The prevalence of Trichomonas Vaginalis in association with other micro-organisms among women with vaginal discharge in Mosul

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ABSTRACT

Objective: To screen women with vaginal discharge for *Trichomonas vaginalis* as well as other pathogens and to follow them up.

Setting: Outpatient Gynaecologic Clinic, Family Planning Clinic, and Antenatal Clinic at Al-Batool Teaching Hospital for Gynaecology and Obstetrics.

Participants: 440 women with vaginal discharge (July 1997 to June 1999).

Intervention: Full medical, gynaecological, sexual and social history was recorded. Clinical examination was carried out. Laboratory investigations including vaginal, cervical and urethral swabs for direct examination, gram-stained smear, as well as culture on appropriate media.

Results: The total number of *Trichomonas vaginalis* infection was 68 (15.5%). The isolated microorganisms in association with *Trichomonas vaginalis* were *Candida albicans* in 19 women (27.9%), *Staphylococcus epidermidis* in 10 women (14.7%), and *Lactobacilli* in six women constituting (8.8%). Both *Diphtheroids* and *Neisseria gonorrheae* showed lower incidences, as only eight women were positive (5.9%) for each. *Escherichia. coli* was present in one patient (1.5%). 24 patients were positive for Trichomonas vaginalis only (35.3%).

The remaining 372 women who were negative for *Trichomonas vaginalis* showed a high rate of infection with Candida species (21.5%), followed by *Staphylococcus epidermidis* (18.3%) and *lactobacilli* (15.3%). Other isolates included *Streptococcus* species (8.6%)., *E.coli* (5.9%), *Klebsiella* (3.8%) ,*Enterococcus fecalis* (3.0%), *Gardnerella vaginalis* (2.6%), *Neisseria gonorrheae* (2.4%), *Proteus* (2.2%), *Diphtheroid* (1.9%), *Staphylococcus aureus* (1.3%) and *Pseudomonas aeruginosa* (0.8%). Moreover, 46 (10.5%) of women showed no growth.

50.0% of infection with *Trichomonas vaginalis* was at age 26-35 years. Considering marital status, the highest rate (76.4%) of infection was among married women followed by divorced (14.7%), widows (5.8%) and singles (2.9%). Pregnant women positive for *Trichomonas vaginalis* represented (45.6%) of cases. 61.7% of infection was among illiterate women.

Metronidazole (flagyl) was given to all in a dose of 250mg orally thrice daily for seven days. Metronidazole was prescribed at the same time to husbands of infected women. Other specific medications (antifungal, antimicrobial agents) were given when indicated either alone or in combination with metropidazole.

Two weeks later only 112 women came for reassessment clinically and by repeating direct smear and culture, that showed fifteen positive cases. Five of them still having *Trichomonas vaginalis*, eight were having *Candida albicans*, and two showed normal smears. Further courses of treatment were given as indicated.

Conclusion: Vaginal discharge is common among women in reproductive life.

Culture is indicated for precise diagnosis

Key wards: Trichomonas vaginalis. Vaginal discharge. Metronidazole.

الخلاصلة

الهدف من اجراء البحث: لتعيين نسبة الإصبابة بالمشعرات المهبلية في النساء اللواتي يعانين من افر از ات مهبلية إضافة إلى البحث عن المسببات الاخرى للافر از ات المهبلية و متابعة الحالات سريريا و مختبريا للبحث في كفاءة المعالجة.

مكان إجراء البحث والإطار الزمني: اجري البحث في العيادة الخارجية للأمراض النسائية والتوليد، عيادة رعنية الحوامل وعيادة تنظيم الأسرة في مستشفى البتول التعليمي للولادة والنسائيات في الموصل.

المشاركون: ٤٤٠ امر أة من النساء اللواتي لديهن افر از ات مهبلية للفترة من تموز ١٩٩٧ إلى حزير ان ١٩٩٩.

طريقة إجراء البحث: تم اخذ المعلومات السريرية المتعلقة بشكوى المريضة وتاريخ المرض والحالة الاجتماعية والتعليمية، ثم اجراء الفحص السريري والفحوصات المختبرية اللازمة.

