

THE INCIDENCE OF TRICHOMONAS AND FUNGUS INFESTATION IN 150 NON-PREGNANT CASES OF LEUCORRHOEA

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The subject of fungus and trichomonas infestations of the vagina can very well be included in any discussion on the recent trends in gynaecology, not because the disease entities are newly identified but because of the resurgence of interest in the subjects due to the sudden increase in incidence of fungus infestation and changes in the relative incidence of trichomonas and fungus infestations as reported from all over the world. It is quite possible, however, that part of this apparent increase is due to the availability of more certain methods of office diagnosis of the disease. Whereas the wet smear still remains the easiest, and nearly as accurate, as the most elaborate cultural method of diagnosis of trichomonas infestation, the fungus does

not lend itself to easy diagnosis with any degree of accuracy by simple smear technique. The diagnosis rests finally on the demonstration of the fungus by cultural methods and it is no simple matter to decide whether the fungus isolated by culture from the vaginal discharge is really the cause of the discharge or merely a harmless parasite. It is from this point of view that we have titled this paper as "Incidence of fungus infestation, etc." and refrained from labelling any case as *Candida vaginitis* as is often casually done. Even when the offending fungus is finally identified to be *Candida albicans*, it is difficult to conclude that it is the cause of symptoms, till the symptoms are finally relieved by the use of one of the fungistatic agents. In our series we have not been able to carry our investigation to the therapeutic stage due to the short period during which we have conducted our studies.

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The fungus infestation of the female genital tract is important not only from the point of view of the local symptoms, which are often distressing, but also from the point of view of the occurrence of oral thrush in the new-born. The relation of the vaginal candidal infection in the mother and oral thrush in the new-born has now been well established. Thrush assumes particularly dangerous proportions in the new-born infants specially when they are premature and debilitated. The danger of systemic dissemination is always greater in these cases constituting a serious threat to life if diagnosis and treatment are not prompt.

Though the response of the host to the presence of *Candida* organisms in the vagina is governed by factors which are still a mystery to us, a few predisposing factors are well-known. Pregnancy and diabetes are two of the most important of these conditions. In pregnancy the increased incidence is supposed to be due to the increased glycogen content of the vagina, as also the occurrence of glycosuria, the incidence of which is usually placed at 15% of the pregnant patients. The increased incidence in diabetes is also attributed to the glycosuria. The third important factor which is supposed to be particularly responsible for the recent sudden increase in the incidence of the disease is the use of antibiotics, particularly broad spectrum antibiotics. Though the usual explanation offered is that the inhibition of most of the vaginal flora allows overgrowth of the already existing fungus organisms, the real mechanism appears to be far more complicated.

The investigations of Frank Roth of the University of Miami have revealed that the broad spectrum antibiotics directly enhance the virulence of the fungus in animals. Further, an interesting toxin has been extracted from *Candida albicans* by sonic vibrations and the toxicity of this extract in animals is found to be greatly enhanced when the toxin is mixed with broad spectrum antibiotics. In this connection it is instructive to recall that Bernstein and Rakoff found the itching and irritation to be out of proportion to the degrees of infection of the fungus in cases where broad spectrum antibiotics have been used. The problem of the mechanism of antibacterial antibiotic potentiation of the fungus organism obviously requires further study.

The use of corticoids and extreme debility are the other factors supposed to predispose to fungus infestation. It is often mentioned that the incidence of fungus is higher in those women who have a habit of frequent vaginal douching, but the reason why this group of women began taking frequent douches has never been mentioned. The importance of increased vaginal acidity and the aciduric vaginal flora is currently discounted.

The relative incidence of fungus and trichomonas infestation is a further point of interest. Lee and Keifer record that whereas before the advent of antibiotics the proportion was 4 cases of trichomonas to 1 of moniliasis, recently the ratio has changed to 1 of trichomonas to 3 of moniliasis. Pace and Schantz find even this ratio too low and report a proportion of 1 to 7 in favour of moni-

liasis in non-pregnant cases and 1 to 15 in pregnant cases. The proportion in our cases is nowhere near these figures. In 150 cases complaining of leucorrhoea the proportion of trichomonas to fungus infestation was only about 4 to 7. Nadkarni et al. have reported the proportion of trichomonas to fungus to be 9:2 in non-pregnant patients.

The overall incidence of fungus and trichomonas infestations of the female genital tract in any particular community is very difficult to determine and very few figures are available on this point. The common type of report is the one where only cases complaining of leucorrhoea have been investigated. The threshold tolerance of the individual patient to the discomfort caused by leucorrhoea before she consults a doctor varies so considerably, that this factor, in addition to the point whether leucorrhoea was the patient's presenting symptom or whether the symptom was elicited only on questioning, makes the various reports of the results in cases of leucorrhoea not strictly comparable; but that is the nearest one can come to under the existing conditions.

