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IMMUNOCHROMATOGRAPHY IN *TRICHOMONAS VAGINALIS* EPIDEMIOLOGY

By

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ABSTRACT

In the study, cases were collected from Gynecology and Obstetric Department in Mansoura and Minia University- Hospitals. The number of cases examined in the period from June 2008 to June 2009 was 600 cases, from these cases 320 (53.3%) were healthy individuals seeking for medical gynecological advice, the other 280 (46.7%) cases were examined and complaining of vaginitis. As regard cases of vaginitis, the non-pregnant cases were 192 (68.6%) and pregnant cases were 88 (31.4 %(. It was found that there was no significant association between pregnancy and *Trichomonas vaginalis* (p=0.730).

In the present study, other microbes were associated with *T.vaginalis* in (38.9%). It was found that the commonest age with *T.vaginalis* infection was in 20-30 years (10.9%), in 30-40 years it was (6.5%), in 40-50 it was (4.4%), in >50 years it was (3.1%).

The P value of *T. vaginalis* was slightly above the critical level (p=0.052) in pregnancy. In diabetic pregnant cases; *T.vaginalis* was (5%), *C.albicans* (40%) and other microbes (55%). In non-diabetic pregnant cases; *T.vaginalis* was (7.3%), *C. albicans* (38.2%) and other microbes (54.4%).

There was no significant difference between pregnant and non-pregnant prevalences of trichomoniasis (p=0.839) also, in candidiasis (p=1.033) and other microbes (p=0.907).

In the present study, *T. vaginalis* was diagnosed using wet mount microscopy, In Pouch culture and OSOM IC test for antigen detection. The sensitivity of the three tests was 27.8, 72.2 and 100% respectively. So,

IC test proved more effective as a screening test than wet preparation. Also, it detected symptomatic and asymptomatic infections and was rapid, objective, easily to interpret *T. vaginalis* infection without microscopy. However, despite the higher sensitivity recorded by IC assay, it is relatively a costly test compared to the other tests.

INTRODUCTION

The cause of vaginitis / vaginosis cannot be adequately determined solely on the basis of clinical symptoms or physical examination. Vaginitis is caused by bacteria, *Candida* spp. and Trichomondas (Metzger, 1998). A definitive diagnosis of trichomoniasis is based mainly on the detection of the trophozoites in vaginal discharge by direct microscopy technique, with or without the aid of vital staining (Spence et al., 1980, Green Wood and Krick- Hillaire, 1981) and by *in vitro* cultivation of the organisms. Although the latter proved to be 20-30% more than sensitive (Mc Millen, 1989 and Ackers, 1994), the wet film microscopy, being fast and inexpensive, remains the most popular clinical diagnostic tool despite its 70% sensitivity (Fouts and Kraus, 1980, Spence et al., 1980). The parasite releases antigens during growth (Mallinson et al., 1994), proteinases (look-Wood et al., 1979), a cell detaching factor (Krieger et al., 1985) and trichomonas surface proteins. These factors were found to be immunogenic (Alderele and Garza, 1984; Garber et al., 1986; Bozner et al., 1992 and Azab et al., 1992). Some of these factors are capable of including pathogenic changes in cell cultures similar to those produced in the presence of trichomonads and may be responsible for the parasite virulence (Manson and Forman, 1980; Krieger et al., 1985; Garber et al., 1989; Gerber and Chunk-Favel,1990).

So, the detection of antigen in trichomonasis may not only be a useful diagnostic tool but may also correlate with clinical presentation. The aim of this work was to use a sensitive and specific method for diagnosis of antigen in epidemiological study of trichomoniasis.

MATERIALS AND METHODS

The study extended from 7/2008 to 9/2009. This work was carried on 600 cases, 320 of which were healthy and 280 showed vaginitis. Out of 280 cases, 192 of the vaginitis were non-pregnant and 88 were pregnant women of different ages ranging from 20->50 years old, with discharge (103) and without discharge (177), attending the out and inpatient clinics of Gynecology and Obstetric Department of Mansoura and Minia University Hospitals.

A history was taken from the patients in a specially prepared sheets including: name, age, number of children, taking antibiotics, antiseptic, vaginal douches or not, past history of trichomoniasis and treatment. All cases were examined gynecologically and accordingly the studied cases were divided into:

Group A: 320 cases. None of the women in this group had any symptoms relevant to infection of genital tract.. Examination revealed a normal whitish, creamy vaginal mucosa.