النتانج: تبين من الدراسة أن نسبة الإصابة بالمشعرات المهبلية هي ٦٨ امرأة ٥٠٥١% من مجموع ٤٤٠ امرأة خضعت للدراسة، وان هنالك عدد من الأحياء المجهرية المصاحبة للمشعرات المهبلية في أربعة وستونّ حالة وهي حسب تدرج نسبة الإصابة كما يلي: فطريات المهبل بنسبة ٢٧,٩%، البكتريا العنقودية بنسبة ٢,٧١%، بكترياً العصيات الحليبية بنسبة ٨٨٨%، الجر اثيم المسببة للسيلان بنسبة ٩٠٥%، ومن نوع الدفترويد ٥٠٩%، أما عصيات

القولون ١,٥%. بينما كانت أربعة وعشرون حالة (٣٥,٦%) من الإصابات مقتصرة فقط على المشعرات المهبلية بدون بكتريا مصاحبة.

ظهرت أعلى نسبة للإصابة بداء المشعرات في الأعمار بين ٢٦-٣٥ سنة بنسبة ٥٠% وان ٧٦,٤% من النساء

المصابات كنّ متزوجات ٥٠٦٠ من النساء المصابات كنّ حوامل، وإن ١١٧ هنهن كن غير متعلمات. تمت المعالجة باختيار جرع مناسبة من دواء فلاجيل في حالة الإصابة بداء المشعرات لكل من النساء وأزواجهن مع اضافة معالجات اخرى في حالة وجود اكثر من جرثومة.

تمت متابعة مئة و اثنى عشر من الحالات بعد اسبوعين او اكثر من ابتداء المعالجة ودر اسة مدى فاعلية العلاج باعادة الفحص السريري والمختبري وتبين ان هناك خمسة عشرة امرأة فقط بقين يعانين من افرازات مهبلية، خمسةً منهن مصابات بداء المشّعرات وثمانية اخريات مصابات بفطريات المهبل، واثنتان فحصهما طبيعي وقد تم الشفاء

بتكر ار المعالجة. التوصيات: تجنب المعالجة العشوائية للنساء اللواتي يعانين من افرازات مهبلية لاحتمال وجود اكثر من جرثومة مسؤولة عن الاصابة حيث ان استعمال نوعين او اكثر من الدواء بدون التعرف على نوعية الحراثيم المسببة قد يؤخر شفاء الحالة.

encountered in every continent and climate. with no seasonal variability. It has a cosmopolitan distribution and has been identified in all racial groups socioeconomic statuses(1). estimated incidence is more than 170 million cases worldwide(2,3). Infection with Trichomonas vaginalis occurs in women and men at any age, and is common during pregnancy⁽³⁾. The knowledge of other genital pathogens in association with Trichomonas vaginalis is useful in planning treatment and understanding the factors predisposing to trichomonal infection and the factors enhancing the pathogenicity of other microorganisms (4). Vaginal trichomoniasis was found to be associated with Gonococci, Streptococci, Mycoplasma, Diphtheroid(5). Micrococci Enterococci (16) Foutes and Krause⁽⁷⁾ pointed the association trichomoniasis with candidiasis. Similar results were obtained in Sudan by Omer et al⁽⁸⁾ who studied the bacterial vaginal flora among women presenting with leukorrhoea. Trichomonas vaginalis may be a true pathogen, it may act synergistically with bacteria, or it may simply thrive under conditions resulting from other causes (9,10) It has been found that most of the patients with vaginitis are suffering from infection that is caused by Candida species, vaginalis, Trichomonas

richomonal infection has been

vaginalis, or Neisseria gonorrheae(11,12,13) The purpose of this study was to screen women with vaginal discharge Trichomonas vaginalis as well as other

Gardnerella

pathogens; also to follow the patients up after treatment. As far as we know, it is the first study in Mosul area to concentrate on follow up of patients with trichomoniasis after treatment.

PATIENTS AND METHODS

Four-hundred and forty women attended Al-Batool Teaching Hospital for obstetrics and gynecology with a complaint of vaginal discharge were included in the study from July 1997 to June 1999.

