Serological diagnosis of infection of the urinary tract by yeasts

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SYNOPSIS Precipitins to mannan and cytoplasmic antigens of *Candida albicans* and *Torulopsis glabrata* were determined in 25 patients with colonization of the bladder urine by yeasts and in 25 control patients with bacteriuria. Precipitins were present in 64% of the patients with funguria and in 56% of the patients with bacteriuria. There was no correlation between the reactions obtained and the clinical significance of the yeast colonization. It is suggested that a single precipitin test is unhelpful in the assessment of the significance of urinary colonization by yeasts in hospital patients.

Yeasts are often isolated from urine specimens from hospital patients. As with bacteriuria, quantitative criteria can be applied (Ahearn *et al*, 1966), and in fresh specimens a count of 10^5 or more colony-forming units per millilitre almost certainly implies that the yeast is not a contaminant but is genuinely colonizing the bladder urine. Most of the patients with significant funguria, however, appear to suffer no ill effects from this, often persistent, colonization (Schönebeck, 1972) and it is only the occasional patient in whom dramatic complications develop—blood stream or tissue invasion, urinary obstruction, etc. In others there may be some clinical indication, symptomatic or other, of harm from the colonization, but these more doubtful intimations of genuine infection may be difficult to assess, particularly against a background of other illness. It is the more important that harmful effects should be discerned now that we have in flucytosine an active and relatively non-toxic oral agent for the treatment of yeast infection of the urinary tract (Speller, 1975). Serological tests have been used in the assessment of such patients (Wise *et al*, 1972).

This investigation was undertaken to assess the usefulness of a single precipitin test in a series of patients with colonization of the bladder urine by yeasts.

Material and methods

PATIENTS AND CONTROLS

The 25 patients included in the funguria series were

hospital in-patients from whom fresh catheter or mid-stream urines had yielded more than 10^5 colonies per ml of a yeast on at least two occasions.

The 25 control patients were from similar units matched by age and sex with the patients in the funguria series but with significant bacteriuria (more than 10^5 colonies per ml).

Ten millilitres of blood was taken from each patient and the serum was separated and stored at -20°C until required.

CLINICAL ASSESSMENT

The clinical and pathological findings in each patient throughout his stay in hospital were analysed. The incidence in both series of factors thought to predispose to urinary colonization and to fungal infection is given in table I. In each case the clinical significance of the yeast infection was assessed and the patient was assigned to one of the following groups:

- Y3 definite evidence of harmful effects of the yeast, eg, fungaemia, obstruction by yeast, evidence of tissue invasion
- Y2 some evidence of harmful effect, eg, urinary symptoms, fever, difficulty of diabetic control, etc, for which no cause other than the yeast could be discovered; favourable response to eradication of the yeast
- Y1 benign colonization by yeasts; no evidence of any harmful effect of colonization
- Y0 no urinary colonization by yeasts.

LABORATORY ASSESSMENT

Urine specimens were cultured for bacteria and

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	Funguria series	Bacteriuria controls
Total no.	25	25
Male	11	11
Female	14	14
Age—mean	68.8 years	68.4 years
—range	36–91 years	35–87 years
Urinary isolates	16 <i>Candida albicans</i> 8 <i>Torulopsis glabrata</i> 1 <i>Candida parapsilosis</i>	10 coliform (lactose-fermenting) 2 <i>Pseudomonas aeruginosa</i> 2 <i>Streptococcus faecalis</i> 1 <i>Proteus</i> sp
Related urinary symptoms	5	12
Pyuria	16	17
Previous catheterization	24	13
Previous antibacterial agents	25	17
Previous adrenocorticosteroids	4	3
Diabetes mellitus	10	1
Malignant disease	5	8
Fungal infection elsewhere	6	0

Table I Composition of series and controls: urinary and clinical data

Sabouraud's glucose peptone agar for yeasts by a semiquantitative standard loop method; the specimens were refrigerated and the counts were subsequently checked by the method of Miles and Misra (1938). Pyuria was defined as more than 10 leucocytes per high-power microscope field in a centrifuged deposit of the urine resuspended in one-tenth of its original volume.

Yeast isolates were identified first by the serum germ-tube test (Taschdjian *et al*, 1960). Germ-tube-positive isolates were assumed to be *Candida albicans*, and those giving a negative result were further identified by morphology in corn meal agar and by sugar assimilation and fermentation reactions (English, 1974).

