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Observations on the use of the double diffusion test in the diagnosis of vaginal candidiasis

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SUMMARY Precipitins to mannan and cytoplasmic antigens of three *Candida* species were determined in an unselected series of 289 non-pregnant women. Precipitins were present in 20% of sera of women with vaginal candidiasis, in 23% of women harbouring yeasts in the vagina without clinical signs of infection, and in 21% of women harbouring yeasts in sites other than the vagina. Of the 47 patients who reacted with *Candida albicans* mannan or cytoplasmic antigens, 98% reacted with the mannan antigen but only 13% with the cytoplasmic antigen. The inclusion of mannan and cytoplasmic antigens of *C. guilliermondii* and *C. parapsilosis* did not increase the specificity or sensitivity of the test in the diagnosis of vaginal candidiasis. It is suggested that the double diffusion test is of doubtful value as an adjunct to the diagnosis of vaginal candidiasis.

The prevalence of vaginal infection with *Candida albicans* has led to interest in the use of serological tests as adjuncts to the usual clinical and microbiological methods of diagnosis of this condition. In particular, the double diffusion test using mannan and cytoplasmic antigens of *C. albicans* has been reported to be useful for pregnant women (Stanley *et al.*, 1972; Stanley and Hurley, 1974). In this paper we describe the results of an investigation designed to determine the incidence of precipitating antibodies to three *Candida* species in an unselected series of non-pregnant women and the relationship of these antibodies to clinical signs of vaginal infection and the commensal presence of yeasts in the vagina and digestive tract.

Methods

PATIENTS

Altogether 289 non-pregnant women aged between 16 and 45 who were attending the Department of Venereology, Bristol Royal Infirmary for the first time were investigated. High vaginal, oral, and rectal swabs were taken from each patient; the methods used for the isolation and identification of yeasts are described in detail elsewhere (Hilton and Warnock, 1975). Five millilitres of blood were taken from each patient and the serum was separated and stored at −20°C until required.

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ANTIGEN PREPARATION

C. albicans group A 3153 (London School of Hygiene and Tropical Medicine), *C. guilliermondii* CBS 566, and *C. parapsilosis* CBS 604 (Centraalbureau voor Schimmelcultures, Delft, The Netherlands) were cultured for 48 hours at 37°C on a medium containing 2% glucose, 1% peptone, and 2% agar. Cytoplasmic antigens were prepared from washed cells disrupted for 8 minutes in a Braun MSK homogeniser. The disrupted cells were centrifuged at 5000 *g* to sediment particulate material and the supernatant fluid was lyophilised. Mannan antigens were extracted and purified according to the method of Peat *et al.* (1961) as modified by Kocourek and Ballou (1969).

DOUBLE DIFFUSION TEST

The mannan and cytoplasmic antigens were reconstituted in McIlvane's citrate buffer pH 7·0. The mannan antigens were used at concentrations of 1 and 0·1 mg/ml and the cytoplasmic antigens at 30 and 3 mg/ml in the double diffusion test which was carried out in 1·5% Noble agar (Difco) in citrate buffer pH 7·0 containing 0·9% sodium chloride and 0·05% sodium azide. Agar was layered to a depth of 1·5 to 2 mm on glass plates and a template was used to cut antigen wells of 4 mm diameter arranged 6 mm (circumference to circumference) from a 12·5 mm diameter central serum well. Diffusion was car-

ried out in moist chambers at 28°C for five days after which the plates were washed in repeated changes of 0·9% saline, dried, and stained in 0·5% Coomassie Blue BL.

Results

The principal mycological findings for the 289 patients in this investigation are summarised in Table 1. On the basis of these findings, the patients were divided into four groups:

1. 25 subjects with vaginal candidiasis; these patients had clinical signs of vaginitis, with or without concomitant vulvitis, accompanied by the isolation of yeasts from the vagina;
2. 57 subjects who were free from clinical signs of vulvovaginitis but were harbouring yeasts in the vagina;
3. 105 subjects who were free from signs of vulvovaginitis and were not harbouring yeasts in the vagina but were harbouring yeasts in the mouth and/or the rectum.
4. 102 subjects who were free from signs of vulvovaginitis and were not harbouring yeasts in the vagina, mouth, or rectum.

The incidence of precipitating antibodies to Candida antigens in the different groups of patients is compared in Table 2. Of the 289 patients, 52 (18%) had precipitins. Of the 25 patients with vaginal candidiasis, 20% had precipitins compared with 23% of the women harbouring yeasts in the vagina without clinical signs of infection and 21% of the women harbouring yeasts in sites other than the vagina. Of the 102 women from whom no yeasts were isolated, 12% had precipitins.

Most of the precipitin-positive patients (46 of 52) had antibodies to the mannan antigen of *C. albicans;* only six patients had antibodies to the cytoplasmic antigen. A similar distribution occurred with *C. guilliermondii* antigens, but with *C. parapsilosis* antigens the reverse situation was found, seven patients giving reactions with the mannan antigen and 11 with the cytoplasmic antigen.

