

## Immunofluorescence of Yeast in Urine

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A study of immunofluorescence of yeast in the urine was carried out in 18 patients with funguria in an effort to correlate gamma globulin coating with evidence of tissue invasion or as an indicator for institution of therapy. Positive immunofluorescence of yeast in urine is common but is neither indicative of upper urinary tract yeast invasion nor a useful guideline for beginning treatment.

Immunofluorescence of bacteria in the urine has been reported to be reasonably reliable in localization of urinary tract infections (1, 2). The specificity of this technique is thought to result from local antibody production by the kidney.

Because of the difficulty in interpreting the significance of positive urine cultures for yeast, usually *Candida* species, we decided to apply this method to yeast in the urine in an attempt to utilize gamma globulin coating as a clue to tissue invasion or as a criterion for initiation of treatment.

A fresh urine sample was obtained from patients who were reported to have urine cultures positive for yeast colony counts of 50 to  $\geq 100,000/\text{ml}$  (20 of 21  $\geq 100,000$ ). Urine was examined immediately or refrigerated for 24 to 48 h prior to examination by the method of Thomas et al. (3). Five-tenths milliliter of urine was centrifuged at  $1,500 \times g$  for 10 min and the supernatant was discarded. The sediment was washed with phosphate-buffered saline and centrifuged twice. Two-tenths milliliter of a 1:5 dilution of fluorescein-conjugated equine antihuman globulin (Progressive Laboratories, Inc.) was added to the washed sediment and incubated at 37 C for 30 min. The specimen was then centrifuged at  $1,500 \times g$  for 10 min, and the sediment was washed with phosphate-buffered saline and centrifuged twice. Smears of the sediment were made on glass slides, allowed to air dry, and then examined under fluorescent microscopy.

Patients were then followed from 1 to 3 months by repeated urine cultures to ascertain their course or to the autopsy table to determine the presence of tissue invasion. Fungal isolates from the urine were identified in the microbiology laboratories of the Colorado General Hospital and the Denver Veterans Admin-

istration hospitals. Techniques for identification were slightly different in the two hospitals but in general were as follows. Yeast were inoculated into serum for determination of germ tube formation and if detected were called *Candida albicans*. If no germ tube formation was present then species were subsequently cultured on chlamyospore agar and, depending on the presence or absence of pseudohyphae, were subjected to various testing including temperature tolerance, urease production, microscopy morphology, sugar fermentation, and assimilation.

A total of 21 adult patients' urines were examined. Prior to study no patient had received antifungal therapy and the majority had Foley catheters in place. Three of the patients, despite having a report of 100,000 or greater colonies of yeast per ml of urine, had no yeast detectable by careful microscopy of a centrifuged urine sediment, perhaps due to faulty collection (e.g., allowing the urine to sit before culturing), but an explanation was not avidly sought. In any case, the population studied consisted of the other 18 patients. Of these 18 patients, one had *Torulopsis glabrata*, one had *Candida tropicalis*, and the remainder had *C. albicans* in the urine. Fifteen of these 18 patients had positive immunofluorescence of yeast and/or pseudomycelia in the urine. The three patients with negative immunofluorescence all came to autopsy. Two had no evidence of urinary tract candidiasis while one, the patient with positive cultures for *C. tropicalis*, had tissue invasion of the kidneys by fungi, morphologically consistent with *Candida*. One of the patients had received treatment with flucytosine and amphotericin B shortly before her demise.

Adequate follow-up data are available on 13 of the 15 patients with positive immunofluorescence of yeast in the urine. Five of these pa-

tients died and three of these underwent post-mortem examination. None had evidence of urinary tract candidiasis and none had received therapy with antifungal agents prior to autopsy.

Of the survivors, four patients (one with *T. glabrata*) who had positive yeast immunofluorescence spontaneously cleared the fungi from their urine. Three patients were treated with systemic antifungal agents resulting in sterile urine. One of these three patients had evidence of upper urinary tract involvement with *Candida* at the time of surgery. The remaining patient continues to have asymptomatic candiduria 2 months after detection of gamma globulin coating of yeast in his urine.

Data accumulated from this study indicates that gamma globulin coating of yeast in the urine is common (15/18 patients). However, there is no useful clinical correlation between gamma globulin coating as an indicator for treatment or of tissue invasion. This is sup-

ported by the lack of visceral involvement at the time of autopsy in three patients with positive immunofluorescence of yeast in the urine and by spontaneous clearing of candiduria in four of eight untreated patients with positive immunofluorescence.

The findings in our study are in agreement with a recent report by Harding and Merz, who were unable to correlate gamma globulin coating of yeast with the site of urinary tract involvement (S. A. Harding and W. G. Merz, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, F8, p. 86).

#### LITERATURE CITED

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