PREPARATION OF ANTIGENS

Soluble cytoplasmic (somatic) antigens were obtained from *C. albicans* group A and *Torulopsis glabrata* yeast cells grown in glucose peptone broth for 24 hours at 37°C. The cells were disrupted in a Braun MSK homogenizer. Solid debris was removed by high-speed centrifugation and the supernatant was lyophilized.

Mannan antigens were extracted and purified according to the method of Peat *et al* (1961) as modified by Kocourek and Ballou (1969).

The cytoplasmic antigens were reconstituted in saline containing 0.1% sodium azide to concentrations of 30 and 3 mg per ml. The mannan antigens were reconstituted to 1.0 and 0.1 mg per ml.

PRECIPITIN TEST

Double diffusion tests were carried out in 1.5% Noble agar containing 0.1% sodium azide layered to a depth of 1.5 mm on glass plates. The central serum well was 12.5 mm in diameter and the peripheral wells were 4 mm in diameter and 6 mm from the central well. Diffusion was carried out in moist

chambers for up to one week at room temperature. The plates were rinsed in 0.1M saline overnight, dried, and stained in 0.5% Coomassie Blue BL.

NATURE OF PRECIPITIN REACTION

Two types of precipitin reaction have been observed in double diffusion tests with *C. albicans* antigens (Faux, 1968):

1 A broad diffuse 'H' type reaction attributable to heat-stable polysaccharide antigen. The concentration of antigen used is critical, for if the concentration is too high the reaction will disappear.

2 A well-defined 'R' type reaction attributable to heat-labile protein antigen. For this reaction higher concentrations of the antigen are needed, and the reaction is less readily soluble in excess antigen.

The cytoplasmic antigens, containing both protein and some mannan, were used at higher concentrations than the mannan antigens in order to detect 'R' type precipitin reactions.

Results

CLINICAL AND MICROBIOLOGICAL FINDINGS

The principal clinical features of the 50 patients in this investigation, together with the microbiological findings, are summarized in table I. The analysis of the two series of patients in terms of funguria and its clinical significance is given in table II. No patient had incontrovertible evidence of clinically significant yeast infection, and most of those with funguria had no evidence of harm from the colonization. The two patients in the control series who were assigned to the Y1 group developed funguria later in their hospital stay after the bacteriuria had been noted and the serum specimen obtained.

All seven patients in the funguria series, who were assigned to the Y2 group of probable significant infection, were diabetics; four had *T. glabrata* and

Series	Clinical significance group			
	Y3	Y2	Y1	Y0
Funguria	0	7	18	0
Control bacteriuria	0	0	2	23

Table II Clinical assessment of significance

Y0 = no funguria; Y1 = benign funguria; Y2 = probable significant infection; Y3 = definite significant infection (see Methods section).

three had *C. albicans* colonization. Four of these seven patients were treated with flucytosine (100 mg per kg daily for one week) with successful eradication of the yeast in three patients. Resistance to flucytosine with persistence of the yeast occurred in one patient.

SEROLOGICAL FINDINGS

The incidence of precipitating antibodies to *C. albicans* and *T. glabrata* is summarized in table III. Of the 25 patients with funguria, 16 gave 'H' type reactions to *C. albicans* mannan antigens; four of these patients also gave 'H' type reactions with the *C. albicans* cytoplasmic antigen. In addition, two patients gave 'R' type reactions with the *C. albicans* cytoplasmic antigen. Eleven of the 16 patients who reacted with the *C. albicans* antigens also gave 'H' type reactions with the *T. glabrata* antigens.

Of the 25 control patients, 12 gave 'H' type reactions with the *C. albicans* antigens; six of these patients also gave 'H' type reactions with the *T. glabrata* antigens. Two more control patients gave 'H' type reactions with the *T. glabrata* mannan antigen. There was no significant difference in the incidence of precipitins in the two series of patients (χ^2 test, $P > 0.5$).

The incidence of precipitins in the diabetic and non-diabetic funguria series patients is compared in table IV. Of the 10 diabetic patients in this series, six had precipitins to *T. glabrata* as compared with five of the 15 non-diabetic patients.

The incidence of precipitins in relation to the class of clinical significance in the 24 patients with either *C. albicans* or *T. glabrata* colonization is shown in table V. Of the 13 patients with *C. albicans* colonization included in the Y1 group, nine had precipitins to *C. albicans* and five also had precipitins to *T. glabrata*.

