TRICHOMONIASIS: A CLINICAL AND LABORATORY EVALUATION

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SUMMARY

Clinical specimens (high vaginal swabs and urine) were collected from 325 women attending a gynaecologic clinic to determine the incidence of trichomoniasis and other infections of the female genital tract. The use of three diagnostic techniques viz., wet smear, Giemsa smear and culture, in detecting Trichomonas vaginalis from clinical specimens was evaluated. T. vaginalis was detected in 25.33% of 225 cases designated as the study group and in 10% of 100 healthy women designated as the control group. Although the majority of positive findings was achieved using all the three diagnostic methods, the culture method was found to be most sensitive.

Introduction

Trichomoniasis is a frequently encountered condition in both, gynaecologic and venereologic clinics. It has been estimated that about 180 million women all over the world are affected by it annually (Honigberg, 1978). There is a varied symptomatology associated with infection of the genitourinary tract of men and women with *Trichomonas vaginalis* (Natrajan, 1967). Asymptomatic carriership is also quite a common condition. Hence, accuracy of diagnosis is extremely important in the detection and subsequent eradication of the parasite.

The aims of the present investigation were to isolate and identify *Trichomonas vaginalis* from the lower genital tract of women attending a gynaecologic clinic and to assess the asymptomatic infestation rate. We have also compared the efficacy of three diagnostic procedures viz. wet smear, Giemsa staining and culture, in the detection of *T. vaginalis*.

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Material and Methods

The patient population studied, comprised 325 women attending the gynaecologic out-patient department of the L.T.M.G. Hospital, Sion, Bombay 22. Of these, 225 patients represented the study group and 100 healthy women with no gynaecologic complaints represented the control group. Most of the patients belonged to the lower socio-economic strata of society.

Four high vaginal swabs and freshly voided midstream urine were collected from each patient. Two swabs were dipped into modified AC medium (Chatterjee and Ray, 1979), for the cultivation of *T. vaginalis* and the other two were col-

lected in Stuart's transport medium for the preparation of smears and for culturing of mycoplasmas, bacteria and fungi. The pH of the vagina was checked using pH indicator strips. Relevant details were recorded from each patient regarding age, clinical history etc.

Wet mounts were scrutinised under low power and high power magnification for motile *T. vaginalis* trophozoites, clue cells and yeast cells. Giemsa-stained smears were scanned under oil immersion objective for *T. vaginalis* trophozoites and inclusion bodies of *Chlamydia trachomatis*. Subcultures were made from the specimen tubes into fresh AC medium after 24 and 48 hours in all cases and all the tubes were incubated at 37°C, aerobically upto one week.

The sensitivity of modified AC medium in supporting the growth of *T. vaginalis* was evaluated by preparing serial 10 fold dilutions of the organisms in the medium until the last tube theoretically received only 1 cell. The tubes were incubated aerobically at 37°C until macroscopically visible growth was observed.

Mycoplasms, bacteria and fungi were all cultivated using standard techniques (Sonnenwirth and Jarett, 1970).

Results

T. vaginalis was isolated from 57 of the 225 study cases (25.33%) and 10 of the 100 control cases (10%). The difference between the isolation rates in the above two groups was statistically significant ($p < .01$). The overall positivity of *T. vagisalis* in the 325 cases was 20.62% (67/325). Table I gives an account of the various microorganisms isolated from the female genital tract in the study and control groups.