Group B: 280 patients had advice on contraception or wanted a health check-up. All women in this group were free from any gynecological lesion . These

were subdivided according to the following categories:

(1) Pregnancy: 192 cases non-pregnant and 88 cases pregnant.

(2) Discharge: 103 cases with discharge and 177cases without discharge.

The category of pregnancy was subdivided into:

a. Diabetic: 25 cases non-pregnant and 20 cases pregnant.

b. Non-diabetic; non-pregnant: 167 cases and pregnant: 68 cases.

Collection of samples

The patients were instructed to have no vaginal douching or local treatment for at least 3 days before collection of samples. With the patient in lithotomy position, a sterile non-lubricated speculum was inserted into the vagina. The vagina was examined for signs of vaginitis. For determination of the cause of vaginitis, two swabs were taken for bacteria and two swabs for demonstration of *Candida* and three swabs for identification of *Trichomonas* organisms and antigens.

a. Microscopic detection of *T. vaginalis* in wet preparation of vaginal samples: The vaginal specimen was diluted with a drop of normal saline solution, covered by a cover-slip and examined by a light microscopy by 10x to screen for *Trichomonas* motility. If indicated, materials were examined by 40x for confirmation. The examination was continued for three minutes (Krieger et al., 1988 and Beal et al., 1992).

b. **Culturing of *T. vaginalis* in In pouch TV (Biomed diagnostic Santa Clara, Calif.):** The In pouch TV culture medium contained trypticase, protease, maltose, amino acids, salts and antimicrobial agents in phosphate-buffered saline. The In pouch consists of a high barrier, oxygen-resistant plastic bag with two V-shaped chambers connected by a narrow passage. The bag was besides inoculated squeezing the fluid from the top of the In Pouch bag downward to the bottom to avoid fluid leakage, then cotton swab was admitted, the swab was swirled in the upper chamber, mixed in the medium, forcing the inoculum into the lower chamber and the swab was discarded. The bags were sealed with metal tabs to prevent reopening and transported to the laboratory. The pouches were inoculated at 37 °C in a 5% CO_2 incubator. Direct microscopic examination of the plastic pouch was performed in the same way as wet mount at 24 hours. If the result was negative, re-evaluation was done on the 2nd and 5th days.

c. **Immunochromatographic lateral flow antigen detection vaginal swabs (OSOM Kit for *T. vaginalis*, Genzyme diagnostic Cambridge, Mass)**: Swabs were stored immediately at 4°C until processed within 24 hours (EI-Moamly and Rashad, 2008).

The qualitative assay test used the capillary flow dipstick technology. Swab samples were brought to room temperature before examined. Only 0.5 ml of phosphate-buffered saline pH 7.4, containing 0.5% Triton X-l00x 0.01% $NaNO_3$ was put in a test tube. The vaginal swab was inserted into the tube, reentered 10 times vigorously and allowed to soak for one minute. To express as much liquid as possible from the swab, it was squeezed against the tube's sides and

swab was withdrawn. The test dip-stick was placed into the soluble sample. The results were read at 10 minutes. The positive results were proved by the appearance of two lines (test and control zones). Negative results, appearance of only one red line at the central area. The nonvalid results showed no line at the central zone (Peruski and Peruski, 2003).

Statistical analysis:

Non-parametric statistical methods were used. Frequency, mean, standard deviation and standard error of mean were used to describe data.

P value was considered significant if less than 0.05.These tests were run on an IBM compatible personal computer using the Statistical Package for Social Scientists(SPSS) for windows 10 (SPSS Inc. Chicago IL.USA).

RESULTS AND DISCUSSION

It could be noticed in our study that few features of the history and physical examination which were taken as guides by the physician to identify the etiologic agent were not always correct and many cases diagnosed by gynecologist as candidiasis revealed on microbiological investigation that it was not candidiasis but trichomonaisis or non-specific vaginitis (NSV).

Thus depending on symptoms and signs alone is not adequate for diagnosis of the type of vaginitis and smear and culture must be done.

