

RESEARCH ARTICLE

***Trichomonas vaginalis* Infection and Risk Factors among Infertile Women in Gaza, Palestine**

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ABSTRACT

Objectives: Objectives: *Trichomonas vaginalis* is a sexually transmitted disease and can infect a wide range of human beings. The present study was conducted to determine the prevalence and risk factors associated with Trichomoniasis infection among infertile women in Gaza city, Palestine.

Methods: A descriptive-analytical cross-sectional study was conducted between December 2013 to April 2014 in Al Basma medical center in Gaza city. Ethical approval was obtained from the Helsinki Committee in Gaza to conduct the study, and all participants gave consent. A total of 120 endocervical swabs were collected from females attending the center to manage delayed conception. The samples were processed using the PCR technique with Tv1-Tv2 primers in Islamic Universities laboratories. In addition, a structured questionnaire was conducted with all participants regarding socio-demographic data, risk factors, and symptomatology.

Results: The prevalence of *T. vaginalis* was 5.8% among the participants. There was a significant relation between infection and age of the patients, marriage age, the number of previous vaginal infections, and inguinal erythema ($p=0.01$). A higher infection rate was found among unemployed, low educated, mid-zone residents, and live in crowded houses, and married women with smoker husbands.

Conclusion: We concluded that the prevalence of Trichomoniasis is not attributed mainly to personal hygiene, so in future studies, the examination tools used in the clinics should be included /tested to sort the source of infection. *J Microbiol Infect Dis* 2021; 11(4):209-216.

Keywords: Prevalence, *Trichomonas vaginalis*, infertility, Risk Factors, Gaza

INTRODUCTION

Trichomoniasis is a sexually transmitted disease (STD) caused by *Trichomonas vaginalis* (*T. vaginalis*). Trichomoniasis is one of the most common sexually transmitted diseases (STD) in young, sexually active women [1]. According to the World Health Organization (WHO), there are 170-190 million cases of Trichomoniasis annually worldwide [2]. Humans are the only hosts that harbor *T. vaginalis*. In contrast to other protozoa, it present as trophozoite form only with no cyst stage. Transmission of the infection occurs mainly through sexual intercourse. The

parasite is usually isolated from female vaginal and male urethral secretions [3]. Women infected with *T. vaginalis* may complain of variable symptoms as frothy yellow-green vaginal discharge and vulvar irritation. Men with Trichomoniasis may complain of nongonococcal urethritis [4]. Several studies have highlighted that at least 80% of *T. vaginalis* infections are asymptomatic. In addition to the risk of transmission to sex partners, *T. vaginalis* infection has been associated with as much as a 2.7-fold increase in the risk of Human Immunodeficiency Virus acquisition, a 1.3-fold increase in the risk of preterm labor, infertility as well as a 4.7-fold

increase in the risk of pelvic inflammatory disease [5], prostate cancer [6] and cervical cancer [7]. Trichomoniasis is usually underdiagnosed due to multiple factors, including the absence of routine testing [8] (the low sensitivity of a commonly used diagnostic technique (wet mount microscopy) and nonspecific symptomatology. Two studies on Trichomoniasis among Palestinian women in Gaza Strip and West Bank were carried out [9-10].

The present study was conducted to determine the prevalence and risk factors associated with Trichomoniasis infection among infertile women in Gaza city, Palestine.

METHODS

Design and population

The study population was married Palestinian women who attended Al Basma Medical Center in Gaza from December 2013 to April 2014. A total of 120 females agreed to participate in this cross-sectional study. The participants were aged 18-45 years, who were non-menstruating at the time of sample collection, attended the center for attempting conception mainly through assisted reproductive techniques. The patients who received antibiotics were excluded from the study. Ethical approval was obtained from Helsinki Committee, and the Palestinian Health Research Council dated 8-12-2014 and informed consent from each participant. A questionnaire that included information like age, residence area, level of education, infertility status, and symptoms complained by each patient was used to determine the risk factors.

After speculum insertion, the endocervical swab was obtained using a Dacron-tipped by a specialized gynecologist. The swab was inserted 1–2 cm into the endocervical canal, followed by two or three rotations. A swab was collected according to CDC guidelines and as indicated by the manufacturer [11]. The swab was placed in a tube and labeled with the patient's name and date of collection and then sent to the lab for DNA processing.

