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Trichomonas Vaginalis and Associated Microorganisms in Women with Vaginal Discharge in Kerkuk-Iraq

Kerkük'de Vajinal Akıntılı Kadınlarda Trichomonas vaginalis ve Eşlik Eden Mikroorganizmalar

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Abstract

Objectives: Trichomonas vaginalis is an anaerobic flagellated protozoan parasite which is the causative agent of trichomoniasis. Our aim is to show the frequency of Trichomonas vaginalis and other microorganisms among women with vaginal discharge and their husbands attending Kirkuk hospitals during the period from the beginning of July 2007 till the end of May 2008.

Materials & Methods: Laboratory investigations included vaginal, cervical swabs and urine for direct examination; culture of swabs and urine on appropriate media was applied and blood samples for serological test were carried out.

Results: In women with vaginal discharges, the rate of T. vaginalis was 2.8%. The associated organisms were Candida albicans 8.0%, Neisseria gonorrhoea 0.8%. The mixed infections of T. vaginalis and C. albicans were 1.6%. The frequency of co-infection of T. vaginalis and associated Escherichia coli was 1.6%, Staphylococcus aureus 0.8%, and proteus 0.4%.

The highest frequency of pathogenic microorganisms in vaginal swabs was Staph. aureus 12.4%, followed by E. coli 11.6% and C. albicans 8.0%.

The rate of pathogenic agents in urine samples was lower than those in vaginal swabs. In urethral discharge, the rate of N. gonorrhea was 35.0% while E. coli was 5.0%. The highest rate of infections in vaginal swabs and urine was at the age group 15-29 years of age.

Conclusions: The pathogenic infections associated with trichomoniasis are more common in females than males, their rate were highest among 15-29 years of age. The rate of gonorrhea was high in urethral discharge.

Key words: Trichomonas vaginalis, vaginal discharge, Iraq

Özet

Amaç: Trichomonas vaginalis trikomoniazisin etkeni olan anaerobik flajellalı protozoan bir parazittir. Amacımız, Temmuz 2007 -Mayıs 2008 aralığında Kerkük hastanelerine başvuran vajinal akıntılı kadınlarla eşlerinde Trichomonas vaginalis ve diğer mikroorganizmaların sıklığını araştırmaktır.

Materyal ve Metod: Vajinal ve servikal sürüntü ve idrar örnekleri üzerinde laboratuar incelemeleri yapıldı, uygun ortamda sürüntü ve idrar kültürleri ve kan örneklerinde serolojik testler yapıldı.

Bulgular: Vajinal akıntılı kadınlarda T. vaginalis oranı %2.8 iken, eşlik eden mikroorganizmalar %8.0 Candida albicans ve %0.8 Neisseria gonorrhoea olmuştur. T. vaginalis and C. albicans'ın mikst enfeksiyon oranı %1.6, T. vaginalis ve Escherichia coli koenfeksiyon oranı %1.6, Staphylococcus aureus ile % 0.8, proteus ile %0.4 olarak bulunmuştur.

Vajinal sürüntüdeki en yaygın patojen mikroorganizma %12.4 Staph. Aureus, ardından %11.6 E. coli ve %8.0 C. albicans olmuştur.

İdrar örneklerindeki patojen ajan oranı daha düşük bulunmuştur. Üretral sürüntüde N. gonorrhea oranı %35.0, E. coli oranı %.5.0 bulunmuş, vajinal sürüntü ve idrarda en yüksek enfeksiyon oranı 15-29 yaş grubunda bulunmuştur.

Sonuç: Trikomoniazisle ilgili patojen enfeksiyonlar bayanlarda daha sık izlenir, oranı 15-29 yaş grubunda en yüksektir ve idrarda gonore oranı yüksektir.

Anahtar Kelimeler: Trikomonas vajinalis, vajinal akıntı, Irak

Introduction

Trichomonas vaginalis is a parasitic protozoan, that is the cause of trichomoniasis, a sexually transmitted disease of worldwide distribution with variant prevalence in different countries. It is transmitted directly because the trophozoite does not have cyst.[1]

Bacterial vaginosis is a type of vaginal infection that results from the overgrowth of one of several types of bacteria normally present in the vagina, upsetting the natural balance of vaginal bacteria. It is a common condition characterized by a polymicrobial disorder with several anaerobic or facultative bacteria with a reduction or absence of lactobacillus colonization.[2]

The balance of vaginal flora is maintained by the Lactobacilli and it's protective role in preventing vaginal infection by their production of antagonizing compound such as hydrogen peroxide and lactic acid.[3]

The three most common causes of discharge of an infectious etiology are bacterial vaginosis, candidiasis and trichomoniasis.[4] Vaginal discharge is the most commonly observed symptom in women with candidiasis and in women with trichomoniasis.[5]

Materials and Methods

The study was carried out from I st of July 2007 till 30 th of May 2008 on patients attending Kirkuk hospitals (Azadi Teaching Hospital and K1 hospital in North Oil Company) for detection of Trichomonas vaginalis and associated sexually transmitted microorganisms among 250 females and 225 males. The age of patients were ranging from 15-60 years old.