Our 150 cases were all patients from the gynaecological out-patient department complaining of leucorrhoea as either their main or subsidiary symptom, which they complained of either by themselves or on questioning. After eliciting a detailed gynaecological history with special reference to the use of douches, contraceptives, vaginal medications, and the presence of other symptoms like pruritus and burning micturition, a complete gynaecological examination was made and the findings on

speculum examination particularly noted. Material collected from the appropriate sites was examined directly in a drop of normal saline for the defection of trichomonads and also in a drop of 10% potassium hydroxide for the detection of fungus. Smears were made for staining by Gram's method and by Carbol-fuschsin, the latter particularly for the demonstration of trichomonads.

Initially Nickerson's medium was used for the primary culture of the material for the detection of fungal infection. Since the specificity of the medium for the growth of *Candida* organisms proved to be doubtful a new medium was used in our laboratory for the same purpose. The medium named Wheat-flour-Dextrose Agar Medium (WFDA), from its constituents, had many advantages for primary culture. It was simple to prepare and cheap, did not require any special chemical constituents and hence was available in plentiful supply. In addition as against the 3-4 days incubation at room temperature required with the Nickerson's medium, the WFDA medium yielded a luxuriant growth of the fungus in positive cases within 24-48 hours. The medium however shows no specificity and the identification of the type of fungus has to be made by subsequent investigation.

The positive cultures are transplanted on a Saboraud-dextrose agar slant and if a pure culture is obtained the material is transplanted to Saboraud-dextrose broth. It is incubated at 37°C. for 48 hours to note the presence and type of growth.

The broth culture is shaken to suspend the organisms and then streak-

ed on a blood agar plate and incubated at 37°C. for 2-4 days. The blood agar colony is then hand planted to a plate of rice infusion agar and the streak is covered with a cover-slip to produce partially anaerobic conditions to favour the growth of mycelia. These are incubated for 24 hours at 37°C. and then examined directly under the microscope.

The blood agar colonies are also inoculated on carrot plugs for the detection of ascospores and into 2% solutions of dextrose, maltose, fructose and lactose in peptone water for the detection of fermentation with either acid or gas production. The tubes are incubated for 10 days at 37°C. and seen daily.

The cases which yielded positive primary culture on the WFDA medium were investigated further as described above and the results are summarised in Table VIII.

Cultural methods for the growth of trichomonads were not successful as some of the essential ingredi-

ents of simplified Trypticase serum medium were not available. The diagnosis of trichomonas in this series was therefore based on wet smear demonstration of the organism.

Here we summarise our results in 150 non-pregnant cases complaining of leucorrhoea examined for the detection of fungus and trichomonas infestations. It will be seen that the incidence of fungus infestation is much more than that recorded in previous Indian reports; for example, Pandya and Nadkarni have recorded an incidence of 6% while Satyavati and Reddi have recorded an incidence of only 2% in 100 cases of leucorrhoea. The incidence of trichomonas is slightly low, 21.9%, as against 27% reported by Pandya and colleagues.

The higher incidence of both fungus and trichomonas infestations in private patients as compared to hospital patients is likely to be due to the greater frequency of the use of systemic and local antibiotics or more

TABLE I
*Incidence of Fungus and Trichomonas Infestations in 150 Cases
Complaining of Leucorrhoea*

Total no. of cases	Positive for fungus infestation		Positive for trichomonas infestation	
	No. of cases	%	No. of cases	%
150	54	36.0%	32	21.9%

TABLE II
Incidence in Hospital and Private Patients

Group	Total no. of cases	Positive for fungus infestation		Positive for trichomonas infestation	
		No. of cases	%	No. of cases	%
Hospital	130	41	31.5%	27	20.8%
Private	20	13	65.0%	5	25.0%

frequent use of douches, and what is more, an unconscious selection by the referring consultants in referring particularly these cases to the Research Centre where this investigation of moniliasis and trichomonas infestation is under way.

Tables IV and V suggest that religion, caste and obstetric status seem to make no significant difference to the incidence of both trichomonas and moniliasis. The occurrence of fungus infestation in 4 of the 5 single women complaining of leucorrhoea is

TABLE III
Incidence According to Age

Age in years	Total no. of cases	Positive for fungus		Positive for trichomonas	
		No. of cases	%	No. of cases	%
Below 17	1				
17-34	116	43	37.1%	23	19.8%
35-44	21	7	33.3%	7	33.3%
45 & Over	12	4	33.3%	2	16.6%

33.3% incidence of fungus infestation in menopausal patients over 45 years of age is surprising when it is recalled that a higher level of glycogen content of the vagina is supposed to be a predisposing factor in the causation of fungus infestation. The incidence in our series is almost equal to that in the child-bearing age group.

noteworthy but the number is too small to draw any conclusions.

