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Serodiagnosis of Trichomoniasis in a Nigerian Population

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Summary. **Vaginal swabs, endocervical secretions, and sera were obtained from 270 Nigerian women aged between 18 and 40 years and screened for trophozoites of *Trichomonas vaginalis* by microscopy and assay of antitrichomonad antibodies by the passive haemagglutination method. Forty-five females (16.6%) yielded *T. vaginalis* in their vaginal swab samples, and antitrichomonal antibodies were found in the endocervical secretions of 48 females (17.7%) of whom 93.7% were confirmed by microscopy to have trophozoites of *T. vaginalis* in their vaginal swabs. No specific antibodies of *T. vaginalis* were detected in sera of any individuals screened. The determined total and differential white blood cells of both *T. vaginalis* infected and noninfected women showed variations from normal levels, but the presence of trichomoniasis increased the deviation, particularly the development of lymphocytosis. The presence of eosinophilia, lymphocytosis, and specific antibodies in endocervical secretions of cases of trichomoniasis without any presence of corresponding antibodies in sera of the individuals screened present a peculiar immunological response.**

Key words—*Trichomonas vaginalis*, Immune response, Nigerian women.

INTRODUCTION

Trichomonas vaginalis has a worldwide distribution with an incidence ranging from 10 to 30% in different regions[1]. The intra-population variations in the incidence of trichomoniasis known to exist have been related to such factors as sex, age, socio-economic class, and personal hygiene[2,3]. However, it is not well understood whether the incidence of trichomoniasis is on the decline following the introduction into the market of effective chemotherapeutic agents.

T. vaginalis, one of the commonest causes of vaginitis in women[4], has a capacity to invade the vagina during childhood, old age, or pregnancy. It has not been fully determined whether previous exposure to *T. vaginalis* infection provides any acquired immunity against subsequent infection by this parasite, although there is information on the pathogenesis of this organism[5,6]. The present study was therefore initiated to determine the serological implications of trichomoniasis with a view to provide an insight into the immunological response of an affected individual.

MATERIALS AND METHODS

Two hundred and seventy Nigerian women aged between 18 and 40 years drawn from the Ante-natal and Post-natal clinics, Sexually-Transmitted Diseases Clinics and General Out-Patient Departments of the University of Benin Teaching Hospital (UBTH) and Central Hospital, Benin City, were investigated. Detailed clinical histories of the participants were obtained. Those on oral contraceptives or steroid treatment were excluded from this study.

Vaginal, high vaginal, and endocervical swab samples were collected from each female before a physical vaginal examination. The endocervical swab, collected with the aid of a sterile speculum, was immediately broken into bijou bottles containing 1.0ml of phosphate buffered saline (PBS) pH 7.2. For the vaginal and high vaginal swabs, 1.5ml of normal saline (0.85g NaCl/100ml sterile distilled water) was added directly into their plastic containers. The samples in bijou bottles were centrifuged at 1,000 × g for 5 min to remove suspended particles. The resultant

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clear supernatant was stored at −20℃ until required for the antibody assay. The vaginal and high vaginal swab samples were processed within one hour after collection for microscopic examination. Follow up samples were collected from each subject. Two sets of blood samples were collected, the first set (5ml each) being collected into a plain plastic blood tube and allowed to clot at room temperature (28 ± 1℃), while the second set consisting of 2.5 ml each was collected in ethylene diamine tetracetic acid (EDTA) tubes and used for cytology. Clotted samples were centrifuged at 1,000 × g for 5 min, and the sera were separated and stored frozen at −20℃ until required for antibody analysis.

Microscopy

One drop each of vaginal and high vaginal swab samples was placed on a clean microscope slide, covered with a cover slip and examined under a Leitz-labourlux 11 microscope using the × 10 and × 40 objective lenses.

Each vaginal mucous sample (0.1 ml) was mixed with 0.01 ml of 1.0% brilliant cresyl blue stain on a clean microscope slide, covered with a cover slip, and examined under the microscope. *T. vaginalis* resists staining by 1% brilliant cresyl blue, while epithelial cells and yeasts are easily stained.

Cytology

Blood samples in EDTA anticoagulant containers were processed for total white blood cells and differential leucocyte counts following the methods outlined by Dacie and Lewis[7].

Serology

Anti-trichomonas antibodies in the endocervial secretion and sera were determined by the passive haemagglutination method outlined by Campbell et al.,[8] with slight modifications: Human group 'O', Rhesus negative erythyocytes were used instead of sheep erythrocytes.

Vaginal swab samples containing *T. vaginalis* were pooled together in a watch glass under a stereomicroscope (Olympus SZH, Gallen Kamp, Loughborough, England) at a magnification of × 160. *T. vaginalis* were pipetted with a Pasteur pipette into a bijou bottle. They were further purified by washing in three changes of phosphate buffered saline (pH 7.2) by centrifugation at 500 × g for 5 min each time. The supernatants were carefully pipetted off and the final deposit was resuspended in 1.0 ml of PBS (pH 7.2). Approximately 4.6 × 10^8 cells / ml of *T. vaginalis* (monitored with improved Neubaur haemocytometer) were frozen immediately at −20℃ and thawed rapidly under running tap water. Freezing and thawing were carried out through three cycles to completely rupture the cells of the *T. vaginalis* present. The resultant preparation was used as a trichomonas antigen in the passive hemagglutination test after determining the protein content by the method according to Lowry et al[9].