The number of cases examined was 600. From these cases 320 (53.3%) were healthy individuals seeking for medical gynecological advice, the other 280 (46.7%) cases were complaining of vaginitis. As regard cases of vaginitis, the non-pregnant cases were 192 (68.6%) and pregnant cases were 88 (31.4%).

The cases with discharge were found to be 3 cases infected with *T. vaginalis*, 18 cases with *C. albicans*, 29 cases with other microbes and in 53 cases nothing was detected. The cases without discharge were found to be 2 cases with *T. vaginalis*, 34 with *C. albicans*, 46 with other microbes and in 95 cases nothing was detected (Table 1).

In the present study, *T. vaginalis* was diagnosed using wet mount microscopy, In Pouch culture and OSOM IC test for antigen detection. The sensitivity of the three tests was 27.8, 72.2 and 100% respectively. The sensitivity of wet-mount microscopy declined even in short time intervals between collection and examination (Kingston et al., 2003). Also, Huppert et al. (2005) found that the OSOM IC test was more sensitive than wet mount. Pillany et al. (2004) found that the sensitivity and specificity of the Xenostrip TV, and IC- antigen detection assay, were 66.6 and 100% respectively compared to gold standard PCR assay. But, sensitivity and specificity of wet mount were 48.1 % and 100% respectively. So, IC test proved more effective as a screening test than wet preparation. Also, it detected symptomatic and asymptomatic infections and was rapid, objective, easy to interpret *T. vaginalis* infection without microscopy. However, despite the higher sensitivity recorded by IC assay, it is relatively a costly test compared to the other tests.

T. vaginalis is the most widely prevalent non-viral STD world wide disease (Weinstock et al., 2004). But, the prevalence was underestimated without

accepted guidelines for screening women, as the clinician often depended upon insensitive diagnostic tests. El-meomly and Rashad (2008) showed that the antigen detecting IC assay provided better sensitivity than wet preparation of vaginal samples.

In spite of the easy spread of infection, 50% of trichomoniasis were asymptomatic (Meri et al., 2000). Vaginal discharge was the most common complaint associated with vaginal trichomoniasis (Markell et al., 1999). Women with a trichomoniasis history experienced tubal pregnancy l.7 times as those without a history of infection (Sherman et al., 1988, El-Shazly et al., 2001). It has been associated with several complications in pregnancy (Draper et al., 1995, Cotch et al., 1997). Trichomoniasis has been associated with vaginitis, cervacitis, urethritis and pelvic inflammatory disease (Swygard et al., 2004). Afifi et al. (2000) stated that infection with tichomonads increased the risk of pelvic inflammatory disease, tubal infertility and cervical cancer. Cervical mucosa acts as a barrier against ascending infection above cervix (Hanigpberg, 1978). However, Tyndall et al. (1978) found parasites in the body of the uterus and fallopian tubes. Also, trichomoniasis increases the risk of HIV acquisition and transmission in women (Wang et al., 2001) (Tables 2& 3).

Aizabagi et al. (2005) reported that an infection rate among pregnant women of 66.6%, was more or less near the results of Fernandez et al. (2004) who recorded trichomoniasis among pregnant women in 46% in Cuba. On the other hand, Begum et al. (2003) recorded a 1.4% incidence of trichomoniasis among pregnant women in Bangladesh. This difference may be explained on the basis of the difference in culture used, socio-economic status and hygienic education during pregnancy.

Pregnant women showed a higher infection rate (15%) in Iraq (Mahdi, 1996). A high infection rate (13%) was also evident during menstrual years (11-40 years). Women near or post-menopause (over 40 years) showed an incidence of 3.8%. This may be attributed to the hypertrophy and hyperplasia of the vaginal epithelium as well as the increase of glycogen deposits in such cells, produced by high estrogen level (Lazar, 1970).

T. vaginalis was present in a low frequency (6.4%) in cases of vaginitis including non-pregnant cases. It was found that there was no significant association between pregnancy and *T. vaginalis* (p=0.730).

Other microbes were associated with *T. vaginalis* in (38.9%) of cases. The total number of cases infected with *T. vaginalis* was 18 cases, 5 cases infected with *T. vagi*nalis alone (27.7%), 6 cases infected with *T. vaginalis* and *C. albicans* (33.3%) and 7 cases infected with *T. vaginalis* and other microbes (38.9%).