All patients were reviewed clinically, i.e. a standard medical, gynecological, sexual, obstetric and social history was taken. General medical examination was carried out, with local examination. Samples for laboratory investigations were taken from vagina, cervix and urethra. The patients were in the age range of 19-60 years, 244 were married (55.45%), 72 were divorced (16.36%), 16 widowed (3.63%) and 8 singles (1.8%).

Specimens were collected during genital examination in the lithotomy position. A cusco's bivalved speculum was inserted and the discharge from the posterior fornix and also from endocervix was collected using sterile cotton-tipped swabs. Low vaginal swabs were taken from unmarried women.

Vaginal Samples:

Direct wet mount preparation [where a drop of vaginal secretion was mixed with a drop of normal saline and examined under the microscope(40 objective)] for

Trichomonas vaginalis, yeast, epithelial cells and bacteria

Amine test: [was performed where the

vaginal secretion was mixed with 2 drops of (10%) KOH. The result was considered positive if a characteristic fishy smell appeared directly after adding KOH], suggesting infection with Gardnerella

vaginalis and also T. vaginalis. · Gram's stained smears for the presence of microorganisms. polymorphonuclear leukocytes (PMN), epithelial cells and clue cells. Finding cells was reported

semiquantitatively as (+, ++, +++) per field indicating few, moderate, or large.

Microbiological methods:

vaginalis. medium

appearance

The specimens were inoculated duplicate of blood agar plates, Gardnerella

agar, MacConkey's agar, Thayer Martin agar, Sabourand's agar and T. vaginalis, medium (CM 1161, Oxoid) (14,15).

One of the blood agar plates was

incubated aerobically in 5%-10% CO2. while the other was incubated anaerobically using Gas Pak Generating Kit (Oxoid). Gardnerella agar was incubated anaerobically, Thayer Martin medium was 5%-10% CO2.

MacConkey's agar, Sabouraud's agar and

were

biochemical

aerobically All plates were incubated at 37°C for twenty-four hours, with a further twenty-four hours incubation when there was no

growth. Cultures for anaerobes incubated for 48 - 72 hours. The isolates were identified by their morphology, colonial Gram's

and standard

tests, in addition to the API identification

system (Bio Merieux, France); T. vaginalis was identified microscopically by observing their motility on wet mounts and by culture. Treatment of infected women directed according to laboratory results. metronidazole (Flagyl) was given to women positive for Trichomonas vaginalis as an oral dose of 250mg three times per day for seven days. Treatment

of the male partner with metronidazole was

recommended at the same time, as a seven

days oral course of 500mg twice daily or as

Other (antifungal and antimicrobial) drugs were given either alone or in combination with metronidazole depending on the pathogen identified. For Candidiasis, either nystatin 100,000U

vaginal tablet once at night for fourteen days, or miconazol cream for seven days, miconazol 200mg vaginal suppository three times /day for three days, or clotrimazol 100mg vaginal tablet once a day for seven days, or twice per day for three days were prescribed; beside advice on local hygiene. choice between these agents depended on their availability and patient's cooperation.

For bacterial vaginosis (increased prevalence of Gardnerella vaginalis, Gram negative rods, Bacteroids, Mycoplasma and mobiluncus), metronidazole was used in the doses for **Trichomonas** same as vaginalis. The alternative for this treatment was either tetracycline, erythromycin or local triple sulfa cream. Different antimicrobial agents were used

alone or in combination with other preparations to treat other types of vaginal infection depending on laboratory results and after excluding contra-indications (drug hypersensitivity, harmfu! effect pregnancy); of these are cephalosporines, gentamycin, amoxycillin, etc. For women with vaginal discharge in

whom no pathogen is isolated, non-specific

treatment was given after excluding local

causes, e.g; cervical erosion. Tetracycline was prescribed for such women for the possibility of having Chlamydia, Mycoplasma or viral infection which were not tested for in our study. Statistical Analysis: Data were analyzed using the(X2) or Two-Tailed probability test to show significant difference between groups. P value of 0.05 or less was

RESULTS

considered to be significant.