Preparation of erythrocytes

Three milliters of human blood group 'O', Rhesus negative, freshly drawn into a container with EDTA anticoagulant was transferred into 10.0 ml of normal saline in a McCartney bottle. The cell suspension was mixed by gentle shaking for 15−30 sec and centrifuged at 500 × g for 5 min, and the recovered sediment was washed in three changes of normal saline. At the last washing, the cell suspension was centrifuged at 1000 × g for 5 min to obtain packed cells. Then 0.5 ml of packed cells was transferred into 20.0 ml of PBS (pH 7.2), with the cell suspension mixed thoroughly by gentle shaking. Following this, 0.3 ml of the cell suspension was mixed with 3.0 ml of 50 μg/ml tannic acid solution and kept at 37℃ in a water bath for 10.0 min. The cell suspension was centrifuged at 500 × g for 5 min and the packed cells obtained were re-suspended in 3.0 ml PBS (pH 7.2) and mixed thoroughly using a Pasteur pipette. This process was repeated twice to produce tanned red blood cells, 1.0 ml of which was coated with 1.0 ml of Trichomonas antigen preparation suspended in 4.0 ml of PBS (pH 6.4) in a McCartney bottle. The bottle of antigen-coated cells was kept at room temperature for 10 min.

The test

Serially numbered test tubes measuring 13 × 100 mm were set up in a rack and each filled with 0.5 ml of each sample (Endocervical secretions or sera) and 0.05 ml of antigen-coated red blood cells. The negative control consisted of 0.5 ml diluent plus 0.05 ml antigen coated red blood cells. Another control consisted of 0.05 ml of negative control red blood cells and 0.5 ml of each test sample. The rack was shaken to effect complete mixing, and tubes were covered with parafilm and allowed to stand at room temperature for 3 h. At the end of the incubation, the tubes were examined visually for agglutination and incubated further at 4℃ for 12−18 h. The tubes were re-examined for agglutination visually and later

microscopically, noting the pattern of settling for the red blood cells at the bottom of the tubes.

Serial dilutions of test samples were made in PBS for quantitative analysis to determine the titre of antibodies present.

RESULTS

The age distribution of individuals screened and the incidence of *T. vaginalis* among the age groups are shown in Table 1. The highest incidence of *T. vaginalis* occurred in the 25 year-old age group (23.25%) and the least incidence was found in the 35 year-old age group (9.67%); individuals in the 40 year-old age group recorded zero incidences of *T. vaginalis*.

The categorization of individuals on the basis of their clinical symptoms and the incidence of *T. vaginalis* in each group are shown in Table 2. Pregnant women with vaginal discharges had the highest incidence of *T. vaginalis* (54.6%), while cases of puerperium with vaginal discharges had the lowest incidence (16.6%). Individuals drawn from the Sexually Transmitted Diseases Clinics (STD) with clinical symptoms ranging from vaginal discharges, low abdominal pains, pruritus vulva, and vulvovaganitis showed a high incidence of *T. vaginalis* (an average of 32.8%).

Table 3 shows the blood cytology of individuals investigated in this study. The presence of *T. vaginalis* infection was associated with high eosinophils, high lymphocytes, and high total leucocytes count (though within normal range).

Table 4 shows the anti-trichomonad antibodies titre recorded in the groups of women screened. Non-pregnant females with gynecological problems yielded the highest titre of antibodies in their endocervical secretions (1 : 128). A low level of antibodies (1 : 4) was recorded in three females with no clinical symptoms.

DISCUSSION

The incidence of *T. vaginalis* infestation observed in this investigation showed wide variation with respect to both age (Table 1) and clinical symptoms (Table 2). The highest incidence of *T. vaginalis* observed in the 25 year-old age group (Table 1) may be related to their active sexual life, which may increase the rate of exposure of individuals to infection. This view is supported by the zero incidence of the parasite in the 40 year-old age group, who fall into a segment when sexual activities-especially with multiple partners-may be low, except amongst those involved in prosti-

Table 1. Percentage distribution by age of *Trichomonas vaginalis* among the individuals screened

	Age range in years and groups				
	A	B	C	D	E
	20-24	25-29	30-34	35-39	40-44
Number studied	47	96	92	31	14
Number with *T. vaginalis* infection	10	20	15	3	0
Percentage incidence	21.29	23.25	16.30	9.67	0

Table 2. Relationship of clinical symptoms to percentage incidence of *Trichomonas vaginalis*

Clinical symptoms	Groups of individuals screened*				
	Pregnant women	Peurperium	Non-pregnant	STD cases	Others
Vaginal discharge	13 (7., 53.9)	12 (2., 16.7)	10 (3., 30.0)	20 (6., 30.0)	-
Low abdominal pains	9 (0., 0.0)	13 (0., 0.0)	9 (1., 11.1)	10 (4., 40.0)	-
Pruritus vulva	7 (1., 14.8)	5 (0., 0.0)	3 (0., 0.0)	7 (2., 28.6)	-
Vulvo-vaginitis	1 (1., 0.0)	6 (1., 16.7)	7 (2., 28.6)	3 (1., 33.3)	-
Others	-	-	-	-	135 (15., 11.1)
Total	30 (8., 26.7)	36 (3., 8.3)	29 (6., 20.7)	40 (13., 32.5)	135 (15., 11.1)

*Number with *T. vaginalis* infection and the percentage incidence in brackets.