Vaginal swabs, cervical swabs and urine samples were collected from 250 women with vaginal discharges; 225 urine samples and 20 urethral discharges from males.

The vaginal examinations were done by gynecologist, two high vaginal swabs were taken from 250 women and a cervical swab from some women with vaginal discharge. Samples for laboratory investigations were taken from vagina, cervix and urethra. Specimens were collected during genital examination in the lithotomy position.

A cusco's bivalved speculum was inserted and the discharge from the posterior vaginal fornix and also from endocervix was collected using sterile cotton-tipped swabs

Laboratory examinations: Direct wet mount technique was carried out by mixing a drop of vaginal secretion with a drop of normal saline and examined under microscope (X40 objectives) for Trichomonas vaginalis, bacteria, epithelial cells and yeast.

Smears were made from the specimens stained with Gram stain, examined for presence of microorganisms, epithelial cells and polymorphonuclear leukocytes.

Amine test (Whiff test) was done by adding several drops of 10% KOH solution on clean microscopic slide containing a drop of vaginal discharge. The result was considered positive if the characteristic fishy smell appeared, suggesting infection with Gardnerella vaginalis and Trichomonas vaginalis. The slide was left for about 5-15 minutes for examination of candida hyphae. [6,7]

Culture methods: The specimens were inoculated onto duplicate of blood agar plates, Gardnerella agar, MacConkey's agar, Sabourand 's agar and T. vaginalis medium.Some of blood agar plates were incubated aerobically in 5%-10% CO2 while the others were incubated anaerobically using Gas Pak Generating kit (Oxoid). Gardnerella agar was incubated anaerobically. MacConkeys agar, Sabouraud 's agar (Merck, Germany) and T. vaginalis medium (Diamond modified broth (Que-Bac Laboratories-Technical Data 2491/ Canada and CM1161, Oxoid) were incubated aerobically.All plates were incubated at 37 0C for twenty-four hours, with a further twenty-four hour incubation when there was no growth.

Cultures for anaerobes were incubated for 48-72 hours. The isolates were identified by their colony morphology. Gram's stain appearance and standard biochemical tests, in addition to the API identification (Bio Merieux, France); T. vaginalis was identified microscopically by observing their motility on wet mount and by culture .[8] Urine samples were cultured using bacteriological artificial culture media under aerobic condition of cultivation,[9] then urine samples centrifuged, the deposit were examined under microscope for detection of T. vaginalis in wet preparation.[10]

Detection of Chlamydia trachomatis in vaginal swabs, urethral swabs and urine was done by rapid serological test using Chlamydia Rapid Test Device (Chlamydia kit, Acon, USA). Diagnosis of Treponema pallidum was done by detection of immunoglobulin IgG & IgM in serum using rapid chromatography method (TPHA) (Syphilis kit, Acon, USA).

Statistical analysis: The data collected was subjected to statistical analysis. Chi-square ($\chi 2$) test was used to find

statistical difference between the study group, P value less than 0.05 was considered to be significant .[11]

Results

Table 1, shows the distribution of microorganisms in vaginal swabs in direct smears examination and cultures. The highest rate of single infection was Candida albicans (8.0%) followed by Trichomonas vaginalis (2.8%). No Treponema pallidum, Chlamydia trachomatis were detected. In mixed infections, only T. vaginalis and C. albicans were detected at the rate of 1.6%. The overall rate of infection was 13.2%. Statistical analysis showed significant differences between infection rates of microorganisms (P<0.05).

Table 1. The positivity rate of microorganisms associated with vaginal discharge causing STD among 250 samples.