Trichomonas vaginalis infection was detected in 68 cases out of 440 women examined (15.5%).

The prevalence of Trichomonas vaginalis infection among different age groups is shown in Table I. The highest rate of infection (50%) was seen at age group 26-35 year.

Table (1): The prevalence of Trichomonas vaginalis infection among different age groups.

Age group	Total number	Number of positive cases	Percent of
(years)	examined		Positive cases
19 – 25	116	14	20.6
26 35	208	34	50.0
36 45	94	18	26.5
46 – over	22	2	2.9
Total	440	68	100
$E = 3 \ V^2 = 2.909 \ D = 0$	422		

DF = 3, $X^* = 2.808$, P = 0.422

single dose regime of 2g orally.

Table (2): The relation of marital status with Trichomonas vaginalis infection.

Marital status	Number of patients examined	Number of Positive cases	Percent of Positive cases
Married	244	52	76.47
Divorced	72	10	14.75
Widow	16	4	5.83
Single	8	2	2.95
total	440	68	100

DF = 3, $X^2 = 1.839$, P = 0.607

Table(3) · The relation of menstrual history with Trichomonas vaginalis infection

Menstrual history	Number examined	Number of positive cases	Percent of positive cases	
Menstruating	102	28	41.3	
Pregnant	263	31	45.6	
lactating	64	7	10.2	
Menopausal	11	2	2.9	
Total	440	68	100	

DF =3, $X^2 = 15.004$, P = 0.002

Table (4): The distribution of Trichomonas vaginalis according to educational levels.

Educational level	Number examined	Number of positive cases	Percent of positive cases
Illiterate	316	42	61.8
Primary	82	14	20.5
Secondary & above	38	12	17.7
Others*	4	0	0.0
Total	440	68	

^{*} college education and higher

Table (5): Microorganisms isolated from vaginal discharge in association with Trichomonas vaginalis in order of frequency.

Isolated microorganisms	Number of positive cases	Percent (%) of positive cases	
No growth (except T.V*)	24	35.3	
Candida albicans	19	27.9	
Staphylococcus epidermidis	10	14.7	
Lactobacilli	6	8.8	
Diphtheroids	4	5.9	
Neisseria gonorrheae	4	5.9	
Eschereshia coli	1	1.5	
Total (T.V)	68	100	

^{*} T.V = Trichomonas vaginalis

Microorganisms isolated

Table 2 shows the prevalence of Trichomonas vaginalis infection in relation to marital status. The highest rate (76.5%) was among married women.

The relationship between Trichomonas vaginalis infection and menstrual history is presented in Table 3. Highest prevalence

was noted among pregnant women (45.6%).

Table 4 shows infection with *Trichomonas* vaginalis in relation to educational level. Illiterate women had the highest rate (61.7%).

Percent (%)of positive cases

Table (6): Microorganisms isolated from vaginal discharge of 372 women without Trichomonas vaginalis infection. Number of positive cases

more gamerne le orace a	Transci or positive edees	1 CICCIII (70)01 positivo cases
No growth of pathogen	46	12.4
Candida albicans	80	21.5
Staphylococcus epidermidis	68	18.3
Lactobacilli	57	15.3
B. haemolytic Streptococcus	18	4.8
Anaerobic Streptococcus	14	3.8
Enterococcus faecalis		3.0
Eschereshia. coli	22	5.9
Klebseilla	14	3.8
Proteus	8	2.2
Gardnerella. vaginalis	10	2.6
Neisseria gonorrhoeae	9	2.4
Diphtheroids	7	1.9
Staphylococcus aureus	5	1.3
Pseudomonas aeroginosa	4	0.8
Total negative trichomoniasis	373	100

Table (7): Microscopical findings of wet mount preparation

Gram stain	Infected with Trichomonas. Vaginalis		Infected with other micro organisms		
	No	%	No	%	
PMN					
(pus cells)					
-	28	41.2	176	47.3	
+	19	27.9	111	29.8	
++	12	17.6	49	13.2	
+++	9	13.3	36	9.7	
	68	100.0	372	100.0	
pithelial cells				<u></u>	
-	30	44.1	126	43.5	
+	22	32.4	141	37.9	
++	9	13.2	42	11.3	
+++	7	10.3	2 7	7.3	
	68	100.0	372	100.0	
Ciue cells				<u> </u>	
_	66	97.1	351	94.4	
+	2	2.9	21	5.6	
	68	100.0	372	100.0	