Genomic DNA was isolated from the specimens using a DNA extraction solution (Epicentre QuickExtract™, Madison, USA). The extracted DNA can be used for PCR analysis, genetic or environmental research, and human screening. The primer set Tv1

(TAA TGG CAG AAT CTT TGG AG), and Tv2 (GAA CTT TAA CCG AAG GAC TTC) was used is designed to amplify a DNA product of 312 bp, according to Dwivedi et al., [12]. After preparation of the PCR reaction (20 µl), amplification was carried out in a thermocycler (BioRad, USA Biometra). The amplification procedure included 4 min of denaturation at 94°C, followed by 40 cycles each consisting of 10 s of denaturation at 94 °C, 45 s of annealing at 55.8°C, and 15 s of extension at 72 0C. A final extension step at 72 0C for 5 min was included. The quality of the isolated DNA was determined by running 8 µl of each sample with a 50 bp ladder on 2% agarose gel containing Ethidium bromide, then the DNA sample was visualized on a Gel Documentation System.

Statistical analysis

Statistical analysis was carried out using the statistical package for social sciences (SPSS) version 21 for windows. Frequency means and standard deviation were calculated. Confidence intervals (95%) were reported when appropriate. Chi-square test (χ^2) and Fisher exact tests were applied to compare socio-demographic, infertility status, risk factors, and signs and symptoms with *T. vaginalis* infection. Multivariate analysis (logistic regression) was used to study the risk factors.

RESULTS

The present study results showed that 7 (5.8%) of women were positive for *T. vaginalis* using the PCR technique.

Frequency of T. vaginalis infection

The infection rate of *T. vaginalis* infection was higher among women aged 31-45 years old and in cases from Mid-zone governorate. In contrast, women from Gaza North and Rafah showed no infection. The infected subjects had no history of working. The infection rate was highest in primary and preparatory educational levels regarding education. Some infertile women lived in a house shared with their husbands' families. The infection rate was (7%) compared to (5.2%) in women living in a separate house. Infection rates were highest among families with more than two subjects living in the same house. Residency status details are shown in Table 1.

Most infertile women were married at 15-20 years old. The highest infection rate was observed in women married at the age >30 years old with a significant difference and marriage duration for more than ten years ($p=0.001$). All the infected infertile women were married to a non-relative husband. It was found that infertile women with delayed conception before completing the first year of marriage had a higher rate of *T. vaginalis* (14.2%) (Table 2).

The highest rate of *T. vaginalis* Infection was among passive smoker infertile women and among patients who denied a history of previous vaginal infection with a statistically significant relationship ($P=0.04$) or UTI in the last month. None of the infected infertile

women were using vaginal wash. Multivariate analysis (logistic regression) was used to study the possible risk factors among the infected infertile women and are listed in Table 3.

Out of seven infertile women who had no abnormal vaginal discharge, one patient complained of yellow discharge with a bad smell. The highest infection rate was among patients who complained of pruritus and dyspareunia. The presence of vulvar erythema was statistically significant ($P=0.01$). All infected infertile women denied any complaint of lower abdominal pain, dysuria, and bleeding after intercourse. Symptoms associated with *T. vaginalis* infection are shown in Table 4.

Table 1. Frequency of *T. vaginalis* infection according to the socio-demographic characters

Variable	Positive PCR results No./n	(%)	χ^2 , P-value
Age group (yrs)			
16-20	0/15	0	3.441, 0.179
21-30	3/71	4.2	
31-45	4/34	11.8	
Governorates:			
Gaza North	0/26	0	6.060, 0.195
Gaza	3/57	5.3	
Mid zone	3/18	16.7	
Khanyounis	1/12	8.3	
Rafah	0/7	0	
Occupation:			
Working	0/17	0	1.227, 0.333
Not working	7/103	6.8	
Education:			
Primary and Preparatory	2/15	18.2	2.406, 0.300
Secondary	3/43	7	
University	2/62	3.2	
Residency status:			
Independent (separate)	4/77	5.2	0.159, 0.488
Shared with husband family	3/43	7	
Number of subjects in the same house			
2	2/48	4.2	0.405, 0.416
>2	5/72	6.9	

n=total number of items.

Table 2. Frequency of *T. vaginalis* infection according to marriage details and infertility status.