Types of Microorganisms	Positive cases	
	No.	%
Trichomonas vaginalis	7	2.8
Candida albicans	20	8.0
Neisseria gonorrhoeae	2	0.8
Treponema pallidum	0	0
Chlamydia trachomatis	0	0
T. vaginalis & Candida albicans	4	1.6
T. vaginalis & N. gonorrhoeae	0	0
Chl. Trachomatis & C. albicans	0	0
Chl. Trachomatis &T. pallidum	0	0
Total	33	13.2

$\chi 2=53.449$, d.f.=9, (P<0.05)

Table 2. Frequency of Trichomonas vaginalis and associated microorganisms among 250 samples.

Microorganisms	Positive cases	
	Number	Percentage
T. vaginalis & *E. coli*	4	1.6%
T. vaginalis & *Staph. aureus*	2	0.8%
T. vaginalis & Proteus	1	0.4%
Total	7	2.8%

Table 2, shows the frequency of T. vaginalis and associated microorganisms. The rate of T. vaginalis with Escherichia coli was highest (1.6%) followed by T. vaginalis and Staphylococcus aureus (0.8%) and T. vaginalis with Proteus (0.4%) respectively. The overall rate of T. vaginalis and associated microorganisms was 2.8%. Statistically there was no significant difference between infection rates of microorganisms (P>0.05).

Table 3, shows the distribution of pathogenic microorganisms in vaginal swabs. The highest rate was Staph aureus (12.4%) followed by Escherichia coli (11.6%) and Candida albicans (8.0%), β-hemolytic streptococcus 2.8%, Proteus 2.4%, Klebsiella 1.2%, N. gonorrhea 0.8%, Pseudomonas 0.4%. No other pathogenic microorganisms were detected.

Regarding the age groups, at age of 15-29 years old women, the highest rate of infection was Staph. aureus and E. coli (16.9%) followed by C. albicans 8.4%; at 30-44 years old, the highest rate was E. coli (8.7%) followed by Staph. aureus (7.9%), C. albicans 7.0%, while at 45-60 years the highest rate was Staph. aureus (17.5%) followed by E. coli and C. albicans (10%). The overall rate of infections was 16.8% at age of 15-29, followed by 15.6% at age 30-44 years and the lowest was 7.2% at age 45-60 years.

The total rate of infections among different age groups was 99(39.6%). Statistically there was significant difference between groups.

Table 4 shows the distribution of microorganisms in urine samples of females with vaginal discharge. It was found the highest frequency of microorganisms in urine samples was Escherichia coli (6.8%) followed by Staphylococcus aureus (4.8%), B-hemolytic Streptococcus and Proteus (3.2%), Candida albicans (2.0%), Klebsiella (1.2%), Pseudomonas (0.4%) and Neisseria gonorrhoeae (0.8%).

No Gardnerella vaginalis, Chlamydia trachomatis and Treponema pallidum were detected. The overall frequency of microorganisms in urine samples were (22.4%). The highest rate was at age group 15-29 years old (9.2%), followed by 30-44 years (8.0%) and 45-60 years (5.2%) respectively. Statistically there was significant difference between different age groups (P<0.05).

Table 3. Distribution of pathogenic microorganisms in vaginal swabs according to age groups among 250 samples.

Age groups	15-29		30-44		45-60		Total	
No. samples	83		127		40			
Types of Microorganisms	Positive						Positive Cases	
	No.	%	No.	%	No.	%	No.	%
Staph. aureus	14	16.9	10	7.9	7	17.5	31	12.4
Escherichia coli	14	16.9	11	8.7	4	10.0	29	11.6
β-hemolytic streptococcus	2	2.4	4	3.1	1	2.5	7	2.8
Proteus	4	4.8	2	1.6	0	0	6	2.4
Klebsiella	0	0	2	1.6	1	2.5	3	1.2
Pseudomonas	0	0	0	0	1	2.5	1	0.4
N. gonorrhea	1	1.2	1	0.8	0	0	2	0.8
Gard. vaginalis	0	0	0	0	0	0	0	0
Chlamydia trachomatis	0	0	0	0	0	0	0	0
Trep. Pallidum	0	0	0	0	0	0	0	0
Cand. Albicans	7	8.4	9	7.0	4	10	20	8.0
Total	42	16.8	39	15.6	18	7.2	99	39.6

χ^2=8.886, d.f.=2, (P<0.05)

Table 4. Distribution of pathogenic microorganisms in urine samples according to age groups in women with vaginal discharge.