⁻⁼ no cells , += few , ++ = moderate , +++ heavy

PMN= polymorphonuclear leukocytes

Table (8): The distribution of Trichomonas vaginalis according to clinical symptoms (apart from vaginal discharge)

Symptom	Positive	symptoms
	Number	Percent (%)
Irritation and pruritus	36	53.0
Dyspareunia	21	30.9
dysuria	11	16.1
Total	68	100

Table (9): Results of follow up after treatment.

Period of observation	Number of patients	Results of treatment			
		Success	%	Failure	%
None	340		-	-	
2 weeks	112	97	86.6%	15	13.4
4 weeks	15	11	73.3%	4	26.7

 Table (10): Bacteriological results in patients with residual symptoms after treatment.

Microorganism	Residual symptom			percent (%)
	Discharge	Pruritus		
Trichomonas vaginalis	4	1	5	33.3
yeast	3	5	8	53.3
No pathogen	2		2	13

The isolated microorganisms from vaginal discharges in association with Trichomonas vaginalis infection are presented in Table 5. This table shows that Candida albicans was the dominant pathogen which represented (27.9%). Trichomonas vaginalis was the only pathogen isolated from 24 cases out of the 68 women positive with Trichomonas vaginalis, representing (35.3%). Table 6 shows the frequency of other microorganisms isolated in the remaining 372 women with vaginal discharge who were negative for Trichomonas vaginalis. Table 7 shows the microscopical findings of wet preparation. Clinical symptoms due to Trichomonas vaginalis (apart from vaginal discharge) are presented in Table 8. The data showed that irritation and pruritus were evident among 36 women (53.0%), and dyspareunia was a complaint among 21

women (30.9%), while dysuria was found in 11 women only (18.2%).

After prescribing the therapy that we adopted, patients were instructed to return two weeks later for follow up where only one hundred and twelve of them attended: Table 9. The reassessment included clinical evaluation and repeating laboratory procedures including culture. Fifteen of them (13.4%) were still suffering from vaginal discharge and associated local Trichomonas vaginalis problems. detected in 5(33.3%) women. Candida albicans in 8(53.3%). Negative smear and culture results were seen in 2(13%) women. Metronidazole was given again in the same longer course of courses. antimycotic agents was advised eradicate fungal infection. Broad-spectrum antibiotics were prescribed for those in

whom no pathogen was isolated; an antibiotic course like tetracycline or erythromycin was prescribed for the possibility of Chlamydial infection. Of the fifteen women, eleven were seen three weeks later, where only direct wet mount smear examination was carried-out which was negative.

DISCUSSION

The prevalence of Trichomonas vaginalis infection in our study was (15.5%), however other studies from Iraq recorded different results. In Baghdad the prevalence rate varies between 3.9% to $19.5\%^{(16-20)}$, in Mosul 12.5% and $14\%^{(21.14)}$ respectively, in Basrah 11.3% ⁽²²⁾, in Erbil 10.0%⁽²³⁾ and 7.5% in Kirkuk ⁽²⁴⁾. Higher incidences were reported in London and Georgia^(25,7) respectively. The incidence trichomoniasis is as high as 56% in patients attending STD clinics (26). This rate depends on many factors including age, sexual activity, number of sexual partners, other STD, sexual customs, phase of menstrual cycle, technique of examination, specimen collection and laboratory technique. The higher incidence of Trichomonas vaginalis in the Western countries than those obtained in Iraq could be attributed to the local habits; also the promiscuous sexual practice which is common in the western countries and unusual in our community. Hence there is much less chance of spreading the parasite in our community.

The highest incidence (50.0%) of infection by *Trichomonas vaginalis* was reported among females at age group 26-35 year. This finding is in accordance with those reported by other workers (27,23,18) who emphasized that infection occurs mostly at ages of greatest sexual activity. However lower rate (2.9%) of infection was noted among age group 46 years and over, which is in agreement with Al-Najar (20).