Table 3. Leucocyte, lymphocyte and eosinophil profile of cases with and without *Trichomonas vaginalis*

		Mean leucocyte count (To the nearest whole number) ±S. E.				Percentage eosinophil (Mean±S. E.)				Percentage lymphocyte (Mean±S. E.)			
		3.0×10^3	4.0×10^3 ±0.21	5.0×10^3 ±0.26	2.0±46	3.0± 0.317	4.0± 0.241	5.0± 0.241	48.0± 0.462	50.0± 1.041	55.0± 1.342	68.0± 1.324	70.0± 2.171
Individuals with *T. vaginalis* infection	(a)	16	8	21	7	4	21	13	9	11	-	2	23
Individuals without *T. vaginalis* infection	(b)	24	12	9	25	8	5	7	9	0	30	6	-
Percentage of total	(a)	35.6	17.8	46.6	15.6	8.9	46.7	28.9	28.9	24.4	-	4.4	51.6
Number of each group:	(b)	53.3	26.7	20.0	55.6	17.8	11.1	15.6	20.0	-	66.7	13.3	-

Table 4. *Trichomonas vaginalis* load, antibody titre and duration of clinical symptoms in women investigated

Groups and number of individuals	*T. vaginalis* load mean (Count/ml±S. E.)	Antibody titre	Duration of symptoms (in days)
Group I: 10 Women pregnant for the first time	$3.2\times10^4\pm0.012$	1 : 64	<21
Group II: 29 Non-pregnant women with gynaecological problems (Nuliparous)	$2.4\times10^3\pm0.014$	1 : 128	>32
Group III: 3 Non-pregnant women with no clinical symptoms (Multigravida)	Nil	1 : 4	Nil
Group IV: 6 Non-pregnant women from STDs clinics	$2.0\times10^4\pm0.016$	1 : 64	Uncertain

Nil, Endocervical secretions sera from blood yielded no detectable level of antibodies.

tution. This view is not at variance with the report by Osoba and Kean[2,10], who associated promiscuity with an increased incidence of sexually transmitted diseases, including trichomoniasis.

On the basis of clinical symptoms, pregnancy and vaginal discharges showed the highest incidence of *T. vaginalis*. Pregnancy is known to lower resistance to infection and has been associated with an increased incidence of trichomoniasis[4,11]. It was, however, observed that puerperium cases had a significantly lower incidence of *T. vaginalis*. This may be related to antimicrobial properties of the amniotic fluid which had recently washed the vagina during childbirth[12].

The blood picture of individuals with trichomoniasis suggests the presence of eosinophilia and lymphocytosis (Table 3). Increases in eosinophils have been associated with parasitic infection[13], and the increase in lymphocytes may indicate a celluar immune response to the presence of *T. vaginalis* infection. However, some of the individuals without trichomoniasis also showed high eosinophils and lymphocytosis (Table 3) which may be due to the presence of other parasitic infection that were not excluded since Nigeria is in the tropics where parasitic infections are known to be endemic[2].

Antibody production in individuals with trichomoniasis was detected in endocervical secretions of females without a corresponding presence of antibodies in their sera. This finding is contrary to the reports by Hoffmann[14] and Honigberg[13], who reported the presence of low titres of antibodies in sera of women chronically infected by *T. vaginalis*. The differences in our findings may be due to differences in the sensitivity of assay methods employed or the type of individuals screened, since many of the indi-

viduals investigated in this study were not chronically infected with *T. vaginalis* (Table 4). On the other hand, if the absence of antibodies in the sera of individuals investigated represents an inability of *T. vaginalis* to elicit sera circulating antibody formation, then the method of presentation of the parasite antigen and the dose level may be among the determining factors in the immune response of an infected individual. Until these considerations are resolved, it seems the main immunological response to *T. vaginalis* is limited to tissue surfaces, possibly involving secretary IgA. This response seems to diminish quickly as soon as the infection is eliminated. These factors may cause a lack of acquired immunity against *T. vaginalis* infection[15,16].

Despite the existence of effective chemotherapeutic agents, the incidence of *T. vaginalis* is not decreasing. This may be due to its high infectivity, particularly for females, and its mode of transmission, which is mainly by sexual contact[1,3]. There is therefore, a need to explore other means of containing the parasitic infection such as increasing personal resistance to infection by *T. vaginalis*. This calls for more studies on the immunological events associated with the pathogenesis of the parasite with a view to commence vaccine production for mass immunization.

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