Age groups	15-29		30-44		45-60		Total Positive Cases	
No. samples	83		127		40			
Types of Microorganisms	Positive							
	No.	%	No.	%	No.	%	No.	%
Escherichia coli	9	10.8	5	3.9	3	7.5	17	6.8%
Staph.aureus	4	4.8	4	3.2	4	10.0	12	4.8%
β-hemolytic streptococcus	3	3.6	3	2.4	2	5	8	3.2
Proteus	1	1.2	5	3.9	2	5	8	3.2
Klebsiella	2	2.4	0	0	1	2.5	3	1.2
Pseudomonas	0	0	0	0	1	2.5	1	0.4
N. gonorrhea	1	1.2	1	0.8	0	0	2	0.8
Gard. vaginalis	0	0	0	0	0	0	0	0
Chl.trachomatis	0	0	0	0	0	0	0	0
Trep. Pallidum	0	0	0	0	0	0	0	0
Cand. Albicans	3	3.6	2	1.6	0	0	5	2.0
Total	23	9.2	20	8.0	13	5.2	56	22.4

χ^2=8.886, d.f.=2, (P<0.05)

The frequency of microorganisms in urethral discharge in males is indicated in Table 5. Among 20 urethral discharges examined, it was found that the rate of N. gonorrhoeae was (35%) and E.coli was (5%). The highest rate was among the age group 30-44 years (20.0%), followed by 15-29 years (15.0%) and 45-60 years (5.0%).

Table 6 shows the distribution of microorganisms in urine samples in males. The highest rate was N. gonorrhoeae and Staph. aureus (2.7%) followed by E. coli and Proteus (1.7%), C. albicans (0.9 %), Klebsiella (0.4%) respectively.

The highest rate of infection at age group 15-29 years old was N. gonorrhoeae (8.5%); at 30-44 years old was proteus (2.7%), while at at 45-60 years old was Staph aureus (4.4%). The overall rate of infections in urine of males was (10.2%), the highest rate was at 30-44 years old (4.0%) followed by 15-29 (3.5%) and 45-60 years old (2.7%).

Discussion

The frequency of T. vaginalis in vaginal discharges was 2.8%, however, while other studies recorded different results in different provinces in Iraq. In Mosul, 15.5%, [7] showed 6.9%,[8] 14%,[12] 33%.[13] In Baghdad 9.46% ; [14] in Basra 13% , [15] in Najaf 58.4%,[16] in Erbil 16.4% ,[17] 0.39%, [18] in Sulaimaniya 1.6%.[19] The variation in the percentage of infection in different studies may be due to sample size, period of studies, socioeconomic status of population and techniques used in identification of infection.

Regarding the distribution of T. vaginalis in vaginal discharge in different countries, there are also variations in the rate of infection recorded in different parts of the world. In Saudi Arabia, [20] it was shown that 4.5% were positive among women with vaginal discharge who attended Primary Care Clinic in Riyadh City, while Al-Zanbagi[21] reported the rate of T. vaginalis was 0.7% among women with vaginal discharge in 6 cardinal hospitals in Jeddah city. In Turkey, Yazar et al[22] reported 8.3% among 1613 patients attended Obstetrics and Gynecology Department at Ege University in Izmir. In Republic of Korea, 10.4% of women complaining of vaginal signs and symptoms were found to be infected with T. vaginalis.[23] In Nepal, the vaginal discharge was studied among 300 studied populations T. vaginalis infection was found in 44 cases, the incidence being 14.7%. [24]

A study in Mozambique studied 182 female who suffered from vaginal discharge, found the prevalence of T. vaginalis alone as 2% and it was 11% mixed with other sexually transmitted infections. [25]

In USA, [26] the rate of T. vaginalis was reported as 28% among women with vaginal discharge who attended Sexually Transmitted Diseases Clinic in Alabama University, while it was reported as 23.4% in women suffering from vaginitis. [27] The lower rate of infection in Muslim countries than others may be related to culture and Islamic behavior, regarding cleanness and single partner.

In Sulaimania, Kadir and Fattah[19] found, out of 600 women with vaginal discharge examined, 1.6% were infected.

Table 5. Distribution of microorganisms strains in 20 urethral discharges of males according to age groups

Age groups	15-29		30-44		45-60		Total positive cases (20)	
No. samples examined	7		6		7			
Types of microorganisms	Positive cases							
	No.	%	No.	%	No.	%	No.	%
Neisseria gonorrhea	3	15	4	20	0	0	7	35
Escherichia coli	0	0	0	0	1	5	1	5
Total	3	15	4	20	1	5	8	40

χ^2=3.730, d.f.=2, (P>0.05)

Table 6. Distribution of microorganisms isolates from urine of males according to age groups.