Variable	Positive PCR results No./n	%	χ^2 , P-value
Age at marriage (yrs)			
15-20	4/64	6.3	15.322, 0.001*
21-30	0/47	0	
> 30	3/9	33.3	
Duration of marriage (yrs)			
< 1	2/17	11.8	4.884, 0.087
1-10	2/80	2.5	
> 10	3/23	13	
Consanguinity			
Not relative	7/92	7.6	2.262, 0.147
Relative	0/28	0	
Infertility status:			
Delayed conception for < 1 yr	2/14	14.3	2.185, 0.335
Primary infertility	3/72	4.2	
Secondary infertility	2/34	5.9	

*0.001 is significant

Table 3. Frequency of *T. vaginalis* infection according to risk factors.

Risk factors	Positive PCR results No./n	%	χ^2 , P-value
Smoking			
Active	0/0	0	0.733, 0.318
Passive	4/50	8	
Husband non smoker	3/70	4.3	
History of vaginal infections			
Yes	0/41	0	3.858, 0.049**
No	7/79	8.9	
History of UTI in the last month			
Yes	1/21	4.8	0.053, 0.646
No	6/99	6.1	
Weekly vaginal wash use			
Yes	0/8	0	0.531, 0.609
No	7/112	6.3	

** 0.049 is significant

Table 4. Frequency of *T. vaginalis* infection according to symptoms reported by the infertile women.

Symptoms	Positive PCR results, No./n	%	χ^2 , P-value
Abnormal vaginal discharge			
Yes (n=35)	1/35	2.9	0.797, 0.340
Color:			
White	0/21	0	2.974, 0.396
Green	0/4	0	
Yellow	1/9	11.1	
Black	0/1	0	
Smell:			
No odor	0/23	8.3	1.973, 0.343
Bad odor	1/12	7.1	
No	6/85		
Pruritus (vulvar itching)			
Yes	1/15	6.7	0.022, 0.617
No	6/105	5.7	
Lower abdominal pain			
Yes	0/19	0	1.398, 0.289
No	7/101	6.9	
Dysuria (burning urination)			
Yes	0/12	0	0.826, 0.469
No	7/108	6.5	
Dyspareunia (painful intercourse)			
Yes	2/21	9.5	0.631, 0.354
No	5/99	5.1	
Contact bleeding (bleeding after intercourse)			
Yes	0/3	0	0.191, 0.834
No	7/117	6	
Vulvar erythema			
Yes	2/4	50	14.695, 0.017
No	5/116	4.3	

Multivariate analysis (logistic regression) of the data showed the infection has a significant relationship with duration of marriage (0.016) and dyspareunia (0.038).

DISCUSSION

This study is the first study in Palestine assessing *T. vaginalis* prevalence using molecular method (PCR) and showed a prevalence of 5.8% among infertile women attending infertility management center in Gaza strip.

When comparing this study with other studies conducted in neighboring countries, the prevalence is higher than in Jordan, with a prevalence rate of 0.7% [13].

The prevalence in this study was similar to Tunisia (5.6%) [14]. However, in Gaza, the prevalence is higher than Kingdom of Saudi Arabia at 1.43% [15]. This difference can be attributed to many factors, including sample size, study setting, techniques used, population socioeconomic status, and religion.

To make the comparison easier, looking for a study that matches our study methodology will be a suitable way. Kelestemur et al. reported a 4.5% prevalence from Turkey among infertile women (n=160) [16].

The prevalence rate found in the present study is expected, as Palestine is considered an Islamic country where sexual relationships can be achieved by marriage according to religion and law. This also decreases the possibility of sexual transmission as a route of infection but does not prevent indirect transmission as toilet seats and medical specula. Indirect transmission is usually linked to poor hygiene levels, and poverty observed in Gaza Strip. These issues highlight the importance of considering social and cultural background differences before interpreting different communities' results.

In this study, the rate of *T. vaginalis* infection was higher in women aged 31-45 yr old, similar to the result reported by Stemmer et al. [17], where the infection rate increases as age increases. This prevalence may be due to reproductive hormone levels in older women, especially postmenopausal [18]. The marriage age was found also considerable importance. Statistically significant results showed higher infection rates among infertile women of marriage at <20 yrs old and >30. Kaestle et al. reported that higher odds of STI were found in younger ages at first intercourse compared with older ages [19].