In the present study the highest rate of infection was recorded among married patients (76.5%) which is in agreement with those reported by Mohammed and Al-Mallah^(21,23)

The study also showed that the highest incidence (45.6%) of infection was seen among pregnant women. A prevalence of (29.0 %) was reported in Kirkuk⁽²³⁾, and (18.6%) in Basrah. High prevalence was also reported by Poria⁽²⁹⁾. It seems that pregnancy encourages the growth of the parasite through: high estrogen, high pH, large amount of glycogen in the vaginal epithelium and the generally reduced body immunity during pregnancy^(30,31). In addition doctors are usually reluctant to prescribe

metronidazole empirically (without proved indication) during pregnancy because of possible teratogenicity.

The highest rate (61.47%) of infection was seen among illiterate women which was in accordance with those reported by others^(16,19,23).

Many reports have been published on the association of *Trichomonas vaginalis* infection and other microorganisms in patients with vaginal discharge. In the present study *Candida* species was the commonest microorganism isolated (27.9%). This is similar to the results obtained by others^(23, 27) who reported an incidence of (30.0%) and (20.3%) respectively. However Morton ⁽³²⁾ found that the association rate between trichomoniasis and candidiasis was only (7.4%).

In the current study an association was found between isolation of *Trichomonas vaginalis* and *Neisseria gonorrhoeae* (5.9%). This result was higher than that obtained by Omer *et al*⁽²⁷⁾ of 2.5%. It was different from that reported by Morton⁽³²⁾ of 42-46%.

Metronidazole (Flagyl) continued to be the drug of first choice to treat trihomoniasis and bacterial vaginosis since its introduction in 1960⁽³³, 34). Considering treatment of our 68 *Trichomonas vaginalis* infected women the cure rate obtained was (92.6%). The result is comparable to those collected by Dunlop & Wisdom⁽³⁵⁾, and Gsonka, (36) who reported (90.0%), and (92.1%), respectively.

We have chosen the longer course of Flagyl (250 mg tds for seven days) as we believed that the majority of women infested with *Trichomonas vaginalis* are also harboring anaerobic bacteria which respond adequately only with prolonged courses (33, 34). For the male partner, the single dose regimen was chosen as it looked more convenient and practical.

A second course of metronidazole (same initial dose) was repeated for those women who continued to suffer from the infection. This treatment was quite successful in eradicating the parasite. Treatment failure is explained by re-infection by the parasite or due to presence of resistant strains of *Trichomonas vaginalis*. Similar failure rate (11 of 101 women) was registered by Gsonka⁽³⁶⁾.

Metronidazole was completely successful in treating bacterial vaginosis, given in the same dose used for *Trichomonas vaginalis*.

Considering *Candidiasis*, ninety nine positive cases (22.5%) were identified. With appropriate treatment, we achieved a cure rate of 91 cases, (91.9%).

Eight cases (8.1%) positive with Candida were seen at follow up, five of these women were previously positive for Trichomonas vaginalis. This comes in agreement with Gsonka⁽³⁶⁾ who concluded increased incidence of fungi seen in vaginal secretions after successful eradication of Trichomonas vaginalis which may be due to unmasking of these organisms rather than real increased incidence. That is why routine prophylactic measures against fungi are not recommended.

Combination chemotherapy was used in many of our patients as recommended by results of culture, and as suggested by the gynaegologist.

Conclusion: Depending on the results obtained in the current study it was found that the prevalence of *Trichomonas vaginalis* infection in Mosul area was (15.5%). The highest rate of infection was detected among women at child-bearing age (19-35)year, and among illiterate women.

Different microorganisms were found in association with *Trichomonas vaginalis*, like *Candida* species, S. epidermidis, *Lactobacilli*, *Diphtheroids*, *N.gonorrhoeae*, and *E schereshia*.coli.

Clinical symptoms and signs alone are insensitive predictors of the causative pathogen of vaginal discharge, beside the possibility of the presence of more than one pathogen in the same woman. Treatment should be based on results of culture.

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