TABLE I

Isolation Rates of the Various Vaginal Microflora in the Study and Control groups

Organism	Study Group 225 cases		Control Group 100 cases		p value
	No.	%	No.	%	
1 Trichomonas vaginalis	57	25.33	10	10.00	<.01
2 Candida spp.	46	20.4	14	14.00	>.05
3 The Mycoplasmas	80	35.6	16	16.00	=.001
(a) Unreaplasma urearlyticum	67	29.8	15	15.00	=.01
(b) Mycompasma hominis	13	5.7	1	1.00	>0.05
4 Chlamydia trachomatis (inclusion bodies only)	16	7.1	1	1.00	<0.05
5 Bacteria					
(a) Gardnerella vaginalis (by clue cell identification)	31	13.8	3	3.00	<.01
(b) Neisseria gonorrhoeae	11	4.9	0	0.0	>.05
(c) Staph. aureus (Coagulase +)	25	11.1	8	8.00	NS
(d) Gr. B streptococci	11	4.9	4	4.00	NS
(e) Streptococcus viridans	2	0.9	0	0	NS
(f) Escherichia coli	32	14.2	10	10.0	NS
(g) Klebsiella supp.	7	3.1	2	2.00	NS
(h) Proteus spp.	2	0.9	0	0	NS

NS: Not significant.

In the present study, the presence of a vaginal discharge of an abnormal texture or volume, erosion on cervix and the presence of a pronounced leucocytic reaction i.e. the presence of ⩾ 10 WBCs/HPF were all frequently encountered in cases positive for *T. vaginalis*. The difference between their occurrence in *T. vaginalis* positive and negative groups was statistically significant. An elevated pH value of the vagina was also frequently encountered along with *T. vaginalis* infestation. Pruritis and dysuria were encountered with comparable frequencies in groups positive and negative for *T. vaginalis*. Pathogenic and opportunistic micro-organisms such as Beta-haemolytic streptococci, *N. gonorrhoeae* and the mycoplasms were found to co-exist with *T. vaginalis* in a significant number of positive cases. For details, refer to Table II.

Comparison of the three methods of diagnosis

T. vaginalis was detected by any of the three methods in 67 out of 325 cases studied on the whole. 95.5% of the positive findings (64/67) were detected by cultural methods. The wet mount was positive in 82.1% (55/67) of the positive cases, while Giemsa smear could detect only 64.2% (43/67) of the positive cases. A glance at Table III shows that 17.9% of the cases would have been reported as negative had the culture method not been employed. Although organisms could be detected in culture by about 24-48 hours of incubation, high cell counts of the order of 6-7 million parasites per ml were obtained after a few subcultures (three to five).

We found that the modified AC medium of Chatterjee and Ray (1979)

TABLE II

Association of Various Clinical Conditions and micro-organisms alongwith T. vaginalis

Variables	T. vaginalis +ve (n = 67)	T. vaginalis —ve (n = 258)	Signficance
1. Mean age	27.7	25.5	NS
2 Mean pH	5.8	4.5	p <.001
3 Presence of abnormal discharge	50 (74.6)	83 (32.2)	p <.001
4 Pruritis	16 (23.9)	52 (20.2)	NS
5 Dysuria	12 (17.9)	31 (12.01)	NS
6 Erosion on cervix	14 (20.9)	28 (10.9)	p <.001
7 Leucocytic reaction	41 (61.2)	58 (22.5)	p <.001
8 Incidence of other micro-organisms			
(a) C. albicans	14 (20.9)	46 (17.8)	NS
(b) Mycoplasmas	30 (44.8)	66 (25.6)	p <.01
(c) N. gonorrhoeae	7 (10.4)	4 (1.56)	p <.001
(d) G. vaginalis	11 (16.4)	22 (8.5)	NS
(e) Staph. aureus (Coagulase +ve)	7 (10.4)	26 (10.1)	NS
(f) Beta haemolytic streptococci	9 (13.4)	8 (3.1)	Normal Range
(g) Enterobacteraceae	12 (17.9)	41 (15.9)	NS

Key: NS—Not significant.
Figures in brackets indicate %.

