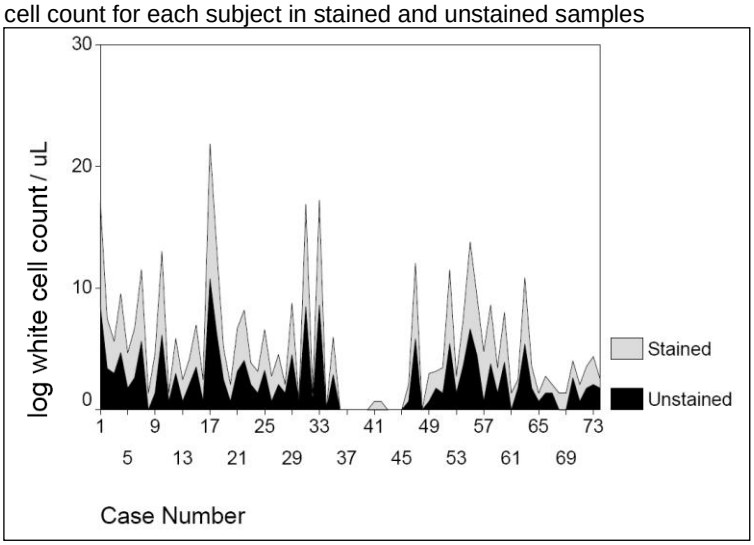
Study design, materials and methods

Urine samples from adults with OAB symptoms were obtained by MSU or CSU. A randomisation process was carried out so that at any point in time the microscopist would be blinded from which sample he was testing. A fresh aliquot was examined unstained in a haemocytometer and the white cell count evaluated. Another aliquot was stained using Sternheimer-Malbin reagent. The commercial preparation of the Sternheimer-Malbin stain, called Kova stain, was used. The quantity and concentration of stain added to a urine sample, and the time it was left to stand before microscopic examination, was optimised through preliminary investigations carried out before samples for the main study were obtained. Samples were kept in the fridge unless they were being stained or analysed. It was found that the optimum quantity and concentration of stain for the staining of leukocytes in urine was 3 drops of neat, undiluted stain per ml of urine being tested. Hence in order to stain a sample, 3 drops of neat stain were added to the 1 ml volume of sample using a disposable pipette. The lid of the sample bottle was then replaced and it was left to stand for 10 minutes, as preliminary studies found that this was the optimum time to let the mixture stand before microscopy. Samples that were left unstained were removed from refrigeration and left to stand for 10 minutes before microscopy. This was carried out in order to reduce any differences in the way in which stained and unstained samples were handled, so as to ensure that a valid comparison between results could be made. 70 pairs of data provided 93% power to detect a 30% difference between methods, α=0.05. Analysis was achieved by the paired t-test applied to the log count and the differences were then described proportionately because of the wide variance in counts between patients (See figure)

Results

74 urine samples from adults (mean age 56, sd=17) with OAB symptoms were obtained by MSU or CSU. The Sternheimer-Malbin stain resulted in consistently higher white cell counts compared to unstained preparations at all levels of count (Mean propionate diff = 104%: 95% CI = 2 % to 200%, t=3.9, p<0.001). See figure:

Figure 1 Area plot of log white cell count for each subject in stained and unstained samples



Interpretation of results

These data demonstrate that the use of a stain to enhance the visibility of white blood cells in the urine can improve the detection rate even when an experienced operator is making the counts. It also shows that current data, gleaned from studies applied to unstained samples of urine, must be underestimating the white cell numbers. The stain proved very effective in discriminating white cells but a differential lymphocyte/neutrophil evaluation was not an option. Micrographs obtained from these preparations would be very amenable to automated, microcomputer image analysis.

Concluding message

The Sternheimer-Malbin stain, by enhancing the visibility of urinary white cells significantly increases the ease and sensitivity of microscopic pyuria screening. These data are encouraging to the aspiration of introducing routine, clinic-based, urine microscopy into the management of patients with LUTS.

References

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Was the Declaration of Helsinki followed?	**Yes**
Was informed consent obtained from the patients?	**Yes**