Patients	Positive reactions to antigens of					
	C. albicans			T. glabrata		
	M	M + C	Per cent positive	M	M + C	Per cent positive
Diabetic (10)	6	1	70.0	5	1	60.0
Non-diabetic (15)	6	3	60.0	5	0	33.3

Table IV Incidence of precipitating antibodies in diabetic and non-diabetic patients

	16 patients with C. albicans colonization		8 patients with T. glabrata colonization	
	C. albicans precipitins	T. glabrata precipitins	C. albicans precipitins	T. glabrata precipitins
Y1	9 (69%)	5 (38%)	2 (50%)	2 (50%)
Y2	1 (33%)	1 (33%)	4 (100%)	3 (75%)

Table V Incidence of precipitating antibodies in relation to clinical signs of infection

pitins to *T. glabrata*. Of the three diabetic patients with *C. albicans* colonization assigned to the Y2 group of probable significant infection, one had precipitins to both *C. albicans* and *T. glabrata*.

Of four Y1 patients with *T. glabrata* colonization, two had precipitins to both *T. glabrata* and *C. albicans*. Three of four diabetic Y2 patients with *T. glabrata* colonization had *T. glabrata* precipitins but all four of these patients also reacted with *C. albicans* antigens.

Discussion

The yeasts isolated in this investigation were those commonly encountered in such specimens (Ahearn *et al*, 1966; Speller and Davies, 1973). The funguria series and the control bacteriuria series differed in several important clinical features. The funguria series had a greater incidence of diabetes mellitus, previous treatment with antibacterial agents, and previous urethral catheterization. The control series had a greater incidence of urinary symptoms. Six of the eight instances of *T. glabrata* colonization occurred in the 10 diabetic patients; this is a well-known association (Marks *et al*, 1970).

Series	Numbers of patients giving positive reaction to antigens of						Per cent positive
	C. albicans			T. glabrata			
	M only	C only	M + C	M only	C only	M + C	
Funguria	12	0	4	1	0	10	64.0
Bacteriuria controls	9	0	3	5	0	3	56.0

Table III Incidence of precipitating antibodies to mannan (M) and cytoplasmic (C) antigens

This investigation has shown that precipitating antibodies to mannan and cytoplasmic antigens can be detected in most hospital patients with fungal or bacterial colonization of the lower urinary tract. Indeed, there was no significant difference in the incidence of precipitins between the two groups. The presence of precipitins to *T. glabrata* mannan antigens in patients with *C. albicans* colonization is not surprising. Benham (1935) found that *T. glabrata* possessed antigens in common with *C. albicans*, and more recent studies have confirmed this observation (Hasenclever and Mitchell, 1960; Tsuchiya *et al.*, 1961).

Precipitins were present, more commonly, against the mannan than the protein antigens in both series of patients, and reactions to protein antigens only were not found. Antibodies to the protein component of the *C. albicans* cytoplasmic antigen were, however, detected in two of the funguria series patients: this may be a significant finding. The detection of precipitins to protein antigens has been thought to indicate deep-seated candidal infection (Taschdjian *et al.*, 1972), but at least one recent investigation has shown that antibodies giving rise to 'R' type reactions can occur in patients in the apparent absence of visceral infection (Stanley *et al.*, 1972). Our findings lend further support to this observation.

The reason for the appearance of the antibodies to the protein component of the *C. albicans* cytoplasmic antigen is uncertain: Murray *et al.* (1969) have suggested undetected candidaemia or transient deep-seated infection as possible reasons for the appearance of precipitins to cytoplasmic antigens. These two possibilities cannot be completely excluded in the two patients in our series giving 'R' type reactions, but in both cases the funguria appeared to be a transient, benign episode related to urethral catheterization and antibacterial therapy. Yeasts were not isolated from other sites in these patients, nor was there a previous history of fungal infection.

The incidence of precipitins in both our series of patients is higher than has been found in some other investigations (Stanley *et al.*, 1972; Stanley and Hurley, 1974). The reasons for this are uncertain; it might be due to differing test methods and antigens used in the different investigations (Faux *et al.*, 1975). It is probable that the failure to obtain reactions in previous investigations has been due to the use of too high concentrations of mannan antigens and that reactions have been obtained in patients with deep-seated infection because larger amounts of antibodies were present. It is likely, however, that the higher incidence of precipitins in our patients is due to the type of patient included in this investigation. Our patients were in hospital, mostly elderly, all

with other conditions, and most had been treated with antibacterial drugs. These factors could have contributed to an increasing colonization of the mouth or intestinal tract with yeasts and this in turn may have stimulated the production of antibodies.

In conclusion the high incidence of precipitin reactions in both series of patients, together with the lack of correlation between clinical and serological findings in the patients with funguria, leads us to suggest that the precipitin test has little to offer in the assessment of the significance in such patients of urinary tract colonization by yeasts.

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