TABLE III

Comparison of Wet Mount, Culture and Giemsa Smear in the Detection of T. vaginalis *in the Study and Control Groups Combined*

Methods of Detection			T. vaginalis +ve (67 cases)	
Culture	Wet Mount	Giemsa smear	No.	%
+	+	+	42	62.7
+	+	—	10	14.9
+	—	—	12	17.9
+	—	+	0	0
—	+	—	2	2.99
—	+	+	1	1.5
—	—	+	0	0
*64/67 (95.5)	55/67 (82.1)	43/67 (64.2)	67	(100)

* Total No. of positive findings (%) by each of the three methods of detection.

was highly sensitive, as it was capable of initiating growth from a single cell of *T. vaginalis*. It was also highly efficient as growth could be detected after 24-48 hours of incubation (with an initial inoculum of $\geqslant 10^3$ cells). A longer incubation period (72-96 hours) was required if growth was to be initiated from 1-10 cells. Hence, it follows that even if the clinical material contain a very low number of parasites, the medium was capable of supporting their growth.

Discussion

The reported incidence of *T. vaginalis* infestation varies widely among various studies. This could be attributed partly to differences in diagnostic techniques and partly to the selection of patient population.

In the present study, the incidence of trichomoniasis showed no trend with age among women of the age group 20 through 49 years. *T. vaginalis* was isolated most frequently from vaginas with a pH more than 5.5 and in those with the presence of $\geqslant$ 10 WBC/HPF. Hence, these two factors should serve as useful pointers to the presence of *T. vaginalis*. We also observed that clinical diagnosis of trichomoniasis was not always reliable and that the use of laboratory methods is of utmost importance. This is due to the fact that *T. vaginalis* was isolated in only 74.55% of the cases clinically diagnosed as trichomonal vaginitis. The parasites were also recovered from 16 cases (9.41%) in which the classical symptoms of trichomoniasis were absent and which were not diagnosed as trichomonal vaginitis. These findings are also augmented by the 10% isolation rate in the control group with essentially normal pelvic findings. The importance of using laboratory methods has also been discussed by other workers (Hughes *et al*, 1966; Kulkarni *et al*, 1981; McLellan R. *et al*, 1982). According to McLellan *et al* (1982), the absence of the typical signs and symptoms of trichomoniasis does not diminish the possibility of infection with the organism.

Regarding the ideal method for the detection of *T. vaginalis*, our findings suggest that the simultaneous employment of wet smear and culture methods should be quite satisfactory. While the wet smear has the advantage of specificity and rapidity of diagnosis, the culture method is superior in its sensitivity. Nearly one-fifth of our positive findings apart from the control group would have gone undetected had the culture method not been used. The Giemsa staining method did not prove to be very advantageous. In fact, it is possible that in several cases which were negative by Giemsa but positive by the other two methods, the organisms may have been too scarce or may have undergone dis-

tortion or lysed during the staining procedure and thereby escaped detection.

The failure of *T. vaginalis* to grow in 3 cases (which were positive by microscopic methods) could probably be due to the extreme liability of these strains and/or an overgrowth of antibiotic resistant bacteria or Candida in the modified AC medium—as experienced by us in this study.

References

1. Chatterjee, D. K. and Ray, D. K.: J. Parasitol, 65: 815-816, 1979.
2. Honigberg, B. M.: In: Parasitic Protozoa, Vol. II, 1978, P. 275-424, Ed. Julius P. Kreier. Academic Press Inc. New York, San Francisco, London.
3. Hughes, H. E., Gordon, A. M. and Barr, G. T. D.: J. Obstet. Gynaec. Brit. C'wlth. 73: 821-827, 1966.
4. Kulkarni, R., Bhatia, V. N., Oumachigni, A. and Nayak, P. N.: J. Obstet. Gynaec. India, 31: 143-148, 1981.
5. McLellan R., Spence, M. R.,, Brochman, M., Raffel, L. and Smith, J. L.: Obstet. Gynaec. 60: 30-33, 1982.
6. Najarian, H. H.: Textbook of Medical Parasitology, 1967, Pp. 91-93. The Wiliams and Wilkins Co., Baltimore.
7. Sonnenwirth, A. C. and Jarett, L.: In: Gradwohl's Clinical Laboratory Methods and Diagnosis, Vol. 2, 7th Edn. 1970. The C. V. Mosby Co., St. Louis Toronto, London.