

Candida precipitins in pregnant women: validity of the test systems used

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SYNOPSIS Sera from 200 pregnant women, with symptoms suggestive of vaginitis and harbouring yeast in the vagina, were examined for precipitating antibodies to three antigens of *C. albicans*, using a gel double diffusion test. A high overall incidence of precipitin-positive sera (47.5%) was found compared with an incidence of 18% in the unselected pregnant population previously studied (Stanley, Hurley, and Carroll 1972).

Using the clinicopathological criteria of Carroll, Hurley, and Stanley (1973), a final aetiological diagnosis of *C. albicans* mycosis was reached in 75 cases and precipitins were demonstrated in 64%. Forty-eight women harbouring *C. albicans* responded favourably to a single course of antifungal treatment, and probably had mycotic vaginitis. The incidence of precipitins in this group was 42%. *C. albicans* was isolated from a further 55 of 62 patients, in whom the incidence of precipitins was 32%.

'Booking' sera were investigated from 50 of the 200 women studied. Sixty-four per cent of women had symptoms of vaginitis at booking and 32% were precipitin positive. Twenty-eight per cent had precipitins on both occasions, and a further 24% acquired candida precipitins during pregnancy.

None of the seven newborn with oral or skin thrush had precipitins to *C. albicans*.

The results indicate that the detection of precipitating antibodies to *C. albicans*, particularly to all three of the antigens described in this paper, would be a useful additional criterion in the diagnosis of candida vaginitis, particularly if the vaginitis were persistent, recurrent, or unresponsive to therapy. The sensitivity of the test system used was 64%, and its specificity 87%; as such, the test is valid and may be reasonably useful as a screening procedure.

In a previous study on an unselected pregnant population, Stanley, Hurley, and Carroll (1972) showed that precipitins to three antigens derived from *Candida albicans*, tested at two concentrations, occurred in 18% of sera from women booking at an antenatal clinic, and that these occurred significantly more frequently in women with probable mycotic vulvovaginitis. By analysis of prospectively recorded clinical data pertaining to this group, Carroll, Hurley, and Stanley (1973) established clinicopathological criteria on which the diagnosis of mycotic vulvovaginitis, or vaginitis, might be soundly based.

We now describe the incidence of candida precipitins in a further group of 200 pregnant women whose sera were selected for examination because of the probability of a high incidence of mycotic vaginitis amongst them. After analysis of the validity of the test systems used, we suggest that the tests in

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their present form may be used as an adjunct to the diagnosis of candida vaginitis, or for population screening for early detection of disease.

Materials and Methods

Five ml clotted blood was requested from 200 women who had been found, on laboratory examination of vaginal swabs sent for diagnosis of pregnancy vaginitis, consecutively to be harbouring yeasts. Sera collected at 'booking' were also examined for 50 of these patients.

Subsequent swabs were examined from 96 women, mainly from those who failed to respond promptly or completely to treatment.

The methods used for examination of vaginal swabs and for isolation and identification of yeasts are described elsewhere (Hurley, Leask, Faktor, and de Fonseka, 1973; Merritt and Hurley, 1972).

The sera were tested for the presence of precipitating antibodies to *C. albicans A* (LSHTM no. 3153). Gel double diffusion was carried out in 1% Oxoid Ionagar no. 2 buffered to pH 8.6 with borate buffer, at a depth of 1 mm. A pure cell wall mannan antigen (1 mg/ml), a culture filtrate antigen (20 mg/ml), and a Mickle-disintegrated cytoplasmic antigen (20 mg/ml) were prepared as described by Stanley *et al* (1972). Antigen wells of 6 mm and 2 mm diameter were separated by 6 mm from a central serum well of 14 mm diameter. The smaller antigen wells were calculated to give a volume ratio about one-tenth of the larger to assist in the detection of antibodies in weakly reacting sera (Murray, Buckley, and Turner, 1969) or reactions that might be masked by excess mannan (Pepys, Faux, Longbottom, McCarthy, and Hargreaves, 1968). The plates were incubated at 30°C for five days. The agar was washed for three days in changes of borate buffer, dried, and stained with Coomassie Brilliant Blue R (5g dissolved in 450 ml methanol, 450 ml distilled water, and 100 ml glacial acetic acid). Differentiation was in the dye solvent. A positive precipitin result was recorded from the stained agar if a band was produced to any of the three antigens.

Results

MICROBIOLOGICAL FINDINGS

Two hundred and ten yeast isolates were cultured from the initial specimens. Ninety-five per cent (190) of the 200 women harboured *C. albicans*. *C. albicans* occurred in association with *C. krusei* (1), with *Saccharomyces cerevisiae* (1), with *Torulopsis glabrata* (6), and with other *Torulopsis* species (2). In nine women the only yeast isolated was *T. glabrata* (6), or *S. cerevisiae* (2), or *C. stellatoidea* (1). *Neisseria gonorrhoeae* was isolated with *Trichomonas vaginalis* and *C. parapsilosis* from one patient. *T. vaginalis* was observed simultaneously with yeasts in seven women (3.5%); in six of these the associated yeast was *C. albicans* (3%) (table I). *T. vaginalis* was seen in specimens from a further six of 96 women whose vaginal flora was subsequently studied.