There was no significant difference between pregnant and non-pregnant prevalence of trichomonaisis (P=0.839), in candidiasis (P=1.033) and other microbes (P=0.907). Browen (1972) in United Kingdom considered that vaginal infection with *Trichomonas* may be more prevalence among gravid women, but Hurley (1979) in United Kingdom reported an incidence of 6% in (1031)

gravid patients. McCormack et al. (1974) study isolated *T. vaginalis* from (19%) of healthy pregnant women and *C. albicans* form (33%) from the same women. The incidence of *T. vaginalis* was high in McCormack et al. (1974) study because *T. vaginalis* was established by cytology which yielded a much higher incidence than either culture or wet film examination.

In the present study, it was found that the commonest age with *T. vaginalis* was in 20-30 years (10.9%), in 30-40 years it was (6.5%), in 40-50 years it was (4.4%), in >50 years it was (3.1 %). The P value of *T. vaginalis* is slightly above the critical level (P=0.0052). In diabetic pregnant cases; *T. vaginalis* was (5%), *C. albicans* (40%) and other microbes (55%). In non-diabetic pregnant cases; *T. vaginalis* was (7.3%), *C. albicans* (38.2%) and other microbes (54.4%) (Tables 4& 5).

Diabetes mellitus is a well known risk factor for candidiasis. However in this sample of pregnant women no difference was observed in candidiasis and trichomoniasis prevalence between diabetic and non-diabetic pregnant women by the primary health care service in controlling their plasma glucose levels (de Leon et al., 2002). As regard the logistic regression of vaginal discharge on the other variable risk factors, it was found that every 10 years; the hazard ratio of vaginal discharge increased by about 25%. Diabetes increases the hazard ratio of vaginal discharge by about 60%. *T. vaginalis* increases the hazard ratio of vaginal discharge by about 49%, *Candida* increases the hazard ratio of vaginal discharge by about 58% and other microbes significantly increase the hazard ratio of vaginal discharge by 23 times (Table 6).

Table (1): Organisms in vaginitis cases in wet mount with and without discharge.

Organism	With discharge		Without discharge		Total	
	N	%	N	%	N	%
T. vaginalis	3	60	2	4	5	100
C.albicans	18	34.6	34	65.4	52	100
Other microbes	29	38.7	46	61.3	75	100
Non-detected	53	35.8	95	64.2	148	100
Total	103	36.8	114	63.2	280	100

Table (2): Trichomoniasis cases by different techniques.

Wet mount		In pouch TV		Antigen detection	
N	%	N	%	N	%
5	27.8	13	72	18	100

Table (3): Organisms isolated from 280 cases with vaginitis by the specific tools.

Organisms	**N**	**%**
T. vaginalis	18	6.4
C. albicans	112	40
Other microbes	150	53.6
Non-detected	20	7

Table (4): Prevalence of *Trichomonas vaginalis* in relation to age.

Age group	**With *T. vaginalis***		**Without *T. vaginalis***		**Total**	
	N	%	N	%	N	%
20-30	8	10.9	65	89.1	73	10
>30-40	5	6.5	71	93.5	76	100
>40-50	3	4.4	64	95.6	67	100
>50	2	3.1	62	96.9	64	100
Total	18	6.4	262	93.6	280	100

Table (5): Trichomoniasis and pregnancy.

Cases	**Positive**		**Negative**		**Total**	
	No	%	No	%	No	%
Non-Pregnant	13	6.7	179	93.3	192	100
Pregnant	5	5.6	83	94.4	88	100
Total	18	6.4	262	93.6	280	100

Table (6): Logistic regression of vaginal discharge on other variable risk factors.

Variable	P.R	S.E of PR	Walχ2	P	Hazard ratio	95% C.I for hazard ratio	
						Lower	Upper
Age(decade)	0.219	0.145	2.279	0.131	1.245	0.973	1.654
Pregnancy	-0.035	0.351	0.010	0.921	0.966	0.485	1.923
DM	0.472	0.421	1.261	0.261	10604	0.703	3.657
Trichomonas	0.399	0.683	0.341	0.559	1.491	0.391	5.687
Candida	0.459	0.412	1.241	0.265	1.583	0.705	3.552
Other microbes	3.148	0.705	19.925	0.001	23.279	5.844	92.722
Constant	-4.736	1.041	20.688	0.001	0.009		

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