The incidence of fungus and trichomonas infestations is almost similar in various symptom groups shown here. The incidence of fungus is apparently lower in those complaining of leucorrhoea and burning micturition but the number of cases in this group is much smaller than the

TABLE IV
Incidence According to Religions and Caste

Group	Total no. of cases	Positive for fungus		Positive for trichomonas	
		No. of cases	%	No. of cases	%
Maharashtrian	68	28	41.2%	12	19.9
Gujarati	24	9	36.5%	5	22.1
Christian	17	4	23.5%	4	23.5%
Bhaiya	16	5	31.2%	3	18.9%
Muslim	7	2	—*	—	*
Sindhi	7	3	*	3	*
Punjabi	4	3	—*	1	*
Madrasi	3	—	—*	2	*
Parsi	2	—	*	2	*
Jew	1	—	*	—	*
Gurkha	1	—	*	—	*

* Percentages not calculated due to very small no. of cases.

TABLE V
Incidence According to Parity

Obstetric status	Total no. of cases	Positive for fungus		Positive for trichomonas	
		No. of cases	%	No. of cases	%
Single ..	5	4		1	
Nulliparous ..	44	17	38.6%	11	25.0%
Parous ..	101	33	34.0%	20	19.9%

TABLE VI
Incidence According to Symptoms

Symptoms	Total no. of cases	Positive for fungus		Positive for trichomonas	
		No. of cases	%	No. of cases	%
Leucorrhoea	52	17	32.6%	10	19.2%
Leucorrhoea + Pruritus	41	18	43.97%	10	24.37%
Leucorrhoea + Pruritus	44	17	38.6%	9	20.4%
Leucorrhoea + Pruritus + Burning micturition	13	2	15.4%	3	23.7%

other groups and it is unfair to draw conclusions from such a small number of cases.

It will be seen from this table that the incidence of fungus is greatest in cases with vulvitis and is lowest in

TABLE VII
Incidence According to Local Pathology

Local pathology	Total no. of cases	Positive for fungus		Positive for trichomonas	
		No. of cases	%	No. of cases	%
Vaginitis	50	24	48.0%	17	34.0%
Vulvitis	15	12	80.0%	4	26.6%
Endocervicitis + Erosion	43	5	11.6%	9	20.9%
No local pathology	67	26	38.9%	9	13.4%

cases with endocervicitis and erosion, while the incidence of trichomonads appears to be greatest in cases with vaginitis. It is also interesting to note that 26 of the total 54 cases which were positive for fungus, i.e. 48% of the positive cases, had no local pathological appearance demonstrable to the naked eye though they certainly complained of leucorrhoea.

the other with thick curdy discharge and pseudo-membrane formation. These appearances, when present, certainly leave little doubt about the diagnosis. But it is unusual to get the text-book clinical picture in practice. We would venture to say that apart from these few classical clear-cut cases the diagnosis of various types of vaginitis from the clinical

TABLE VIII
Nature of Fungus in 54 Positive Cases.

Name	Total no. of cases	Vaginitis & vulvitis	Endocervicitis and erosion	No. local pathology
<i>Candida</i>				
<i>albicans</i>	39	20	1	18
<i>C. krusei</i>	1			1
<i>C. stellatoidea</i>	4	1	1	2
Fungus other than <i>C.</i>	14	3	2	5

This table demonstrates the results of the tests for further classification of the isolated fungi. As already stated 10 of the 54 cases were found not to belong to the *Candida* group at all. As against the reports of Dawkins et al., and Rauramo, the *stellatoidea* species was demonstrated in 4 of the 44 cases harbouring the *Candida* group of organisms. As stated above, the various species were quite indistinguishable on primary culture or direct smear.

Comments

All students of gynaecology after reading the standard text-book descriptions of various types of vaginitis are apt to harbour an impression that these are quite clear-cut easily distinguishable entities, some, with the thin frothy irritating foul smelling discharge in a flea-bitten vagina with eccentric erosion of the cervix, and

picture alone is nothing short of guesswork. Add to these two the newly recognised entity of *Haemophilus* vaginitis without any specific symptoms or characteristic local appearances and the clinical confusion is complete. The assertion of some authors that trichonomas and monilial infestations rarely occur together has been belied by our findings where we have 6 cases showing both the organisms. The corollary is, that the demonstration of the trichomonads in wet smear may not be the complete diagnosis of a case of leucorrhoea and it is incumbent on us to culture the discharge on a suitable medium to exclude associated fungus infection. Further, the specificity of Nickerson's medium for *Candida* is open to doubt as all the 10 cases subsequently proved not to belong to the *Candida* group and those proved to belong to the species *stella-*

toidea and krusei yielded the typical brown or black colonies on the Nickerson's medium. On the other hand two of our cases which yielded cream coloured colonies on the Nickerson's medium were subsequently found to belong to the *Candida albicans* species.

Some few points of practical importance emerge from this study. It is clear that only cultural methods can be relied on for the diagnosis of fungus infestation except in a few cases showing a text-book clinical picture. Further, the process of identifying the species of the isolated fungus is so cumbersome even in a well equipped laboratory, that the empirical use of fungistatic agents in all cases of leucorrhoea in whom a fungus has been demonstrated appears at present to be the more practical course of action.

It would thus appear that the study of vaginal fungus and trichomonas infestation deserves greater attention from both the practising gynaecologist and the research worker.

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