CLINICAL FINDINGS

The incidence of signs and symptoms relevant to vulvovaginitis in this group of women is given in

	No.
Vaginitis	68 (34.0%)
Vulvitis	0 (0.0%)
Vulvovaginitis	5 (2.5%)
Plaques	3 (1.5%)
Irritation	80 (40.0%)
Discharge	163 (81.5%)
Diabetes	4 (2.0%)
Glycosuria	12 (6.0%)

Table II Relevant recorded clinical data in 200 symptomatic pregnant women harbouring yeasts in the vagina

table II. In 75 patients the clinicopathological criteria of Carroll *et al* (1973) were used to establish the diagnosis of *C. albicans* vaginitis. These are the presence of vaginal thrush plaques, irrespective of isolation of the thrush fungus, and signs of vaginitis, with or without concomitant vulvitis, coupled with isolation of *C. albicans*. Discharge and irritation are ignored as being of no probative value. These criteria were supplemented, and women with diabetes, from whom *C. albicans* was isolated, were regarded as having candida vaginitis (Hesseltine, 1933). The frequency of observation of *Trichomonas vaginalis* relative to candida, throughout the pregnancy, together with the response to specific therapy, was taken into account when deciding whether vaginitis was primarily mycotic or trichomonadic. Of the remaining women, prompt response to antifungal therapy, together with isolation of *C. albicans*, was regarded as indicative that the vaginitis was probably of mycotic origin (48 patients) and the isolation of *C. albicans* from 55 of 62 women, for whom no final diagnosis was reached, also indicated probable mycosis.

Thus, in 75 patients the diagnosis of candida vaginitis was established for certain, while in 103, it was probable. Predominantly trichomonadic vaginitis was diagnosed in nine patients; concurrent candida and trichomonadic vaginitis in three; gonorrhoea in one; and no firm aetiological diagnosis in nine.

INCIDENCE OF CANDIDA PRECIPITINS

Ninety-five of the 200 requested sera tested (47.5%) contained precipitins to at least one of the three antigens tested. The incidence of precipitins in the

Total <i>C. albicans</i> Isolated	<i>C. albicans</i> Isolated Associated with Other Yeasts	Yeasts Isolated Unassociated with <i>C. albicans</i>	<i>Neisseria gonorrhoeae</i> Isolated	<i>Trichomonas vaginalis</i> Seen
190	10	10	1	7

Table 1 Principal microbiological findings at first examination (VS) of 200 pregnant women with symptoms and signs of vulvovaginitis harbouring vaginal yeasts

different clinical groups is shown in table III. Sixty-four per cent of the 75 women diagnosed on clinicopathological criteria as having *C. albicans* vaginitis, and 42% of the 48 women with probable *C. albicans* vaginitis, diagnosed on response to antifungal therapy, had precipitating antibodies to *C. albicans*. The three women with mixed candida and trichomonadic vaginitis and 32% of the remainder had precipitins in their sera.

The analysis of results obtained with each individual antigen is given in table IV. It can be seen that the reactions detected by the pure mannan and cytoplasmic antigens do not differ appreciably in any of the clinical groups. However, tests were not carried out to determine whether the patients' sera were reacting with the mannan or protein components of the cytoplasmic antigen, although certain sera gave multiple bands with the latter. Most reactions were detected with the culture filtrate antigen.

Final Aetiological Diagnosis of the Vaginitis	No.	<i>C. albicans</i> Isolated	<i>Candida</i> Precipitins Present
<i>C. albicans</i> ¹	75	75	48 (64%)
<i>C. albicans</i> ²	48	48	20 (42%)
Other mycotic ¹	0	0	0
Other mycotic ²	2	0	1
Mixed mycotic and trichomonadic	3	3	3
Predominantly trichomonadic	9	9	3
Gonorrhoea	1	0	0
Not certainly established	62	55	20 (32%)

Table III Incidence of candida precipitins in 200 pregnant women with symptoms or signs of vulvovaginitis and harbouring yeasts in the vagina

Diagnosis based on clinicopathological criteria of Carroll *et al* (1973).
¹Cases diagnosed on favourable response to antifungal therapy.

As previously described by Stanley *et al* (1972), no single antigen, or single concentration of an antigen, detects all positive reactions. Until the diagnostic significance of reactions to particular components of antigenic extracts of *C. albicans* is determined we recommend the use of all three antigens as an aid to the diagnosis of *C. albicans* vaginitis. Reactions to all three antigens appear more frequently in mycotic and probable mycotic vaginitis than in other clinical groups described in this paper and may relate to weight or chronicity of infection or overgrowth of the fungus.

Sera from 50 of the 200 women, stored since the routine antenatal booking tests had been made, were also examined, and the results of the two serological examinations were compared. Thirty-two per cent of sera contained candida precipitins at booking, and 52% contained precipitins on serological examination following the first isolation of yeasts from the vagina. In 28% precipitins were present in both the booking and in the requested serum. Thus, 24% of patients had acquired candida precipitins during pregnancy, Half of the women who had acquired precipitins had had symptoms at booking, and in a quarter of them, the predicted clinical diagnosis had been mycotic vaginitis.