As in many conservative Arabic countries, in Palestine, early age marriage below 20-year-old is common. According to the Palestinian Central Bureau of Statistics (PCBS), the median age of marriage for Palestinian females is 19.5 years [20]. The high infection rate among this group can be explained by the biological predisposition of the immature cervix to infection if exposed [21].

On the other hand, usually in old age marriage, women usually try to get pregnant soon after marriage which may lead here to attend conception consultation clinics earlier. Subsequently, they will undergo gynecological investigations (as hysteroscopy, IUI, IVF), which favor transmission of the parasite by inoculation through infected instruments. In the present study, all infected women were married to a relative husband, which is common in Palestine.

A higher detection rate of infection was among infertile women who attended the center before completing the first year of marriage. This may be due to a change in the vaginal environment (mainly after intercourse) which decreases the acidity of the vaginal PH favoring the growth of such parasites [22].

The highest infection rate was among women living in the mid-zone of Gaza. According to our knowledge, there are no infertility management centers in the Mid-zone, so most infertile women from the Mid-zone attend the centers in Gaza city, which is closer to them than other governorates.

A higher detection rate of *T. vaginalis* infection was found in women with preparatory educational levels by Mbizvo et al.. They found a positive association between reproductive tract infections (as *T. vaginalis*) and lower educational levels [23]. Thus, it may be possible that better education was associated with the early seeking of confirmation of diagnosis and treatment for STDs, as explained by Fernando et al. [24].

When discussing residency, the infection rate was higher in women living in houses shared with their husband's families. This may favor indirect transmission via common towels and toilet seats in such houses. This also may explain the increased risk of infection when the number of subjects living together in the same house is increased. As reported by Scheidell, [25] crowding was associated with STDs.

Poverty and crowding is a major problem in the Gaza strip. According to Safi and Elnamrouy, [26] poverty in Palestine was associated with household size (large number of children less than 18 yrs old or a large number of unemployed adults more than 18 yrs) and with a high dependency ratio (No. of family members/No. of bedroom). In addition, the low economic status and unemployment of the husbands are common. All these factors prevent the couples from having an independent house. Instead, they will live in a room (in the father of law' house) sharing a kitchen and bathroom with minimal privacy and minimal hygienic conditions.

Although the cross-sectional design is not the perfect design for defining risk factors, it can draw attention to some risks. For example, smoking is considered a risk factor for Trichomoniasis [27]. Our results showed that

as high infection detection among women married to a smoker partner.

Statistically significant results showed denial of any history of previous vaginal infection in women infected with *T. vaginalis*. This may be due to inaccurate diagnosis of these episodes or insufficient information delivered from physicians to the patients and knowledge status of those patients. All infertile women denied any history of STDs. However, knowledge of STDs is limited in the community. There are neither electronic health recording systems nor STDs clinics in MOH in the Gaza strip, making it impossible to get such information from patient files.

All infected patients denied usage of vaginal wash weekly. This was different from the results by Sutton et al. [28], that they found that the infection rate is higher among females using vaginal douching. As explained by Rosenberg and Phillips, [29] douching reduces the density of normal vaginal flora, so there may be a predisposition to colonization by such sexually transmitted pathogens.

Our finding is different from Sivaranjini et al., who found that among the clinical signs in women with vaginal discharge, having a profuse, malodorous, frothy, and mucopurulent or purulent discharge was significantly associated with Trichomoniasis [30]. Similarly, Petrin et al. recorded that the classic presentation of *T. vaginalis* is a purulent, foul-smelling vaginal discharge associated with pruritis dysuria and dyspareunia [31]. However, these classic symptoms are only seen 20% of the sickness time. Statistically significant results showed that vulvar erythema is a significant complaint among infected patients, as reported by the findings of Wølnner-Hanssen et al., [32].

This study has also some limitations. Although PCR is considered a rapid, sensitive technique as their results can be available within four hrs. of sample collection, it is still expensive compared to the conventional methods' cost concerning the limited resources of the MOH in the Gaza strip.

Conclusion

There was no significant correlation between *T. vaginalis* infection and female personal hygiene practice and residency conditions, which may favor iatrogenic cause as a source of infection.

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