Age groups	15-29		30-44		45-60		Total	
No. sample Examined	47		110		68		Positive Cases	
Types of Microorganisms	Positive							
	No.	%	No.	%	No.	%	No.	%
Neisseria gonorrhoeae	4	8.5	2	1.8	0	0	6	2.7
Staphylococcus aureus	1	2.1	2	1.8	3	4.4	6	2.7
Escherichia coli	2	4.3	1	0.9	1	1.5	4	1.7
Proteus	1	2.1	3	2.7	0	0	4	1.7
Klebsiella	0	0	0	0	1	1.5	1	0.4
Candida albicans	0	0	1	0.9	1	1.5	2	0.9
Total	8	3.5	9	4.0	6	2.7	23	10.2

χ^2=3. 011, d.f.=2, (P>0.05)

The overall rate of T. vaginalis was (2.8%) in females only, while N. gonorrhoeae was 35.8% (35% in males and 0.8% in females).

It is low in comparison to other studies. This indicates that there are a lower number of females in this society harboring the parasites acting as the main reservoir and transmitter to other people and it is representing a real problem that should not be neglected and must receive attention.

They reported high prevalence of T. vaginalis among age group 26-35 years of age followed by 16-25 and 36-45 years, the lowest in 46-55 years of age, this is identical with the study of that proved the prevalence of T. vaginalis was higher in women over age 25 years. [28] This result may be due to the ability of the parasite to alter at the vaginal environment for its survival. In the present study the distribution of microorganisms was high among different age groups; the highest was in urine culture of 15-29 years old.

A study done by Mahdi et al[29] showed that the highest infection rate (12.6%) was found in women of reproductive age. The majority of positive cases were in the age group (20-40) years, while women near or post-menopause showed low incidence of infection, and this is probably due to absence of suitable environment for growth of T. vaginalis.

The overall rate of microorganisms in vaginal swabs was 13.2%. The rate of Candida albicans among patients with vaginal discharges was highest 8.0%. This rate of infection is accordance with Morton[30], who reported that the association rate between trichomoniasis and candidiasis was 7.4%, but lower than that was reported in Mosul city[8], in which the rate of C. albicans was 19.4%., and 27.9%.[7]

In comparison the rate of T. vaginalis and Neisseria gonorrhea in males and females, it was 2.8% in females only. While N. gonorrhea was 33.8% (35% in males and 0.8% in females). It may be related to the presence of symptoms in male and easy detection.

The rate of T. vaginalis in vaginal swabs in women was 2.8%, while mixed infections of T. vaginalis and Candida albicans was 1.6%. No infection was found in urine samples of women with vaginal discharges; urine of males and urethral discharge of males, this may be due to sample size in this study.

The lower rate of infection in urine samples than vaginal swabs is reported in many studies.

In Tikrit, Kadir and Kadir[31] found that the rate of infection in vaginal swab was 3.04% and in urine samples 1.3%; in Kirkuk, Dawood et al[32] found the rate of infection in vaginal swab and urine samples were 1.2% and 0.2% respectively.

The lower rate of infection in urine samples than vaginal swabs is expected as the genital tract is the normal habitat of T. vaginalis[33], therefore higher rate of infection was found in the vaginal secretion than in urine. In addition, the type of eastern toilets used by our people, differ from that used by European people reduces the chance of contamination and transmission of infection.

Because Trichomonas trophozoites can survive in urine for up to several hours [34] and survival rates in urine were less than 10% after 24 hours of exposure, therefore contact with toilet contaminated with parasite may have constituted a source of infection .[35]

Most studies diagnose T. vaginalis on vaginal discharge examination as it is superior to urine samples examination, but some studies showed that using molecular method such as polymerase chain reaction technique (PCR) are able to detect T. vaginalis in the presence of one cell at least from urine sample and vaginal discharge.[36]

In southern Iraq it was recorded that PCR increases the sensitivity of urine samples about 90% and vaginal discharge 100% for detection of T. vaginalis. [37] Therefore, it is recommended to use PCR technique for detection of microorganisms in urine and vaginal discharge.

Conclusion

From the results of this study, it was found the rate of T vaginalis in patients with vaginal discharge was 2.8%, it was associated with C. albicans, N. gonorrhea, Staph aureus and proteus. No T. vaginalis was detected among males. The distribution of microorganisms in females was higher than males, except N. gonorrhea was higher in males. Their rate in vaginal swabs was greater than in urine and are highest among 15-29 years old.

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