The distribution of precipitins in the 52 patients

Table 1 *Distribution of yeasts in 289 women*

Patient category	*Number of patients*	*Number of isolates of:*		
		C. albicans	C. parapsilosis	C. tropicalis
Vaginal candidiasis	25 (9%)	24	1	0
No infection, yeast in vagina	57 (20%)	56	1	0
No infection, yeast in sites other than vagina (mouth, rectum)	105 (36%)	101	2	2
No infection, no yeast isolated	102 (35%)	—	—	—
Totals	289 (100%)	181	4	2

Table 2 *Incidence of precipitins to mannan and cytoplasmic antigens of* C. albicans, C. guilliermondii, *and* C. parapsilosis

Patient category	*Number of patients*	*Number of sera with precipitins*	*Number of sera with precipitins to:*								
			C. albicans			C. guilliermondii			C. parapsilosis		
			M	C	M+C	M	C	M+C	M	C	M+C
Vaginal candidiasis	25	5 (20%)	5	2	2	3	2	2	0	1	0
No infection, yeast in vagina	57	13 (23%)	10	1	1	7	2	2	2	6	1
No infection, yeast in sites other than vagina (mouth, rectum)	105	22 (21%)	19	2	1	13	3	3	2	3	0
No infection, no yeast isolated	102	12 (12%)	12	1	1	5	1	1	3	1	0
Totals	289	52 (18%)	46	6	5	28	8	8	7	11	1

M = mannan; C = cytoplasmic antigen; M+C = mannan + cytoplasmic antigens

Table 3 *Distribution of precipitins to antigens of* C. albicans, C. guilliermondii, *and* C. parapsilosis

Patient category	*Number of sera with precipitins*	*Number of sera with precipitins to only:*						
		CA	CG	CP	CA+CG	CA+CP	CP+CG	CA+CG+CP
Vaginal candidiasis	5	2	0	0	2	0	0	1
No infection, yeast in vagina	13	3	1	2	2	1	0	4
No infection, yeast in sites other than vagina (mouth, rectum)	22	7	1	1	9	1	0	3
No infection, no yeast isolated	12	4	0	0	4	3	0	1
Totals	52	16	2	3	17	5	0	9

CA = *C. albicans*; CG = *C. guilliermondii*; CP = *C. parapsilosis*

who gave positive reactions to the *C. albicans*, *C. guilliermondii*, and *C. parapsilosis* antigens is shown in Table 3. There was no distinctive pattern of reactions to the various combinations of antigens of the three species with sera of any particular patient group. None of the 52 patients reacted only with the *C. guilliermondii* and *C. parapsilosis* antigens. The inclusion of these antigens revealed five patients who did not react with the *C. albicans* antigens; three of these patients were women with no clinical signs of infection and yeasts in the vagina and two were women harbouring yeasts in sites other than the vagina.

It should be mentioned that in order to detect all positive reactions, the antigens had to be used at both concentrations. No definite pattern of reactions to the different concentrations of the individual antigens emerged when the different patient groups were compared. In most instances, reactions were obtained with one or other antigen concentration but not with both concentrations.

Discussion

The demonstration of precipitins in sera of pregnant women was first reported by Stanley *et al.* (1972), who found an incidence of 18% in an unselected series of patients. This is identical with the incidence found in the present investigation of non-pregnant women. However, in this investigation only 20% of women with proven vaginal candidiasis had precipitins, and this incidence was similar to that (23%) observed in women who were harbouring yeasts in the vagina without clinical signs of infection, and also to the incidence (21%) observed in women who were harbouring yeasts in sites other than the vagina.

The presence of precipitins to the mannan antigens rather than the cytoplasmic antigens in the patients in this investigation is not a surprising finding. Antibodies to cytoplasmic antigens have been found in most patients with deep-seated candidiasis but have not often been detected in normal persons or patients with superficial cutaneous or mucosal infection (Taschdjian *et al.*, 1967). This finding has been attributed to differences in antigenic stimulation: for cytoplasmic antigens to be released, phagocytosis of the yeast cell appears to be essential (Taschdjian *et al.*, 1971). This does not occur in superficial infections (Montes and Wilborn, 1968). In this situation it is the mannan antigens, present on the surface of the intact yeast cells (Hasenclever and Mitchell, 1964), that provide the antigenic stimulation. Indeed, antibodies to these mannan antigens can be detected in most normal persons as well as in patients with mucocutaneous candidiasis (Chew and Theus, 1967). In this respect our results confirm that the carriage of yeasts on mucosal surfaces is sufficient to stimulate the formation of antibodies in the absence of clinical signs of infection.

Murray *et al.* (1969) regarded the presence of precipitins to more than one *Candida* species as being of greater diagnostic significance than the presence of precipitins to only one species. The inclusion of *C. parapsilosis* and *C. guilliermondii* antigens to increase the antigenic spectrum in this investigation did not produce a more sensitive or specific test for the diagnosis of vaginal candidiasis compared to the use of *C. albicans* antigens alone. In no particular patient group did the incidence of reactions to *C. guilliermondii* or *C. parapsilosis* antigens indicate any particular significance.

The incidence of precipitins in our patients with vaginal candidiasis is lower than has been found in previous investigations. Stanley and Hurley (1974) detected precipitins in 64% of pregnant women with this condition compared with 20% of non-pregnant women in this investigation. The reason for this difference is uncertain but it might be due to the differing test methods and antigens used. Faux *et al.* (1975) compared a number of different methods of performing double diffusion tests for *C. albicans* antibodies and concluded that the variation in results obtained using different test methods and antigens was such that direct comparison of the incidence of precipitins in groups of patients is not possible.

In contrast to the present investigation, Stanley and Hurley (1974) did not determine the incidence of precipitins in pregnant women harbouring yeasts as commensals in the vagina or other sites. This omission, if taken in conjunction with the findings of the present investigation, casts some doubt on their suggestion that the double diffusion test is a useful adjunct to the diagnosis of vaginal candidiasis.

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