Finally, sera were obtained from seven of the babies with thrush born to the 200 women, but none contained precipitating antibodies to *C. albicans*.

Discussion

Stanley *et al* (1972) reviewed the literature pertaining to candida precipitins, commenting that they believed that theirs was the first study of the distribution and probable significance of candida precipitins in pregnant women. They commented on the difficulties

Final Aetiological Diagnosis of the Vaginitis	Positive Reactions to Antigens of <i>C. albicans</i>										
	M	S	CF	M+S+CF	M Only	S Only	CF Only	M+S Only	M+CF Only	S+CF Only	Total +ve
<i>C. albicans</i> ¹ (75)	29	28	42	21	3	0	15	3	2	4	48
<i>C. albicans</i> ² (48)	11	14	17	8	1	2	3	0	2	4	20
Other mycotic ¹ (0)	0	0	0	0	0	0	0	0	0	0	0
Other mycotic ² (2)	1	1	1	1	0	0	0	0	0	0	1
Mixed mycotic and trichomonadic (3)	1	1	3	1	0	0	2	0	0	0	3
Predominantly trichomonadic (9)	2	2	3	2	0	0	1	0	0	0	3
Gonorrhoea (1)	0	0	0	0	0	0	0	0	0	0	0
Not certainly established (62)	13	13	17	9	2	0	4	1	1	3	20
Total +ve	57	59	83	42	6	2	25	4	5	11	95

Table IV Incidence of candida precipitins to three antigens of *C. albicans* in 200 selected pregnant women

^{1,2} See table III

M = mannan; S = cytoplasmic; CF = culture filtrate

of achieving an accurate aetiological diagnosis of vaginitis using uncritical clinical and laboratory criteria, and they considered that a serological test might prove of value in the diagnosis of pregnancy thrush. They showed that the number of patients from whom *C. albicans* is isolated, in the absence of signs of plaque formation, or of vaginitis, is small (15%) but not negligible (Carroll *et al*, 1973), and, conversely, that 84% of women from whom *C. albicans* is isolated during pregnancy have signs of vaginitis. The patients who were the subjects of the present study were selected because, as a group harbouring yeasts, they were likely to have a high incidence of *C. albicans* vaginitis, and thus to afford a large number of sera suitable for the assessment of the specificity and sensitivity of precipitin tests in the diagnosis of mycotic vaginitis.

The identity of the vaginal yeasts from the population selected did not differ from that previously noted in other studies from our hospital, and the predominating fungus was the pathogen *C. albicans* which was isolated from 95% of the patients.

A high overall incidence (47.5%) of precipitating antibodies to antigens of *C. albicans* was found in the selected women, all of whom had symptoms ascribed to vaginitis, and harboured yeasts in the vagina. This is significantly higher than the 18% recorded for an unselected pregnant population (Stanley *et al*, 1972). Applying clinicopathological criteria previously enunciated (Carroll *et al*, 1973) to validate the diagnosis, candida precipitins occurred in 64% of 75 women with undoubted candida vaginitis, and in 42% of 48 women, whose response to therapy suggested specific fungal vaginitis. *C. albicans* was isolated from 55 of a further group of 62 patients and the incidence of precipitins, although lower (32%) than in women with undoubted candida vaginitis, was still almost double that occurring in an unselected population, suggesting that the aetiology of the vaginitis was mycotic. Stanley *et al* (1972) showed that the presence of precipitins was significantly associated with acute, recurrent, or relapsing candida vaginitis ($P < 0.001$).

These observations encourage us to propose that serological examination of pregnant women for the presence of candida precipitins may prove a useful test for candida vaginitis, and an adjunct to culture. It may also be of use as a screening procedure for early detection of candida vaginitis. With the methods and criteria of disease that we have used, its sensitivity, according to the formula (Wilson and Jungner, 1968):

Sensitivity =

$$\frac{\text{Diseased persons with positive test}}{\text{All persons in population with disease}}$$

is 64%.

Its specificity, according to the formula:

Specificity =

$$\frac{\text{Non-diseased persons with negative test}}{\text{All persons in population without disease}}$$

is 87%, this figure being based on studies on an unselected population (Stanley *et al*, 1972).

Thus, the 'false negative' rate is of the order of 36% and the 'false positive' rate of the order of 13%. The test systems used are, therefore, valid, since they separate those who have candidosis from those who do not.

There is every hope that the sensitivity of the serological test can be improved, by variation of the antigens used, and the techniques employed. For example, it may be possible to elaborate more highly specific antigens, or to improve sensitivity without loss of specificity by concentrating the serum. In this study, as before, we have made no attempt to correlate positive findings with the presence of other superficial candida mycoses, and the 'false' positives may relate to candidosis elsewhere. Meanwhile, the test methods described may still prove useful, if serological examination encourages early and more accurate diagnosis and prompt treatment of candida vaginitis during pregnancy.

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